DRUG COMBINATIONS IN PALLIATIVE CARE: A COMPATIBILITY STUDY

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ABSTRACT

End of life care often involves the use of combinations of more than one drug in a syringe for administration by a continuous subcutaneous infusion using a syringe driver or pump. This is considered to be an effective method of symptom management and can provide a good control of symptoms.

The combining of more than one drug however raises the question of compatibility. Even though many combinations of drugs have been successfully used in clinical practice in specialist areas without supporting laboratory data, the dangers of this practice are unknown and it is important to study the chemical and physical compatibilities of combining more than one drug for administration. The publication of National Patient Safety Alert 20: 'Promoting safer use of injectable medicines' by the NPSA in March 2007 addressed this issue.

Assessment of the Marie Curie Hospice database (Liverpool) has identified ten supportive drug combinations that have been used for continuous subcutaneous infusion and their associated dose. In this study the compatibility of these supportive drug combinations was assessed with each of the following opioids: morphine, diamorphine, hydromorphone, oxycodone and alfentanil.

The preparation of the combinations replicated clinical practice as close as possible. The combinations were prepared in BD syringes and a CME T34 syringe pump together with its administration set was used for the infusion of the prepared combinations at ambient temperature. Assessment of the combinations, including appearance, pH and compatibility assays, was performed at time zero, then 3 hours, 6 hours and 24 hours after the start of the infusion. High performance liquid chromatography (HPLC) was the principal technique for compatibility assessment.

This study proposed 50 combinations for compatibility assessment. Of the 45 combinations tested, the results identified 40 combinations as compatible. At the stated concentrations these 40 combinations were considered compatible with the diluents, the syringes and administration sets used. No incompatibility was evident in any of the combinations tested however the data obtained could not be used to confirm compatibility in five of the combinations tested.

The results from this study are a step in the right direction in providing healthcare professionals with data on compatibility of drug combinations used in end of life care. Further work in this area is required to fully support current and future practices where multiple drugs are combined in single administration forms to ensure effective treatment and patient safety.

CONTENTS

1. INTRODUCTION

The World Health Organisation (WHO) defines palliative care as improving the quality of life of patients with life threatening illness, along with that of their families. This process involves identifying, assessing and treating the pain and also any other potential problems in order to prevent and relieve suffering *(WHO)*. One of the aims of palliative care is to provide relief from pain and other distressing symptoms *(NCPC)*. In order to treat this pain and other symptoms in end of life care, different drugs are often combined and administered using a syringe driver or pump. It is the combining of these drugs and their compatibility that is the focus of this research.

For patients that are at the end of life through illness, it is the delivery of a high standard of care in the last few days or hours of life that involves palliative care. In order to achieve this level of care, injectable medicines are sometimes combined and used to either treat a single symptom, or multiple symptoms that need intervention simultaneously *(Rose and Currow, 2009)*. It is often found that the oral route of administration is no longer an option, which could be due to vomiting or a decreased level of consciousness. Alternative routes of administration include rectal administration, transdermal, intravenous injections or infusions *(McLeod and Flowers, 2006)*. The preferred method, however, is subcutaneous administration, which can be bolus injections via an indwelling subcutaneous cannula or through a battery operated syringe driver for continuous infusion *(Rose and Currow, 2009)*. The use of a syringe driver or pump to deliver a continuous subcutaneous infusion (CSCI) is the route that is widely used in the treatment of end of life patients. It is considered to be an effective method of symptom management, is less invasive than other methods and can provide good control of symptoms.

1.1. Drug Combinations

The combining of injectable medicines for CSCI in end of life care could potentially result in interactions between and amongst the different drugs, which health care staff may not be aware of. The publication of Patient Safety Alert 20: 'Promoting safer use of injectable medicines' by the NPSA in March 2007 actually addresses this issue. It includes the recommendation that healthcare staff need to have full technical information about drug stability in solution and compatibility information for commonly used drug mixtures in specialist areas only, which covers the mixing of injectable medicines in the same syringe *(NPSA, 2007)*.

The availability of compatibility data however is limited and therefore necessitates this study. Healthcare staff have access to reference sources to investigate the combining of particular drugs to see if they are compatible. The reference sources include: The Syringe Driver book *(Dickman & Schneider, 2011)*, the Palliative Care Formulary, the website www.palliativedrugs.com used to access the Syringe Driver Survey Database (SDSD) and local guidelines *(Centre for Pharmacy Postgraduate Education Hospital Pharmacy Learning Programme)*. These sources mainly concern the visual compatibility of drug combinations. This however, cannot rule out chemical and physical incompatibilities within the syringe, which may potentially lead to decreased drug concentrations or the possibility of degradants occurring. For example, from clinical observations the following mixture is incompatible; diamorphine 40mg, cyclizine 200mg and metoclopramide 60mg in water for injection in a final volume of 17ml *(Dickman et al, 2005)*. Many combinations have been successfully used in clinical practice without supporting laboratory data; however, laboratory testing is the best confirmation of stability and compatibility. The compatibility data from reference sources may state the dose of the drug but the final volume may vary, hence, concentration is what needs to be considered, and of course, drug combinations may only be compatible at certain concentrations.

For treating symptoms in end of life care, it is common that at least two or three drugs are mixed in the same syringe and, on occasion, a fourth may be required. However, even though the drugs have product licences, the majority are unlicensed for administration via a CSCI, but most pharmaceutical companies are aware that 'off label' usage occurs *(Dickman et al, 2005)*. Not all drugs are suitable to be given by continuous subcutaneous infusion. This is because the formulation can be too acidic or too alkaline or contain particular excipients leading to site irritation *(Dickman et al, 2005)*. The drugs are chosen based upon the specific therapeutic effect they exert in controlling common end of life symptoms. There are numerous combinations of two, three or four drugs that can be combined in syringes to control relevant symptoms. So, where does one start to determine which combinations, and at what concentrations, to assess for compatibility?

The Marie Curie Palliative Care Institute Liverpool (MCPCIL) is a collaboration between the voluntary sector, academia and the NHS. It is a 'leading organisation in the field of palliative care, with a specific focus on end of life care and care of the dying' *(MCPCIL)*. One of the sites it operates from is the Marie Curie Hospice in Liverpool. The hospice developed a database that recorded combinations of drugs administered by CSCI over at least a three year period. Therefore, this database was used to determine which

drug combinations should be tested in this current work. Assessment of the database identified that over 90% of the combinations consisted of an opioid plus at least one other supportive drug. The supportive drugs are chosen for specific symptoms, which are aided by practice guidelines. The supportive drugs were selected as they were the most frequent combinations encountered in practice, and for the opioid it was based on the frequency of prescribing of the available opioids. This assessment also identified the appropriate doses to study. For the supportive drugs, the most commonly used doses were employed, but in the case of the opioids the dose representing the 75th percentile were used. The database identified a broad range of opioid doses, therefore, this range was split into percentiles and the 75th percentile was taken to represent the maximum dose most likely to be encountered in clinical practice. The supportive drug combinations and associated doses are listed in table 1.1. The dose is the total amount of each drug stated in the combination that is required to be added to the syringe being used to administer the CSCI. These are then diluted to a final volume of 20ml, therefore, the actual concentration of drug in the syringe is the dose divided by the final syringe volume.

Table 1.1. The supportive drug combinations and associated doses

Combination Number	Supportive Drug Combination
1	Cyclizine 150mg and haloperidol 5mg
2	Cyclizine 150mg and midazolam 30mg
3	Levomepromazine 25mg and metoclopramide 60mg
4	Haloperidol 5mg and midazolam 30mg
5	Hyoscine 120mg and levomepromazine 12.5mg
6	Levomepromazine 50mg and midazolam 30mg
7	Metoclopramide 30mg and midazolam 30mg
8	Cyclizine 150mg, glycopyrronium 1.2mg and haloperidol 5mg
9	Cyclizine 150mg, haloperidol 5mg and midazolam 30mg
10	Glycopyrronium 2.4mg, levomepromazine 50mg and midazolam 30mg

In clinical practice the supportive drugs are often, but not always, combined with one of several available opioids before administration. The database search has also identified the opioids that have been used and their associated doses; these are listed in table 1.2. Again, the dose is the total amount of each opioid required to be added to the syringe for administering the CSCI.

Table 1.2. The opioids and their associated doses

Opioid	Dose
Morphine	120mg
Diamorphine	100mg
Oxycodone	50mg
Alfentanil	10mg

Apart from the four opioids mentioned above, it is known that the opioid hydromorphone is used in other countries. Currently, this drug is not used in the United Kingdom (UK), due to the availability of the other opioids, but there is the prospect that it will soon be available in the UK. Therefore, to broaden the potential of this research, this opioid was considered as well. The dose of 50mg of hydromorphone was chosen based on practical experience.

From the combinations identified it can be seen that there are 12 different drugs to consider. They are: alfentanil, cyclizine, diamorphine, glycopyrronium, haloperidol, hydromorphone, hyoscine butylbromide, levomepromazine, metoclopramide, midazolam, morphine and oxycodone. Each of these drugs will have been formulated as a salt of the weak acid or weak base of that drug. For example, morphine tartrate and morphine sulphate are salts of morphine. Most drug formulations will contain the salt that is the most appropriate for its intended purpose.

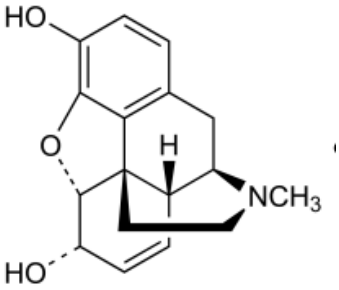
1.2. Drugs Used in End of Life Care

Assessment of the Marie Curie Hospice database has essentially provided a starting point of which combinations to assess for compatibility. It has identified the most commonly encountered supportive drug combinations and the dose of different opioids that are also used, so the logical starting point would be to assess each of the supportive drug combinations in combination with each of the five different opioids. The drugs used in end of life care vary in their mode of action and they are all used to control a variety of symptoms that may have developed in the patient.

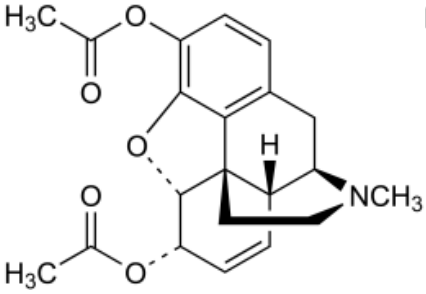
Patients receiving end of life care often experience pain. In order to control this pain, by either reducing or eliminating it, an opioid analgesic is used. Alfentanil, diamorphine, hydromorphone, morphine and oxycodone are all classed as opioid analgesics. Their structures can be seen in figure 1.1.

Figure 1.1. Structures of opioid analgesics

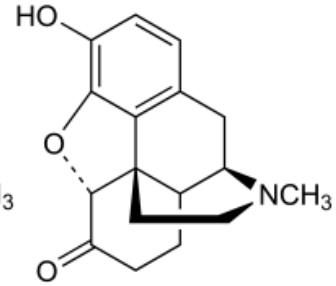
Morphine



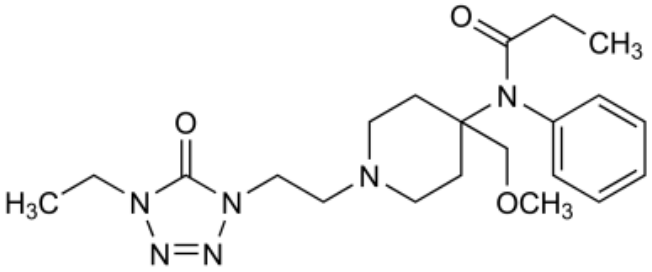
Diamorphine



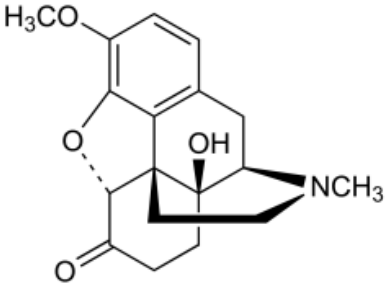
Hydromorphone



Alfentanil



Oxycodone

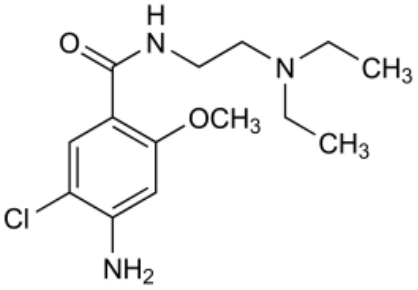


Alfentanil is a short acting opioid analgesic. It is related to fentanyl and is known to be more lipid soluble than morphine *(Martindale)*. Diamorphine is an acetylated morphine derivative. It is used for relieving severe pain and is a more potent analgesic than morphine. It is preferred by some as the opioid of choice because of its high water solubility. Hydromorphone is a phenanthrene derivative and is used for the relief of moderate to severe pain. It is related to morphine and has greater analgesic potency, which again allows it to be a suitable alternative to morphine due to its greater solubility in water allowing for a smaller dose volume to be used *(Martindale)*. Morphine is a phenanthrene derivative and is used to relieve moderate to severe pain and associated anxiety *(Martindale)*. It is now becoming the opioid of choice because it is the least expensive *(Dickman et al, 2005)*. Oxycodone is also a phenanthrene derivative and is used for relieving moderate to severe pain. The choice of opioid is also dependent on other conditions or symptoms the patient may have.

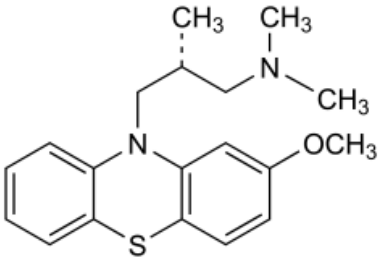
Nausea and vomiting is a common symptom in end of life care patients and in order to stop, prevent or relieve it, an antiemetic is used. The drugs cyclizine, haloperidol, levomepromazine and metoclopramide have antiemetic properties. Refer to figure 1.2 for their structures.

Figure 1.2. Structures of antiemetics

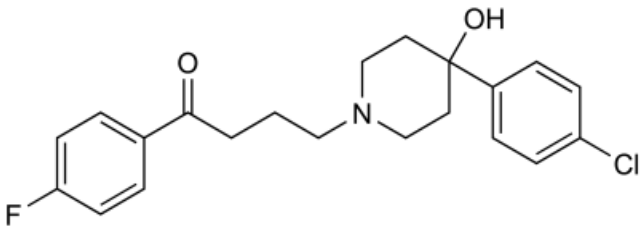
Metoclopramide



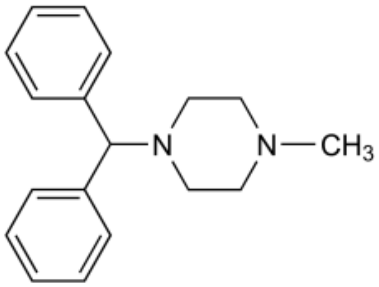
Levomepromazine



Haloperidol



Cyclizine

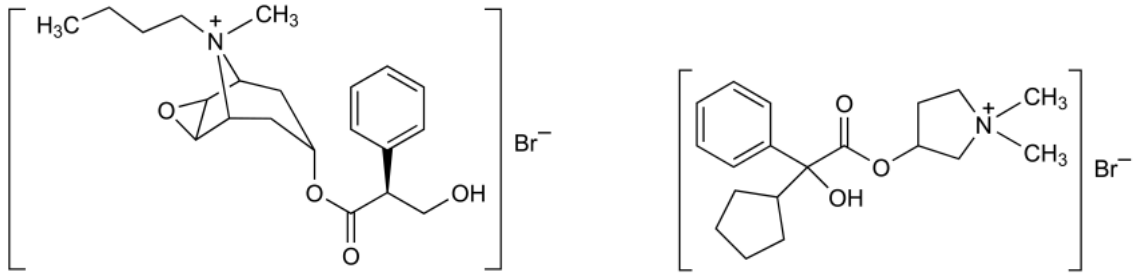


Cyclizine is a piperazine derivative. It is a sedating antihistamine with antimuscarinic activity which makes it effective against nausea and vomiting *(Martindale, BNF)*. Haloperidol is a butyrophenone. It is therapeutically classed as an antipsychotic but is used in the management of nausea and vomiting in end of life care, along with the treatment of restlessness and confusion *(Martindale)*. In the case of levomepromazine it is a phenothiazine that has antihistaminic actions and antiemetic activity; hence allowing it to be used for the treatment of nausea and vomiting but due to its sedative properties the dose is limited *(BNF, Martindale)*. Clinically it is the most effective antiemetic, even at low doses, but sedation can be an issue. Metoclopramide hydrochloride is a substituted benzamide and has prokinetic and antiemetic properties, allowing it to be used in the treatment of nausea and vomiting, particularly if gastro-intestinal disorders are apparent *(BNF, Martindale)*.

In order to control bowel colic and excessive respiratory secretions, glycopyrronium bromide and hyoscine butylbromide are used *(BNF)*. They are both quaternary ammonium derivatives and are classed as antimuscarinic drugs (anticholinergic), see figure 1.3 for their structures.

Figure 1.3. Structures of antimuscarinic drugs

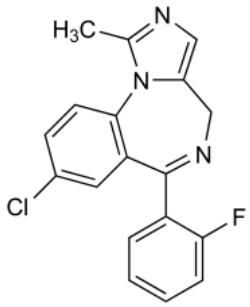
Hyoscine butylbromide **Glycopyrronium**



In addition, a sedative and antiepileptic agent such as midazolam (figure 1.4) may need to be administered.

Figure 1.4. Structure of midazolam

Midazolam



Midazolam is a benzodiazepine, which is used in the treatement of restlessness, confusion and convulsions *(BNF, Martindale)*. Both levomepromazine and, to a lesser extent, haloperidol exhibit sedative effects and are also used for restlessness and confusion *(BNF)*.

Patients receiving end of life care may experience a variety of symptoms that are addressed by different drugs. In practice, combining these drugs allows multiple symptoms to be controlled via a single administration point using CSCI.

The chemistry of the drugs being combined needs to be considered. When drugs are combined together and administered by CSCI, there is the possibility that the drugs interact together possibly resulting in the activity of the drugs being affected e.g. their affect can be increased or decreased. This interaction is not limited to in the body as it can actually occur when combining the different drugs in a syringe prior to administration. However, unless there is a physical change in the appearance of the solution it would not

be known that there is any interaction and this may only become apparent by the therapeutic affects in the patient. However, laboratory analysis can help identify possible interactions.

The pH and pK_a values of the drug components can give us some information on how we expect the different drugs to react on combination. The pH, which is a measure of the acidity of a solution, of the drug components being combined can affect the stability of the mixture and/or solubility of the individual drugs *(Dickman et al, 2005)*. The pK_a value indicates the acidic or basic properties of the drug component and the pH at which the concentration of the ionised and non-ionised forms of the drug component are at equal concentrations. For example, a value less than 2 indicates a strong acid, a value between 2 and 7 a weak acid, a value between 7 and 10 a weak base and those with pKa values greater than 10 are strong bases. This ability to behave as an acid or a base is not only reliant on itself but on the hydrogen ion concentration in the environment it is in. The combining of different drug components in a syringe is often used for continuous subcutaneous infusions where the syringe contents are infused directly into the blood. It is known that the concentration of hydrogen ions in different body fluids varies but blood has a pH range of 7.35 to 7.45. The degree of ionisation of the drug components may vary considerably under different conditions of pH.

The drug components can exist as weak acids or bases and subsequently in an ionised and non-ionised form. The ratio of the two forms varies with pH. All the drugs being used in this research are salts of weak bases i.e. they are associated with negative ions like chloride or sulphate (e.g. oxycodone hydrochloride and morphine sulphate), and typically have a pH below 6.0. As the drug components are weak bases, in acidic environments they are highly ionised, whereas in basic environments they are predominantly non-ionised. During syringe preparation their ionisation states therefore are dependent on the presence of the combination drugs and the salts they are supplied in, as well as the diluent used.

HPLC uses a UV detector at a fixed wavelength to detect changes in the mobile phase as it flows through the system. Different drug components being analysed by HPLC will absorb to varying degrees dependent on the wavelength selected. The extent to which they absorb can also be affected by the solvent being used. Each drug component in the combination will have a maximum wavelength (λ_{max}) at which it absorbs but an optimum

wavelength across the drugs in the combination would have to be selected. Table 1.3 shows the pK_a and λ_{max} for the drug components in this research.

Table 1.3. pKa and λ_{max} values of the drugs used in the current study

Drug Component	pKa	λ max
Morphine	8.0, 9.9	285nm (aqueous acid) 298nm (aqueous alkali)
Diamorphine	7.6	279nm (aqueous acid) 299nm (aqueous alkali)
Hydromorphone	8.2	280nm (aqueous acid) 290nm (aqueous alkali)
Alfentanil	6.5	258nm (isopropyl alcohol)
Oxycodone	8.9	280nm (aqueous acid), no alkali shift
Metoclopramide	9.3	273nm, 309nm (aqueous acid)
Levomepromazine	9.2	250nm, 302nm (aqueous acid) 259nm, 323nm (aqueous acid)
Haloperidol	8.3	245nm (methanolic acid)
Cyclizine	2.4, 7.8	257nm, 262nm (aqueous acid) 260nm (aqueous alkali)
Hyoscine butylbromide	-	252nm, 258nm, 264nm (aqueous acid)
Glycopyrronium bromide	-	252nm, 258nm, 264nm (aqueous acid)
Midazolam	6.2	235nm

(Moffat et al, 2004)

1.3. Analytical Methods

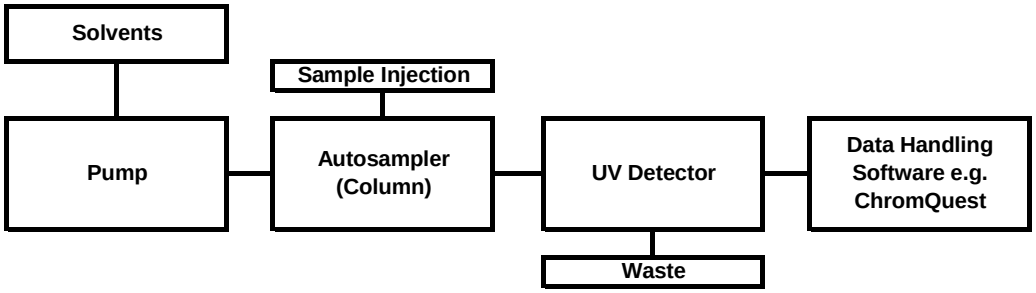
Once the combinations and the diluents had been identified, the methods that could be used to assess the compatibility of these drug combinations were considered. When multiple drugs are being combined in a syringe, as the number of drugs increases, maintaining their compatibility becomes more critical *(McLeod and Flowers, 2006)*, and appropriate methods should be in place to assess this.

Drug compatibility can be assessed by visual observations, or by monitoring the occurrence of physical or chemical changes and also through changes in their therapeutic effects *(Rose and Currow, 2009)*. Each of these areas has the potential to overlap. Observing a change in the therapeutic effect was beyond the scope of this research; however the possible indications of incompatibility could be the patient not responding to the treatment or potential toxic and adverse side effects developing. Visual incompatibility can be concluded if any of the following occur: precipitate formation, haziness, turbidity, viscosity changes, colour change, layer formation and effervescence *(Rose and Currow, 2009)*. The syringe, as well as the administration set should be regularly checked for any visual changes. With regard to physical and chemical changes, some of the visual

observations are due to chemical reaction but there is also the possibility of formation of unseen new chemical entities. Numerous combinations of drugs have been assessed for compatibility by including visual observations in the assessment *(Peterson et al, 1998; Negro et al, 2005)*. To assess any chemical instability in the form of new entities or degradants of the initial drug compounds, selective and specific analytical techniques needed to be used, such as high performance liquid chromatography (HPLC) and liquid chromatography mass spectrometry (LCMS).

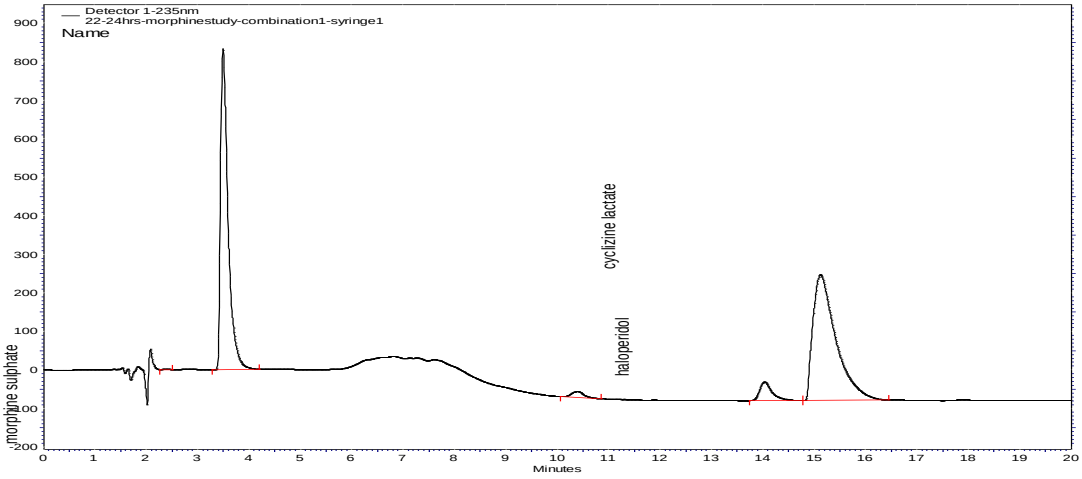
HPLC is a sensitive chromatographic technique which allows the separation and analysis of components. Figure 1.5 shows a schematic diagram of a typical HPLC system.

Figure 1.5. Schematic diagram of a HPLC system



Reversed phase chromatography was used in this research. The mobile phase was polar (e.g. methanol, water, acetonitirile) and the stationary phase of the column was non-polar (e.g. bonded phases - long hydrocarbon chains like C18). This results in polar components of the sample passing more quickly through the column due to their affinity for the polar solvent, whereas non-polar components pass more slowly due to their attraction and retention to the stationary phase. The detector measures the UV absorption of the mobile phase which changes in response to a component of the sample being eluted. An alternative detector is a diode array detector (DAD) which allows the UV spectrum range to be scanned. The detector response is acquired by a data handling system, which records the absorbance response against time. This pictorial representation is known as a chromatogram, see figure 1.6.

Figure 1.6. A typical chromatogram obtained from analyzing a sample on a reverse phase HPLC system. Peaks are subsequently identified and labelled.



If a chromatogram is unsatisfactory, for example, insufficient separation of the components, the parameters could be optimised. This could include:

- Varying the proportions of the solvents in the mobile phase in order to alter its characteristics and potentially improve separation and resolution of components.
- Changing the column i.e. a different stationary phase, length and particle diameter, potentially allowing for improved separation, resolution and peak shape. This could also reduce the retention time of the components resulting in a shorter analysis time.
- Reducing or increasing the volume of sample introduced onto the column. An 'off-scale' and very broad peak is indicative of too much sample.
- The use of a gradient for a sample containing components with a wide range of chemical properties. The variation in the ratio of the mobile phase solvents throughout the analysis could lead to enhanced separation and reduction in analysis times.
- The pH of the mobile phase and temperature can also aide the separation of components.

The process of varying the parameters in HPLC is known as analytical method development and allows the optimal conditions for separating the components of a sample to be obtained. The final combination of these factors is known as the HPLC method. After optimisation of the methods, analysis of the samples could occur. The HPLC methods were optimised for the drug combinations used in this study by ensuring the drug components were separated from each other and that they could be quantified. During the analysis of the samples the data handling software, linked to the detector, recorded and generated the chromatograms of the samples. These chromatograms were used to quantify the amount of each drug component in the sample, through the assessment of peak areas.

The quantification of the drug components was used to ascertain whether there were any changes in dose over a period of time. Overall the HPLC method was used to look for additional peaks, peak shifts and concentration changes, which allowed the compatibility of the combination to be determined.

HPLC can be used as a technique on its own but can also be combined with mass spectrometry (MS), known as liquid chromatography mass spectrometry (LCMS). LCMS combines the separation abilities of HPLC with the detection capabilities of mass spectrometry, which could potentially be used if problems are encountered with the HPLC technique. LCMS has a number of applications across a variety of different fields; however, in relation to this work this technique can potentially be used to identify unknown peaks obtained in the HPLC method through structure elucidation.

The HPLC part of LCMS is in principle the same as using the technique on its own. The difference in the two techniques occurs after the mobile phase has passed through the column. In LCMS, the mobile phase then enters the mass spectrometer, where it is sprayed into an atmospheric region. This is the atomospheric pressure ion source and interface, where the separated components of the sample being analysed are removed from the mobile phase and ionised to produce gas phase ions. At this stage most of the mobile phase is pumped to waste. Ionisation involves the addition or removal of electrons to the molecule or atom to produce ions by using strong electric fields in the vapour or condensed phase. This process can occur by different methods, for example, electrospray ionization (EI), atomospheric pressure chemical ionization (APCI) and atmospheric pressure photo ionization (APPI). The following stages are carried out under high vacuum. The ions then pass into the mass analyser where they are separated based on their mass to charge (m/z) ratio using electrical or magnetic fields. There are a number of different analysers including quadrupole, time-of-flight, ion trap and magnetic sector. The ions then pass to a detector. It 'counts' the ions from the mass analyzer and plots their abundance against their m/z ratio to produce a mass spectrum of the component. There are different detectors available: electron multiplier, dynode, photodiode and multi-channel plate *(EARL)*.

The techniques mentioned above have been used to detect the individual drugs investigated in this research with success *(Al Tannak et al, 2012; Barcia et al, 2003)*. Their application in assessing drug compatibility of a number of components requires optimisation and validation of the methods *(Grassby and Hutchings, 1997; Trabelsi et al, 2002)*.

1.4. Impact of Work

In end of life care there is a need for compatibility data for the drug combinations being administered to patients. Analytical assessment of the drug combinations is the preferred source of compatibility information and therefore, this work is the starting point in providing specific analytical data on some of the drug combinations being used in end of life care.

1.5. Aims

The aims of this research were to assess each prepared drug combination for physical and chemical incompatibility.

1.6. Objectives

In order to achieve the aims identified, the following objectives were addressed:

- Development of analytical methods to determine the optimal conditions to separate the components of the drug combinations using HPLC or LCMS.
- Used the developed analytical methods (HPLC or LCMS) to investigate individual drug solutions and identify possible degradants.
- Prepared the drug combinations as close to those in clinical practice as practically possible.
- Simulated an infusion, using a syringe driver, of the prepared drug combination to replicate clinical practice.
- Monitored syringe driver and drug combinations for any visual precipitations or alterations.
- Determined the concentration of each drug in each combination at four time points over the 24 hour infusion period using the developed analytical methods and monitored the appearance of any additional peaks if present.
- Determined whether the drug combinations were compatible from the data obtained.

2. METHODS

Drug combinations have been identified in the introduction that require testing for chemical and physical compatibility. The combinations can be split into two sets; the supportive drug combinations and the opioids. Each of the five opioids, where possible, was tested with each of the ten supportive drug combinations, giving a total of fifty combinations.

2.1. Materials

The following drug components, along with 0.9%w/v NaCl (Baxter Healthcare Ltd) were purchased from Stockport Pharmacy (Stepping Hill Hospital, Stockport):

- Morphine sulphate 30mg/ml injection BP (CP Pharmaceuticals)
- Diamorphine hydrochloride 100mg injection (Wockhardt)
- Rapifen intensive care, alfentanil hydrochloride 5mg/ml (Janssen-Cilag)
- Cyclizine lactate 50mg/ml injection (Martindale Pharmaceuticals)
- Haloperidol 5mg/ml injection BP (Antigen Pharmaceuticals)
- Haldol 5mg/ml injection (Janssen-Cilag)
- Midazolam 5mg/ml injection (Hameln Pharmaceuticals Ltd)
- Buscopan ampoules 20mg/ml solution for injection (Boehringer Ingelheim)
- Nozinan 25mg/ml injection (Sanofi aventis, Archimedes, LINK)
- Metoclopramide 5mg/ml injection (Hameln Pharmaceuticals Ltd)
- Metoclopramide 5mg/ml injection BP (Antigen Pharmaceuticals)
- Glycopyrronium bromide 0.2mg/ml injection (Martindale Pharmaceuticals)
- Glycopyrronium bromide 200µg/ml injection (Taro Pharmaceuticals)

Oxycodone hydrochloride injection 50mg/ml and Palladone 50mg/ml (hydromorphone hydrochloride) were kindly donated by Mundipharma Research Ltd. The analytical reagents haloperidol, metoclopramide hydrochloride and (-)-scopolamine N-butylbromide were purchased from Sigma-Aldrich (Gillingham, Dorset). WFI was purchased from Hameln. Ammonium acetate and acetonitrile were purchased from VWR International Ltd (Lutterworth, Leicestershire). The CME T34 syringe pumps and administration sets were kindly donated by CME McKinley Ltd.

2.2. The Syringe Driver

In order to assess the drug combinations a syringe driver was required. A syringe driver or pump is a small, portable, battery driven infusion device. It is used to gradually administer medication subcutaneously over a fixed time period, usually 24 hours, to a patient via a syringe. There are a number of different syringe drivers or pumps available, for example, Micrel MP Daily and MP mlh, Graseby MS26 and MS16A and CME T34 *(Dickman & Schneider, 2011)*. The rate of delivery is the main difference between them. The rate of delivery can be based on millilitres (ml) or millimetres (mm) of syringe plunger travel. In the healthcare setting, there is the possibility of finding both types of rate of delivery, which could potentially result in errors. This was addressed in the National Patient Safety Agency (NPSA) alert issued in December 2010, 'Safer ambulatory syringe drivers'. It includes the recommendation that the syringe driver should be used with a rate setting in millilitres per hour (ml/hr) *(NPSA, 2010)*. Of the devices available, only the CME T34 was suitable for use in palliative care because it met the criteria set out by the NPSA in its safety report.

At the time this research was being considered, of the syringe drivers or pumps available, it was the CME T34 Syringe Pump that was being adopted by many National Health Service (NHS) trusts nationwide. Hence, the CME T34 was used for this research to ensure reproduction of the clinical setting. The CME T34 used to be called the McKinley T34 Syringe Pump. The CME T34 Syringe Pump was launched in the United Kingdom (UK) in May 2005 *(Costello et al, 2008)*. This model eliminates measurements being made by the user, thus, reducing the risk of programming errors. The user only has to confirm the brand and size of the syringe being used, as the device is programmed for a fixed 24 hour period and using the data stored in its memory and the volume in the syringe to calculate the infusion rate in ml/hr *(CME McKinley)*.

2.3. Choice of Syringe Diluent

In the healthcare setting, there are a number of diluents that can be used to dilute drugs to the required concentration for administration. However, for use in the syringe driver, water for injections (WFI) or saline solution (0.9%w/v sodium chloride) are the diluents of choice. In some countries however, dextrose is also used *(McLeod and Flowers, 2006)*. A survey carried out in 1992 of specialist palliative care units in the United Kingdom and Eire revealed that in 90% of cases water was usually used as the

diluent in the syringe *(O'Doherty et al, 2001)*. The use of a diluent should optimize the stability of the solution and the drugs to be infused, along with enabling the administration of the combination over a prescribed time *(McLeod and Flowers, 2006)*. The drugs in the syringe should be diluted to maximum volume, usually 20ml, and be delivered over a maximum time of 24 hours for assurance of chemical stability. For this research, the diluent of choice was NaCl solution (0.9%w/v) in order to ensure the solution is as close to physiological tonicity as possible *(Dickman et al, 2005)*. However, WFI was used for the combinations containing cyclizine. This is because cyclizine may precipitate as the amount of chloride ions from the NaCl solution increases *(BNF)*.

In order to assess the compatibility of each combination a syringe preparation protocol, a sample collection protocol and a sample testing protocol were devised. This ensured that each combination was assessed in the same way.

2.4. Syringe Preparation Protocol

For each combination, polypropylene syringes (30ml, BD Plastipak) were prepared in duplicate. The preparation used in clinical practice was replicated as far as practically possible. In the case of morphine with combination 10, a 50/60ml BD Plastipak syringe was used. This was because on calculating the volume of each drug required to make the combination it was greater than the maximum fill limit of a 30ml BD Plastipak syringe on the CME T34 syringe pump. It is worth mentioning here that the CME T34 syringe pump is not limited to just BD Plastipak syringes, these were chosen in order to replicate clinical practice. The CME T34 syringe pump is pre-programmed with a range of different syringe brands and sizes, so it can detect through sensors when attaching the syringe as to what syringe size and type it is.

The numbers of ampoules corresponding to the dose in the combination for each drug were drawn up into the syringe and any air expelled. The syringe was further diluted to a maximum fill volume of 20ml with 0.9%w/v NaCl (Baxter Healthcare Ltd) and capped with a Luer-lok cap (Baxa). Water for Injection (WFI) (Hameln Pharmaceuticals Ltd) was used for cyclizine containing combinations instead of 0.9%w/v NaCl. Further dilution was not required when the syringe contents exceeded 20ml. Refer to table 2.1 for the diluent used for each combination. The syringes were inverted ten times to ensure a homogenous solution. In a laboratory environment, the above preparation process for analytical assessment would have been performed using grade A 20ml volumetric

glassware, but this was not done due to this research replicating the processes used in the healthcare setting, which combines the drug components directly into a syringe.

Table 2.1. Diluent used for each combination

Combination	Opioid + Combination				
	Morphine	**Diamorphine**	**Hydromorphone**	**Oxycodone**	**Alfentanil**
1	WFI	WFI	WFI	WFI	WFI
2	WFI	WFI	WFI	WFI	WFI
3	NaCl	NaCl	NaCl	NaCl	NaCl
4	NaCl	NaCl	NaCl	NaCl	NaCl
5	NaCl	NaCl	NaCl	NaCl	NaCl
6	NaCl	NaCl	NaCl	NaCl	NaCl
7	NaCl	NaCl	NaCl	NaCl	NaCl
8	WFI	WFI	WFI	X	X
9	WFI	WFI	WFI	WFI	WFI
10	N/A	X	NaCl	X	X

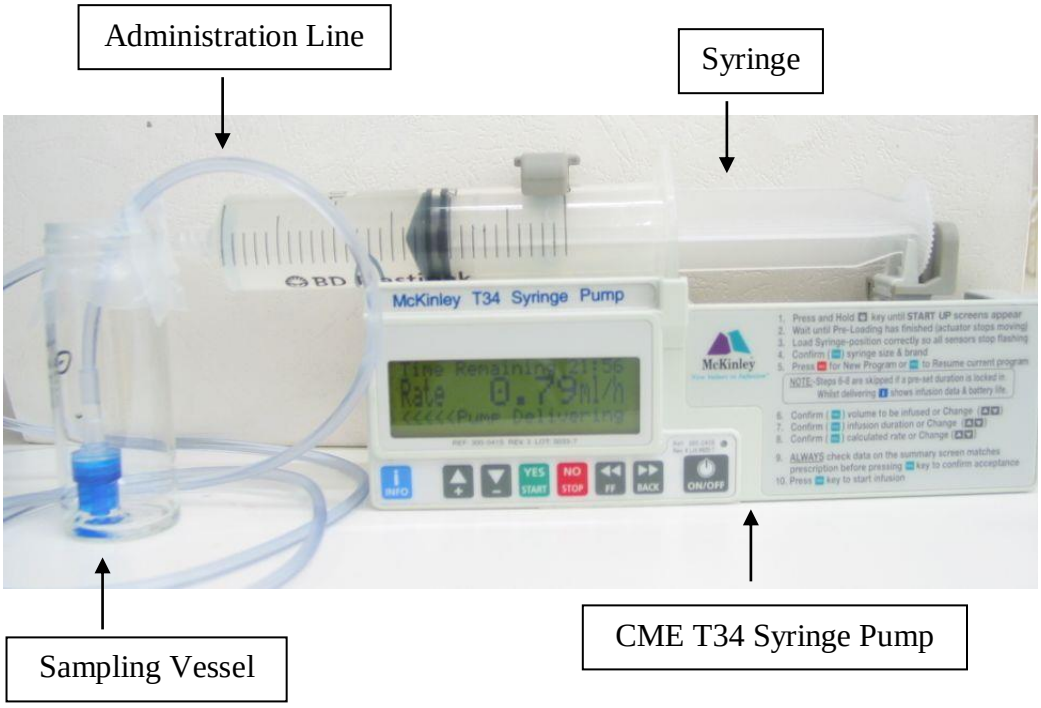
Note: WFI = Water for Injection
NaCl = 0.9%w/v sodium chloride
N/A = contents exceeded 20ml, therefore, no diluent required
X = combination not tested

2.5. Sample Collection Protocol

An administration line was attached to the syringe and manually purged. This aliquot was collected for the initial time point (0 hours). The filled syringe was fitted to the CME T34 syringe pump and the instructions on the syringe pump screen were followed before starting the infusion, for example, the correct brand and size of syringe were confirmed. The volume in the syringe, infusion duration and infusion rate were also displayed. The 'giving' end of the administration line was placed in a vessel at the start of the infusion and after 1 hour transferred to a sampling vessel for a period of two hours (3 hour time point). This time period was required to collect sufficient sample for analytical testing due to the infusion rate. The infusion rates varied between 0.73ml/hr and 0.79ml/hr for the combinations tested in 30ml BD Plastipak syringes and between 0.97ml/hr and 1.00ml/hr for the combination tested in a 50/60ml BD Plastipak syringe. This variation was caused by the difference in the volume collected for the initial time point during the purging of the administration line. The volume collected was based on dispensing 1.5ml using the graduation marks on the syringe. Therefore, possible inaccuracies could have occurred when preparing to the final volume of 20ml and then purging 1.5ml because it was reliant on visual interpretation. In clinical practice, the purge option on the CME T34 syringe pump would be used. The infusion rate was then calculated from the volume remaining in the syringe after purging and dividing it by the infusion duration of 24 hours.

After 3 hours the administration line was returned to the vessel until 4 hours had elapsed, at which point it was transferred to another sampling vessel for a two hour period (6 hour time point). After 6 hours the administration line was returned to the vessel. At 22 hours the administration line was transferred to another sampling vessel until the infusion was complete at 24 hours (24 hour time point). The simulated infusion was carried out at ambient laboratory conditions (18-25ºC). The infusion set-up is shown in figure 2.1.

Figure 2.1. CME T34 Syringe Pump Set-up



2.6. Sample Testing Protocol

The assessment of compatibility for each combination involved analytical tests at each of the four time points (0, 3, 6 and 24 hours) identified in section 2.5. At each time point, the pH of the contents of each sampling vessel from the two syringes was tested and the individual drug concentrations, along with any additional peaks, were determined by HPLC and in some instances by LCMS. The set-up was also inspected for visual appearance. The tests are described below.

2.6.1. Visual Appearance

The syringe, its contents, the attached administration line and sampling vessel were visually observed *in situ* for any evidence of colour change or precipitate formation at each of the four time points.

2.6.2. pH

The pH of the contents of each sampling vessel at each time point was measured with a glass combination electrode (Thermo Russell) at ambient temperature. The pH meter (InoLab WTW Level 1) was calibrated using buffers at pH 4 and 7 (AVS Titrinorm).

2.6.3. High Performance Liquid Chromatography (HPLC)

In order to obtain the individual concentration of each drug component in the combinations, analytical methods were developed in order to obtain the optimal chromatographic conditions for separating each drug component. However, it was not just each drug component of the combination that the method had to be able to separate, the excipients in the drug preparation and potential degradants of the drug also had to be considered.

2.6.3.1. HPLC Method Development

A method used by Nassr *et al. (2001)* stipulated the chromatographic conditions used to achieve the separation of seven drugs, which included some used in this research. These conditions were adapted and developed for the methods required to separate the drug components in each of the combinations in this research.

For the development and HPLC analysis of the individual drug concentrations in each combination, the same HPLC system was used. The HPLC system consisted of a vacuum degasser, binary gradient pump, autosampler and diode array detector (DAD) (Thermo Scientific, Hemel Hempstead UK). Chromatographic results were collected by data handling software (ChromQuest 4.1, Thermo Scientific, Hemel Hempstead UK). The chromatographic separation was performed at ambient temperature on a reverse phase column 150mm x 4.6mm, 5µm particle size. Elution was obtained using a gradient with a mobile phase consisting of acetonitrile (Line A) (VWR International) and 0.05M

ammonium acetate adjusted to pH 4.6 with formic acid (Line B) (VWR International). A flow rate of 1ml/min, an ambient column temperature and an autosampler tray temperature of 10°C were maintained throughout the analysis. The chromatographic signal was scanned over the range 190-360nm with the principal signal monitored at 235nm. The injection volume was 10µl.

A 150 x 4.6 mm Platinum EPS C_{18} 100Å 5µm column (Alltech) was selected to start the HPLC method development and a solution of each drug component in the combination was prepared. The drug component solution was first analysed using a 50%A 50%B mobile phase composition and scanned to obtain its optimal absorbance wavelength. The proportions of mobile phases A and B were then adjusted to obtain a suitable chromatogram (good peak shape and a reasonable retention time) of the drug component. In most instances, it was found that one drug component would be eluted at a lower proportion of mobile phase A than the other drug components in the combination. At this point, the individual solutions of the drug components were combined into one solution and analysed using the mobile phase composition at the lowest proportion of mobile phase A. The proportion of mobile phase A was maintained until the drug component at this composition had been eluted and then the proportion of mobile phase A was then increased over a particular time frame to a higher proportion of mobile phase A which eluted the remaining drug components from the combination. This proportion of mobile phase A was maintained until the entire drug components from the made up combinations had been eluted. The time it took to elute all the drug components in the combination was the analysis time, which varied for each combination.

2.6.3.2. Developed HPLC Methods

The analytical method development stage resulted in methods for each combination. Not all the combinations could be separated by the same method due to the varying properties of the drug components in the combination. Each method contained specific column, gradient and wavelength conditions for the combination it had been developed for. The details of the developed methods are given below:

Morphine Combinations

Table 2.2 shows the individual methods developed for each combination with morphine. For morphine combination 6 the drug components midazolam and

levomepromazine eluted at similar retention times on the Platinum column i.e. they could not be baseline resolved. Changing the proportion of mobile phases A and B was not sufficient to separate them, therefore, the column was changed and the development process resulted in a method able to separate all the drug components in this combination.

Table 2.2. Method conditions for morphine combinations 1-10

Combination	1			2 and 9, 4, 5			3, 7		
Column	Platinum			Platinum			Platinum		
Wavelength	235nm			235, 250, 230nm			235nm		
	Gradient Conditions								
	Time (min)	**% A**	**% B**	**Time (min)**	**% A**	**% B**	**Time (min)**	**% A**	**% B**
	0	15	85	0	15	85	0	15	85
	3	15	85	3	15	85	3	15	85
	5	45	55	5	45	55	5	48	52
	20	45	55	28	45	55	25	48	52

Combination	6, 10			8		
Column	Symmetry			Symmetry		
Wavelength	250, 235/250nm			235nm		
	Gradient Conditions					
	Time (min)	**% A**	**% B**	**Time (min)**	**% A**	**% B**
	0	10	90	0	10	90
	3	10	90	3	10	90
	5	40	60	5	30	70
	16	40	60	18	30	70

Morphine combination 10 contained three of the same drug components as combination 6; therefore the method for combination 6 was the starting point. An individual solution of the fourth drug component was prepared and analysed using this method and was found to elute within the analysis time and was separated from the other drug components. The resulting chromatography for this combination showed that the glycopyrronium peak was significantly smaller than the other three drug components. The optimum absorbance for this drug component was at a different wavelength than the other drug components; therefore, the method was set to run at two different wavelengths in order to obtain the optimal chromatography for each of the drug components.

The method for morphine combination 1 was the starting point for morphine combination 8 because it had separated three of the drug components this combination contained (morphine, cyclizine and haloperidol). However, the fourth drug component in combination 8, glycopyrronium, could not be separated from two of the other drug

components, cyclizine and haloperidol, using this method. The column was changed from the Platinum column to the Symmetry column and the analytical method development process was started again. The alternative column separated all four drug components.

Diamorphine Combinations

The Platinum column was the starting point for the diamorphine combinations along with a 15%A and 85%B mobile phase composition. However, the diamorphine peak eluted too close to the solvent front and there was another peak present which had not been resolved either. The column was changed and the analytical method development process started. The degradation pathway of diamorphine was known and therefore, the methods had to be able to elute morphine as well. An individual solution of morphine was analysed by the methods and was found to elute around the solvent front. This was deemed acceptable because it was separated from diamorphine itself and it was not expected that diamorphine would degrade to this extent over a 24 hour period. In aqueous solution diamorphine undergoes hydrolysis to 6-monoacetylmorphine and then to morphine with the rate of decomposition being at a minimum around pH 4 *(Martindale)*. This was confirmed when a solution of diamorphine hydrochloride was heated at 60^{o}C for a period of 24 hours and when analysed after this period no morphine peak was apparent. For all the methods developed for the diamorphine combinations see table 2.3.

Table 2.3. Method conditions for diamorphine combinations 1-7 and 9

Combination	1 (2, 3, 4, 7, 9)			5			6		
Column	Symmetry			Symmetry			Symmetry		
Wavelength	235nm			235nm			235nm		
	Gradient Conditions								
	Time (min)	**% A**	**% B**	**Time (min)**	**% A**	**% B**	**Time (min)**	**% A**	**% B**
	0	20	80	0	20	80	0	20	80
	5	20	80	9	20	80	5	20	80
	7	30	70	11	30	70	7	40	60
	20 (28)	30 (30)	70 (70)	30	30	70	18	40	60

As a result of problems encountered in the HPLC analysis for morphine combinations 8 and 10, there are no HPLC methods for the same combinations with diamorphine. These combinations will be considered in the LCMS analysis section (2.6.4) below.

Hydromorphone Combinations

The starting point for the hydromorphone combinations was using the Symmetry column. The individual solution of hydromorphone showed there was a small peak before the hydromorphone peak and in order to ensure this peak was resolved from the solvent front a longer analysis time was required which affected the shape of the hydromorphone peak. The Platinum column was then used and a suitable chromatogram was obtained. The hydromorphone solution required the same mobile phase composition as the morphine solution to elute it, so the methods used for the morphine combinations were adapted to suit the hydromorphone combinations. See table 2.4 for all the hydromorphone combination methods used. There are no HPLC methods for combinations 8 and 10 with hydromorphone because of the problems encountered with the same combinations with morphine. These combinations will be considered in the LCMS analysis section (2.6.4).

Table 2.4. Method conditions for hydromorphone combinations 1-7 and 9

Combination	1 (2, 4, 9)				3, 5, 7				6		
Column	Platinum				Platinum				Symmetry		
Wavelength	235nm				235nm				235nm		
	Gradient Conditions										
	Time (min)	**% A**	**% B**		**Time (min)**	**% A**	**% B**		**Time (min)**	**% A**	**% B**
	0	15	85		0	15	85		0	10	90
	3	15	85		3	15	85		3	10	90
	5	45	55		5	35	65		5	40	60
	20 (30)	45 (45)	55 (55)		45	35	65		16	40	60

Oxycodone Combinations

The analytical method development process for the oxycodone combinations was carried out on both the Platinum and Symmetry columns. The choice of column for the combination was dependent on the chromatography, table 2.5 shows the methods developed. Combinations 8 and 10 with oxycodone do not have a HPLC method. They will be considered in the LCMS analysis section (2.6.4) because of problems previously encountered with the same combinations.

Table 2.5. Method conditions for oxycodone combinations 1-7 and 9

Combination	1			2, 3, 4, 5, 7, 9			6		
Column	Platinum			Platinum			Symmetry		
Wavelength	235nm			235nm			235nm		
	Gradient Conditions								
	Time (min)	**% A**	**% B**	**Time (min)**	**% A**	**% B**	**Time (min)**	**% A**	**% B**
	0	25	75	0	25	75	0	15	85
	4	25	75	4	25	75	4	15	85
	6	45	55	6	45	55	6	45	55
	18	45	55	28	45	55	16	45	55

Alfentanil Combinations

The analytical method development process showed that the alfentanil peak was retained to a larger extent by the stationary phases of the columns compared to the other opioids and this caused the alfentanil drug component to be eluted at similar retention times to the other drug components in the combinations. In order to overcome this it was found that using an isocratic system rather than a gradient system was more practical in order to obtain a method for each combination. The methods do not change the composition of mobile phases A or B over the analysis and the individual methods for all the alfentanil combinations are shown in table 2.6. There are no HPLC methods for combinations 8 and 10 with alfentanil, they will be considered in the LCMS analysis section (2.6.4), together with the other opioid combinations.

Table 2.6. Method conditions for alfentanil combinations 1-7 and 9

Combination	**Column**	**Wavelength**	**%A**	**%B**	**Run Time (min)**
1	Symmetry	235nm	24	76	25
2, 7	Platinum	235nm	40	60	28
3, 5	Platinum	235nm	40	60	32
4	Symmetry	235nm	30	70	20
6	Symmetry	235nm	26	74	40
9	Symmetry	235nm	24	76	40

2.6.3.3. Preparation of HPLC Standards

To calculate the concentration of each drug component in the combination a standard of known concentration for each drug component was prepared based on the method developed. The concentration of the standard was equivalent to the theoretical concentration of the drug component in the diluted sample preparation. The concentration of each drug component in the sample was calculated from the standard. Each standard

was prepared in duplicate from the salt specified in the ampoule used for preparing the combination. The individual salts of each drug component were sourced as analytical reagents, 'in-house' raw materials or from an ampoule of that particular drug component. Refer to table 2.7 for information on standards used. All standards were prepared in glass volumetrics and diluted with distilled water.

Table 2.7. List of Standards

Drug Component	**Drug Form**	**Source**
Morphine	Morphine sulphate	INH
Diamorphine	Diamorphine hydrochloride	INH
Hydromorphone	Hydromorphone hydrochloride	Amp
Oxycodone	Oxycodone hydrochloride	Amp
Alfentanil	Alfentanil hydrochloride	Amp
Cylizine	Cyclizine lactate	Amp
Haloperidol	Haloperidol	AR
Midazolam	Midazolam hydrochloride	INH
Metoclopramide	Metoclopramide hydrochloride	AR
Levomepromazine	Levomepromazine hydrochloride	Amp
Glycopyrronium	Glycopyrronium bromide	Amp
Hyoscine butylbromide	(-)-Scopolamine N-butylbromide	AR

Note: INH = 'in-house' raw material
Amp = an ampoule of the drug component
AR = analytical reagent

With the exception of haloperidol, all standards were soluble in water. On investigation, the Material Safety Data Sheet (MSDS) for the analytical reagent of haloperidol stated it was insoluble in water but soluble in weak acid. The Summary of Product Characteristics (SPC) for haloperidol listed lactic acid (Fluka Analytical) as one of the excipients; therefore, this was used to dissolve the haloperidol initially and then further diluted with water. For the duration of the HPLC analysis the standard preparations were stored refrigerated (5°C ± 3°C).

2.6.3.4. Preparation of HPLC Samples

The contents of the syringes collected in the sampling vessels were too concentrated to be injected straight onto the column for analysis by HPLC. The sensitivity of the detector and the strength of the UV absorption for the individual drug components resulted in a chromatogram with peaks off-scale. The contents of the sampling vessel were therefore diluted with distilled water to ensure the drug components were on-scale, and this dilution varied between combinations. Single dilutions in distilled water, from the contents of the sampling vessel, were performed for each syringe at each time point. A

micropipette was used to pipette 0.5ml of the sampling vessel contents into a glass volumetric flask and then diluted to volume with distilled water. Table 2.8 lists the dilutions performed for each combination. The flask contents were thoroughly shaken and a portion transferred to a HPLC vial, which was then injected twice by the HPLC system.

Table 2.8. The sample dilution for each combination

Opioid	Combination (s)	Dilution
Morphine	4 and 5	1 in 10
Morphine	1-3 and 7-9	1 in 20
Morphine	6 and 10	1 in 40
Diamorphine	1-5, 7 and 9	1 in 20
Diamorphine	6	1 in 40
Hydromorphone	1-7 and 9	1 in 40
Oxycodone	1-7 and 9	1 in 40
Alfentanil	1-7 and 9	1 in 20

2.6.3.5. HPLC Analysis Protocol

Once the HPLC methods had been developed and the standards and samples prepared a set protocol was followed for the HPLC analyses.

The HPLC system was set up: the mobile phases were prepared and added to the relevant solvent lines, the method for the combination being tested was loaded and the column was conditioned with the mobile phase proportions from the method. The chromatographic software was used to create a sequence to analyse all relevant standard preparations and sample preparations for the drug combination.

Individual standards for each drug component in the combination were prepared in duplicate and six injections of each preparation were analysed. After starting the infusion via the CME T34 syringe pump and collecting the sample over the required time, a single dilution from the sampling vessel contents of each syringe was performed. Each dilution was injected twice, immediately after the standard injections. The 3 hour dilutions and the 6 hour dilutions were the next solutions to be analysed. No standard preparations were analysed between the two time points because there was insufficient time to do this, as once the 3 hour samples had been analysed it was time for the 6 hour samples to be analysed. Therefore, a standard preparation or multiple standard preparations from each drug component were only injected after the 6 hour sample injections because there was sufficient time to complete this before the next samples required analysing. Two injections

from each sample dilution at the 24 hour time point were then analysed and followed by further injections from each individual drug component standard preparation.

Once the analysis was complete, the injections in the sequence were grouped so all the standard injections were together followed by the sample injections for each time point. The integration parameters of the method were amended, where necessary, to obtain optimum integration for peaks in each standard and sample chromatogram. The standard concentrations were entered into the method for the individual standard preparations, along with the multiplier and sample amount for each of the samples. The data was then processed to generate the concentration for the individual drug components of the combination at each time point. For each method, a two point calibration was used and the average response factor from the two levels of standard was used to generate the sample results.

2.6.3.6. HPLC Method Validation

Each HPLC method developed was validated to ensure it was suitable for the intended purpose of its development. The validation of an analytical procedure is covered by an ICH guideline; Validation of Analytical procedures: Text and Methodology Q2 (R1) (*ICH*). The analytical procedure describes the process of how each analytical test is performed in order to complete the analysis. It should include details on the sample, reference standards, reagents and equipment (*ICH*), to name a few. For each combination, a study protocol and method protocol was generated, which covered these details.

The following are characteristics that should be considered for validation of an analytical procedure; accuracy, precision including repeatability and intermediate precision, specificity, detection limit, quantitation limit, linearity and range. Robustness can also be considered (*ICH*). The main validation that was carried out for all the HPLC methods included standard repeatability and sample repeatability, with limited specificity.

In order to correctly identify the individual drug components in each combination a comparison to the reference standard was performed. Individual standards of the drug components present in the combination were prepared and when analysed, their chromatograms depicted a principal peak. The retention time associated with that peak was then assigned to that drug component. In order to identify the peaks in the combination, the retention time of the peaks that occurred in the combination

chromatogram were compared to the retention times of the individual standard preparations. This allowed peaks to be identified based on retention time comparisons.

Each combination was prepared from ampoules of the corresponding drug component injection and it had to be considered whether or not the excipients present in the injection would produce a peak(s) in the chromatograms. The Summary of Product Characteristics (SPC) for each injection listed the excipients present but not the concentration of them. Therefore, the individual standard chromatograms used to identify the drug component were also used to confirm if there were any additional peaks present relating to that drug component i.e. possible excipient peaks. Table 2.9 lists the excipients found in each injection used to prepare the combinations. It can be seen that there are a number of common excipients.

Table 2.9. Excipients present in the injections for each drug component

Preparation	Manufacturer	Excipients
Morphine sulphate 30mg/ml injection BP	CP Pharmaceuticals	Sodium metabisulphite Sodium hydroxide Hydrochloric acid Water for injections
Diamorphine hydrochloride 100mg injection	Wockhardt	Water for injections
Palladone 50mg/ml (hydromorphone hydrochloride)	Mundipharma Research Ltd	Citric acid anhydrous Sodium citrate Sodium chloride Sodium hydroxide soln Hydrochloric acid Water for injections
Oxycodone hydrochloride injection 50mg/ml	Mundipharma Research Ltd	Citric acid monohydrate Sodium citrate Sodium chloride Sodium hydroxide Dil. hydrochloric acid Water for injections
Rapifen intensive care, alfentanil hydrochloride 5mg/ml	Janssen-Cilag	Sodium chloride Water
Cyclizine lactate 50mg/ml injection	Martindale Pharmaceuticals	Lactic acid Water for injections
Haloperidol injection BP 5mg/ml Haldol injection 5mg/ml	Antigen Pharmaceuticals Janssen-Cilag	Lactic acid Sodium hydroxide Water for injections
Midazolam 5mg/ml injection	Hameln Pharmaceuticals Ltd	Sodium chloride Hydrochloric acid Water for Injections
Buscopan ampoules 20mg/ml solution for injection	Boehringer Ingelheim	Sodium chloride Water for injections
Nozinan injection 25mg/ml	Sanofi aventis Archimedes LINK	Ascorbic acid Sodium sulphite Sodium chloride Nitrogen Water for injections
Metoclopramide 5mg/ml injection Metoclopramide injection BP	Hameln Pharmaceuticals Ltd Antigen Pharmaceuticals	Sodium chloride Citric acid monohydrate Sodium citrate Hydrochloric acid Sodium hydroxide Sterile water for injections Nitrogen
Glycopyrronium bromide 0.2mg/ml injection Glycopyrronium bromide 200µg/ml injection	Martindale Pharmaceuticals Taro Pharmaceuticals	Sodium chloride Dil. hydrochloric acid Water for Injections

In order to identify possible degradation peaks, individual drug component solutions were subjected to heat stress conditions over a particular time frame, typically 24 hours and up to 13 days. A stock solution of known concentration of the individual drug component was prepared. A portion of the stock solution was immediately diluted to a concentration acceptable for the method it was being analysed by and injected twice by HPLC. A portion of the stock solution was also transferred to a glass vial and placed in a laboratory oven (Vulcan) that was set in the temperature range 58^{o}C to 80^{o}C. The stock solution was removed from the laboratory oven and allowed to cool before being diluted and analysed by the same method as the initial diluted solution, this was done at intervals up to 24 hours. The resulting chromatograms of the initial solution and the degraded solution were compared and the retention time of any new peaks was documented in the method information.

Another area considered was the possibility of extractables from the syringe or administration line. This was assessed by preparing a syringe containing the most common diluent, 0.9%w/v NaCl, and performing an infusion on this only. The sample collection protocol used for testing the combinations was followed. However, samples were only analysed by HPLC initially and at 24 hours.

The precision of the method was determined by standard and sample repeatability. The ICH guideline states that repeatability should be assessed by using a minimum of six determinations at 100% of the test concentration (*ICH*). For each combination, two standard preparations for each drug component in the combination were prepared at the concentration that the drug component was in the combination. The two standard solutions were each injected six times. Sample repeatability was performed on a homogeneous preparation of each combination. Six preparations of the dilution required for the combination were prepared and analysed.

2.6.4. Liquid Chromatography Mass Spectrometry (LCMS)

Whilst conducting the development and HPLC analysis of morphine with combinations 8 and 10 it was observed that the drug component glycopyrronium was significantly smaller in the chromatograms than the other drug peaks in the combination. This made it difficult to obtain consistent peak areas for this drug component. The reason for this was attributed to its lower dose (1.2mg or 2.4mg) compared to the other drug

components in the combination. To overcome this problem, the alternative technique LCMS was investigated in order to assess the concentration of this drug component.

2.6.4.1. LCMS/MS Method Development

The analytical method development for LCMS/MS was split into two parts; the LC method development and the MS method development.

The LC method development part was essentially the same as that for the HPLC method development detailed above. Combinations 8 and 10 contained the same drug components as combinations 1 and 6 respectively with the addition of glycopyrronium. Therefore, the HPLC methods that had been developed for combinations 1 and 6 were the starting point for the LC methods for combinations 8 and 10. An equilibriation time was added to the gradient conditions in the LC method, in order for the system to settle between injections.

The MS/MS method development process involved analysing individual drug component solutions by MS to obtain the mass to charge (m/z) value of the drug component peak. This m/z value was then entered into the MS method and the solution was then analysed again in MS/MS mode. The individual solutions were then combined to obtain a solution that contained all four drug components of the combination (not at the concentrations present in the samples), which was then analysed by MS. The resulting spectrum contained peaks for all of the drug components. Based on the retention time at which the drug components eluted, the method was split into segments that contained just one drug component peak. For each segment the m/z value for the expected peak was added to the method and changed to MS/MS mode. The combined solution was then reanalysed and a spectrum of the fragmented peaks of the drug components obtained.

The same LCMS/MS system was used for the analysis of combinations 8 and 10. The LC system consisted of a vacuum degasser, binary gradient pump, autosampler and UV detector (HP1100, Agilent Technologies, UK) with the software Chemstation LC 3D systems Rev B.01.03. The chromatographic separation was performed at ambient temperature on a reverse phase column 150mm x 4.6mm, 5µm particle size. Elution was obtained using a gradient with a mobile phase consisting of acetonitrile (Line A) and 0.05M ammonium acetate adjusted to pH 4.6 with formic acid (Line B). A flow rate of 1ml/min and an autosampler tray temperature of 10°C were maintained throughout the

analysis. The injection volume was 4µl. The MS system was an Ion Trap (Agilent Technologies, UK) with the software MSD Trap Control version 5.3. The nebulizer, dry gas and dry temp were set at 60.0psi, 11.0l/min and 350°C respectively. These were the settings specified by the manufacturer when the LC was operating at 1ml/min. The polarity was in positive mode. The capillary voltage, end plate offset voltage and skimmer voltage were -3500V, -500V and 40.0V respectively. These did not change from method to method. The capillary exit voltage varied depending on the m/z value of the drug component, as did some of the octuple voltages.

2.6.4.2. LCMS/MS Methods

The LCMS/MS methods listed below were obtained by the analytical method development process. The methods contained specific conditions relating to the combination it had been developed for. No methods have been developed for oxycodone with combinations 8 and 10 because of laboratory time constraints and availability of the LCMS instrumentation.

Morphine Combinations 8 and 10

These methods have been developed in order to assess the glycopyrronium drug component only. Table 2.10 shows the method conditions for morphine combinations 8 and 10.

Table 2.10. Method conditions for morphine combinations 8 and 10

Combination 8

Column:	Symmetry	
Gradient Conditions		
Time (min)	**A%**	**B%**
0	10	90
3	10	90
5	30	70
16	30	70
16.1	10	90
21	10	90

MS(n) = MS/MS			
Segment	**Component**	**+MS2(m/z) → …**	**Cap Exit (V)**
1	morphine	(286) → 201.0	112.5
2	glycopyrronium	(318) → 116.2	114.8
3	cyclizine	(267) → 167.0	111
4	haloperidol	(376) → 165.0, 358.1	119.2

Combination 10

Column:	Symmetry	
Gradient Conditions		
Time (min)	**A%**	**B%**
0	10	90
3	10	90
5	40	60
16	40	60
16.1	10	90
21	10	90

MS(n) = MS/MS			
Segment	**Component**	**+MS2(m/z) → …**	**Cap Exit (V)**
1	morphine	(286) → 201.0	112.5
2	unknown	(345) → 314.1	116.9
3	glycopyrronium	(318) → 116.1	114.8
4	levomepromazine	(329) → 100.3	115.7
5	midazolam	(326) → 291.1	115.5

Diamorphine Combinations 8 and 10

The methods for diamorphine combinations 8 and 10 have been developed in order to assess all four drug components in the combinations. See table 2.11 for all the methods for the diamorphine combinations that have been developed.

Hydromorphone Combinations 8 and 10

Table 2.12 shows the individual methods developed for combinations 8 and 10 with hydromorphone.

Table 2.11. Method conditions for diamorphine combinations 8 and 10

Combination 8

Column:	Symmetry	
Gradient Conditions		
Time (min)	**A%**	**B%**
0	20	80
5	20	80
7	30	70
17	30	70
17.1	20	80
22	20	80

MS(n) = MS/MS			
Segment	**Component**	**+MS2(m/z) → …**	**Cap Exit (V)**
1	unknown	(286) → 201.0	112.5
2	6-monoacetyl	(328) → 268.1, 211.0	115.6
3	diamorphine	(370) → 268.1, 328.2	118.8
4	glycopyrronium	(318) → 116.1	114.8
5	cyclizine	(267) → 167.0	111
6	haloperidol	(376) → 165.0, 358.2	119.2

Combination 10

Column:	Symmetry	
Gradient Conditions		
Time (min)	**A%**	**B%**
0	20	80
5	20	80
7	40	60
18	40	60
18.1	20	80
23	20	80

MS(n) = MS/MS			
Segment	**Component**	**+MS2(m/z) → …**	**Cap Exit (V)**
1	6-monoacetyl	(328) → 268.1, 211.0	115.6
2	diamorphine	(370) → 268.1, 328.2	118.8
3	unknown	(344) → 315.1	116.8
4	glycopyrronium	(318) → 116.1	114.8
5	levomepromazine	(329) → 100.2	115.7
6	midazolam	(376) → 291.1	115.5

Table 2.12. Method conditions for hydromorphone combinations 8 and 10

Combination 8

Column:	Symmetry	
Gradient Conditions		
Time (min)	**A%**	**B%**
0	10	90
3	10	90
5	30	70
17	30	70
17.1	10	90
22	10	90

MS(n) = MS/MS			
Segment	**Component**	**+MS2(m/z) → …**	**Cap Exit (V)**
1	unknown	(304) → 286.1	113.8
2	hydromorphone	(286) → 185.0	112.5
3	glycopyrronium	(318) → 116.1	114.8
4	cyclizine	(267) → 167	113.5
5	haloperidol	(376) → 165.0, 358.1	119.2

Combination 10

Column:	Symmetry	
Gradient Conditions		
Time (min)	**A%**	**B%**
0	10	90
3	10	90
5	40	60
15	40	60
15.1	10	90
20	10	90

MS(n) = MS/MS			
Segment	**Component**	**+MS2(m/z) → …**	**Cap Exit (V)**
1	unknown	(304) → 286.1	113.8
2	hydromorphone	(286) → 185.0	112.5
3	unknown	(453) → 435.3	125
4	unknown	(344) → 315.1	116.8
5	glycopyrronium	(318) → 116.1	114.8
6	levomepromazine	(329) → 100.2	115.7
7	midazolam	(326) → 291.1	115.5

Alfentanil Combinations 8 and 10

The LCMS/MS methods developed for alfentanil combinations 8 and 10 used an isocratic system. Refer to table 2.13 for the specific method conditions.

Table 2.13. Method conditions for alfentanil combinations 8 and 10

Combination 8

Column:	Symmetry	
Gradient Conditions		
Time (min)	**A%**	**B%**
0	24	76
30	24	76

MS(n) = MS/MS			
Segment	**Component**	**+MS2(m/z) → …**	**Cap Exit (V)**
1	glycopyrronium	(318) → 116.1	112.5
2	alfentanil	(417) → 268.1, 385.2	114.8
3	cyclizine	(267) → 167.0	111
4	haloperidol	(376) → 165.0, 358.1	119.2

Combination 10

Column:	Symmetry	
Gradient Conditions		
Time (min)	**A%**	**B%**
0	26	74
50	26	74

MS(n) = MS/MS			
Segment	**Component**	**+MS2(m/z) → …**	**Cap Exit (V)**
1	unknown	(344) → 315.1, 109.1	116.8
2	glycopyrronium	(318) → 116.1	114.8
3	alfentanil	(417) → 268.1, 385.2	122.3
4	midazolam	(326) → 291.1	115.5
5	levomepromazine	(329) → 100.1	115.7

2.6.4.3. Preparation of LCMS/MS Standards

To calculate the concentration of each drug component of the combination a calibration curve was prepared. A calibration curve for each drug component in the combination was required. The calibration curve was made up of five levels of standard, with one level being equivalent to the theoretical concentration of the drug component in the diluted sample preparation and the other levels being higher and lower than the theoretical concentration. The standards were prepared from the salt specified on the ampoule used for preparing the combination. This was done using one of the following: the analytical reagent, the 'in-house' raw material or an ampoule of that particular drug component. Refer to table 2.7 in section 2.6.3.3 for information on the standards used. All standard preparations were carried out in glass volumetrics and diluted with distilled water.

2.6.4.4. Preparation of LCMS/MS Samples

The contents of the sampling vessels were too concentrated to be injected without dilution onto the column for analysis by LCMS/MS, as was the case with the HPLC methods in section 2.6.3.4. The contents of the sampling vessel for each syringe at each

time point were therefore diluted using distilled water. Table 2.14 lists the dilutions for the combinations analysed by LCMS/MS.

Table 2.14. The sample dilutions for the combinations by LCMS/MS

Opioid	Combination(s)	Dilution
Morphine	8	1 in 20
Morphine	10	1 in 40
Diamorphine	8	1 in 20
Diamorphine	10	1 in 40
Hydromorphone	8 and 10	1 in 40
Alfentanil	8 and 10	1 in 20

The dilution of the sampling vessel contents was performed using a micropipette and was transferred into a glass volumetric flask, which was diluted to volume with distilled water. The contents of the flask were thoroughly shaken and a portion was transferred to an LC vial, which was then injected twice by LCMS.

2.6.4.5. LCMS/MS Analysis Protocol

A set protocol for the LCMS/MS analyses was followed once the calibration standards and the samples had been prepared.

The Agilent LC system was set up: the mobile phases were prepared and added to the relevant solvent lines, the method for the combination was loaded and the column conditioned with the mobile phase proportions from the relevant method. The MS system was switched on and allowed to reach the required temperatures. The instrument controlled software was used to create sequences for each time point. The initial sequence contained a diluent blank, followed by two injections of the diluted sampling vessel contents from each syringe. Once this sequence was complete the 3 hour time point sequence was loaded which contained two injections of the diluted sampling vessel contents from each syringe. On completion, the 6 hour time point sequence was loaded which contained two injections of the diluted sampling vessel contents from each syringe, along with the five calibration standard levels for three drug components. The calibration levels were injected in order of increasing concentration for each drug component. The calibration level equivalent to the theoretical concentration of the drug component was injected six times and all other levels were injected twice. The final sequence to be loaded was the 24 hour time point. This contained two injections of the diluted sampling vessel

contents from each syringe, along with the five calibration standard levels, in order of increasing concentration of the remaining drug component.

Once the analysis was complete the sequences were loaded into the software QuantAnalysis. Individual methods were created for each drug component which contained the concentration of the standard calibration levels in nanograms per ml (ng/ml). It also contained information on: the retention time of the drug component, the target m/z value of the drug component along with its fragment ion m/z value. The data was processed using each of the drug component methods. The results were generated using the calibration curve for each drug component.

2.7. Storage Conditions

Before carrying out an analysis on a particular combination, all standards, reagents and drug component injections were stored in their original containers and at the appropriate storage temperatures. Mobile phases for the HPLC analysis were prepared before each combination was tested and stored in Duran solvent bottles throughout the analysis. Table 2.15 states the conditions applied to the testing of each combination.

Table 2.15 Testing conditions

Component	**Temperature**
Prepared standards	5°C ± 3°C
Prepared syringe, administration line and sampling vessel	Ambient room temperature (18-25°C)
Diluted sample preparation	5°C ± 3°C
Diluted sample preparation (HPLC vial)	10°C

2.8. Assessment of Data

Once the HPLC analysis was complete the results for each drug component in the combination was calculated. This was all done through the data handling software, ChromQuest.

The concentrations of the standards were calculated as follows:

$$\text{Standard concentraton (mg/ml)} = \frac{\text{weight taken (g) x 1000 x purity x (100 - water content)}}{\text{flask volume x 100 x 100}}$$

This was only the stock concentration, which did not account for any subsequent dilutions of the standard.

The response factor (RF) of the standard was obtained by the calculation below:

$$RF = \frac{\text{mean standard area}}{\text{standard concentration}}$$

The concentration of the drug component was obtained in the following way:

$$\text{Drug concentration (mg/ml)} = \frac{\text{sample area x multiplier}}{\text{average RF standard x sample amount}}$$

The multiplier and sample amount accounted for the subsequent standard and sample dilutions that were performed for each combination. The average RF value was obtained from the RF values for the two levels of standards prepared.
In order to obtain a percentage, the following was performed:

$$\text{Percentage}(\%) = \frac{\text{concentration of drug component (mg/ml) x 100}}{\text{theoretical concentration of drug component in combination}}$$

The theoretical concentration of the drug component in the combination was the dose of the drug component divided by the final combination volume (20ml in most instances).
The data obtained was tabulated for assessment. This was done in two formats, one looking at the percentage nominal and the other looking at percentage initial remaining.

The data obtained was assessed by the statistical method Analysis of Variance (ANOVA), which enabled significant differences in the data sets to be identified. A one-way ANOVA was used to compare three or more sets of unpaired measurements and the p value was determined. There was four data sets for each drug component (i.e. the four time points over which the drug component was analysed) and within each data set there was four results (two results from each of the two syringe preparations).

3. RESULTS AND DISCUSSION

In order to assess the combinations for compatibility, the visual appearance of the syringe contents and its pH, along with the concentration of each drug component in the combination were deemed the most important tests to perform. Visual observations of the syringe contents were performed over the infusion period to monitor any changes in the appearance of the contents, which would be the most obvious indication of any incompatibility. In addition, measurements of pH of the syringe contents were performed at each time point, with any significant changes pointing towards incompatibility. In order to determine the concentration of each drug component, HPLC was decided to be the most beneficial analytical technique. It would allow the concentration of each drug component to be calculated and assessed over the study period, along with monitoring the appearance of any additional peaks which could indicate the unsuitability of combining these drug components. As part of the work HPLC methods were developed in order to separate the drug components in the combinations and these were used to assess the concentration of each drug component present at the four time points monitored over a 24 hour period. This chapter will discuss the methods used, along with the results obtained.

3.1. Appearance

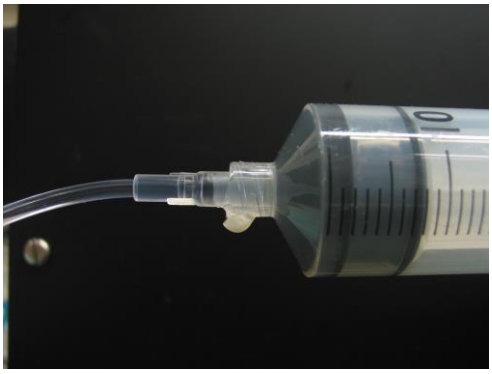
Of the combinations tested it was not expected that there would be any issues with the appearance because these particular drug combinations had been identified from the Marie Curie database as having been administered to patients. The appearance was monitored for any evidence of precipitation occurring within the syringe or administration set. Any change could indicate physical incompatibility between the syringe contents. In literature, researchers mention monitoring the appearance of solutions over the time of the study by means of observation using the naked eye, against black and white background or microscopic evaluation, *(Amri et al, 2010; Chandler et al, 1996; Grassby and Hutchings, 1997; Hines and Pleasance, 2009; Negro et al, 2005)*. Precipitation, change of colour, cloudiness and crystallisation are aspects that have been monitored.

3.1.1. Morphine Combinations

Most of the combinations remained clear, colourless and free from visible particulate matter over the 24 hour study period, with the exception of combination 10. Here, at the end of the study, a residue was observed externally on the syringe and administration set connection (shown in figure 3.1). This residue indicated a leak had occurred during the study and its formation was expected to be due to evaporation of the syringe contents. No precipitate, which would indicate chemical incompatibilty, however, was noticed within the syringe during the administration period. On visual observation at each previous time point, there was no evidence of precipitation in the administration line, syringe or sampling vessel.

In addition, a leak was observed during the infusion of combination 8. It was noticed whilst collecting the 3 hour time point sample and occurred at the connection of the administration line to the syringe. The collection of sample was unaffected and there was no evidence of residue formation at the point of the leak.

Figure 3.1. Precipitate observed on syringe connection for morphine combination 10



The suppliers of the syringe drivers and administration sets, CME McKinley UK Limited, were contacted about the first observation only. They investigated this issue and responded that the following two outcomes replicated this issue:

1. The syringe connector of the administration set was only partially screwed to the syringe.
2. The syringe connector of the administration set contained a crack *(CME McKinley).*

As the administration lines had been disposed of, and based on the response obtained, it was suspected the administration set had been over-tightened when being attached to the syringe, which had caused a slight crack in the syringe connector of the

administration line. Over the infusion period, this would have resulted in some of the syringe contents leaking and, in the case of combination 10, forming a residue on the connection. Particular attention was paid in future when connecting the administration set to the syringe.

3.1.2. Diamorphine Combinations

It is known that mixtures of diamorphine hydrochloride and cyclizine can precipitate when the concentration of cyclizine is greater than 10mg/ml, when the diluent is 0.9% NaCl and when it is used after 24 hours (*MICROMEDEX® Healthcare Series*). Grassby and Hutchings recommend the use of water for injections not 0.9% NaCl as the diluent for mixtures of diamorphine and cyclizine *(Grassby and Hutchings, 1997)*. In the healthcare setting, WFI is used to dilute combinations containing cyclizine. Hence, in this research in order to reduce the potential of precipitation and to obtain data that is applicable in the healthcare setting, the combinations containing cyclizine (1, 2, 8 and 9) were diluted with WFI and, had a concentration of 7.5mg/ml.

Combinations 1, 2 and 8 remained clear, colourless and free from visible particulate matter over the 24 hour infusion period.

In the case of diamorphine combination 9, a white residue was observed on the underside of the syringe and administration line connection for one of the syringes. The residue was similar to that observed for morphine combination 10 (shown in figure 3.1). The residue was noticed at the end of the study. On observation at each previous time point, there was no evidence of precipitation in the administration line, syringe or sampling vessel. The residue was attributed to the over tightening of the administration line to the syringe, resulting in a slight leak and thus evaporation of the syringe contents formed the residue. Combination 10 had not been tested due to laboratory time constraints and availability of instrumentation.

Combinations 3, 4, 5, 6, 7 and 9 remained clear and free from visible particulate matter. However, on preparation of these combinations, the reconstitution of the diamorphine hydrochloride 100mg injection resulted in a yellow solution (shown in figure 3.2). On completion of the syringe preparation, for combinations 3, 4, 5, 6, 7 and 9, this yellow colour was not apparent due to the reconstituted diamorphine having been diluted to the final combination volume of 20ml. The same batch of diamorphine hydrochloride

was used to prepare each of these combinations. The yellow colour had not been observed when reconstituting the diamorphine hydrochloride for the preparation of combinations 1, 2 and 8, for which a different batch of diamorphine was available.

Figure 3.2. The yellow colour observed in reconstituted diamorphine hydrochloride

In a clinical situation, if a yellow colour was noticed, the diamorphine hydrochloride injection would not be used. The manufacturer of the diamorphine hydrochloride *(Wockhardt)* was contacted. Their response included the following:

1. There can be variation in colour at the higher strengths due to a concentration issue of the freeze dried plug.
2. Diamorphine hydrochloride is a white to off-white powder which, when dissolved can give a pale straw colour.
3. The particular batch in question was coming to the end of its shelf life.
4. In a clinical situation a sample would be requested to be sent to them along with its packaging. The appropriate assays would be performed and compared to the release and end of shelf life specifications.
5. Once the product has left them they cannot state whether it has been stored correctly.

The batch used for combinations 3, 4, 5, 6, 7 and 9 was within its expiration date and had been stored as directed, below 25°C and within the outer carton. The Summary of Product Characteristics (SPC) for the diamorphine hydrochloride used states its pharmaceutical form as a white to off-white, sterile, freeze dried powder. The yellow colour was therefore suspected to be due to variation in the freeze dried plug. However, it could not be ascertained as to whether the batch had previously been stored correctly.

3.1.3. Hydromorphone Combinations

All ten combinations remained clear, colourless and free from visible particulate matter over the 24 hour infusion period. However, in the case of combination 8, one of the syringes was leaking from the point where the administration line connected to the syringe (at the same point as the precipitate formation shown in figure 3.1). This was noticed after 1 hour and meant the syringe contents were not being infused through the administration line. In order to collect a sample at each time point, the sampling vessel was placed underneath the connection to collect the contents for analysis. There was no evidence of precipitate formation. Ideally, this combination would have been repeated but due to the nature of the drugs and time constraints this was not possible at the time. However, no observable difference would be expected between the sample being collected from the syringe and not the end of the administration line.

3.1.4. Oxycodone Combinations

Combinations 1-7 and combination 9 remained clear, colourless and free from visible particulate matter over the 24 hour infusion period. As with diamorphine combination 10, oxycodone combinations 8 and 10 were not tested due to instrument availability and laboratory time constraints.

3.1.5. Alfentanil Combinations

The combinations 1-7 and combination 9 were the only combinations tested in this set. Combinations 8 and 10 have not been tested due to laboratory time constraints and availability of instrumentation. The eight combinations tested remained clear, colourless and free from visible particulate matter over the 24 hour infusion period.

3.1.6. Overall Comments on Appearance Results

Overall, the combinations tested reveal that they are physically compatible for infusion over a 24 hour period only. This can be concluded because there was no evidence of precipitation or discolouration within the syringe or administration line from visual observations during the infusion period. This research has not reported any visual changes in appearance, but work carried out by other researchers, not specifically on these combinations, have documented changes in visual appearance. Grassby and Hutchings reported that combinations containing high concentrations of diamorphine hydrochloride and cyclizine lactate precipitated immediately on preparation *(Grassby and Hutchings, 1997)*. They looked at combinations in the range 5-100mg/ml for diamorphine hydrochloride and 5-50mg/ml for cyclizine lactate. They do not state what strength the precipitate occurred at but from this work, combining 100mg diamorphine and 150mg cyclizine to a final volume of 20ml with either 5mg haloperidol or 30mg midazolam did not result in any precipitation. Observations were reported by another group that a mixture of morphine, octreotide, haloperidol, famotidine and midazolam formed precipitate on storage at 4ºC after 24 hours but was stable and compatible at 25ºC *(Nassr et al, 2001)*. The same group also reported that a mixture of morphine, dexamethasone and haloperidol was incompatible at any temperature *(Nassr et al, 2001)*. Even though this research has investigated different strength mixtures and combinations it can still further support the literature that is already available. Also, an admixture of morphine sulphate 100mg and levomepromazine hydrochloride 12.5mg in 17ml at 37ºC showed a change in colour after 48 hours *(Al-Tannak et al, 2012)*. No colour changes were observed in the combinations containing morphine and levomepromazine in this research, but compatibility was only carried out at ambient temperature, not at the mild degradation conditions used in the above work.

3.2. pH

For the combinations tested it was not thought that there would be any significant changes in the pH of the solutions due to all the drugs being acidic. The actual combining of the drugs would be the first point where any issue would have been apparent i.e. possible turbidity of the solution. Apart from being a possible indication of incompatibility, the pH has been documented as it is one of the suggested causes of site reactions in CSCI *(Dickman et al, 2005)*. Blood pH is regulated between 7.35 and 7.45 and infusing an acidic solution could potentially upset the acid/base balance of the blood.

However, the body has buffering processes that can cope with the affect of adding an acidic solution by infusion. Also, infusion is the most effective way of delivering a number of different drugs to treat a wide range of symptoms in one go. The average pH and the relative standard deviation (%RSD) of the pH readings obtained for the two syringes for each combination at each time point was tabulated and are shown below in sections 3.2.1 to 3.2.5. The pH result for the individual syringes of each combination at each time point are documented in the appendix (section 6.1 to 6.5). Any significant and consistent change in pH over the infusion period could possibly indicate chemical incompatibility.

3.2.1. Morphine Combinations

For the morphine combinations there was little evidence of change in pH for any of the combinations over the 24 hour infusion period (table 3.1).

It is known that morphine salts can be precipitated out in an alkaline environment because they are sensitive to changes in pH (*MICROMEDEX® Healthcare Series*). No evidence of precipitation was observed in these combinations and it can be seen that all the combinations have acidic pH values.

An exceptionally high %RSD value has been obtained for the 6 hour time point of combination 5. One of the two readings for this sample was 1 pH unit higher than the other, which was also not consistent with the other readings obtained for the other time points. This high result was suspected to be due to a contaminated pH tube but could not be repeated due to the limited amount of sample available.

Table 3.1. Average pH and %RSD values for the morphine combinations

	Combination									
Time point	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8***	**9**	**10**
0 hours	3.70	3.77	4.16	3.49	4.34	3.57	3.76	3.69	3.69	3.38
%RSD	0.57	0.00	1.02	0.20	4.08	1.58	3.20	1.55	1.34	2.30
3 hours	3.76	3.75	4.67	3.49	4.57	3.81	3.74	3.65	3.71	3.35
%RSD	1.69	0.00	5.45	0.61	2.01	2.60	3.03	1.68	2.67	4.64
6 hours	3.83	3.75	4.32	3.46	4.96	3.74	3.75	3.73	3.67	3.35
%RSD	0.74	0.19	5.24	1.63	13.69	2.84	1.89	2.69	0.77	3.17
24 hours	3.64	3.73	4.42	3.54	4.72	3.76	3.69	3.68	3.74	3.17
%RSD	0.58	0.76	1.28	0.20	5.09	1.50	0.58	3.03	0.00	2.68

*data is based on an average of four readings

Combination 8 had been performed twice; by HPLC and by LCMS and pH readings had been taken for both analyses.

3.2.2. Diamorphine Combinations

There was little evidence of change in the pH readings for any of the diamorphine combinations (table 3.2). At the initial time point for combination 2, one syringe reading was 1 pH unit higher than the other, which resulted in a higher average reading than the subsequent time points. This high reading was not deemed significant due to the other pH readings throughout the study being consistent with each other and no trend in pH variation was observed. This high reading was suspected to be due to a contaminated pH tube.

During the infusion for combination 7, the administration line was not transferred to the sampling vessel for the 6 hour time point. The reading recorded is from the contents of the waste vessel, which was carried out for comparative purposes only.

The pH reading for combinations 7 and 8 at 24 hours are from one syringe only. The contents of the sampling vessel were spilt and there was insufficient sample left to perform the test. Ideally, the infusion would have been repeated but due to the nature and cost of the drugs this was not possible at the time.

Table 3.2. Average pH and %RSD values for the diamorphine combinations

	Combination								
Time point	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
0 hours	3.70	4.21	4.40	3.28	5.08	3.88	3.62	3.74	3.62
%RSD	1.15	15.98	0.48	5.83	11.56	4.01	1.56	0.57	0.20
3 hours	3.72	3.77	4.50	3.35	4.54	3.63	3.57	3.73	3.46
%RSD	0.19	0.75	4.56	0.84	3.59	1.95	0.20	0.95	2.45
6 hours	3.74	3.80	4.36	3.38	4.67	3.65	3.78	3.73	3.59
%RSD	0.00	4.47	0.32	1.47	3.94	0.19	4.68	0.57	0.79
24 hours	3.69	3.69	4.57	3.32	4.59	3.71	3.62	3.69	3.81
%RSD	0.00	0.38	2.32	0.43	0.46	0.19	-	-	0.19

It is apparent that using the reconstituted diamorphine hydrochloride that was yellow in colour has not caused any significant change in pH for combinations 3, 4, 5, 6, 7 and 9.

The rate of decomposition of diamorphine is known to be minimal at about pH 4 (*MICROMEDEX® Healthcare Series*). The pH readings of the combinations tested were all below pH 5 so this suggested that there should not have been significant decomposition of diamorphine in these combinations.

3.2.3. Hydromorphone Combinations

For the hydromorphone combinations there was little evidence of change in pH for any of the combinations, as shown in table 3.3. The high RSD for combination 7 at 3 hours was due to there being a difference of 2.6 pH units between each syringe reading. However, the mean result was typical of the results at previous and subsequent time points.

Table 3.3. Average pH and %RSD values for the hydromorphone combinations

	Combination									
Time point	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
0 hours	3.71	3.76	4.24	3.60	4.35	3.83	3.86	3.77	3.69	3.47
%RSD	0.76	0.38	0.33	0.79	0.98	1.85	0.18	0.38	0.77	0.41
3 hours	3.70	3.73	4.23	3.55	4.35	3.74	3.81	3.72	3.63	3.40
%RSD	0.38	0.00	0.84	1.20	0.49	0.95	4.83	0.76	0.00	0.00
6 hours	3.68	3.71	4.14	3.58	4.33	3.81	3.80	3.75	3.68	3.39
%RSD	0.38	0.57	0.86	0.40	0.16	0.56	0.19	0.38	0.00	0.42
24 hours	3.92	3.78	4.19	3.57	4.38	3.83	3.83	3.82	3.73	3.41
%RSD	0.00	0.56	0.34	0.60	0.48	0.55	1.48	0.93	0.19	0.41

3.2.4. Oxycodone Combinations

There was little evidence of change in the pH readings for any of the oxycodone combinations (table 3.4). Only the repeat results for combination 5 have been reported. It was repeated due to variation in the HPLC results for levomepromazine. The initial pH results did show variation (table A31 in section 6.4) at the initial and 3 hour time point but for consistency of performing all tests at the same time, just the repeat results have been tabulated here. For the repeat results, exceptionally high %RSD values were obtained for the initial, 6 hour and 24 hour time points. This was due to there being a difference of 0.6 pH units or greater between the two readings at each time point. The readings could not be repeated due to insufficient sample.

Table 3.4. Average pH and %RSD values for the oxycodone combinations

	Combination							
Time point	**1**	**2**	**3**	**4**	**5 repeat**	**6**	**7**	**9**
0 hours	3.79	3.71	4.71	3.36	5.46	3.95	3.57	3.73
%RSD	0.19	0.57	1.35	0.21	8.94	0.18	0.40	1.71
3 hours	3.78	3.82	4.53	3.27	4.75	3.76	3.56	3.67
%RSD	0.00	0.19	1.87	0.65	1.79	0.75	1.99	0.19
6 hours	3.83	3.85	4.67	3.28	5.09	3.81	3.59	3.76
%RSD	0.92	0.55	2.42	0.22	7.93	0.19	1.97	2.07
24 hours	3.81	3.76	4.94	3.30	5.44	3.85	3.60	3.77
%RSD	0.37	1.69	6.01	0.43	12.88	2.39	0.39	0.94

3.2.5. Alfentanil Combinations

For the alfentanil combinations there was no notable change in pH for any of the combinations (table 3.5). The pH reading for one syringe was considerably higher than the other at the 3 hour time point for combination 5. There was sufficient sample left to repeat the pH reading and the repeated result was consistent with that of the second syringe. The value represented in the table is a mean of the two concordant readings.

Table 3.5. Average pH and %RSD values for the alfentanil combinations

	Combination							
Time point	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**9**
0 hours	3.76	3.77	4.27	3.47	4.75	3.85	3.80	3.71
%RSD	0.75	0.00	1.82	1.43	0.30	0.55	0.56	0.76
3 hours	3.73	3.74	4.11	3.39	4.64	3.66	3.63	3.65
%RSD	0.38	0.19	0.00	0.42	3.51	0.97	0.39	0.19
6 hours	3.71	3.74	4.18	3.40	4.76	3.74	3.67	3.67
%RSD	0.00	0.76	0.51	0.00	1.34	0.38	0.19	0.00
24 hours	3.77	3.77	4.20	3.41	4.62	3.88	3.76	3.67
%RSD	0.38	0.38	0.17	0.21	2.25	1.46	0.38	0.19

3.2.6. Overall Comments on pH Results

In the literature, it has been noted that researchers have monitored pH in their studies of a similar nature. Mixtures of haloperidol and hyoscine-N-butyl bromide showed acidic pH values in the range 3.00 to 3.42 at 25^{o}C *(Barcia et al, 2003)*. Also, a mean pH value of 3.5 for diamorphine and cyclizine, along with a mean pH value of 3.31 for diamorphine, cyclizine and haloperidol, have been reported *(Grassby and Hutchings, 1997)*. The results in this research are not too dissimilar from these.

It has been noted that the pH results for combinations 3 and 5 were slightly higher than the other combinations. This difference has been attributed to the pH range of the individual drugs in these particular combinations. Hyoscine butylbromide can be in the range pH 3.7-5.5, levomepromazine pH 4.5 and metoclopramide within the range pH 3-5, compared to the other supportive drugs which tend to have pH values less than 4. An increase has not been seen in the other combinations containing these particular drugs, which is probably due to the pH of the other drugs in the combination having a more acidic pH range.

It is known that levomepromazine is incompatible in alkaline solutions *(Martindale)* and it can be seen from each set that for the combinations containing this drug, combinations 3, 5 and 6 and 10, the resulting solutions are all acidic.

In conclusion, changes in pH can indicate that a chemical change has taken place, but from the data obtained it is evident that overall the pH results have showed little evidence of change over the 24 hours. The pH and solubility of drug injections are directly related *(Rose and Currow, 2009)* and combining injections of widely differing pH can result in precipitate formation. In the above combinations, drug injections of similar pH

have been combined, hence, no problems have been experienced as the individual injections of the drug components in each combination have acidic pH values, resulting in an overall acidic solution. Combining drugs of differing pH could potentially result in chemical incompatibility and Good et al reported visual incompatibility in combinations of midazolam (pH 3.5) and dexamethasone (pH 7), which was thought to be due to a pH effect *(Good et al, 2004)*.

3.3. HPLC Assay

With regards to the HPLC assay it was more difficult to predict as to whether interactions would be apparent. Assessment of the concentration of each drug component at the four time points was a starting point, along with appearance of any additional peaks in the chromatograms. The SPC of the individual drug components indicated stability for 24 hours once opened, so a reduction in the concentration of the drugs was not expected.

The individual drug component concentration for each opioid combined with the supportive drug combinations 1-7 and 9 was obtained by HPLC analysis. For morphine combinations 8 and 10, the individual drug component concentrations were assessed by HPLC and LCMS analysis. Diamorphine combination 8 and hydromorphone combinations 8 and 10 were assessed by LCMS analysis only. Unfortunately, the remaining combinations could not be assessed.

The HPLC analysis used methods that had been developed for each combination. Nassr *et al. (2001)* performed a study evaluating the compatibility and stability of five morphine drug mixtures used for palliative care. For the drugs used in their study, they identified a HPLC method capable of separating the seven drugs and their associated preservatives. Their method used a gradient system with the two elution solvents being acetonitrile and 0.05M potassium phosphate buffer at pH 4.6. Four of the drugs separated by this method were present in the combinations for this research project, thus this method was used as the starting point for the HPLC method development.

The chromatographic conditions stipulated by Nassr *et al.* were adapted. For example, the phosphate buffer was changed to ammonium acetate at the same strength and pH. Ammonium acetate (VWR International) was chosen because it is a buffer that is compatible with LCMS instrumentation. This buffer allowed the methods that were developed to be transferred to this instrumentation, if required, without any delay. Nassr *et*

al. used a Zorbax® Eclipse XDB, $C_{18,}$ 3.5µm, 4.6 x 75mm column, however, a longer column with a larger particle size was used because the HPLC systems in the laboratory typically used longer columns and these columns were available for the analysis. A 150 x 4.6 mm Platinum EPS C_{18} 100Å 5µm column (Alltech) was the starting column, however, it did not always give suitable chromatography so an alternative column was used; 150 x 4.6mm Symmetry C_{18} 5µm (Waters Limited). Both columns were reverse phase columns with measurements of 150 x 4.6mm, a pore size of 100Å and a particle size of 5µm. The differences however, occurred in the stationary phase. The Symmetry column contained a spherical particle shape and was end-capped with a carbon load of 19%, whereas the Platinum column contained a monomeric phase which was not end-capped and had a carbon load of 5%. The Platinum column was also EPS (extended polar selectivity), which had controlled silica exposure resulting in an extended selectivity range. These differences enabled levomepromazine and midazolam in combination 6 to be separated. The wavelengths used by Nassr *et al.* were 250 and 285nm; however, as the HPLC system consisted of a diode array detector (DAD) this was set to scan in the range 190 to 360nm to obtain the optimal absorbance wavelength for the drug components in the combinations.

As part of the method development, specificity was performed in order to be able to discriminate the drug component from possible excipients or impurities or degradation products. To identify degradation peaks of the drug components, individual drug component solutions were subjected to heat stress conditions over a period of time and then analysed by the appropriate developed method. Not all of the individual drug component solutions were subjected to this forced degradation or analysed by each method. This was deemed unnecessary as the infusions were only performed over a 24 hour time frame, there were similarities between the methods, and there was some overlap of drug components between combinations. The individual drug component injections used in the preparation of each combination had expiry dates beyond 24 hours and the recommended storage was below 25^{o}C. The drug combinations, once prepared, were infused within 24 hours at a temperature below 25^{o}C, thus minimal degradation was expected due to these conditions. Forced degradation was carried out on a stock standard preparation of the drug component, which was then diluted to the appropriate concentration of the relevant method before being analysed by HPLC. Refer to table 3.6 for results of the degradation work carried out.

Table 3.6. Summary of degradration findings

Morphine Combinations				
Component	**Combination**	**Temp (°C)**	**Duration**	**Findings**
Cyclizine	2	60	24hrs	No degradation peaks
Levomepromazine	3	80	48hrs	No degradation peaks
Morphine	4	60	24hrs	Possible peaks 5-8mins
Haloperidol	4	60	24hrs	No degradation peaks
Midazolam	4	60	26hrs	No degradation peaks
Sample	4	60	24hrs	No degradation peaks
Hyoscine	5	60	24hrs	Peak 2.9min
Levomepromazine	5	60	24hrs	No degradation peaks
Levomepromazine	6	60	24hrs	No degradation peaks
Sample	6	60	24hrs	No degradation peaks
Metoclopramide	7	60	24hrs	No degradation peaks
Midazolam	7	80	72hrs	Peak 2.3min
Cyclizine	8	60	24hrs	No degradation peaks
Haloperidol	8	60	24hrs	No degradation peaks
Diamorphine Combinations				
Diamorphine	1	60	24hrs	Peak 1.9min
Cyclizine	1	58	72hrs	No degradation peaks
Haloperidol	1	58	72hrs	No degradation peaks
Sample	1	58	72hrs	Peak1.5min and 1.9min
Midazolam	2	58	72hrs	No degradation peaks
Sample	2	58	72hrs	Peak 1.9min and 10.7min
Metoclopramide	3	80	48hrs	No degradation peaks
Levomepromazine	3	80	48hrs	Doublet 8.8-9.2min
Hyoscine	5	80	48hrs	Peak 1.9min
Levomepromazine	5	80	48hrs	No degradation peaks
Midazolam	6	80	72hrs	Peak 1.6min
Levomepromazine	6	80	48hrs	Peak 2min
Alfentanil Combinations				
Alfentanil	-	58	48hrs	Monitor peak 1.44min
Oxycodone Combinations				
Oxycodone	2-5, 7, 9	80	48hrs	Peak 2.15min
Midazolam	2-5, 7, 9	80	72hrs	Peak 2.15min
Levomepromazine	2-5, 7, 9	80	48hrs	No degradation peaks
Cyclizine	2-5, 7, 9	80	72hrs	Monitor peak 10.1min
Haloperidol	2-5, 7, 9	80	72hrs	Peak 2.15min
Metoclopramide	2-5, 7, 9	80	48hrs	No degradation peaks
Hyoscine	2-5, 7, 9	80	48hrs	No degradation peaks

NOTE: 'No degradation peaks' refers to the fact that no new peaks had formed

In most instances, no further peaks were identified. Due to the short study period no further methods to force degradation of the drugs was undertaken. Acid-base degradation could have been carried out to identify degradants of the drug components *(ICH)*. From the literature it is known that the use of acid or base hydrolysis is required to achieve some of the drug component degradation products but unfortunately this has not

been carried out so the methods used are limited to separating out thermal degradation products only, which this research has shown can be achieved and was thought to be sufficient for these short studies.

It is known that midazolam requires oxidation or base hydrolysis for significant degradation to occur as only limited degradation happens under thermal conditions (*Amruthraj et al., 2013*). The decomposition products of hydromorphone after heating in acid and alkali occur before the hydromorphone peak in the HPLC method documented by Trissel *et al*. They also have evidence of stability for 2 months at room temperature for hydromorphone hydrochloride 1.5mg/ml and 80mg/ml in 0.9% NaCl in polypropylene syringes (*Trissel et al, 2002*). Treatment of oxycodone with hydrogen peroxide and ozone results in the N-oxide and aldehyde respectively (*Salomies and Salo, 2000*). Diamorphine can undergo hydrolysis to 6-monoacetylmorphine and then to morphine due to changes occurring in temperature and pH (*Hutchinson and Somogyi, 2002*). The appearance of 6-monoacetylmorphine was apparent during these studies. The degradation products of morphine are pseudomorphine and morphine-N-oxide, which can be formed after heating with strong base (*Beaumont and Deeks, 1982*). Panaggio and Greene developed a HPLC method that was able to detect haloperidol and its degradation products. They showed that the degradation products emerged after storage at elevated temperatures, at different pH's and on exposure to light (*Panaggio and Greene, 1983*). Levomepromazine's main degradation product is levomepromazine sulfoxide (*Karpińska et al., 2006*). Maquille and Jiwan have investigated the photodegradation of metoclopramide (*2009*). No detectable changes in chromatography were observed when hyoscine-N-butyl bromide was heated in mobile phase at 40^{o}C for 10 days (*Barcia et al., 2003*).

The combination sample chromatograms were monitored for the presence of any additional peaks. Any peak which could not be attributed to an individual drug component, through comparison with the standard solution, and was seen in the initial time point chromatogram of the combination was monitored in all subsequent time point injections and assessed on completion of the infusion. Different diluents were used to prepare the syringe combinations and also the standard and sample solutions, therefore these were also analysed by the HPLC method. The diluents were: WFI or 0.9%w/v NaCl (the diluents used to prepare each combination) and distilled water, which was used to dilute the standard and sample preparations. This allowed any additional peaks to be discounted from the combination if they also occurred in these injections. The initial (0h) and final (24h) sample chromatograms have been included below for each combination (see figures 3.3 to 3.45). These chromatograms show that there are additional peaks

present alongside the drug components. These peaks also occur in the individual drug component standard chromatograms or are attributed to the diluents used. In most combinations these additional peaks have not increased in size throughout the study.

The ICH guidelines mention a number of characteristics that should be considered for validation of an analytical procedure, however, due to time constraints and the cost of being able to perform certain tests the methods only had limited validation carried out. The method used for each combination was assessed for standard repeatability and sample repeatability. The criteria for standard repeatability stipulated that the areas obtained for the six injections of each standard solution had to have a relative standard deviation (RSD) of less than 2.0% and the response factors for each standard solution had to agree within ±2% of each other. The standard repeatability was carried out using a two point calibration, where two solutions of the same strength were prepared. Perhaps preparing two levels of standards at 90% and 110% of nominal would have been more appropriate due to the number of results obtained being greater than 100%. Compliance with standard repeatability criteria meant only the first standard preparation was used throughout the rest of the analysis and to generate the sample results. The criteria for sample repeatability stated that the areas obtained for each drug component in the combination for each of the six preparations had a RSD less than 2.0% and a maximum acceptable deviation (MAD) of less than 4.0%. However, the criteria for these assessments were not met for each combination. In the case of standard repeatability, if the two standard preparations did not comply, one of the following was done:

1. a third preparation of the standard was prepared and assessed against the first two preparations to deem whether a preparation error had occurred, or
2. both standard preparations were used throughout the rest of the analysis, and in order to generate the results the average response factor of the two standards was used.

The first option was dependent on whether there was sufficient standard of the same batch available to prepare another solution, otherwise the second option was chosen.

The individual standard solutions were prepared and analysed before the infusions were started, between the 6 hour and 24 hour samples and also after completion of the infusion. They were stored refrigerated for the duration of the HPLC analysis. No significant change was observed between the sets of standard analyses indicating that the solutions were stable over the HPLC analysis duration.

Each drug combination was prepared in duplicate. At each time point a sample from each of the two syringes was analysed using the HPLC method developed for that combination. The HPLC system injected each preparation twice, generating four results for each time point. The appendix (sections 6.1 to 6.5) shows the full set of results for each combination. These results were used to determine the concentration of each drug component in the combination. The percentage results (% nominal) in the appendix have been calculated by dividing the sample result by the theoretical result of that particular drug. The theoretical result is the concentration of the drug present in the final volume of the syringe contents. Some of the concentrations presented in the results are greater than 100% nominal, which may be due to an overage in the ampoule or inaccuracies in diluting whilst preparing the syringe. As these methods aimed to identify changes in the drug concentrations during the infusion period, the initial concentrations that were higher than 100% were acceptable and variations from that initial value were monitored. The average result for each drug in the combination, along with the RSD of the four injections, at each of the time points had been tabulated and these are shown in sections 3.3.1 to 3.3.5. The mean results shown in the tables have taken the initial time point result as 100% and the subsequent results have been calculated relative to that value i.e. the percent initial remaining. The high RSD results have been attributed to the results from one syringe preparation being higher or lower than the other syringe preparation. The individual results for syringe 1 (S1) and syringe 2 (S2) have also been included in the tables.

Overall, the aim of the HPLC method was to calculate the concentration of each drug component in each combination to determine whether its concentration was unaffected by combination with other drug components over a fixed 24 hour time frame. The use of the one-way ANOVA statistical test enabled significant differences in the results to be determined. ANOVA compares the means of a set of data to establish if any variation is due to changes in the concentration of the drugs and not simply due to chance of random sampling. A one-way ANOVA was chosen because there is only the variable time that is affecting the samples tested and each set of data obtained at the different time points was compared to the initial (0h) set of data for the combination in question. The criterion for significant difference, the p-value, was set at 0.05. Hence, if the resulting p-value was less than 0.05, the differences in the data sets were deemed significant. However, for p-values greater than 0.05, then there was 95% confidence that the data obtained did not show any significant difference.

In assessing the results of these combinations and drawing conclusions from them, it is necessary to differentiate between the syringe preparations carried out in a clinical setting and those performed in this research. In a clinical setting, the syringe is prepared only once and the healthcare professional would not be measuring concentrations. However, in this research the concentration of each drug component in the syringe was assessed by an analytical technique, and duplicate preparation of each combination was done. This duplicate preparation has identified the inherent differences in drug concentrations between the two syringes prepared. These differences are considered to be solely due to the individual drug component vials used to prepare the syringes having limits and overages associated with them.

3.3.1. Morphine Combinations

It is known that morphine, whether on its own or in combination has been extensively studied in the literature. For example, the following groups have looked at morphine on its own: *Vermeire and Remon,* 1999 and *Hor et al.,* 1997. *Nassr et al., 2001* and *Negro et al., 2006* however have looked at morphine in combination. The following results contribute further to the research available about morphine.

Combination 1

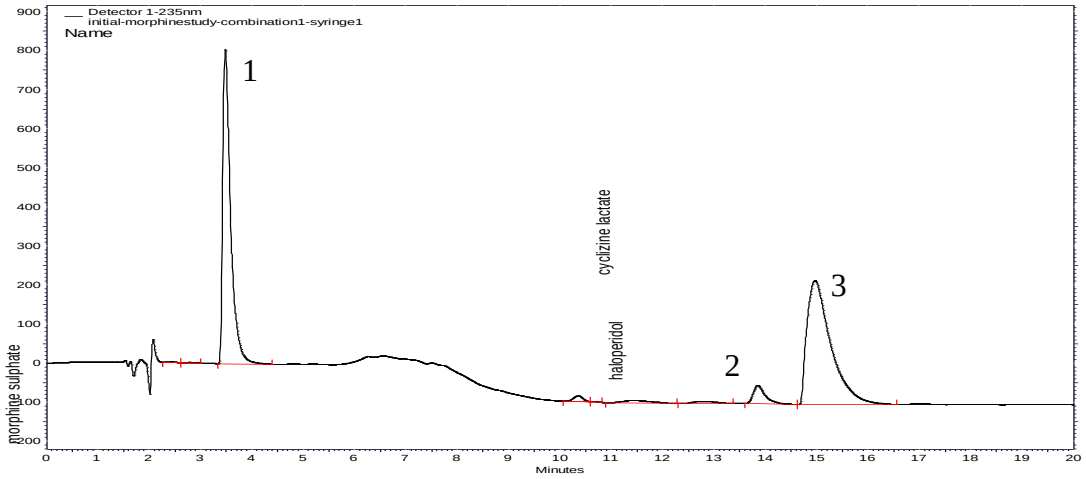
Table 3.7 shows the results and figure 3.3 depict the chromatography for this combination.

Table 3.7. Average results of the HPLC assay for morphine combination 1

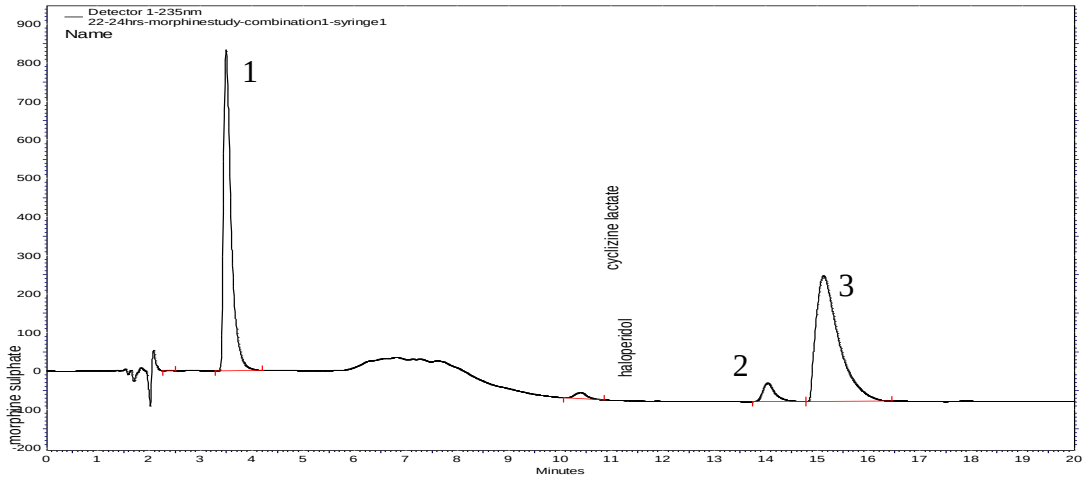
	Morphine % initial			Haloperidol % initial			Cyclizine % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (0.36%)	108.6	108.1	100.0 (0.77%)	102.8	102.3	100.0 (1.09%)	107.5	106.0
3 hours	100.7 (0.45%)	109.5	108.7	103.3 (2.26%)	107.8	104.2	100.7 (1.25%)	108.6	106.3
6 hours	99.7 (0.10%)	108.0	108.1	102.5 (3.45%)	105.0	105.2	99.9 (0.87%)	107.4	106.0
24 hours	101.7 (2.49%)	107.9	112.6	103.7 (2.82%)	107.7	105.1	100.4 (0.78%)	107.4	106.9

Figure 3.3. Representative chromatograms for combination 1 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent morphine sulphate (retention time 3.5min), haloperidol (retention time 13.8min) and cyclizine lactate (retention time 15.0min) respectively

(A)



(B)



Combination 2

Table 3.8 shows the results for this combination. A decrease in midazolam concentration had been observed at 3 hours. Statistical analysis indicated that there was significant difference in this midazolam result. On looking at the data, it was observed that the results for both syringes were lower than the other time points and the decrease was suspected to be due to an error in the sample dilution preparation. An overall decrease in concentration was not observed, so could not be attributed to a 'real' drop in concentration. An instrument error was not suspected because the RSD of the four injections from two separate HPLC vials was 0.66%. Figure 3.4 shows the chromatography for this combination.

Combination 3

Results higher than the other time points had been obtained at 24 hours for morphine and metoclopramide, along with the results for levomepromazine at 6 hours. Statistical analysis reveals that there was significant difference in the results for morphine and metoclopramide. Unfortunately, there is no further time point after 24 hours to determine whether a trend was developing, but possible high results could be due to variation in the chromatographic integration. For levomepromazine no significant difference was apparent. The results for this combination can be seen in table 3.9, along with its chromatograms in figure 3.5.

Combination 4

Table 3.10 and figure 3.6 show the results of combination 4 with morphine and its associated chromatograms respectively.

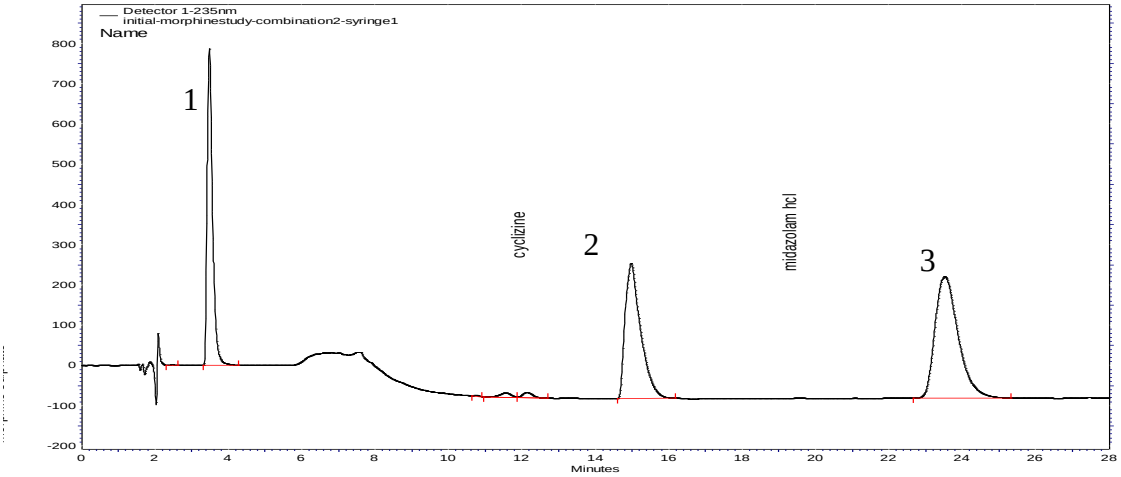
Table 3.8. Average results of the HPLC assay for morphine combination 2

	Morphine % initial			Cyclizine % initial			Midazolam % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (1.80%)	107.9	111.3	100.0 (1.76%)	104.1	107.1	100.0 (2.21%)	129.3	133.3
3 hours	99.6 (1.37%)	108.0	110.4	100.3 (1.04%)	105.0	106.8	92.4* (0.66%)	121.8	120.9
6 hours	99.0 (2.51%)	106.2	110.9	99.9 (1.65%)	104.0	107.0	96.6 (2.23%)	124.6	129.0
24 hours	98.3 (2.85%)	105.1	110.3	99.6 (2.75%)	102.8	107.7	98.5 (2.19%)	127.0	131.7

* denotes where a significant difference was observed (one-way ANOVA, sample size n=4)

Figure 3.4. Representative chromatograms for combination 2 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent morphine sulphate (retention time 3.5min), cyclizine lactate (retention time 15.0min) and midazolam (retention time 23.5min) respectively

(A)



(B)

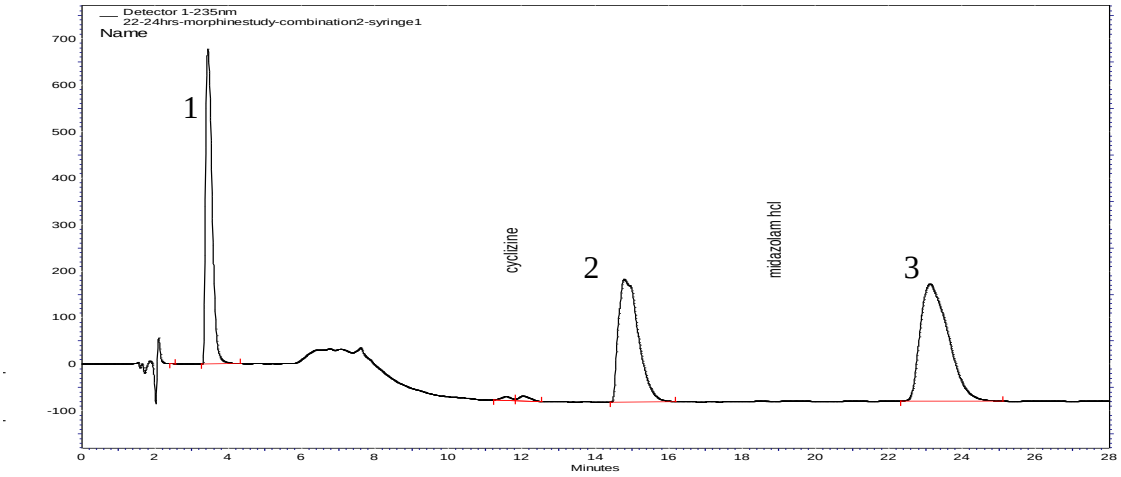


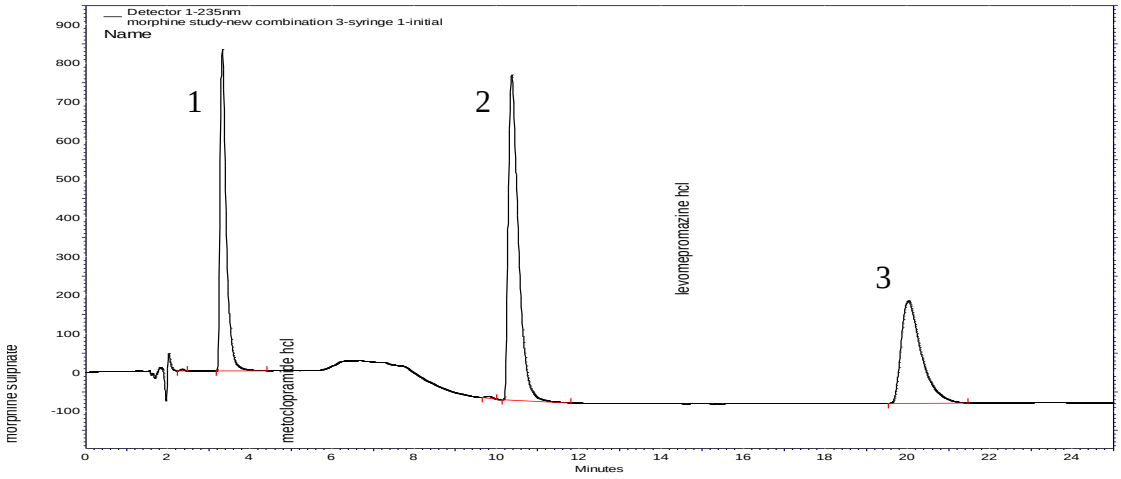
Table 3.9. Average results of the HPLC assay for morphine combination 3

	Morphine % initial			Metoclopramide % initial			Levomepromazine % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (1.87%)	109.0	106.1	100.0 (1.57%)	117.6	115.1	100.0 (1.87%)	133.1	129.3
3 hours	101.6 (1.17%)	108.2	110.3	102.3 (0.83%)	118.3	119.9	102.4 (1.57%)	134.7	133.9
6 hours	101.8 (0.60%)	109.7	109.2	102.9 (0.99%)	120.2	119.1	105.1 (3.81%)	141.5	134.3
24 hours	105.* (1.59%)	114.7	111.7	104.* (1.10%)	123.1	121.0	102.8 (1.33%)	136.4	133.5

* denotes where a significant difference was observed (one-way ANOVA, sample size n=4)

Figure 3.5. Representative chromatograms for combination 3 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent morphine sulphate (retention time 3.3min), metoclopramide hcl (retention time 10.4min) and levomepromazine hcl (retention time 20.0min) respectively

(A)



(B)

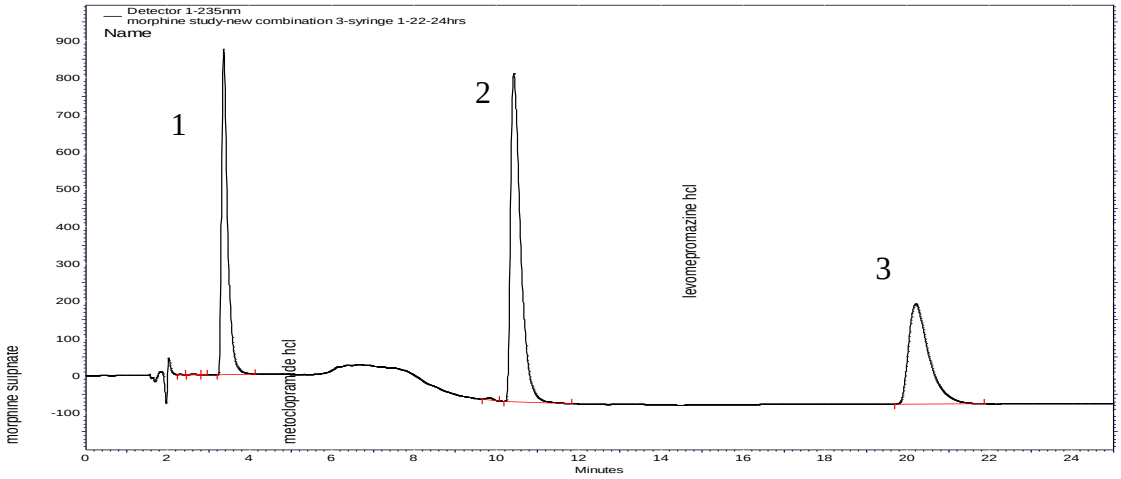
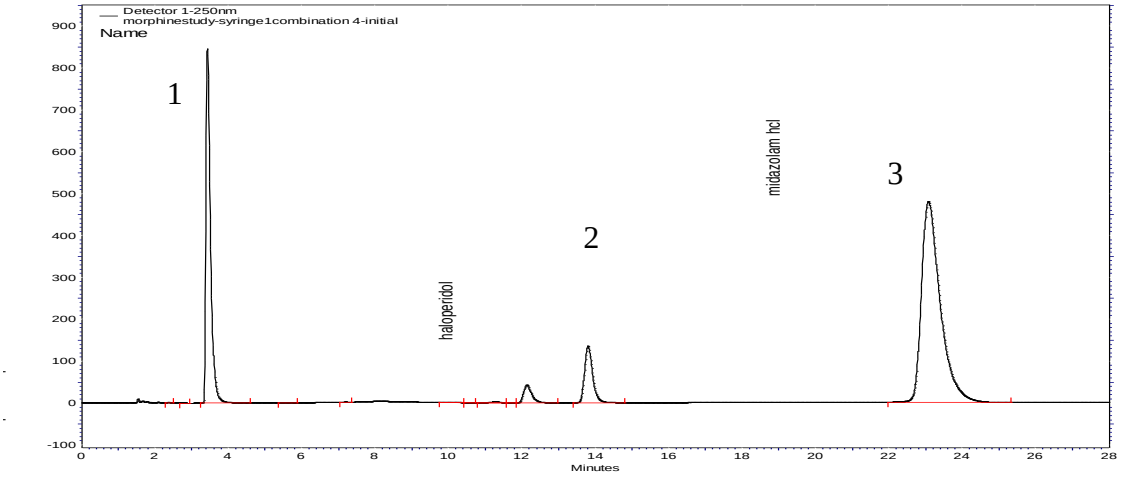


Table 3.10. Average results of the HPLC assay for morphine combination 4

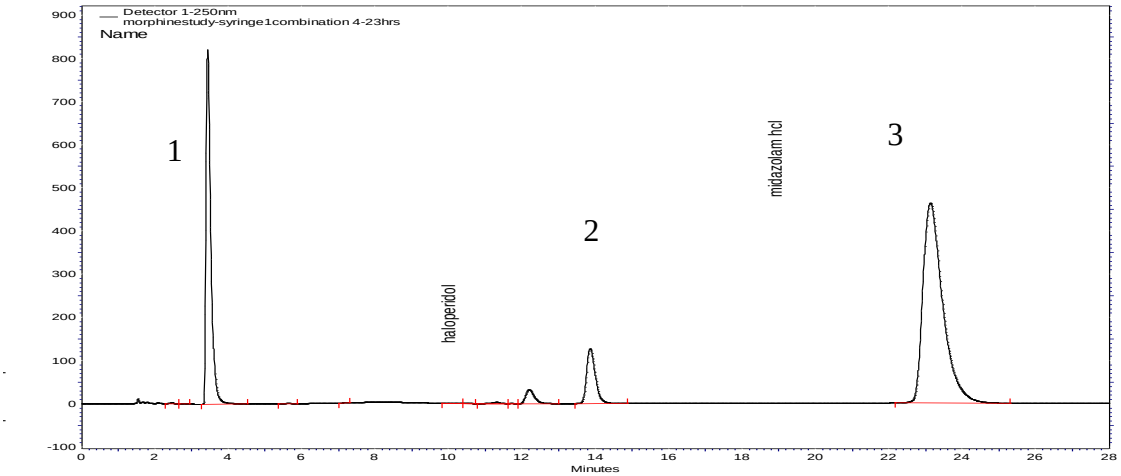
	Morphine % initial			Haloperidol % initial			Midazolam % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (1.45%)	108.0	105.4	100.0 (1.79%)	106.6	103.3	100.0 (1.78%)	124.1	125.5
3 hours	101.3 (1.94%)	109.9	106.3	101.1 (2.26%)	108.1	104.0	97.3 (1.83%)	121.5	121.1
6 hours	100.3 (3.22%)	109.9	104.1	100.2 (3.67%)	108.5	101.9	98.7 (2.15%)	124.3	122.0
24 hours	101.5 (1.51%)	109.7	106.9	101.5 (1.68%)	108.1	104.9	100.9 (1.26%)	125.7	126.2

Figure 3.6. Representative chromatograms for combination 4 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent morphine sulphate (retention time 3.4min), haloperidol (retention time 13.8min) and midazolam hcl (retention time 23.0min) respectively

(A)



(B)



Combination 5

Table 3.11 shows the results for this combination and figure 3.7 the chromatography. At all time points the levomepromazine results had significantly higher RSD values than previously seen. Looking at the individual results for each syringe preparation at each time point, refer to table A5 in section 6.1, it is evident that there is up to a 16% difference between the two syringe preparations depending on the time point. However, if the results for each syringe preparation, i.e. all three drug components, had been assessed separately then there is no significant change in any of the drug component concentrations for either preparation over the infusion period studied. In clinical practice only one preparation of this combination would have been prepared and the concentrations of the drug components would not have been monitored, therefore, any variation would not be reported. However, in terms of this research, this variation is beyond acceptable for the technique used and has highlighted inherent differences between the syringes as a result of overages and limits of the drug component vials. In addition, the technique used to add the levomepromazine drug component to the combination may have contributed to the difference in concentration between the two syringes. The levomepromazine drug component was only supplied in a 1ml ampoule containing 25mg/ml, however, in this combination; 12.5mg of levomepromazine was required. Therefore, half an ampoule was added to the other drug components in one syringe and the other half was added to the other syringe. For some of the other opioids with this combination, problems were also encountered, and advice was sought for the 'best practice' of preparing this combination from the Aseptic Department at Stepping Hill Hospital. On advice, a 1ml syringe with needle was used to draw up 0.5ml from the levomepromazine ampoule and then add the contents to the syringe containing the combination. This process was then repeated for the second preparation. Duplicate syringes were prepared to confirm that the technique used was consistent for each preparation and the results obtained were in line with each other. The result here have identified the extent of variation that may occur between different preparations, nonetheless they do confirm no significant changes in drug concentration over the period of study.

Statistical analysis showed significant difference in the morphine and hyoscine butylbromide results. This is due to the lower results at the 3 hour time point followed by the higher results at the 24 hour time point; but no trends are evident.

Table 3.11. Average results of the HPLC assay for morphine combination 5

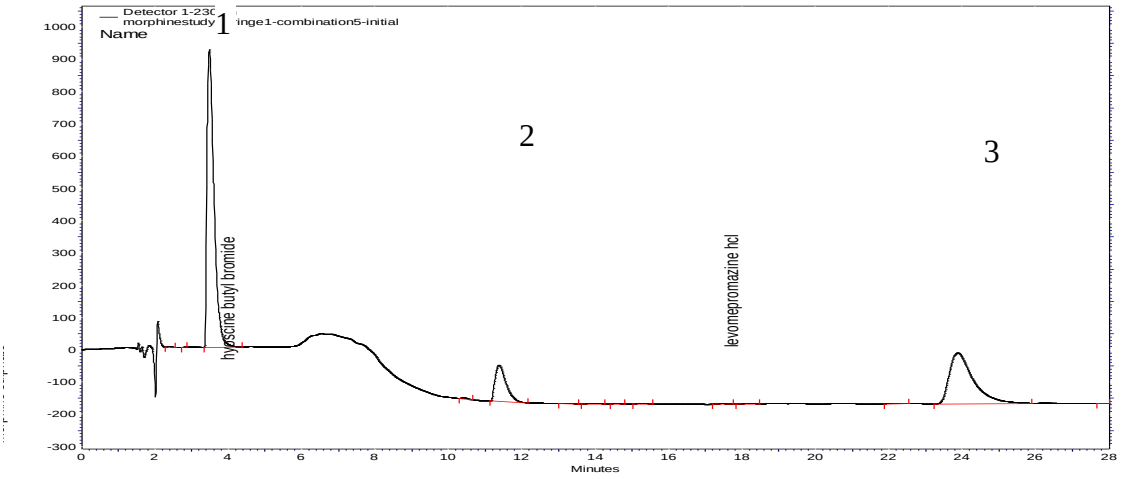
	Morphine % initial			Hyoscine butylbromide % initial			Levomepromazine % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (2.38%)	104.5	101.4	100.0 (0.59%)	105.0	104.9	100.0 (6.44%)	116.0	103.8
3 hours	96.7* (0.43%)	99.9	99.2	98.2* (1.68%)	104.1	102.0	95.6 (6.51%)	111.0	99.1
6 hours	99.4 (0.82%)	101.8	102.8	100.4 (1.29%)	104.7	106.0	98.7 (7.31%)	115.2	101.8
24 hours	103.3* (3.51%)	109.1	103.6	103.9* (0.46%)	109.2	108.9	99.0 (8.68%)	116.9	100.7

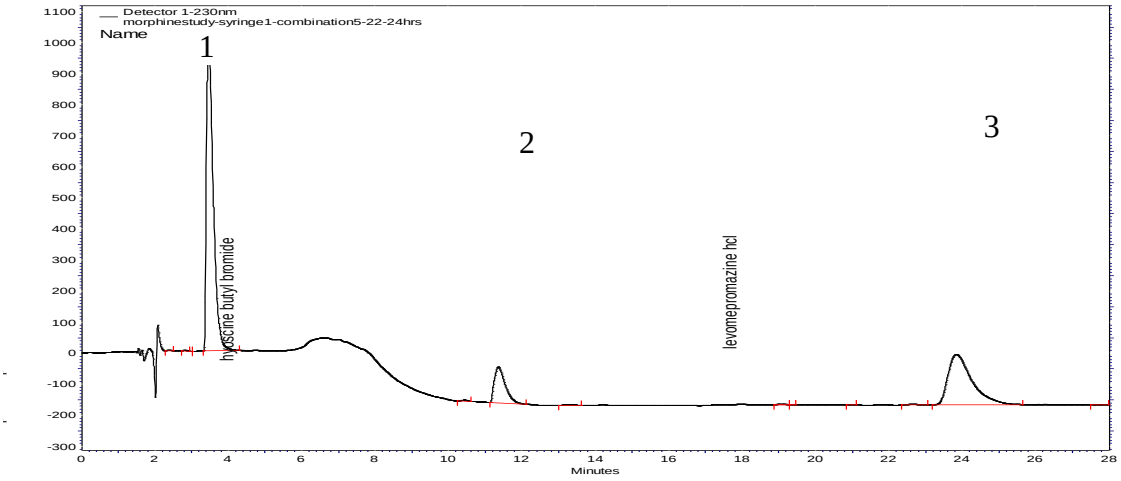
* denotes where a significant difference was observed (one-way ANOVA, sample size n=4)

Figure 3.7. Representative chromatograms for combination 5 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent morphine sulphate (retention time 3.5min), hyoscine butylbromide (retention time 11.4min) and levomepromazine hcl (retention time 23.9min) respectively

(A)

(B)





Combination 6

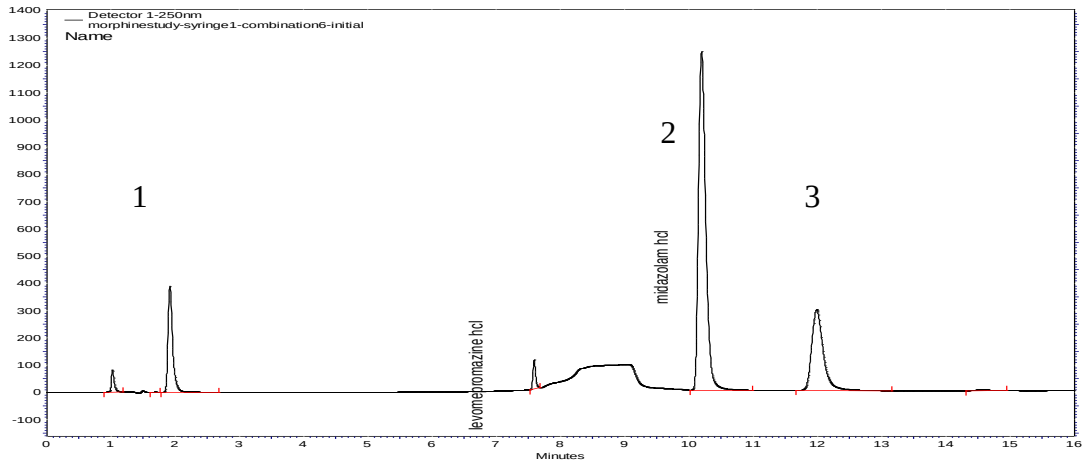
Table 3.12 and figure 3.8 show the results and chromatography for this combination.

Table 3.12. Average results of the HPLC assay for morphine combination 6

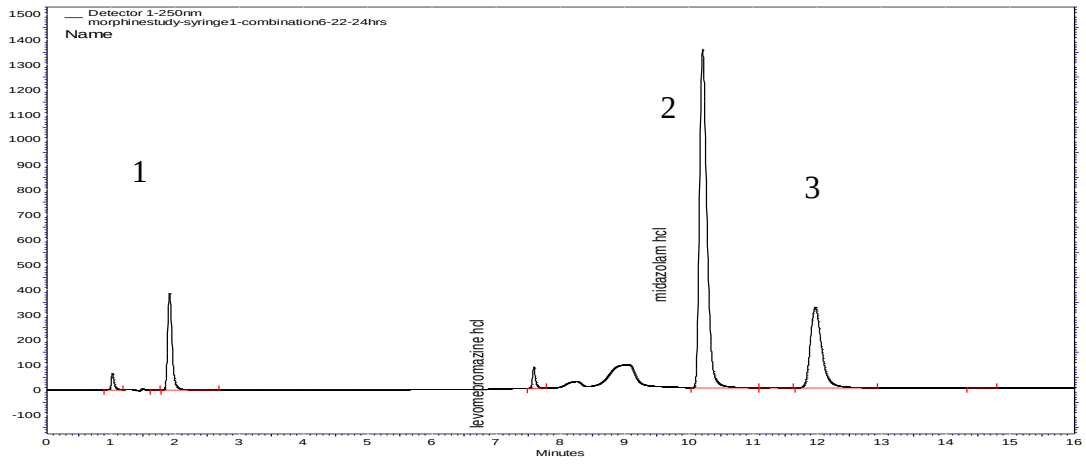
	Morphine % initial			Levomepromazine % initial			Midazolam % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (3.15%)	118.2	111.9	100.0 (4.12%)	113.6	122.0	100.0 (5.26%)	114.9	125.8
3 hours	98.7 (0.52%)	113.0	114.1	104.4 (0.56%)	123.4	122.6	100.4 (1.22%)	120.2	121.4
6 hours	97.9 (1.13%)	111.7	113.7	104.5 (0.45%)	122.9	123.3	103.0 (1.35%)	122.7	125.3
24 hours	98.9 (1.14%)	112.8	114.9	106.4 (0.53%)	125.7	125.0	104.8 (2.12%)	123.9	128.3

Figure 3.8. Representative chromatograms for combination 6 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent morphine sulphate (retention time 1.9min), levomepromazine hcl (retention time 10.2min) and midazolam hcl (retention time 12.0min) respectively

(A)



(B)



Combination 7

The results for combination 7 can be seen in table 3.13 and its associated chromatography in figure 3.9. The 24 hour time point samples were actually analysed 5 hours after being sampled because the column had to be repacked due to poor chromatography, hence, causing the delay. Statistical tests revealed significant difference in the morphine and metoclopramide results. This has been attributed to the decrease at the 24 hour time point. Unfortunately, there are no further time points to see if this is a trend, but the repacking of the column mid analysis could be a contributory factor. Even though there is a decrease in the midazolam results, at the 3 hour and 24 hour time points, the data does not show significant difference because at the 6 hour time point the result was in line with the initial result. The midazolam data shows variation in each set of results causing the high RSD values. The drop in concentration for this combination was not deemed significant.

In the literature, the stability of a mixture of metoclopramide hydrochloride 0.5mg/ml and morphine sulphate 1mg/ml, in different delivery presentations, was dependent on the diluent used and was related to the metoclopramide component *(Nixon et al, 1995).* However, Dickman *et al* reported physical compatibility over 24 hours for a number of concentrations of combination 7 in sodium chloride 0.9% based on clinical observations (*Dickman et al,* 2005). This data is based on a final syringe volume of 18ml. This research has used higher concentrations of these drug components in a final syringe volume of 20ml, but confirms physical compatibility, along with chemical compatibility over 24 hours of this combination.

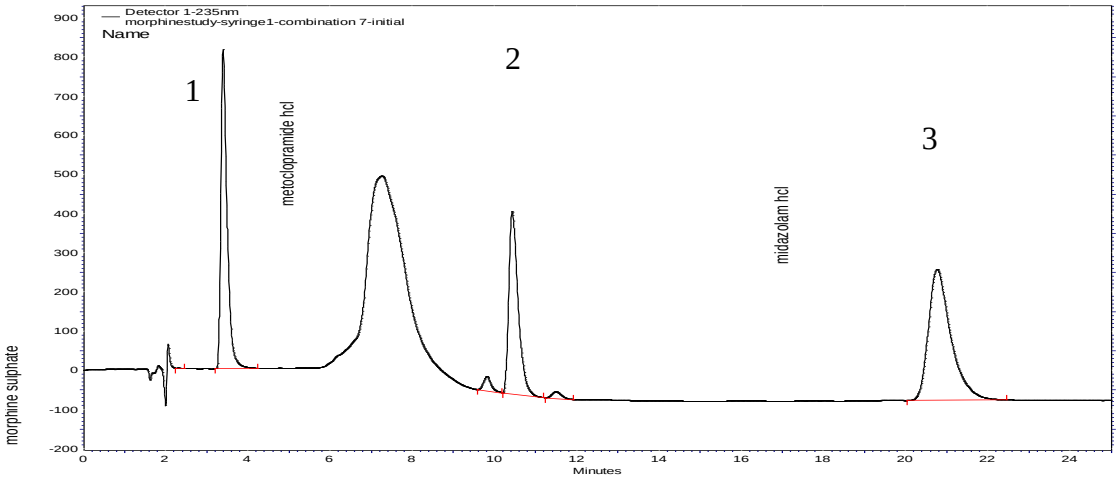
Table 3.13. Average results for the HPLC assay for morphine combination 7

	Morphine % initial			Metoclopramide % initial			Midazolam % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (0.99%)	110.7	109.1	100.0 (0.37%)	124.3	124.4	100.0 (3.65%)	119.7	126.9
3 hours	99.6 (0.62%)	109.2	109.5	100.0 (0.87%)	123.6	125.1	96.3 (3.63%)	115.1	122.3
6 hours	99.1 (0.54%)	109.1	108.6	99.6 (0.51%)	123.6	124.0	99.2 (2.93%)	119.6	125.0
24 hours	95.7* (1.56%)	104.9	105.4	98.5* (0.56%)	122.2	122.8	98.6 (1.44%)	121.0	122.1

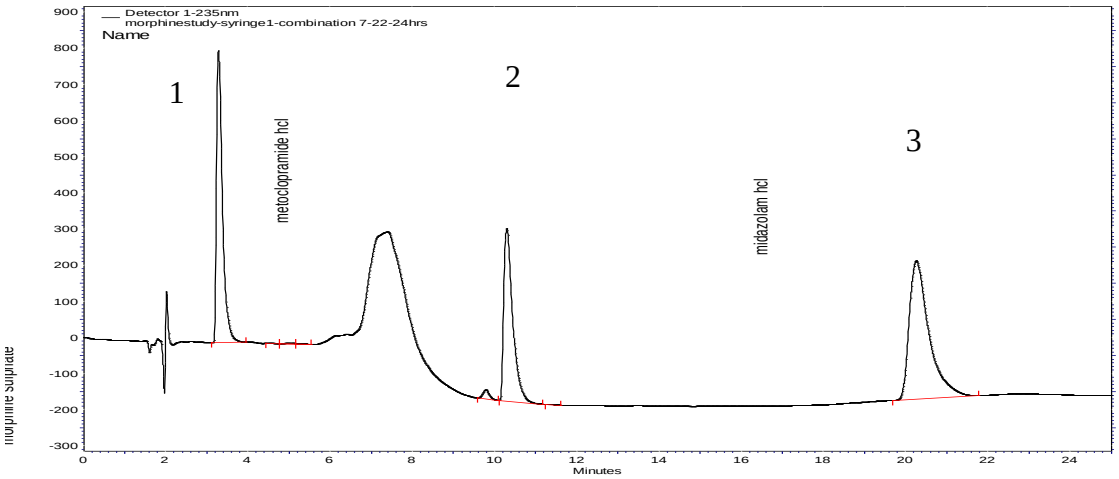
* denotes where a significant difference was observed (one-way ANOVA, sample size n=4)

Figure 3.9. Representative chromatograms for combination 7 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent morphine sulphate (retention time 3.4min), metoclopramide hcl (retention time 10.4min) and midazolam hcl (retention time 20.8min) respectively

(A)



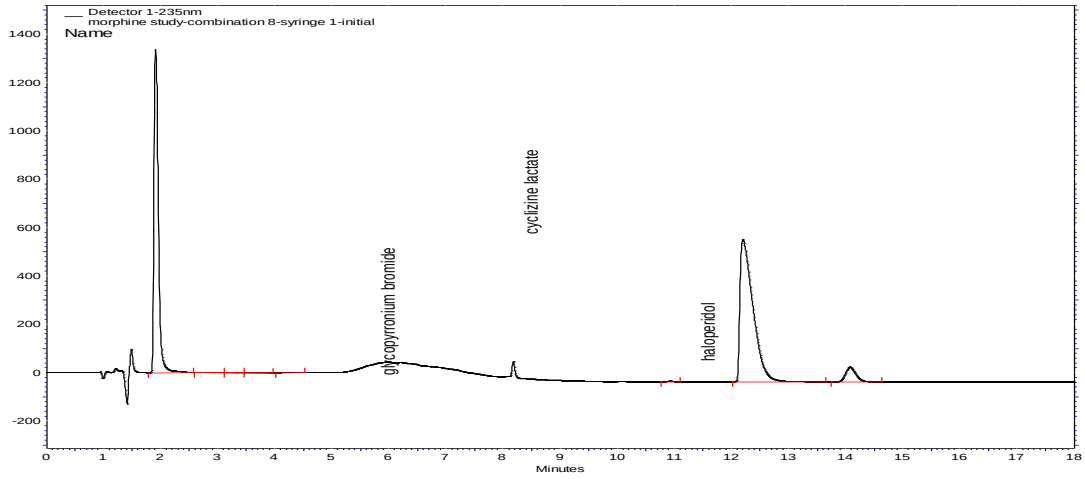
(B)



Combination 8

The sample chromatograms for this combination showed that the glycopyrronium component peak was significantly smaller than the other drug component peaks i.e. could only just be observed. An example chromatogram is shown in figure 3.10.

Figure 3.10. Example chromatogram of morphine combination 8



As a result of this difference, there was variation in the data for this peak due to difficulties in getting consistent integration leading to high RSD values for the results at each time point. The glycopyrronium standard results did not comply with the criteria either, therefore, the glycopyrronium results for this combination was not used. The data from the other drug component peaks was suitable to use and are shown in table 3.14, along with their chromatography in figure 3.11. The analysis only of the glycopyrronium component of this combination was repeated using LCMS/MS. Refer to section 3.4 for the glycopyrronium results analysed by LCMS/MS.

Statistical analysis shows that there is significant difference in the morphine results. This has been attributed to the 6 hour time point having consistent results for both syringe preparations but the 0 hour and 3 hour time points having one syringe preparation higher and the 24 hour time point having both preparations higher.

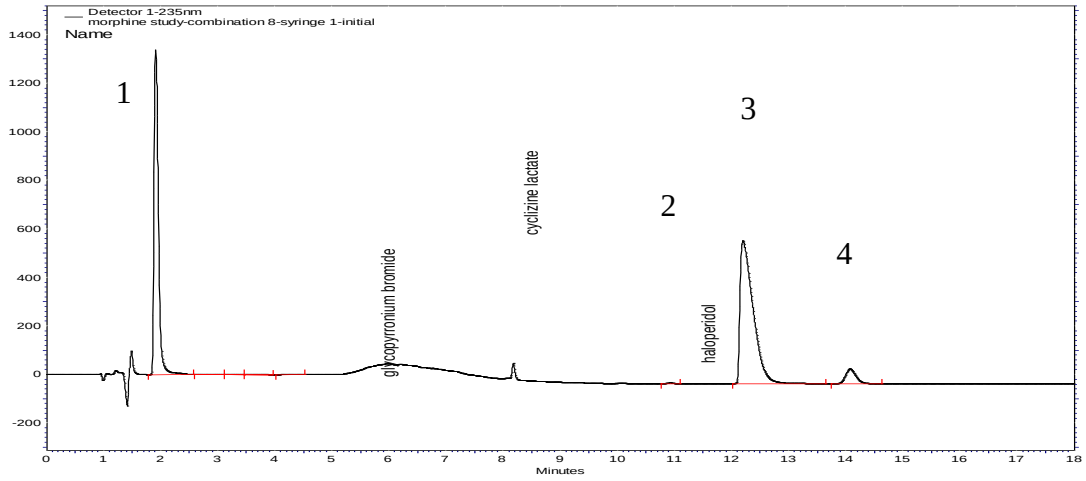
Table 3.14. Average results for the HPLC assay for morphine combination 8

	Morphine % initial			Cyclizine % initial			Glycopyrronium % initial			Haloperidol % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0* (2.32%)	108.4	104.3	100.0 (1.18%)	109.3	107.2	100.0 (4.00%)	113.9	112.9	100.0 (3.91%)	107.4	100.7
3 hours	99.0* (1.17%)	106.3	104.4	100.7 (0.67%)	109.4	108.7	100.9 (5.29%)	113.3	115.5	99.2 (3.40%)	106.1	100.2
6 hours	98.1* (0.56%)	104.3	104.3	99.5 (0.40%)	107.7	107.8	106.0 (8.73%)	126.7	113.8	98.0 (1.72%)	103.5	100.5
24 hours	105.4* (2.71%)	114.6	109.5	101.2 (1.12%)	110.7	108.6	102.6 (7.22%)	114.6	118.0	101.1 (3.61%)	108.4	102.0

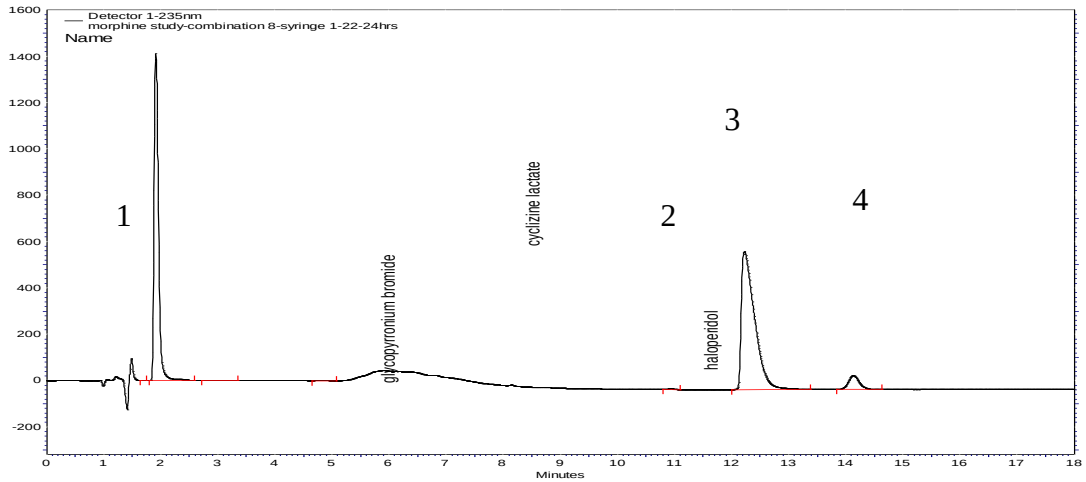
results not used to draw conclusions, included for comparative purposes only
* denotes where a significant difference was observed (one-way ANOVA, sample size n=4)

Figure 3.11. Representative chromatograms for combination 8 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2, 3 and 4 represent morphine sulphate (retention time 1.9min), glycopyrronium bromide (retention time 10.9min), cyclizine lactate (retention time 12.2min) and haloperidol (retention time 14.1min) respectively

(A)



(B)



Combination 9

The results are tabulated in table 3.15 and figure 3.12 depicts the chromatography for this combination. The morphine, cyclizine and midazolam results showed significant difference on statistical analysis. In the case of morphine and cyclizine, the increases at the 24 hour time point and the 6 hour and 24 hour time points respectively, are thought to be due to differences in chromatographic integration. However, for midazolam it is the fact that the results at the 3 hour time point were low compared to the results from the other time points, therefore, this decrease was not suspected to be a real drop in concentration.

Combination 10

The results for combination 10 and its associated chromatography are shown in table 3.16 and figure 3.13 respectively. The sample chromatograms, as with combination 8, showed that the glycopyrronium component peak was significantly smaller than the other drug component peaks. The high RSD values have been attributed to variation in the injection areas between the same sample and between the two syringe preparations. The glycopyrronium standard also showed variation and did not meet criteria. From these observations it was decided to repeat the analysis of the combination by LCMS/MS but only the glycopyrronium component would be assessed because the data for the other three drug components in the combination was acceptable from this analysis.

There was significant difference in the morphine and levomepromazine results after statistical analysis, which was attributed to the lower results for both syringe preparations at the 6 hour time point. As no trends were observed in the data, these did not confirm any real changes in the drug concentrations over the 24h. The midazolam 6 hour time point results were lower than those obtained at the other time points but this was not deemed significant.

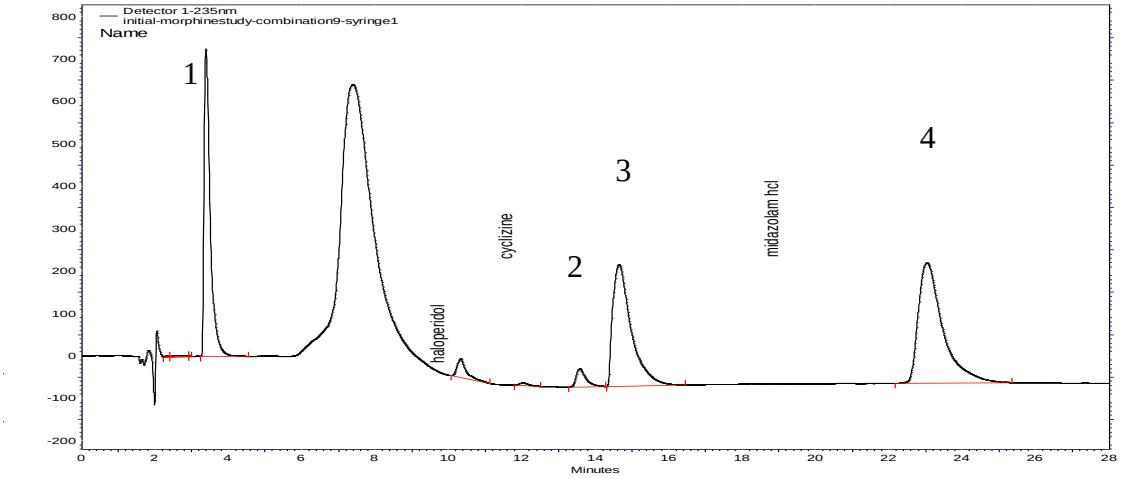
Table 3.15. Average results for the HPLC assay for morphine combination 9

	Morphine % initial			Haloperidol % initial			Cyclizine % initial			Midazolam % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (1.07%)	107.7	105.9	100.0 (1.03%)	99.1	99.8	100.0 (1.17%)	105.7	103.8	100.0 (0.79%)	128.9	127.4
3 hours	99.7 (0.68%)	105.9	107.1	100.4 (3.92%)	96.6	103.1	100.3 (1.13%)	104.5	105.5	94.8* (1.41%)	120.1	123.0
6 hours	101.9 (0.62%)	108.6	109.1	103.7 (1.03%)	102.7	103.6	103.0* (0.32%)	107.8	107.9	100.0 (1.22%)	127.0	129.4
24 hour	106.4* (0.41%)	113.3	113.9	104.5 (2.54%)	102.7	105.2	104.1* (1.06%)	105.1	110.1	102.8 (1.42%)	130.2	133.3

* denotes where a significant difference was observed (one-way ANOVA, sample size n=4)

Figure 3.12. Representative chromatograms for combination 9 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2, 3 and 4 represent morphine sulphate (retention time 3.4min), haloperidol (retention time 13.6min), cyclizine lactate (retention time 14.7min) and midazolam hcl (retention time 23.0min) respectively

(A)



(B)

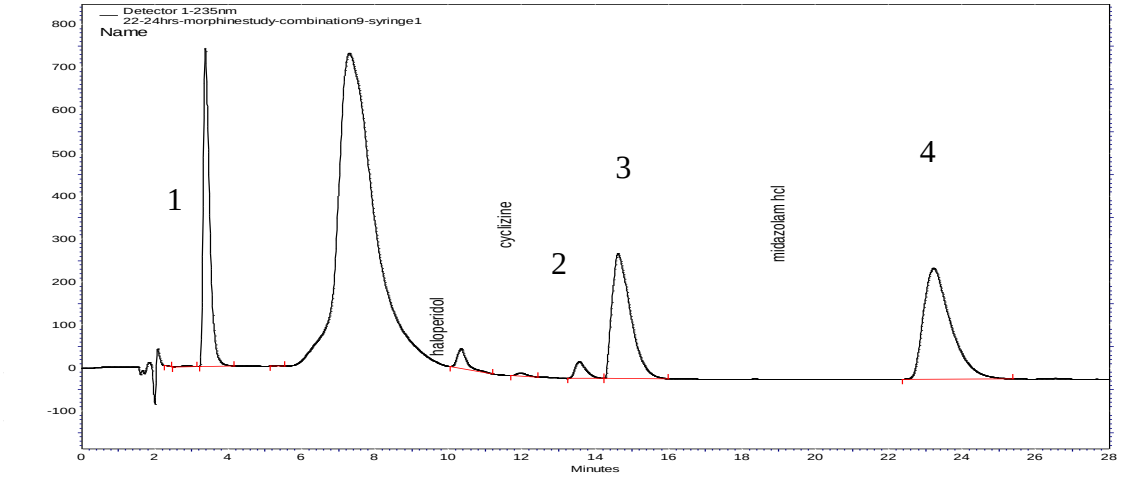


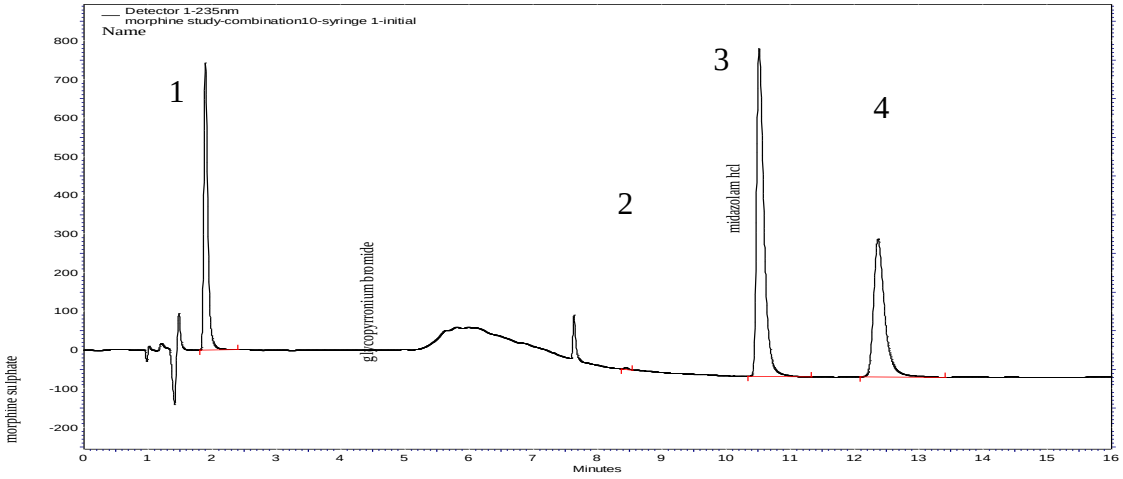
Table 3.16. Average results for the HPLC assay for morphine combination 10

	Morphine % initial			Glycopyrronium % initial			Levomepromazine % initial			Midazolam % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (1.36%)	99.3	101.2	100.0 (5.96%)	102.3	110.9	100.0 (0.80%)	129.0	128.2	100.0 (4.47%)	110.9	118.5
3 hours	102.2 (1.09%)	101.8	103.0	104.8 (9.78%)	111.3	112.1	101.5 (0.53%)	131.0	130.1	100.3 (5.35%)	111.1	119.1
6 hours	98.2* (0.66%)	98.0	98.8	100.9 (9.53%)	107.7	107.5	97.9* (0.70%)	125.4	126.5	94.6 (5.98%)	103.9	113.1
24 hour	103.8 (0.45%)	104.4	103.6	112.0 (5.39%)	123.7	115.2	99.8 (0.49%)	128.5	128.3	99.7 (3.36%)	111.3	117.3

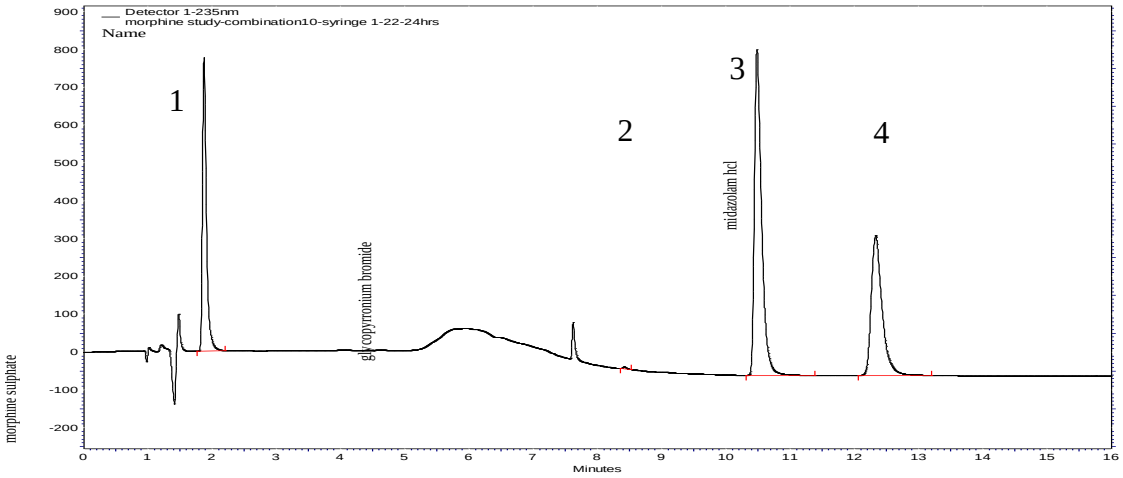
results not used to draw conclusions, for comparative purposes only
* denotes where a significant difference was observed (one-way ANOVA, sample size n=4)

Figure 3.13. Representative chromatograms for combination 9 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2, 3 and 4 represent morphine sulphate (retention time 1.9min), glycopyrronium bromide (retention time 8.4min), levomepromazine hcl (retention time 10.5min) and midazolam hcl (retention time 12.4min) respectively

(A)



(B)



Overall, the above results confirm compatibility of the supportive drug combinations with morphine.

3.3.2. Diamorphine Combinations

In aqueous solution, diamorphine is known to undergo hydrolysis to the monoacetyl derivative (6-monoacetylmorphine) and also to morphine *(Beaumont et al, 1982)*. 6-monoacetylmorphine was detected in each of the combinations and was monitored in each study, where its percentage was calculated relative to the area of the diamorphine peak. It increased in size over the study period, with the increases varying between 0.14% and 0.65%, but in the case of combination 3 an increase of 2.89% occurred. The largest increase occurred between 6 and 24 hours, which was expected because of the time that had elapsed between the two time points. 6-monoacetylmorphine was also present in the diamorphine standard chromatograms. Morphine was not detected in any of the combinations.

As mentioned in section 3.1.2 on preparation of combinations 3, 4, 5, 6, 7 and 9, the diamorphine hydrochloride 100mg injection resulted in a yellow colour on reconstitution. Even though the yellow colour of the reconstituted diamorphine hydrochloride was not visually apparent on completion of syringe preparation it did have an impact chromatographically. In these combinations (3, 4, 5, 6, 7 and 9), a higher initial area percentage of 6-monoacetylmorphine relative to diamorphine was observed, 2.75% compared to 1.17% where the diamorphine hydrochloride was colourless (combinations 1 and 2). Also, the average initial concentration of diamorphine was lower in the combinations where the diamorphine hydrochloride was yellow in colour, 95.4% compared to 103.0% where the diamorphine hydrochloride was colourless. The yellow colour and the associated decrease in diamorphine hydrochloride concentration and the increase in 6-monoacetylmorphine concentration indicated that some degradation had occurred. It is known that the pharmacological activity of diamorphine is attributed to its metabolites *(Hutchinson et al, 2002)*. In clinical practice, a yellow coloured diamorphine hydrochloride would not be used.

<u>Combination 1</u>

The results for combination 1 are in table 3.17 and figure 3.14 shows its chromatography. Grassby and Hutchings have performed compatibility and stability work on combinations of the drug components in this combination. They demonstrated that combination of diamorphine and cyclizine in a 1:1 ratio for concentrations up to 20mg/ml were stable after 24 hours *(Grassby and Hutchings, 1997)*. In this research, the concentration of diamorphine and cyclizine were not in a 1:1 ratio but in the ratio, 2:3 with

concentrations of 5mg/ml and 7.5mg/ml respectively. These concentrations are below the 20mg/ml concentrations reported in the literature but further support the compatibility of combining diamorphine and cyclizine.

Combination 2

Refer to table 3.18 and figure 3.15 for the results and associated chromatography for this combination respectively. Statistical analysis indicated that there was significant difference in the midazolam results, which could be attributed to the decrease at 3 hours. On looking at the data, it was observed that the results at 3 hours were lower than the other time points, so a real drop in concentration was not suspected as an overall decrease in concentration was not observed and the other time point results were consistent with each other.

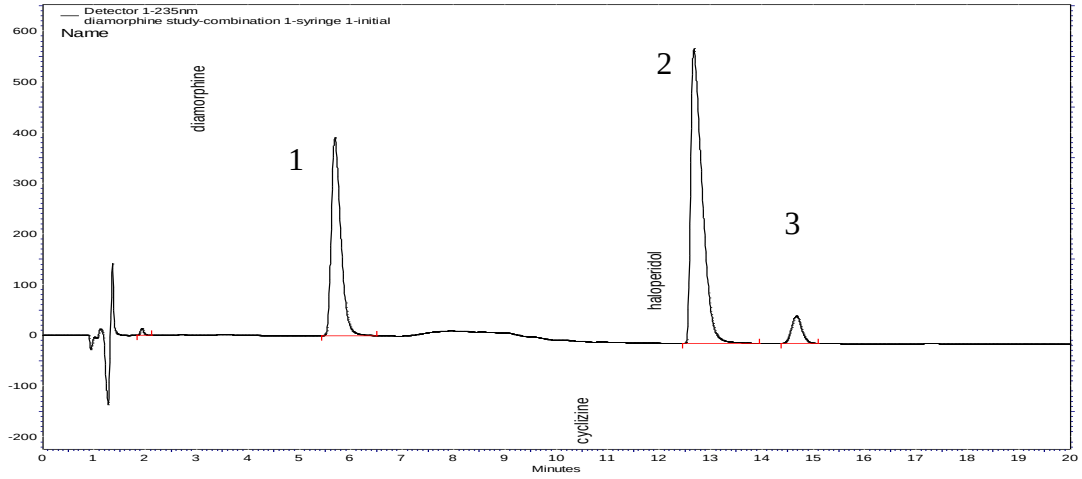
Work conducted by Allwood *et al* reported that mixtures of diamorphine hydrochloride, 10 or 500mg and midazolam 10 or 75mg diluted to a volume of 15ml with WFI in plastic syringes may be assigned a 14 day shelf life on storage at ambient temperature *(Allwood et al, 1994)*. The concentrations of diamorphine and midazolam in this work are not the same as those reported in the literature but the results further support compatibility for combining diamorphine and midazolam.

Table 3.17. Average results for the HPLC assay for diamorphine combination 1

	Diamorphine % initial			Cyclizine % initial			Haloperidol % initial			6-monoacetyl morphine
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	% relative to diamorphine peak area
0 hours	100.0 (2.08%)	106.0	102.4	100.0 (0.50%)	108.0	107.2	100.0 (1.60%)	104.6	102.5	1.18
3 hours	98.8 (1.51%)	104.3	101.8	99.1 (0.18%)	106.8	106.6	98.1 (1.58%)	103.0	100.2	1.26
6 hours	99.5 (1.90%)	105.4	102.0	99.9 (0.75%)	108.2	106.8	99.5 (3.24%)	105.8	100.2	1.27
24 hours	99.4 (1.83%)	105.2	102.0	100.1 (0.70%)	108.2	107.3	99.0 (3.22%)	105.0	101.1	1.59

Figure 3.14. Representative chromatograms for combination 1 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 diamorphine (retention time 5.7min), cyclizine (retention time 12.7min) and haloperidol (retention time 14.7min) respectively

(A)



(B)

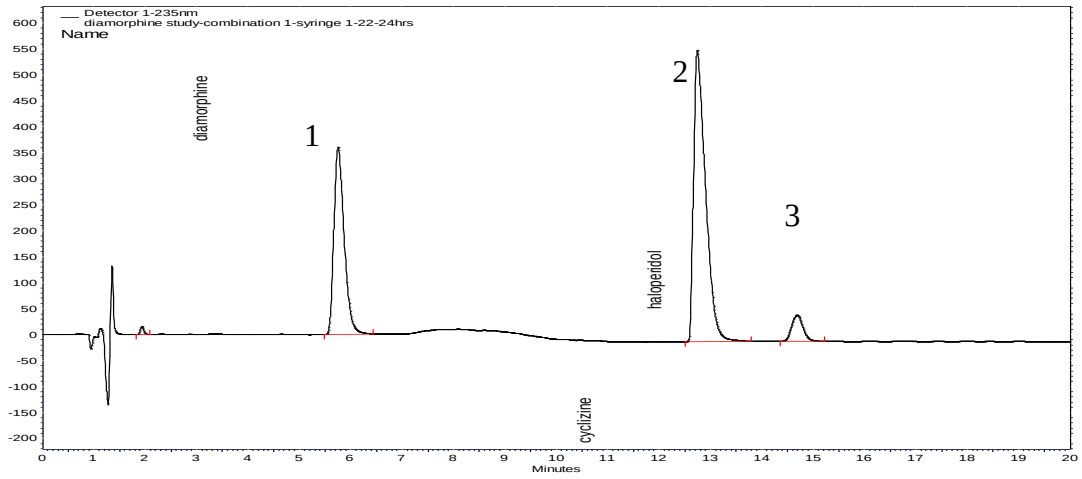


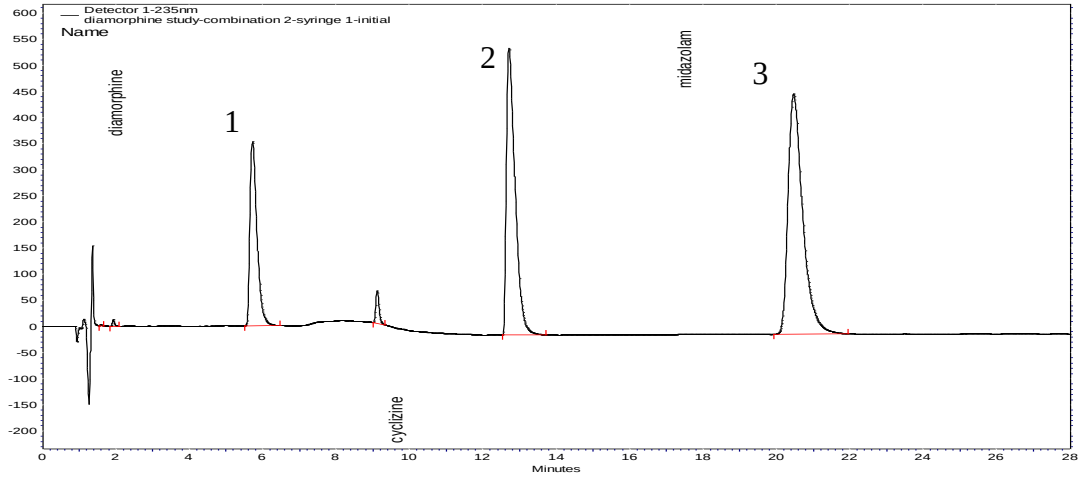
Table 3.18. Average results for the HPLC assay for diamorphine combination 2

	Diamorphine % initial			Cyclizine % initial			Midazolam % initial			6-monoacetyl Morphine
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	% relative to diamorphine peak area
0 hours	100.0 (3.66%)	101.1	107.7	100.0 (0.41%)	110.6	110.3	100.0 (0.97%)	128.4	129.6	1.15
3 hours	99.6 (4.29%)	100.2	107.8	99.6 (0.15%)	109.9	110.1	95.5* (1.32%)	123.3	123.2	1.27
6 hours	99.5 (4.72%)	99.7	108.2	99.8 (1.09%)	109.4	111.2	97.6 (1.81%)	124.3	127.5	1.33
24 hours	100.4 (2.96%)	102.2	107.4	99.6 (1.31%)	111.3	108.8	100.1 (1.13%)	130.0	128.3	1.50

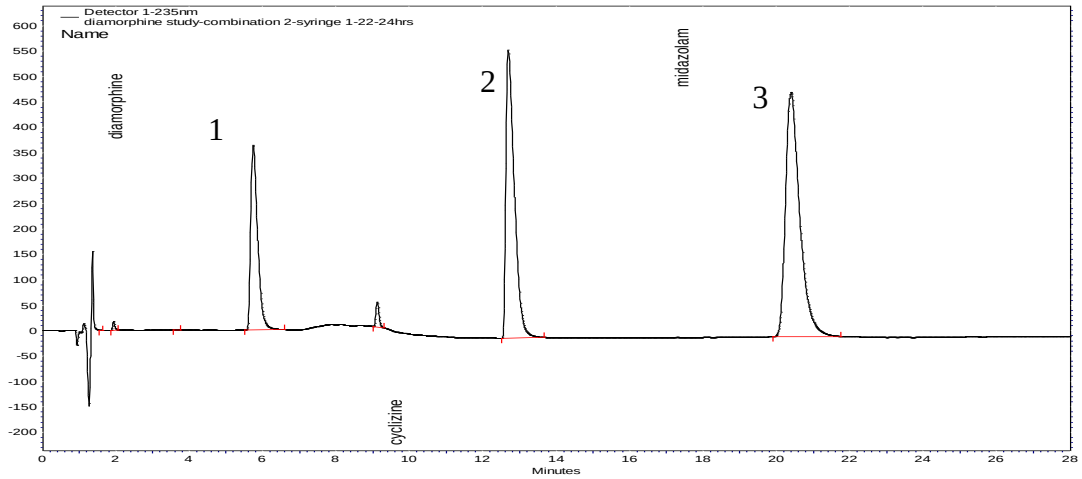
* denotes where a significant difference was observed (one-way ANOVA, sample size n=4)

Figure 3.15. Representative chromatograms for combination 2 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent diamorphine (retention time 5.8min), cyclizine (retention time 12.7min) and midazolam (retention time 20.5min) respectively

(A)



(B)



Combination 3

The results for combination 3 are tabulated in table 3.19 and figure 3.16 shows its chromatography. Statistical analysis indicated significant difference for both levomepromazine and metoclopramide and was attributed to the decrease in the results at the 3 hour time point for levomepromazine and both the 3 hour and 6 hour time points for metoclopramide. The results at these time points are lower than the other time points, so a real drop in concentration is not suspected due to the other time point results being consistent with each other.

Combination 4

Refer to table 3.20 and figure 3.17 for the results and chromatography respectively of this combination. From statistical tests performed there was significant difference in the diamorphine and haloperidol results. This could be due to the diamorphine results at 0 hours and the haloperidol results at 6 hours being lower than the other time point results but this is not deemed significant.

Combination 5

For the results obtained for combination 5 refer to table 3.21 and for the associated chromatography refer to figure 3.18.

Combination 6

For the results for this combination refer to table 3.22 and figure 3.19 shows its chromatography. Statistical analysis revealed significant difference in the levomepromazine and midazolam results. Both these results showed a decrease at the 3 hour and 6 hour time points, with the average result for these time points being lower than the initial and final time points. A real drop in concentration has not occurred as both the 0 hour and 24 hour results were consistent with each other.

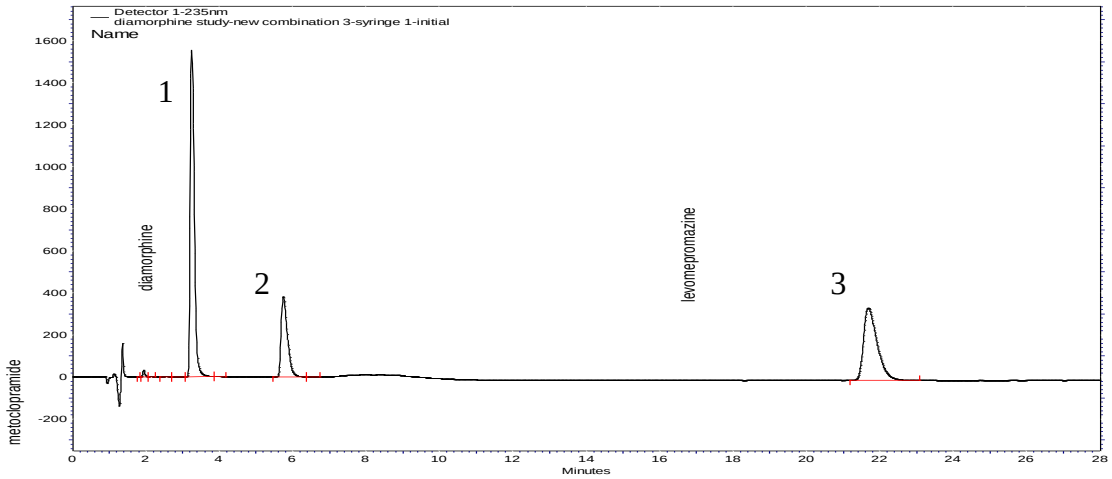
Table 3.19. Average results for the HPLC assay for diamorphine combination 3

	Diamorphine % initial			Levomepromazine % initial			Metoclopramide % initial			6-monoacetyl Morphine
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	% relative to diamorphine peak area
0 hours	100.0 (0.67%)	94.5	94.2	100.0 (1.32%)	126.0	128.2	100.0 (1.36%)	114.5	111.9	2.83
3 hours	99.0 (1.48%)	94.6	92.2	92.9* (1.00%)	117.2	119.1	96.3* (1.07%)	110.0	108.0	3.53
6 hours	100.2 (2.53%)	96.6	92.5	99.1 (1.40%)	127.5	124.5	96.7* (1.40%)	110.7	108.1	3.54
24 hours	98.8 (0.57%)	93.4	92.9	97.8 (3.17%)	120.9	127.8	101.9 (2.90%)	117.8	112.8	5.72

* denotes where a significant difference was observed (one-way ANOVA, sample size n=4)

Figure 3.16. Representative chromatograms for combination 3 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent metoclopramide (retention time 3.3min), diamorphine (retention time 5.7min) and levomepromazine (retention time 21.7min) respectively

(A)



(B)

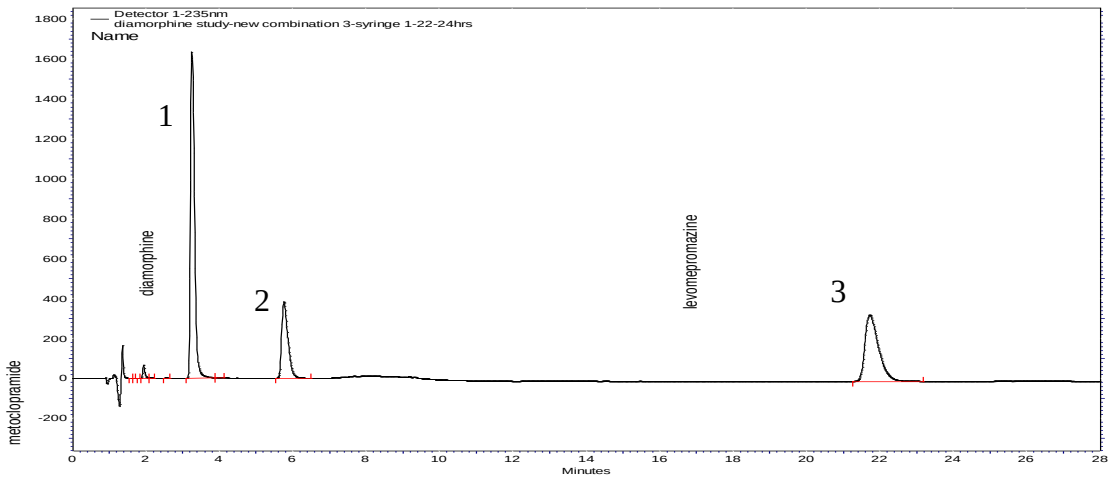


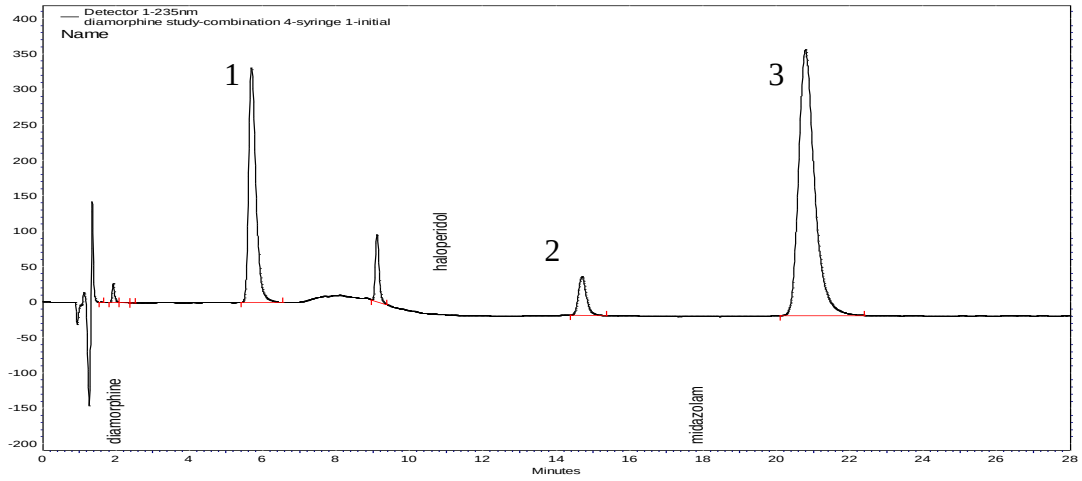
Table 3.20. Average results of the HPLC assay for diamorphine combination 4

	Diamorphine % initial			Haloperidol % initial			Midazolam % initial			6-monoacetyl Morphine
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	% relative to diamorphine peak area
0 hours	100.0* (1.96%)	95.1	98.4	100.0 (1.79%)	112.3	115.2	100.0 (6.81%)	111.4	122.5	2.81
3 hours	102.5 (2.08%)	97.5	100.9	101.9 (1.79%)	114.2	117.7	99.5 (6.72%)	111.2	121.4	2.90
6 hours	101.4 (0.44%)	97.8	98.4	99.1* (2.30%)	110.5	114.9	99.5 (6.00%)	112.3	120.4	2.87
24 hours	104.4 (0.56%)	100.6	101.3	103.0 (1.05%)	117.4	116.8	103.9 (3.74%)	118.7	124.2	3.19

* denotes where a significant difference in the sets of results was observed (one-way ANOVA, sample size n=4)

Figure 3.17. Representative chromatograms for combination 4 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent diamorphine (retention time 5.7min), haloperidol (retention time 14.7min) and midazolam (retention time 20.8min) respectively

(A)



(B)

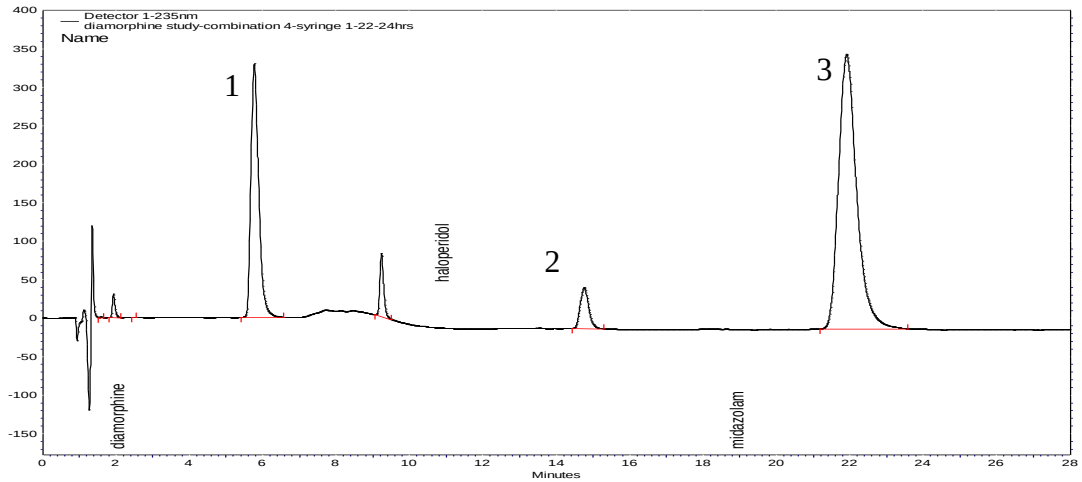
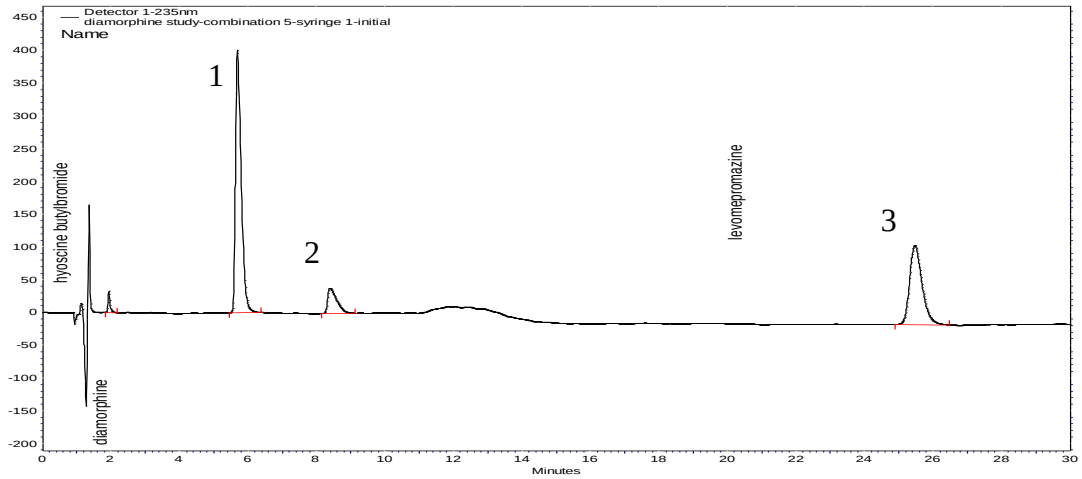


Table 3.21. Average results for the HPLC assay for diamorphine combination 5

	Diamorphine % initial			Hyoscine butylbromide % initial			Levomepromazine % initial			6-monoacetyl Morphine
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	% relative to diamorphine peak area
0 hours	100.0 (2.41%)	92.5	88.9	100.0 (4.21%)	108.9	101.8	100.0 (1.65%)	98.0	100.8	2.85
3 hours	100.3 (1.57%)	92.2	89.8	100.0 (2.68%)	106.6	104.1	99.5 (2.12%)	96.2	99.5	2.88
6 hours	100.0 (1.56%)	91.9	89.4	100.7 (1.96%)	107.1	105.1	99.5 (1.62%)	98.1	100.6	2.92
24 hours	98.7 (1.33%)	90.6	88.5	100.0 (1.90%)	105.1	105.7	103.9 (1.12%)	97.9	99.8	3.39

Figure 3.18. Representative chromatograms for combination 5 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent diamorphine (retention time 5.7min), hyoscine butylbromide (retention time 8.4min) and levomepromazine (retention time 25.5min) respectively

(A)



(B)

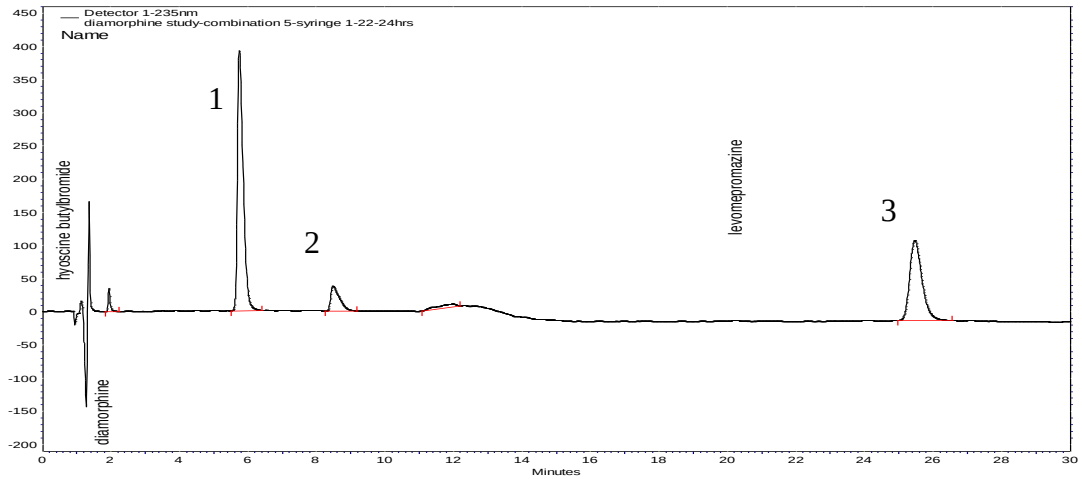


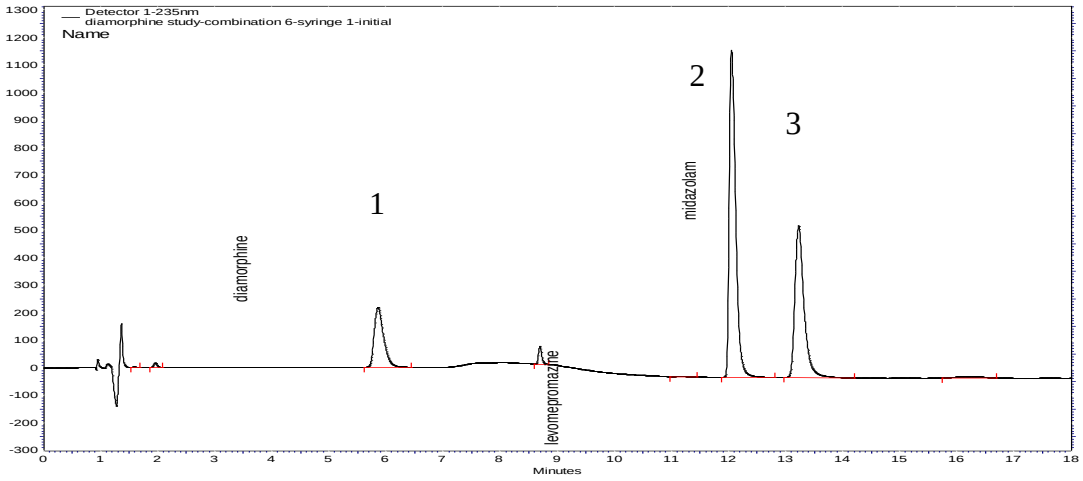
Table 3.22. Average results for the HPLC assay for diamorphine combination 6

	Diamorphine % initial			Levomepromazine % initial			Midazolam % initial			6-monoacetyl Morphine
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	% relative to diamorphine peak area
0 hours	100.0 (3.91%)	104.4	97.6	100.0 (3.86%)	134.3	125.6	100.0 (2.25%)	122.6	124.0	2.62
3 hours	98.8 (1.91%)	101.5	98.2	97.2* (0.71%)	125.7	127.1	95.1* (3.42%)	113.9	120.6	2.80
6 hours	99.0 (2.43%)	102.1	97.9	97.5* (0.78%)	126.0	127.5	96.9* (2.56%)	117.1	122.0	2.98
24 hours	100.8 (2.57%)	104.0	99.5	101.9 (0.97%)	133.5	131.5	100.3 (2.28%)	121.7	125.5	3.24

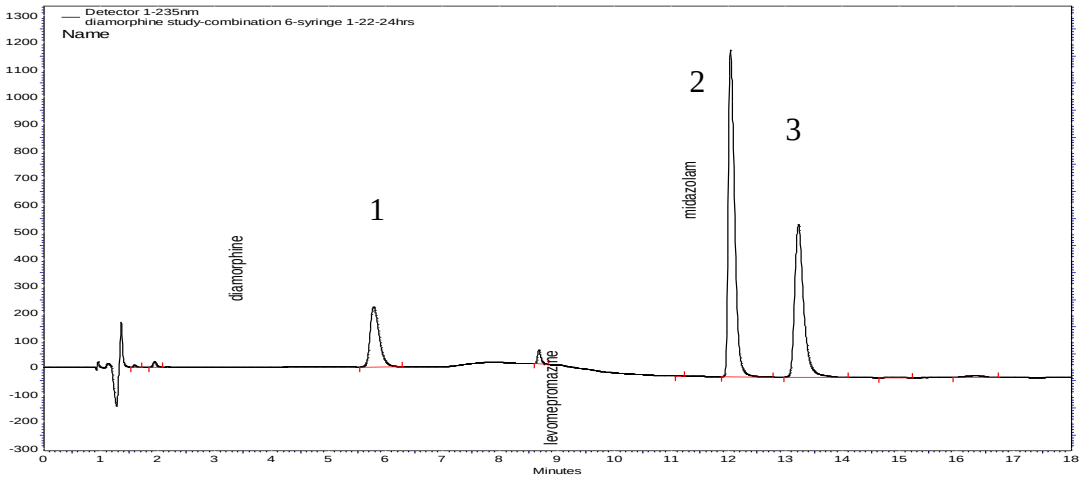
* denotes where a significant difference was observed (one-way ANOVA, sample size n=4)

Figure 3.19. Representative chromatograms for combination 6 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent diamorphine (retention time 5.9min), levomepromazine (retention time 12.0min) and midazolam (retention time 13.2min) respectively

(A)



(B)



Combination 7

Table 3.23 tabulates the results for combination 7 and figure 3.20 shows the chromatography. The results for the 6 hour time point are for comparative purposes only because the administration line was not transferred to the sampling vessel for sample collection. The contents of the waste pot were analysed instead.

Statistical analysis indicated significant difference in the metoclopramide results; however, the likely cause of this was the decrease in concentration at the 6 hour time point but this result is for comparative purposes only.

Combination 9

The results for combination 9 are in table 3.24 and for its associated chromatography refer to figure 3.21. Statistical tests indicated significant difference in the midazolam, diamorphine and cyclizine results. There is a decrease in the midazolam results at 3 hours and 6 hours. As the initial and final time points are consistent with each other, this decrease is not thought to be significant. Possible causes of this decrease include an error in the dilution process or variation in the chromatographic integration.

Physical compatibility over 24 hours, based on clinical observations, is known for varying concentrations of the drugs present in combination 9 using the diluent WFI (*Dickman et al,* 2005). The data is for a final volume of 17ml. The results for the presented work further support physical compatibility of this combination in WFI for 24 hours.

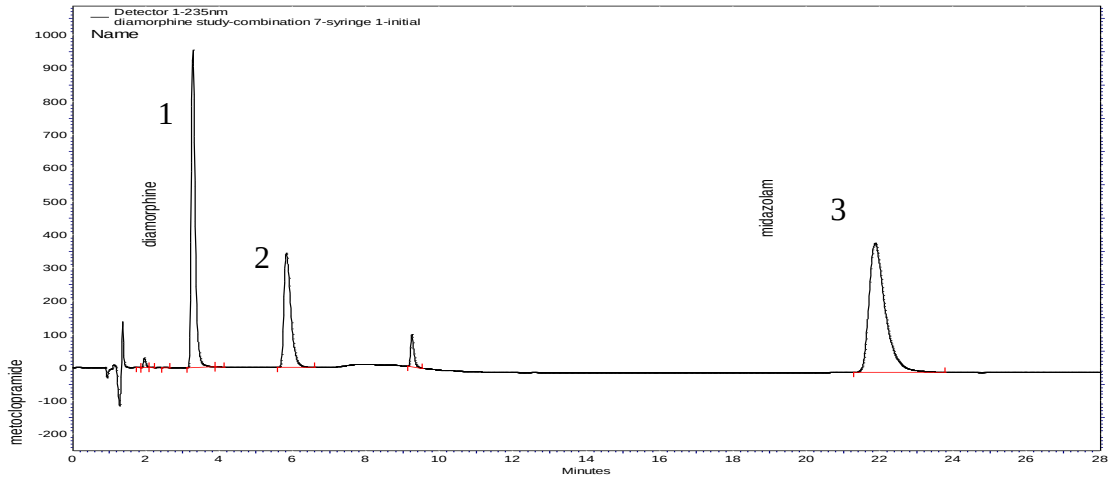
Table 3.23. Average results for the HPLC assay for diamorphine combination 7

	Diamorphine % initial			Metoclopramide % initial			Midazolam % initial			6-monoacetyl morphine
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	% relative to diamorphine peak area
0 hours	100.0 (3.54%)	92.2	98.0	100.0 (0.77%)	123.1	124.0	100.0 (5.74%)	117.2	126.4	2.68
3 hours	100.3 (2.68%)	93.2	97.6	99.5 (0.32%)	123.3	122.7	97.9 (4.89%)	115.2	123.2	2.77
6 hours#	100.1# (3.31%)	92.5	97.9	97.9#* (0.61%)	120.6	121.4	99.0# (2.47%)	118.0	123.1	2.84#
24 hours	99.7 (1.77%)	93.5	96.1	98.9 (1.30%)	123.2	121.2	99.7 (4.88%)	117.2	125.8	3.33

results not used to draw conclusions, included for comparative purposes only
* denotes where a significant difference was observed (one-way ANOVA, sample size n=4)

Figure 3.20. Representative chromatograms for combination 7 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent metoclopramide (retention time 3.3min), diamorphine (retention time 5.8min) and midazolam (retention time 21.8min) respectively

(A)



(B)

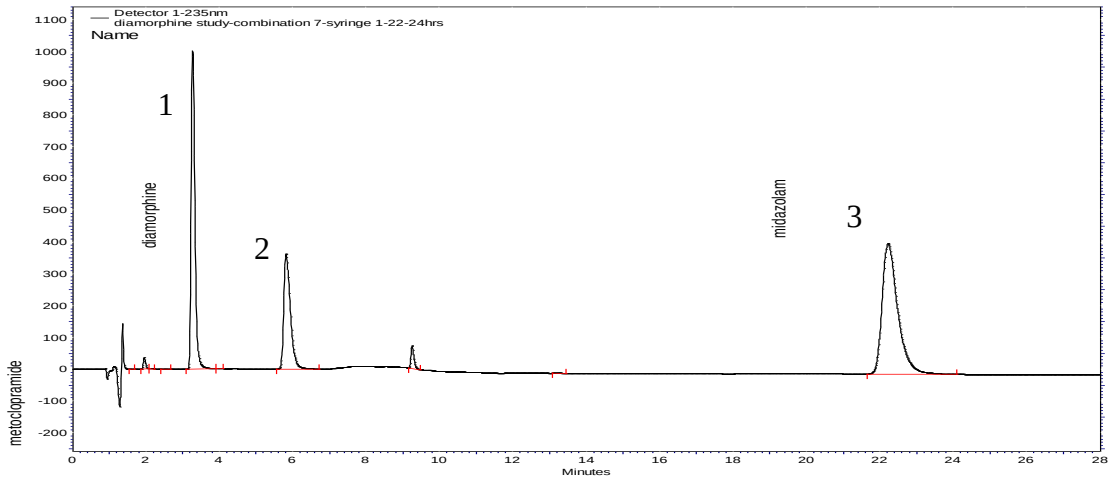


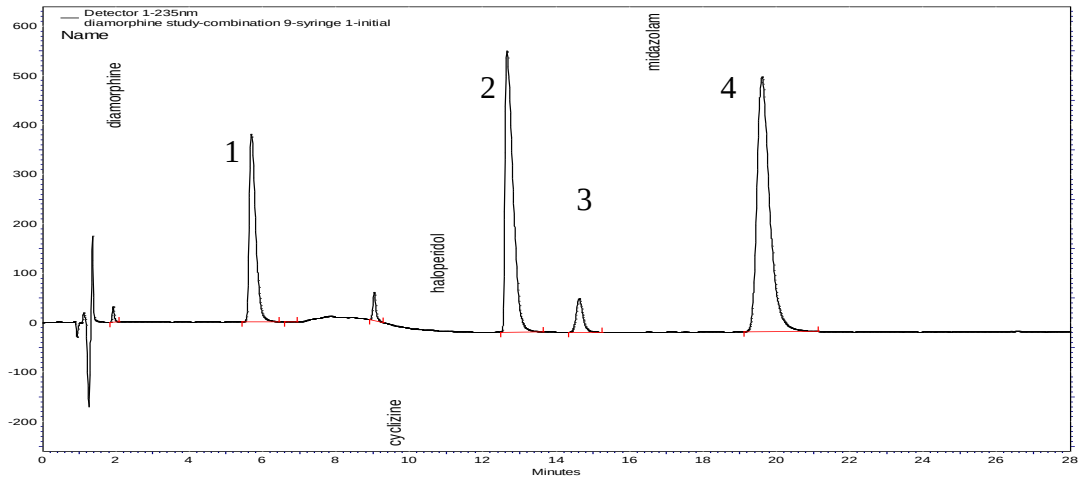
Table 3.24. Average results for the HPLC assay for diamorphine combination 9

	Diamorphine % initial			Haloperidol % initial			Cyclizine % initial			Midazolam % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (1.53%)	95.7	93.8	100.0 (1.69%)	121.3	121.4	100.0 (0.65%)	108.1	107.4	100.0 (0.32%)	122.8	124.2
3 hours	98.9 (0.78%)	93.5	93.3	98.3 (1.77%)	114.4	116.1	98.4 (1.06%)	105.6	106.6	95.0* (2.12%)	119.7	123.1
6 hours	98.5 (0.30%)	93.3	92.8	98.7 (0.93%)	116.0	117.6	98.8 (0.22%)	106.7	106.4	96.3* (1.17%)	121.2	122.5
24 hours	101.0 (1.57%)	96.7	94.2	100.6 (0.62%)	122.1	121.2	101.0 (0.81%)	109.6	108.1	100.2 (0.42%)	124.7	123.7

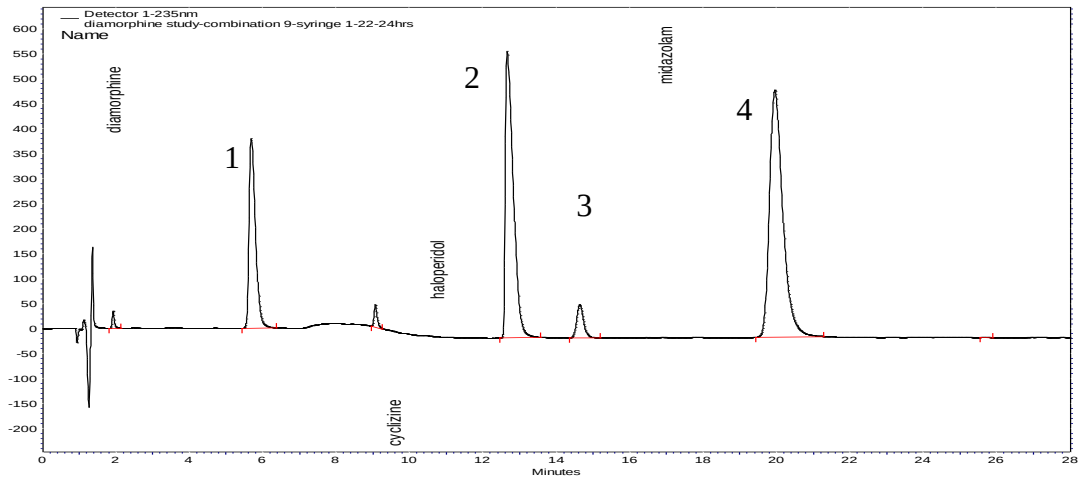
* denotes where a significant difference was observed (one-way ANOVA, sample size n=4)

Figure 3.21. Representative chromatograms for combination 9 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2, 3 and 4 represent diamorphine (retention time 5.7min), cyclizine (retention time 12.7min), haloperidol (retention time 14.6min) and midazolam (retention time 19.6min) respectively

(A)



(B)



In summary, each of the supportive drug combinations tested with diamorphine in this section support compatibility over 24 hours. The HPLC analysis supports minimal decomposition in that the concentration of diamorphine showed no significant changes and there was a less than a 0.7% increase in the known degradant peak over the study period. However, for certain combinations, diamorphine that was yellow on reconstitution was used and this would not have occurred in a clinical setting, but the data obtained for these combinations showed that the diamorphine concentration was unaffected over the study period.

3.3.3. Hydromorphone Combinations

There is evidence in the literature that hydromorphone has been studied for stability. Khondkar *et al* have demonstrated stability and sterility in patient controlled analgesia injectors of a 0.2mg/ml solution of hydromorphone hydrochloride solution in normal saline *(Khondkar et al, 2010)*. Further to that, hydromorphone concentrations of 10mg/ml, 20mg/ml, 50mg/ml and 100mg/ml in 0.9% normal saline and 5% dextrose have been found to be stable for 28 days *(Fudin et al, 2000)*. The hydromorphone in this research is at a concentration of 2.5mg/ml.

In the hydromorphone combination sample chromatograms, an additional peak occurred, which on examination of the standard chromatograms was present in the hydromorphone standard only, indicating it was related to hydromorphone. The peak did not increase in size over the study period. The injection was used to prepare both the standard and combination, indicating that the same peaks would be expected to be seen in both sets of chromatograms.

Combination 1

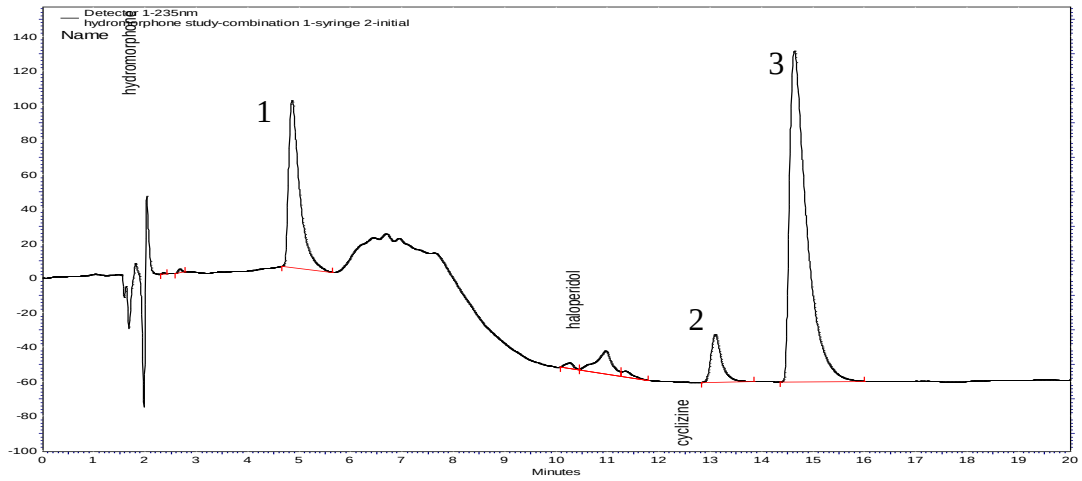
The results for this combination have been tabulated in table 3.25 and figure 3.22 shows its chromatography.

Table 3.25. Average results of the HPLC assay for hydromorphone combination 1

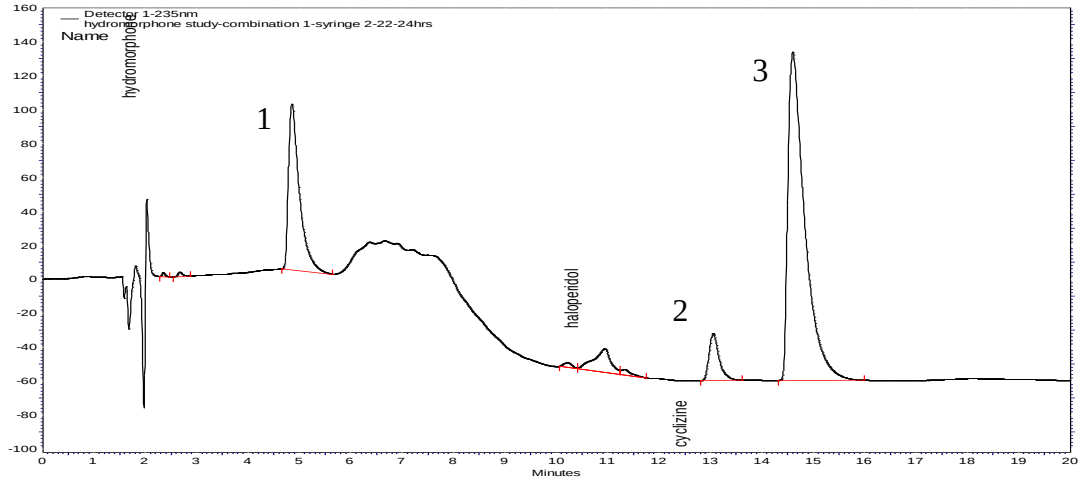
	Hydromorphone % initial			Cyclizine % initial			Haloperidol % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (5.18%)	108.6	99.0	100.0 (4.47%)	102.9	95.6	100.0 (2.39%)	106.0	101.9
3 hours	99.2 (6.10%)	108.1	97.4	100.1 (3.24%)	102.1	96.7	99.4 (4.67%)	107.3	99.3
6 hours	99.8 (5.14%)	108.0	98.8	100.4 (4.60%)	103.5	95.9	97.4 (6.45%)	106.5	95.9
24 hours	99.6 (6.18%)	108.6	97.7	99.8 (4.11%)	102.6	95.6	98.2 (6.20%)	106.3	97.7

Figure 3.22. Representative chromatograms for combination 1 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent hydromorphone (retention time 4.8min), haloperidol (retention time 13.1min) and cyclizine (retention time 14.6min) respectively

(A)



(B)



Combination 2

Table 3.26 contains the results and figure 3.23 shows the chromatography for combination 2. Statistical tests indicated significant difference in the midazolam results. A decrease in concentration was observed at the 3 hour time point; however, this was not deemed a 'real' drop in concentration. The results for this time point were lower than those obtained at the other time points, which were consistent with each other, and thus no trend in the results was notable.

In literature, for the two drug combination of hydromorphone and midazolam prepared in 0.9% sodium chloride or 5% dextrose in water, Walker demonstrated physical compatibility at 4ºC and 23ºC for a period of 23 days at various concentrations *(Walker, 1996)*. The work presented supports compatibility in WFI for the three drug combination.

Combination 3

Refer to table 3.27 for the results and figure 3.24 for the chromatography for combination 3.

Combination 4

The results for combination 4 are in table 3.28 and its associated chromatography is shown in figure 3.25.

Combination 5

The results and chromatography for combination 5 can be seen in table 3.29 and figure 3.26 respectively. The 3 hour time point was based on the average of three injections because the instrument was not started from the correct point in the sequence.

The increase in hyoscine butylbromide concentration at 6 hours and the decrease in levomepromazine concentration at 3 hours have not been deemed significant differences through statistical analyses.

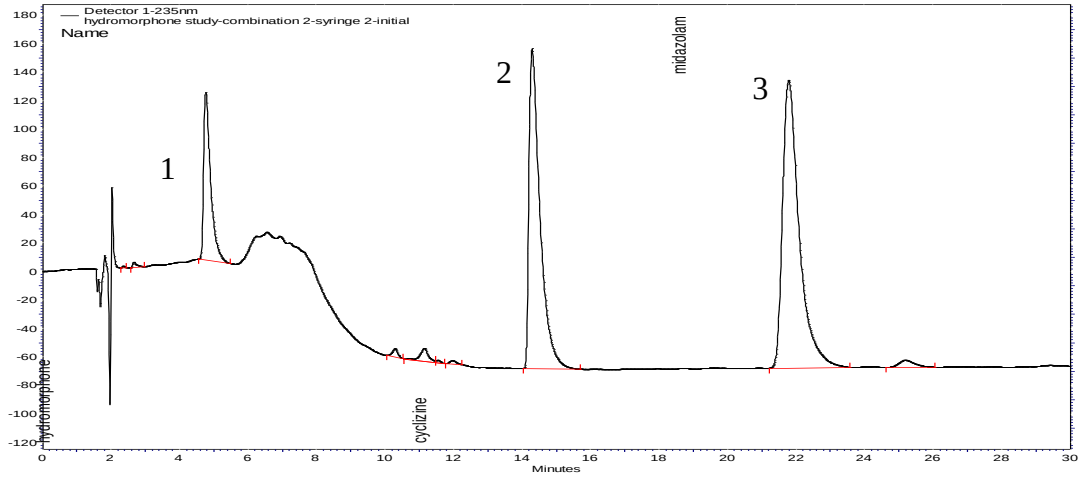
Table 3.26. Average results of the HPLC assay for hydromorphone combination 2

	Hydromorphone % initial			Cyclizine % initial			Midazolam % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (1.73%)	119.7	117.4	100.0 (0.31%)	108.6	109.1	100.0 (1.91%)	134.4	137.0
3 hours	100.4 (1.32%)	119.7	118.3	99.8 (0.34%)	108.4	108.8	96.1* (1.38%)	131.3	129.5
6 hours	99.4 (1.05%)	118.4	117.2	99.6 (0.71%)	107.7	109.1	98.3 (0.62%)	132.9	133.9
24 hours	100.1 (0.71%)	118.9	118.5	100.5 (0.58%)	108.8	109.9	100.0 (0.60%)	135.9	135.5

* denotes where a significant difference was observed (one-way ANOVA, sample size n=4)

Figure 3.23. Representative chromatograms for combination 2 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent hydromorphone (retention time 4.8min), cyclizine (retention time 14.3min) and midazolam (retention time 21.8min) respectively

(A)



(B)

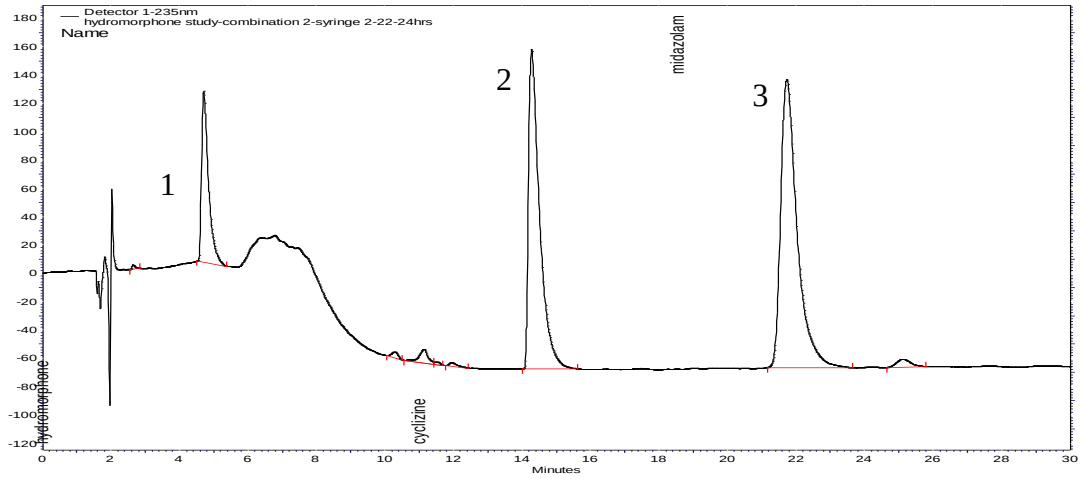
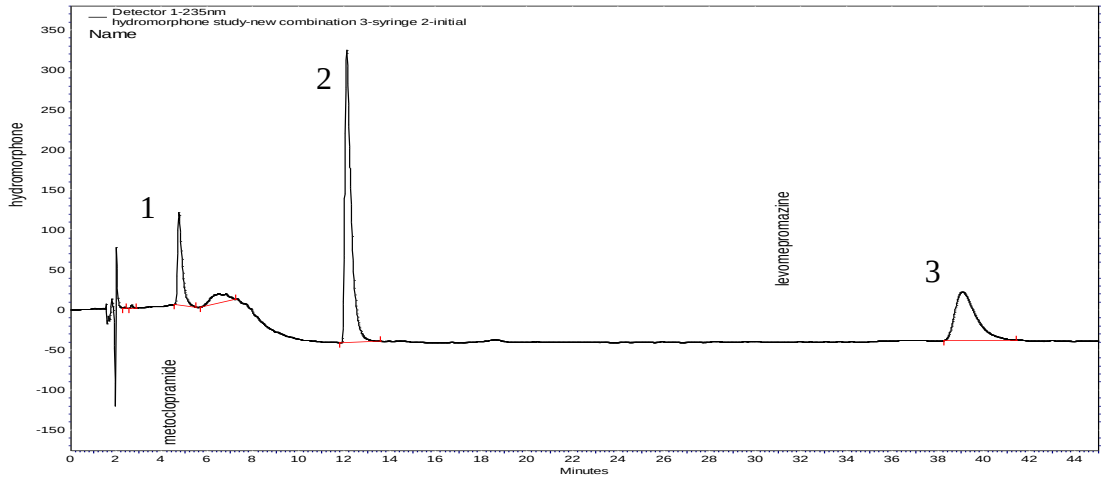


Table 3.27. Average results of the HPLC assay for hydromorphone combination 3

	Hydromorphone % initial			Metoclopramide % initial			Levomepromazine % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (1.35%)	114.9	112.5	100.0 (0.66%)	118.6	117.4	100.0 (1.62%)	131.0	132.9
3 hours	99.4 (1.54%)	114.5	111.6	98.4 (2.02%)	118.1	114.1	98.3 (1.17%)	130.1	129.4
6 hours	98.5 (0.35%)	112.2	111.8	97.9 (0.78%)	116.3	114.8	99.2 (1.56%)	130.2	131.4
24 hours	99.9 (1.24%)	114.6	112.5	99.3 (0.66%)	117.9	116.6	98.6 (1.18%)	128.8	131.3

Figure 3.24. Representative chromatograms for combination 3 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent hydromorphone (retention time 4.7min), metoclopramide (retention time 12.1min) and levomepromazine (retention time 39.1min) respectively

(A)



(B)

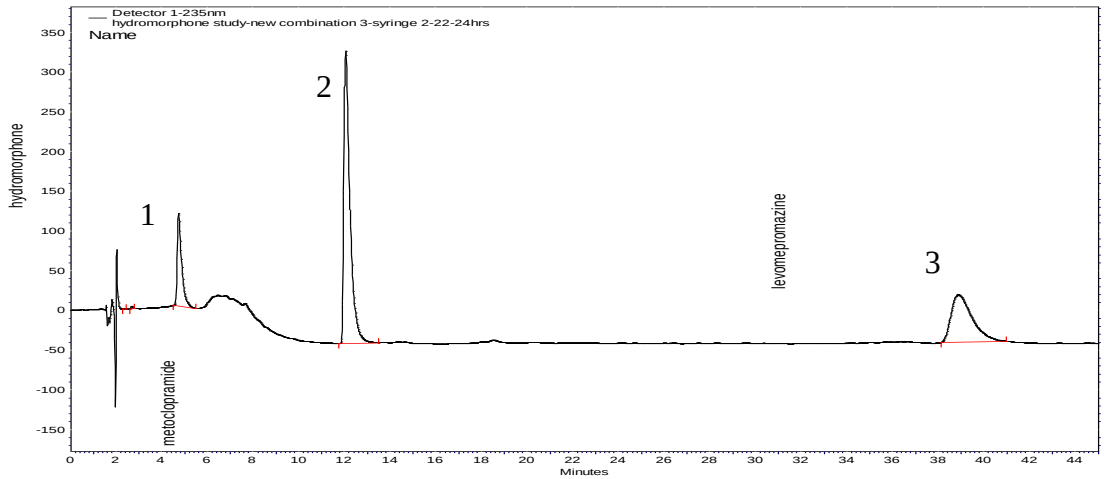
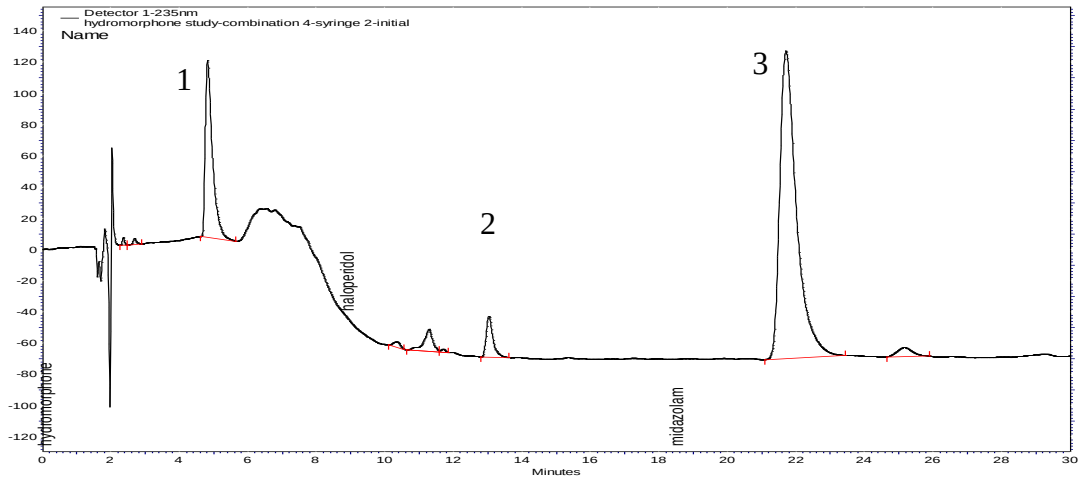


Table 3.28. Average results of the HPLC assay for hydromorphone combination 4

	Hydromorphone % initial			Haloperidol % initial			Midazolam % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (0.48%)	115.9	116.0	100.0 (3.29%)	106.0	100.4	100.0 (2.78%)	130.7	134.6
3 hours	102.1 (0.72%)	118.5	117.8	102.2 (3.49%)	106.6	104.3	97.4 (3.97%)	125.7	132.9
6 hours	101.8 (1.48%)	116.6	119.1	102.6 (3.68%)	108.3	103.4	99.3 (4.76%)	127.4	136.2
24 hours	101.3 (1.17%)	117.9	116.5	97.7 (3.23%)	100.2	101.5	98.0 (3.05%)	127.5	132.5

Figure 3.25. Representative chromatograms for combination 4 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent hydromorphone (retention time 4.8min), haloperidol (retention time 13.1min) and midazolam (retention time 21.9min) respectively

(A)



(B)

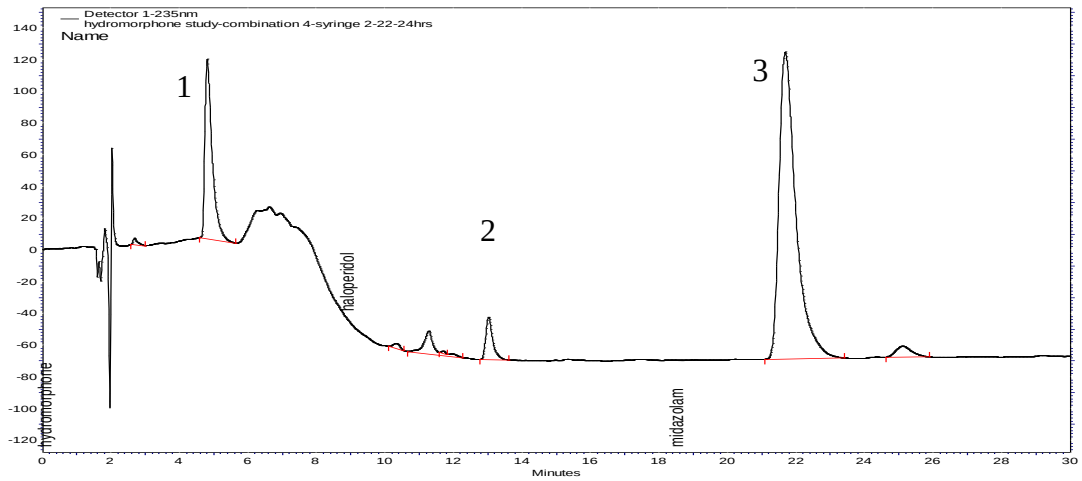
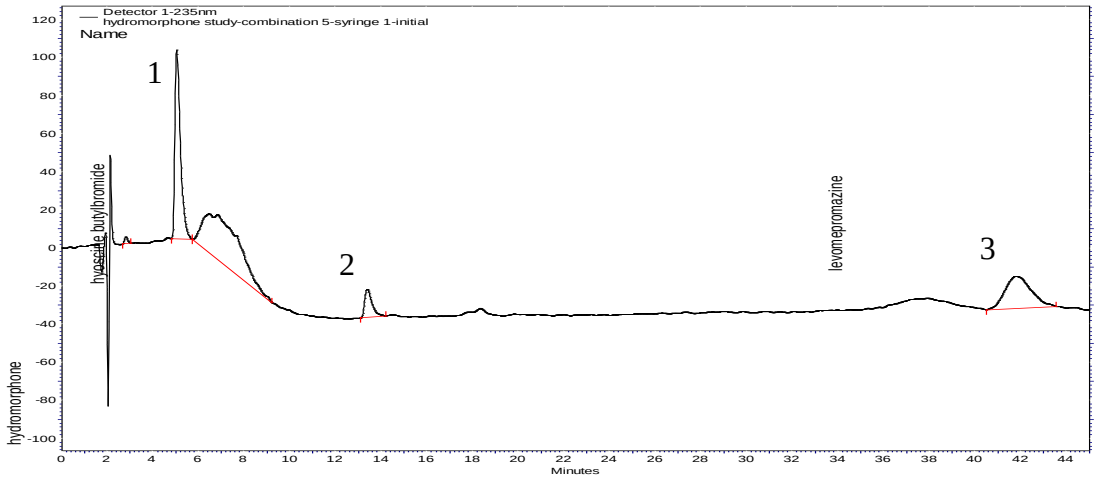


Table 3.29. Average results of the HPLC assay for hydromorphone combination 5

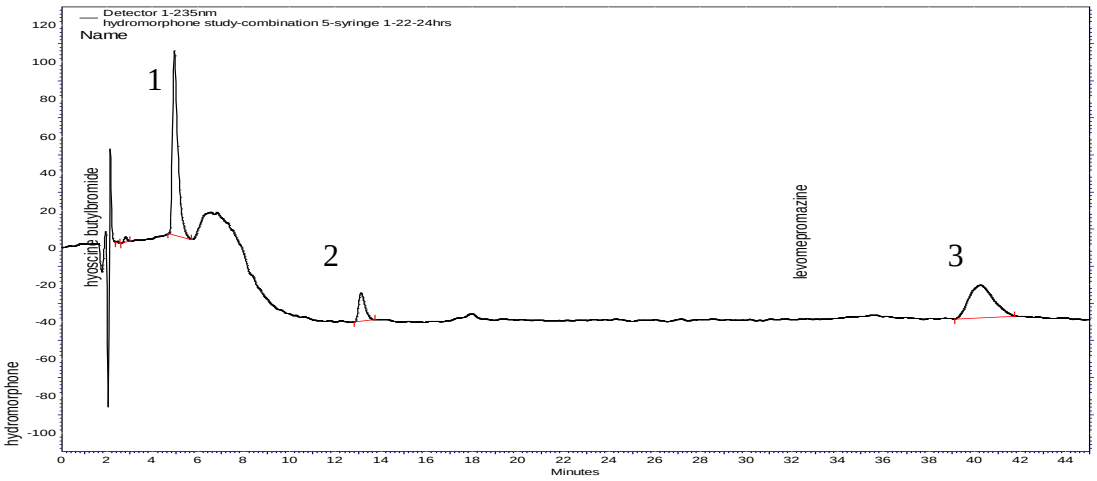
	Hydromorphone % initial			Hyoscine butylbromide % initial			Levomepromazine % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (1.71%)	125.9	122.5	100.0 (3.09%)	113.3	113.8	100.0 (2.45%)	135.5	130.9
3 hours	99.0 (0.75%)	124.1	122.5	100.9 (5.97%)	107.3	118.2	95.9 (2.67%)	126.1	128.4
6 hours	98.4 (2.23%)	124.2	120.2	105.3 (3.41%)	122.6	116.5	97.5 (6.18%)	134.5	125.3
24 hours	97.7 (1.32%)	122.1	120.6	98.7 (3.45%)	110.9	113.1	100.7 (2.52%)	131.9	136.3

Figure 3.26. Representative chromatograms for combination 5 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent hydromorphone (retention time 5.1min), hyoscine butylbromide (retention time 13.4min) and levomepromazine (retention time 41.6min) respectively

(A)



(B)



Combination 6

Refer to table 3.30 for the results and figure 3.27 for the chromatography for this combination. Statistical tests indicated a significant difference in all three drug component results. There was a decrease in concentration for hydromorphone at 6 hours, and for both levomepromazine and midazolam at the 3 hour and 6 hour time points. The results at these time points were lower than all the other time points, but were not considered a real drop in concentration as the initial and final results were consistent with each other.

Combination 7

The results and chromatography for combination 7 are shown in table 3.31 and figure 3.28 respectively. Statistical tests revealed significant difference in the hydromorphone results. This was attributed to the decrease in concentration at the 3 hour time point because one syringe preparation was lower than the other. On comparison with each syringe preparation at every time point, it was apparent that this was the only low syringe preparation result, therefore, a real drop in concentration had not occurred.

Combination 9

Table 3.32 shows the results and figure 3.29 depicts the chromatography for this combination. Statisitical analysis revealed significant difference in the midazolam results, which was attributed to the decrease in concentration at the 3 hour time point. This was not deemed significant because the results from the other time points were consistent with each other.

Hydromorphone 1.78mg/ml, cyclizine 16.67mg/ml, haloperidol 0.11mg/ml and midazolam 0.56mg/ml, based on clinical observation, is known to be physically compatible for 24 hours in WFI (*Dickman et al,* 2005). The data is based on 18ml being the volume in the syringe. Even though this work has assessed different concentrations of these drug components, the results also support physical compatibility in WFI over 24 hours.

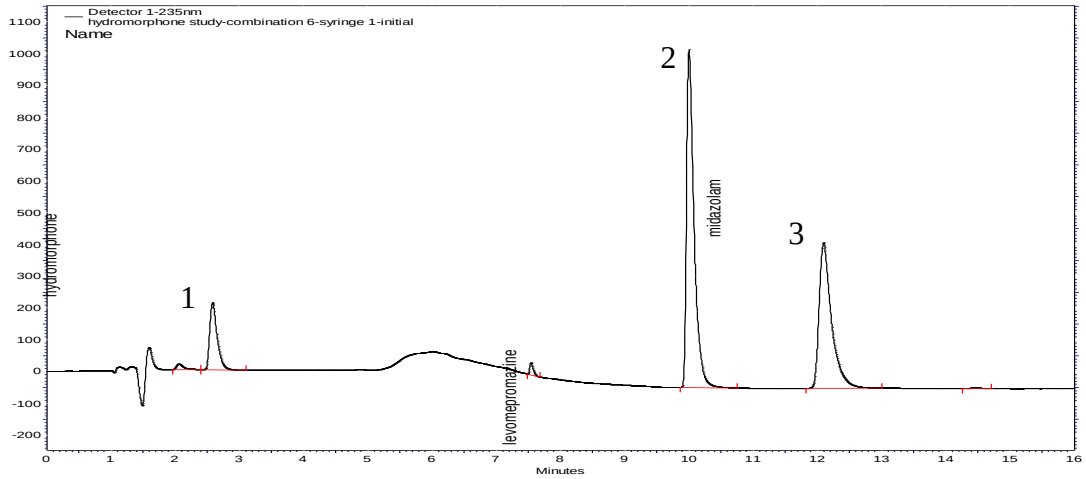
Table 3.30. Average results of the HPLC assay for hydromorphone combination 6

	Hydromorphone % initial			Levomepromazine % initial			Midazolam % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (1.54%)	109.6	111.2	100.0 (0.46%)	129.9	130.3	100.0 (2.24%)	123.7	128.5
3 hours	101.4 (1.01%)	111.2	112.8	97.5* (1.63%)	128.1	125.5	93.2* (0.99%)	116.7	118.3
6 hours	98.3* (1.06%)	108.0	109.1	96.0* (1.02%)	124.4	125.4	94.8* (2.42%)	117.2	121.8
24 hours	100.9 (0.48%)	111.2	111.6	101.3 (0.64%)	132.3	131.3	99.5 (1.94%)	123.7	127.2

* denotes where a significant difference was observed (one-way ANOVA, sample size n=4)

Figure 3.27. Representative chromatograms for combination 6 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent hydromorphone (retention time 2.6min), levomepromazine (retention time 10.0min) and midazolam (retention time 12.1min) respectively

(A)



(B)

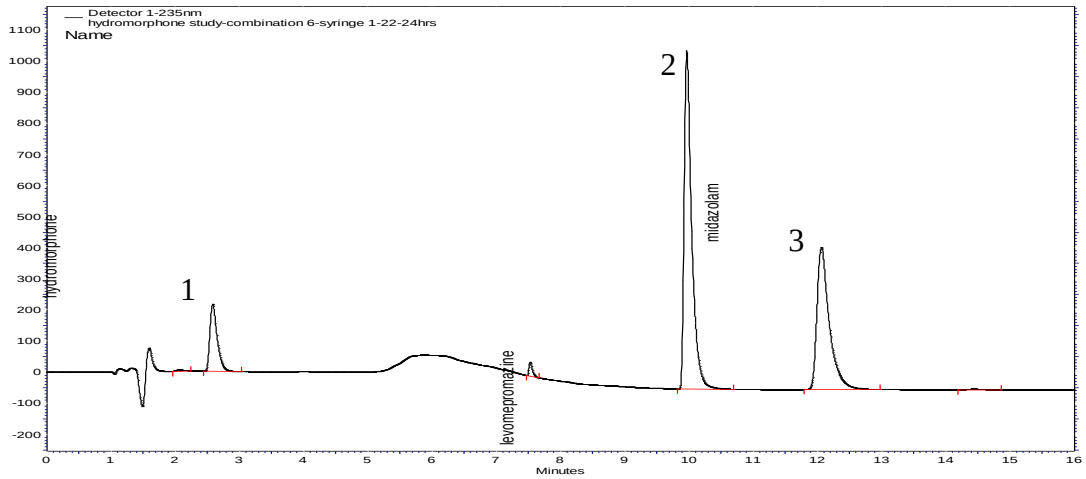


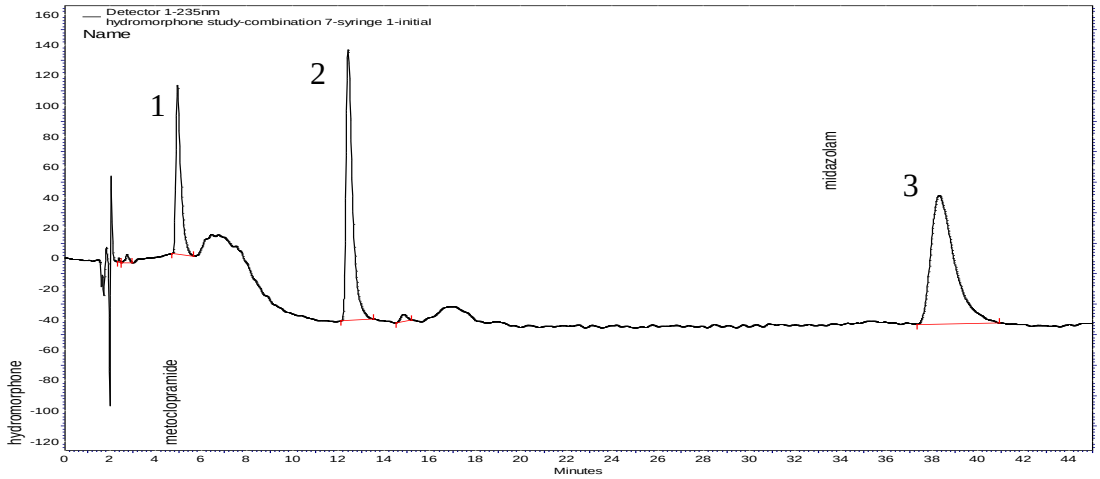
Table 3.31. Average results for the HPLC assay for hydromorphone combination 7

	Hydromorphone % initial			Metoclopramide % initial			Midazolam % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (0.51%)	116.8	117.4	100.0 (0.51%)	128.2	127.3	100.0 (4.49%)	125.9	135.3
3 hours	97.1* (1.43%)	112.3	115.0	98.5 (1.29%)	125.7	126.1	94.0 (3.86%)	119.3	126.2
6 hours	98.8 (0.85%)	114.8	116.4	99.1 (0.47%)	126.7	126.4	98.4 (2.00%)	127.4	129.4
24 hours	99.0 (1.19%)	116.1	115.8	98.4 (0.93%)	126.7	124.8	97.5 (3.72%)	125.3	129.3

* denotes where a significant difference was observed (one-way ANOVA, sample size n=4)

Figure 3.28. Representative chromatograms for combination 7 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent hydromorphone (retention time 4.9min), metoclopramide (retention time 12.4min) and midazolam (retention time 38.1min) respectively

(A)



(B)

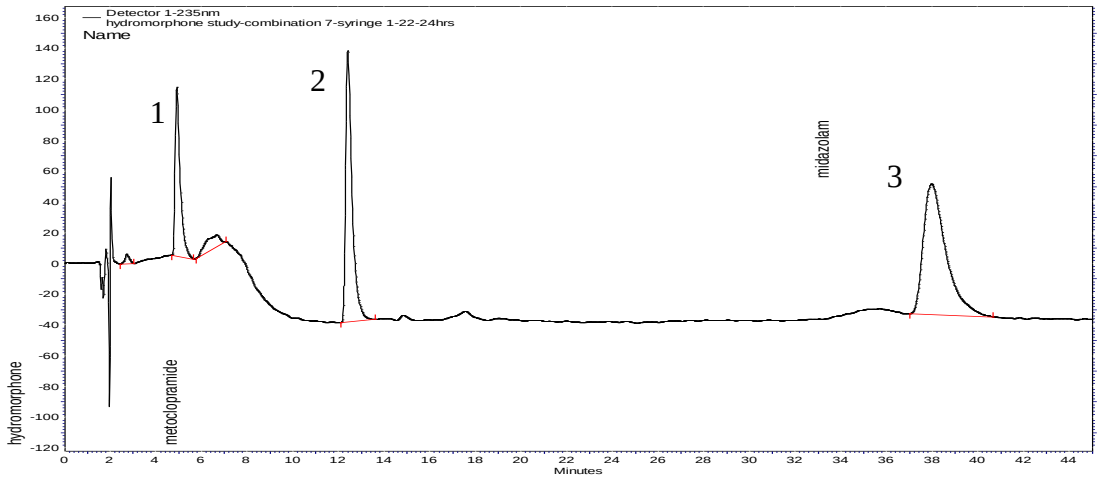


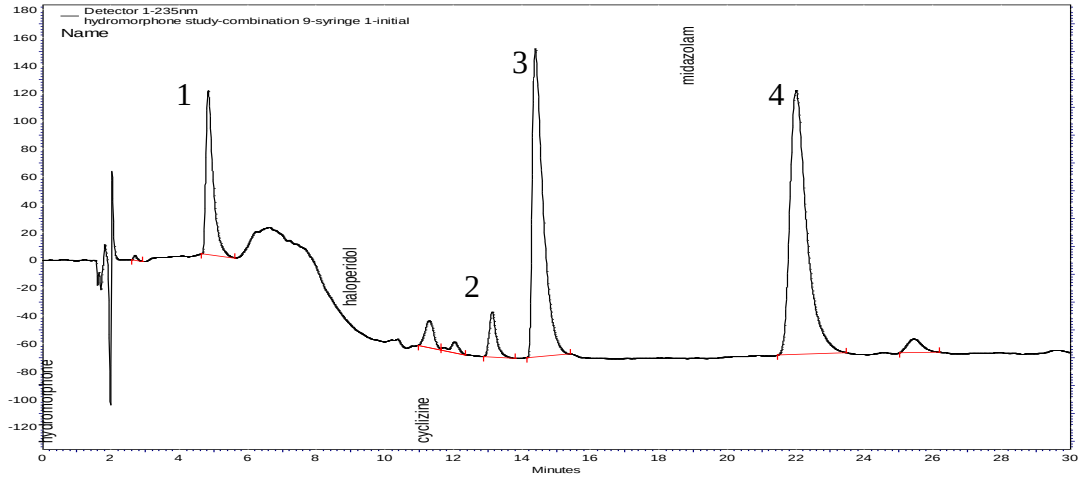
Table 3.32. Average results for the HPLC assay for hydromorphone combination 9

	Hydromorphone % initial			Haloperidol % initial			Cyclizine % initial			Midazolam % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (0.50%)	115.3	114.8	100.0 (3.94%)	128.2	122.4	100.0 (0.23%)	107.4	107.0	100.0 (1.66%)	136.6	133.5
3 hours	100.5 (1.27%)	114.4	116.9	98.6 (1.57%)	124.3	122.9	100.5 (0.81%)	107.2	108.2	96.8* (0.66%)	130.0	131.3
6 hours	101.2 (0.22%)	116.4	116.5	98.7 (1.86%)	124.7	122.7	100.5 (0.56%)	108.2	107.3	98.9 (1.05%)	134.1	133.0
24 hour	100.8 (0.96%)	116.8	115.1	99.3 (1.71%)	125.5	123.4	99.9 (0.86%)	107.6	106.5	98.2 (1.14%)	133.2	132.1

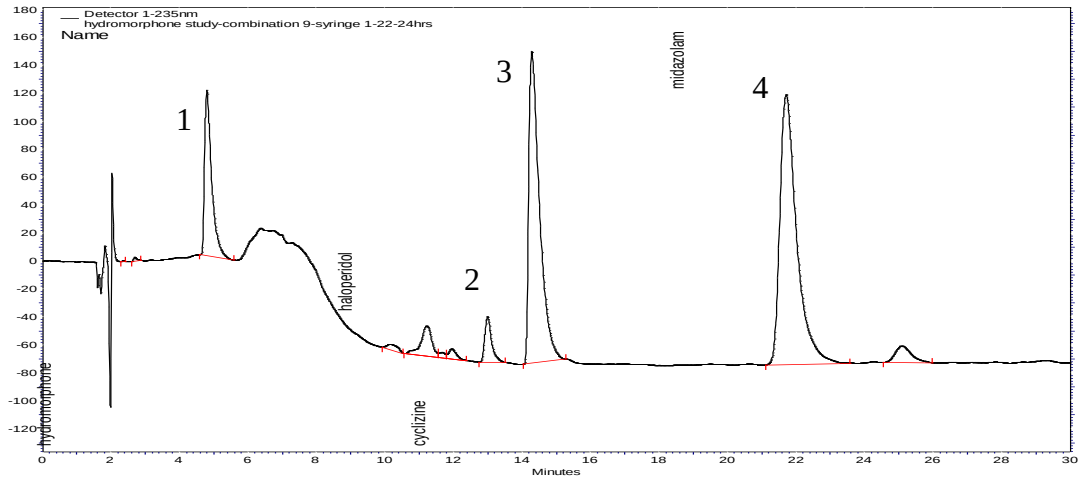
* denotes where a significant difference was observed (one-way ANOVA, sample size n=4)

Figure 3.29. Representative chromatograms for combination 9 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2, 3 and 4 represent hydromorphone (retention time 4.8min), haloperidol (retention time 13.0min), cyclizine (retention time 14.3min) and midazolam (retention time 21.7min) respectively

(A)



(B)



Of the supportive drug combinations tested with hydromorphone, the data supports compatibility over a 24 hour period.

3.3.4. Oxycodone Combinations

In the literature, Amri *et al* demonstrated in patient controlled analgesic devices that pure and diluted oxycodone hydrochloride solutions were stable for 28 days when prepared aseptically and stored at room temperature *(Amri et al, 2010)*.

For all the oxycodone combinations tested in this current work, an additional peak occurred, which was only present at the corresponding retention time in the oxycodone standard chromatograms. The peak did not increase in size over the study period. This peak was thought to be related to oxycodone because both the standard and sample solutions were prepared using the injection of oxycodone and not different materials.

Combination 1

The results for combination 1 are tabulated in table 3.33 and figure 3.30 shows the associated chromatography. Based on clinical observation, it is known that oxycodone 25mg, cyclizine 150mg and haloperidol 5mg is physically compatible for 24 hours in WFI based on a final syringe volume of 17ml (*Dickman et al,* 2005). The concentrations tested in this work are similar to those in the literature, therefore, the results presented for this combination further support physical compatibility in WFI for 24 hours.

Combination 2

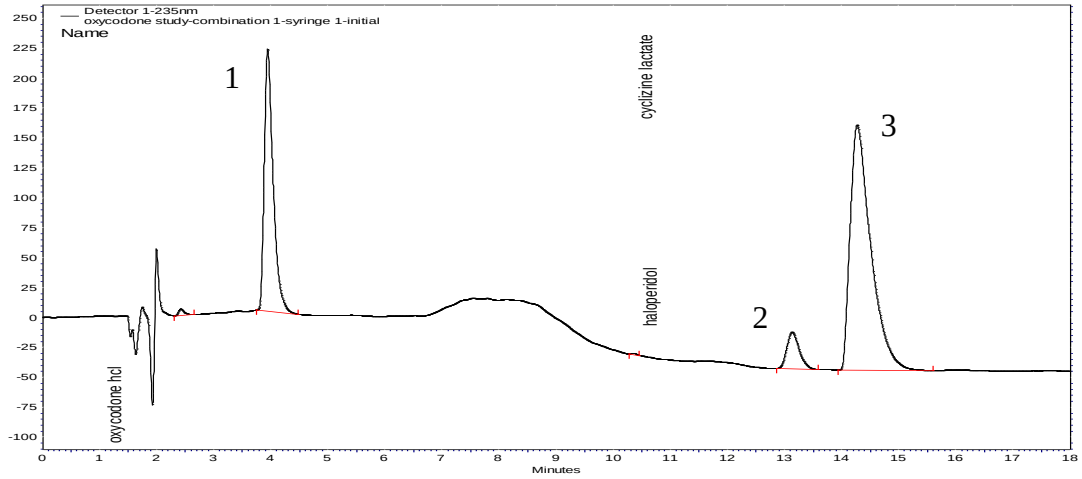
Table 3.34 shows the results and figure 3.31 the chromatography for this combination. Statistical tests indicated significant difference in the midazolam results. This was attributed to the decrease in concentration at the 3 hour time point. A real drop in concentration was not suspected because the results at this time point were lower than all the other time points.

Table 3.33. Average results of the HPLC assay for oxycodone combination 1

	Oxycodone % initial			Cyclizine % initial			Haloperidol % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (3.99%)	122.5	114.4	100.0 (2.44%)	110.8	106.4	100.0 (1.06%)	118.8	119.5
3 hours	99.4 (2.99%)	120.7	114.8	100.4 (2.20%)	110.8	107.2	100.6 (2.44%)	118.6	121.0
6 hours	98.1 (2.69%)	119.0	113.6	98.8 (2.12%)	109.2	105.3	96.8 (2.99%)	117.5	113.2
24 hours	99.3 (3.49%)	121.2	114.1	100.1 (2.02%)	110.5	106.8	101.0 (1.92%)	121.7	118.9

Figure 3.30. Representative chromatograms for combination 1 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent oxycodone hcl (retention time 3.9min), haloperidol (retention time 13.1min) and cyclizine lactate (retention time 14.3min) respectively

(A)



(B)

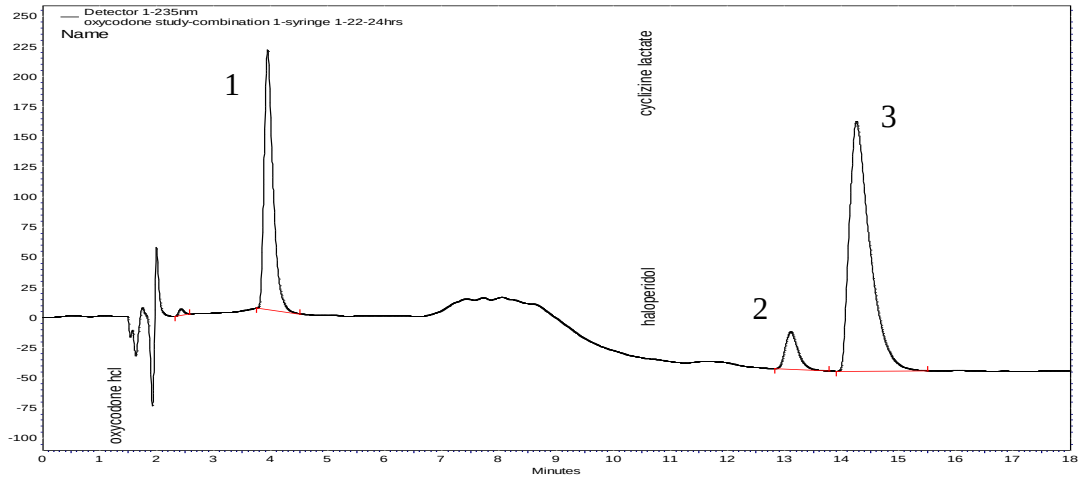


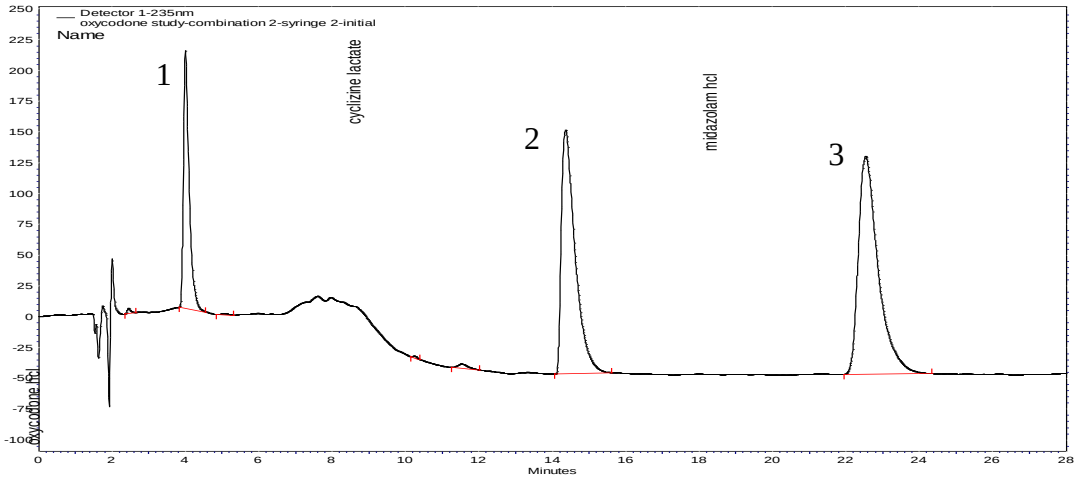
Table 3.34. Average results of the HPLC assay for oxycodone combination 2

	Oxycodone % initial			Cyclizine % initial			Midazolam % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (1.81%)	118.3	120.6	100.0 (1.55%)	110.4	112.4	100.0 (1.76%)	127.4	128.3
3 hours	99.2 (0.35%)	118.8	118.3	100.2 (1.09%)	112.7	110.6	94.9* (1.25%)	122.6	120.0
6 hours	101.7 (0.75%)	121.2	121.7	101.5 (0.92%)	113.7	112.5	100.2 (0.43%)	127.9	128.2
24 hours	100.7 (1.21%)	119.5	121.2	100.9 (0.78%)	112.5	112.3	98.2 (0.89%)	125.6	125.5

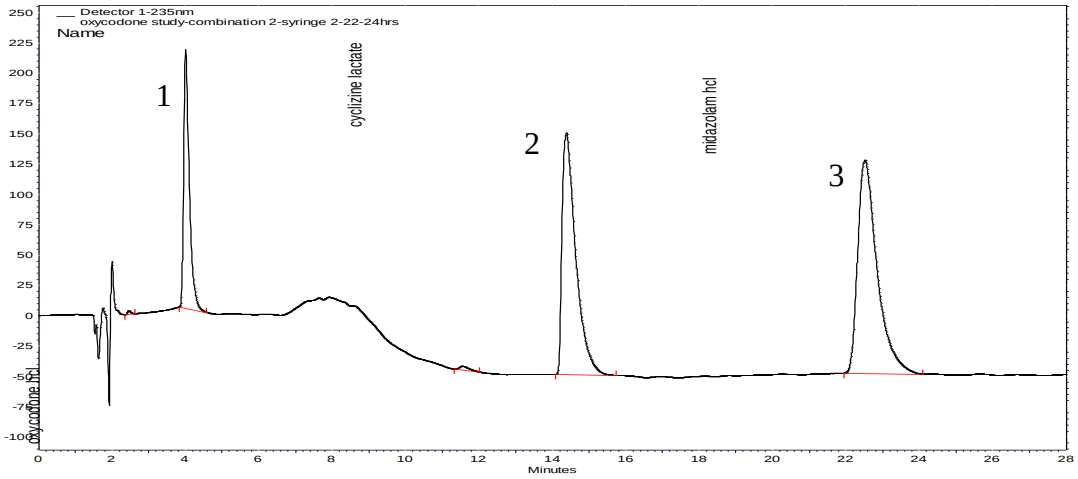
* denotes where a significant difference was observed (one-way ANOVA, sample size n=4)

Figure 3.31. Representative chromatograms for combination 2 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent oxycodone hcl (retention time 4.0min), cyclizine lactate (retention time 14.4min) and midazolam hcl (retention time 22.6min) respectively

(A)



(B)



Combination 3

The results for combination 3 and its associated chromatography are in table 3.35 and figure 3.32 respectively. Statistical analysis revealed significant difference in the levomepromazine results and was attributed to the decrease in concentration at the 3 hour time point. This decrease was not deemed significant because the results from this time point were lower and not consistent with the other time points.

Combination 4

Refer to table 3.36 for the results and figure 3.33 for the chromatography for combination 4. The haloperidol results showed significant difference after statistical tests. This was attributed to the decrease in concentration at the 6 hour time point. It was not deemed a real drop in concentration because the results at this time point were lower than the results at the other time points.

Oxycodone 5mg/ml, haloperidol 0.25mg/ml and midazolam 1mg/ml is known to be physically compatible for 24 hours in both 0.9% NaCl and WFI (*Dickman et al,* 2005). The data is based on 20ml being the volume in the syringe. The concentrations tested in this work are very similar to that reported in the literature and the results presented further support the compatibility of this combination in 0.9% NaCl over 24 hours.

Combination 5

As mentioned in section 3.2.4 combination 5 was repeated due to variation in the results obtained for levomepromazine. Only the results from the repeat analysis have been reported in table 3.37. Figure 3.34 shows the chromatography for this combination.

Statistical tests indicated significant difference in the levomepromazine results. This was attributed to the results having no consistency between each syringe preparation or between the time points. Refer to table A31a in section 6.4. On this basis, and the apparent variation in the hyoscine butylbromide results, which caused the high RSD values, the results obtained were not deemed suitable to determine compatibility of this combination. However, Dickman *et al* have reported physical compatibility for oxycodone 5mg/ml, hyoscine butylbromide 6mg/ml and levomepromazine 1.25mg/ml in both 0.9% NaCl and WFI over 24 hours (*Dickman et al,* 2005).

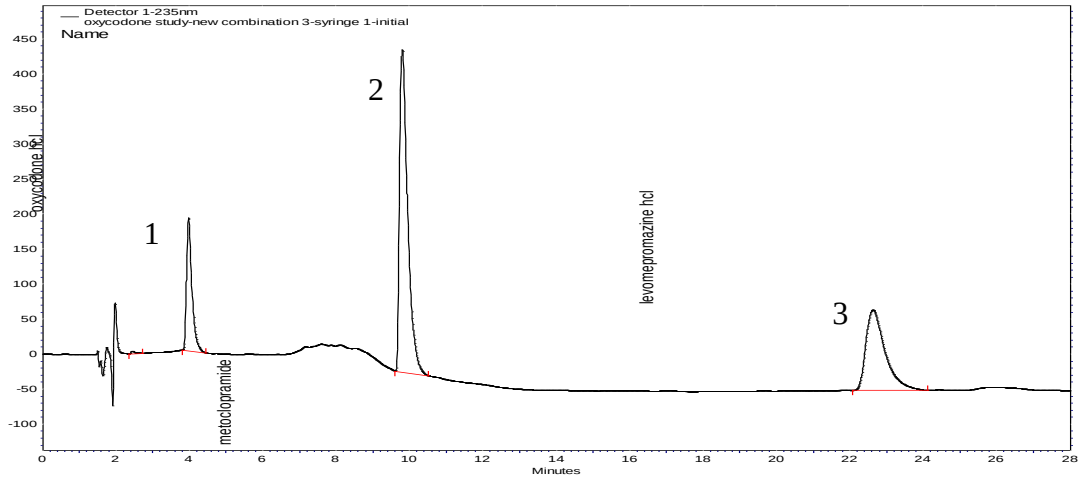
Table 3.35. Average results of the HPLC assay for oxycodone combination 3

	Oxycodone % initial			Metoclopramide % initial			Levomepromazine % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (3.08%)	98.3	103.6	100.0 (1.14%)	120.8	122.7	100.0 (0.68%)	131.1	130.4
3 hours	100.6 (1.83%)	100.1	103.0	100.1 (0.64%)	121.3	122.5	95.5* (1.59%)	126.4	123.2
6 hours	99.6 (2.84%)	98.2	102.8	100.1 (0.86%)	121.3	122.5	98.1 (1.41%)	129.5	127.0
24 hours	101.4 (3.35%)	99.4	105.2	102.4 (1.97%)	122.6	126.7	100.7 (1.95%)	130.0	133.2

* denotes where a significant difference was observed (one-way ANOVA, sample size n=4)

Figure 3.32. Representative chromatograms for combination 3 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent oxycodone hcl (retention time 4.0min), metoclopramide (retention time 9.8min) and levomepromazine hcl (retention time 22.6min) respectively

(A)



(B)

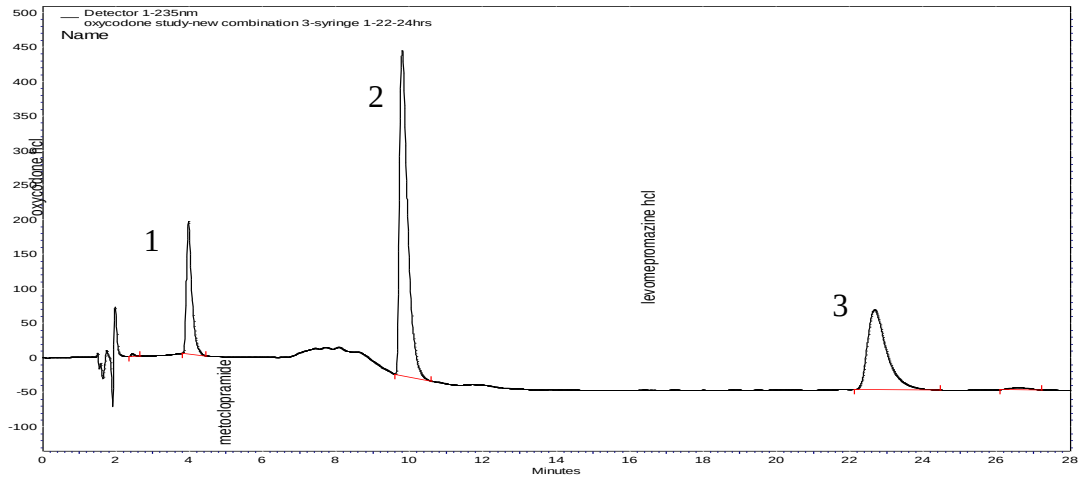


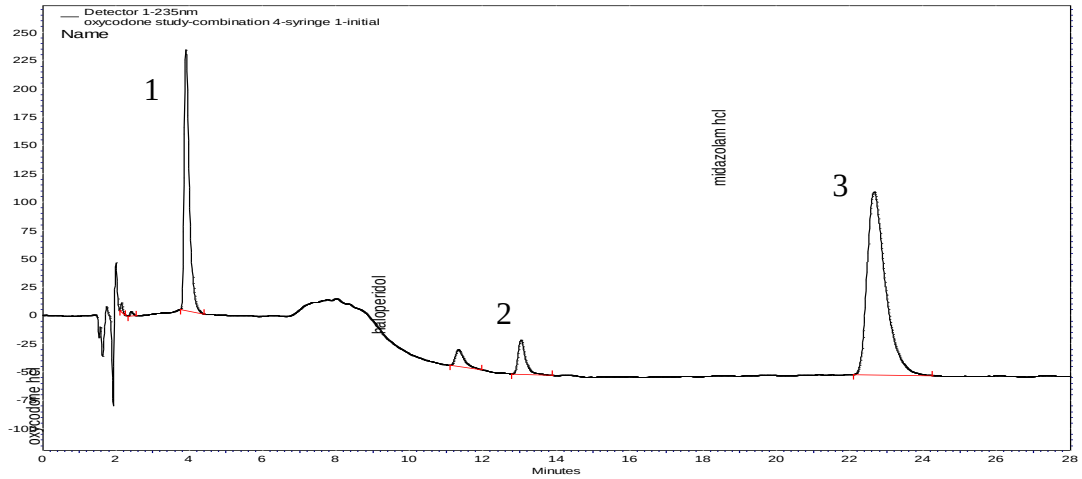
Table 3.36. Average results of the HPLC assay for oxycodone combination 4

	Oxycodone % initial			Haloperidol % initial			Midazolam % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (1.18%)	115.9	114.2	100.0 (3.11%)	111.5	106.0	100.0 (6.36%)	116.7	125.3
3 hours	100.6 (1.14%)	116.8	114.6	99.7 (3.10%)	110.0	106.8	97.6 (5.80%)	113.9	122.2
6 hours	99.9 (0.46%)	115.3	114.6	94.1* (1.94%)	102.1	102.5	97.8 (6.10%)	114.0	122.7
24 hours	99.7 (1.21%)	115.9	113.5	96.8 (3.27%)	108.2	102.4	103.6 (1.35%)	126.5	124.2

* denotes where a significant difference was observed (one-way ANOVA, sample size n=4)

Figure 3.33. Representative chromatograms for combination 4 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent oxycodone hcl (retention time 3.9min), haloperidol (retention time 13.0min) and midazolam hcl (retention time 22.6min) respectively

(A)



(B)

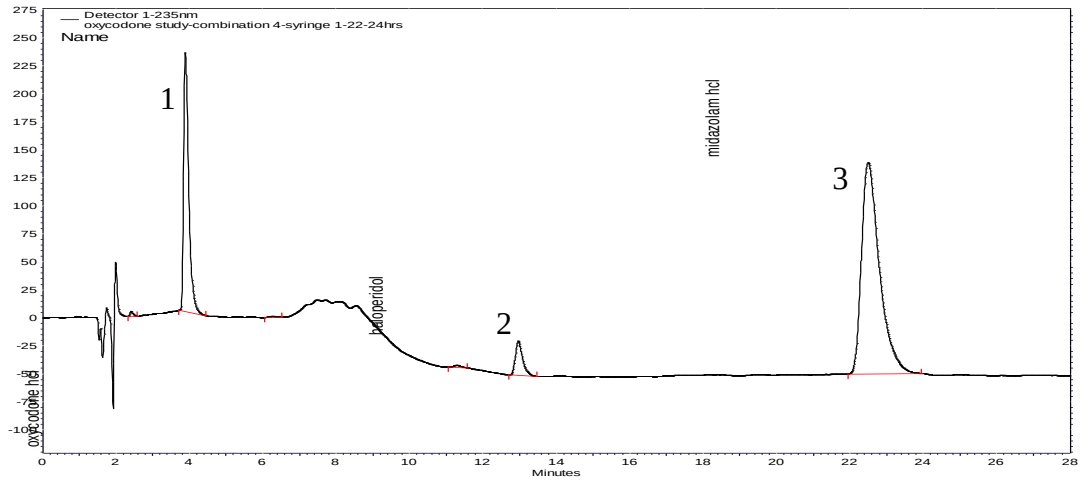


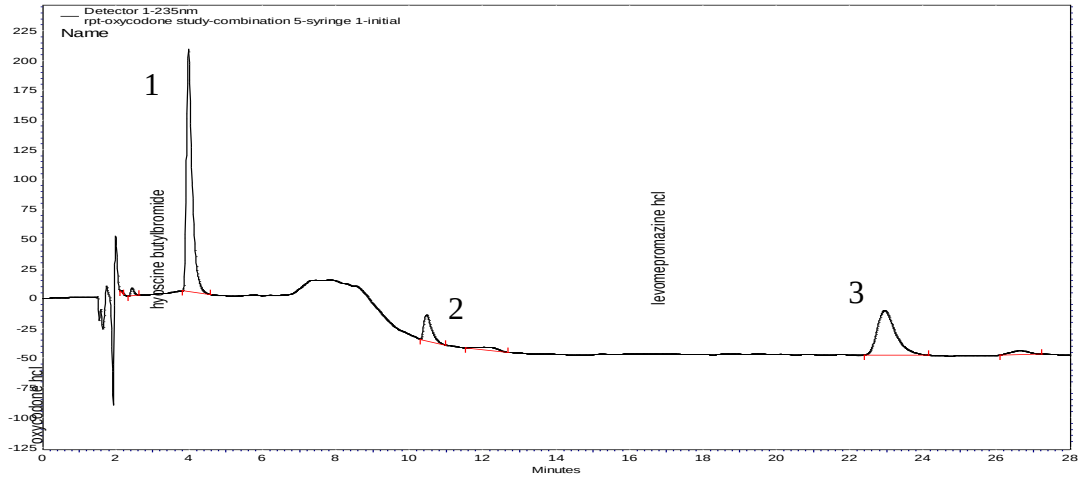
Table 3.37. Average results of the repeat HPLC assay for oxycodone combination 5

	Oxycodone % initial			Hyoscine butylbromide % initial			Levomepromazine % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (0.65%)	114.4	114.1	100.0 (5.14%)	106.8	104.8	100.0* (18.48%)	92.6	127.8
3 hours	95.7 (8.03%)	116.9	101.7	98.2 (9.51%)	114.8	97.9	75.2* (1.81%)	81.6	84.1
6 hours	101.8 (1.08%)	117.3	115.3	106.6 (1.75%)	114.1	116.6	84.3* (3.04%)	90.6	95.3
24 hours	102.3 (0.61%)	117.1	116.8	105.1 (3.47%)	112.6	115.0	86.0* (4.87%)	90.9	98.7

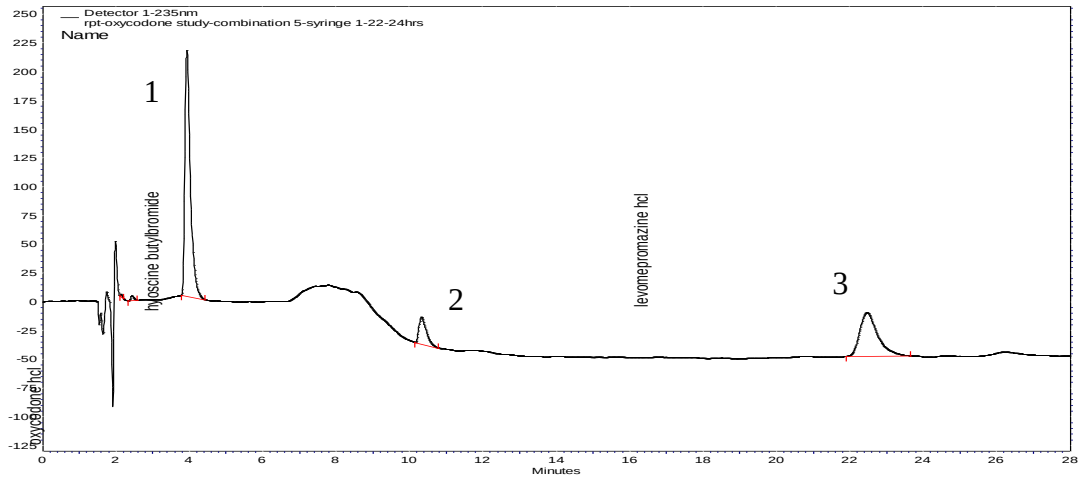
* denotes where a significant difference was observed (one-way ANOVA, sample size n=4)

Figure 3.34. Representative chromatograms for combination 5 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent oxycodone hcl (retention time 4.0min), hyoscine butylbromide (retention time 10.5min) and levomepromazine hcl (retention time 23.0min) respectively

(A)



(B)



Combination 6

Table 3.38 tabulates the results for combination 6 and figure 3.35 shows its associated chromatography. Statistical tests indicated significant difference for both levomepromazine and midazolam. This was attributed to the decrease at the 3 hour and 6 hour time points. A real drop in concentration was not considered because the initial and final time point results were consistent with each other and the results at the 3 hour and 6 hour time points were lower than these results.

Combination 7

The results and chromatography for this combination are shown in table 3.39 and figure 3.36 respectively.

Combination 9

Refer to table 3.40 for the results and figure 3.37 for the chromatography of combination 9. Significant difference in the oxycodone, haloperidol and midazolam results was indicated by statistical tests. They have been attributed to the decrease that has occurred at the 6 hour time point and also the 3 hour time point for haloperidol and midazolam. However, a real drop in concentration was not considered because the results from the other time points were consistent with each other and it can be seen that these time point results are considerably lower than the others.

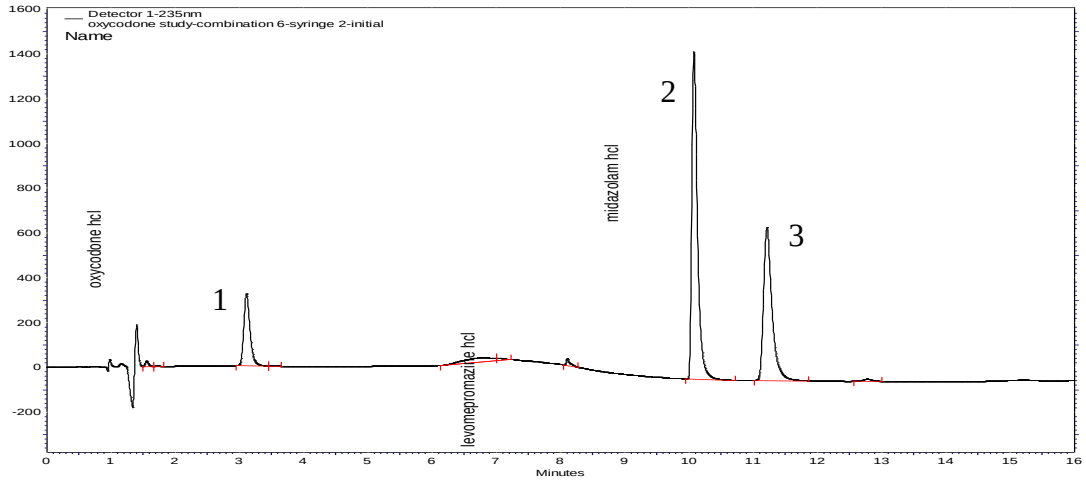
Table 3.38. Average results of the HPLC assay for oxycodone combination 6

	Oxycodone % initial			Levomepromazine % initial			Midazolam % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (0.90%)	112.1	113.3	100.0 (0.76%)	131.2	129.7	100.0 (1.40%)	116.6	118.7
3 hours	99.9 (0.70%)	113.1	112.0	94.2* (0.32%)	122.8	122.8	93.4* (2.63%)	108.5	111.4
6 hours	98.3 (0.40%)	111.1	110.3	93.5* (1.32%)	120.8	123.2	95.1* (3.65%)	109.4	114.4
24 hours	99.1 (0.82%)	112.3	111.1	99.9 (1.37%)	131.7	128.8	99.9 (0.44%)	117.7	117.5

* denotes where a significant difference was observed (one-way ANOVA, sample size n=4)

Figure 3.35. Representative chromatograms for combination 6 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent oxycodone hcl (retention time 3.1min), levomepromazine hcl (retention time 10.1min) and midazolam hcl (retention time 11.2min) respectively

(A)



(B)

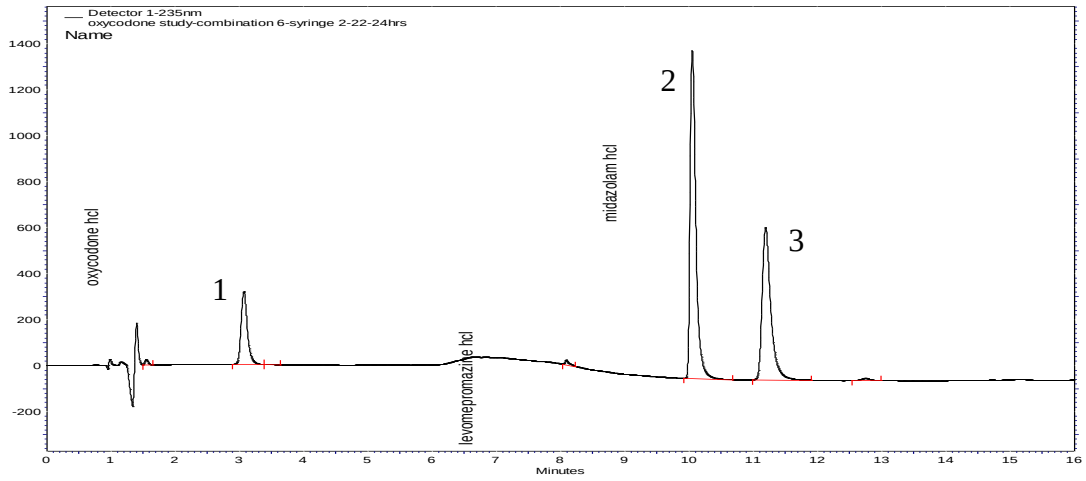
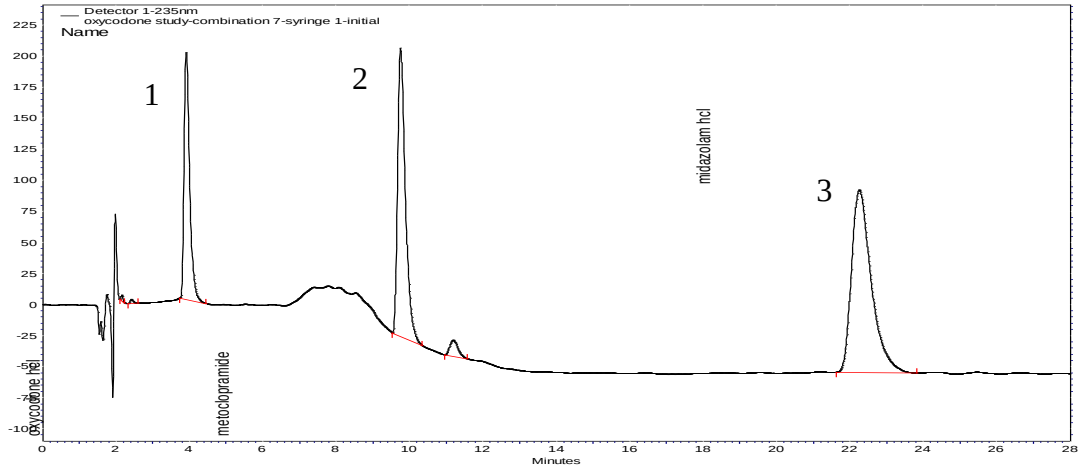


Table 3.39. Average results for the HPLC assay for oxycodone combination 7

	Oxycodone % initial			Metoclopramide % initial			Midazolam % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (1.50%)	109.9	107.2	100.0 (0.83%)	127.4	128.2	100.0 (6.53%)	114.2	126.5
3 hours	96.2 (2.01%)	106.0	102.9	100.6 (2.40%)	126.1	131.0	98.6 (7.77%)	111.3	126.1
6 hours	98.8 (0.38%)	107.6	107.0	99.7 (1.17%)	126.0	128.6	98.7 (6.49%)	112.6	124.9
24 hours	99.2 (3.49%)	110.9	104.5	101.4 (0.43%)	129.3	129.9	100.9 (2.89%)	119.0	123.9

Figure 3.36. Representative chromatograms for combination 7 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent oxycodone hcl (retention time 3.9min), metoclopramide (retention time 9.8min) and midazolam hcl (retention time 22.2min) respectively

(A)



(B)

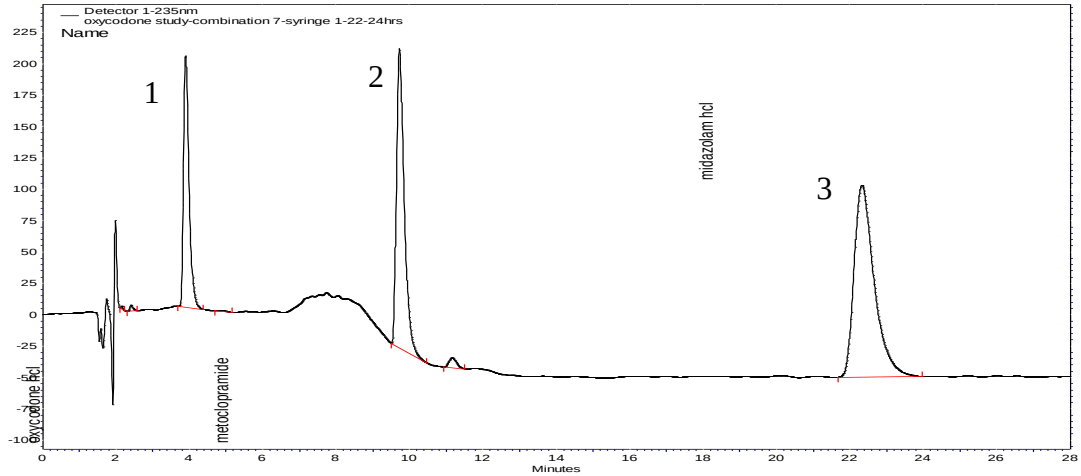


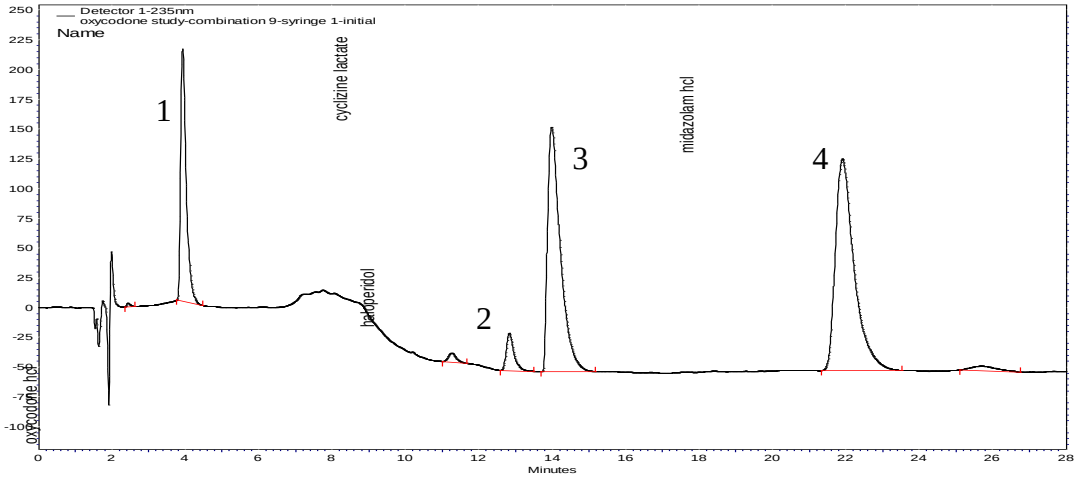
Table 3.40. Average results for the HPLC assay for oxycodone combination 9

	Oxycodone % initial			Haloperidol % initial			Cyclizine % initial			Midazolam % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (0.42%)	118.3	117.6	100.0 (0.75%)	121.3	120.5	100.0 (0.90%)	111.3	111.6	100.0 (1.97%)	131.8	133.0
3 hours	97.3 (2.81%)	114.8	114.9	96.3* (1.50%)	115.9	116.9	97.8 (2.55%)	109.7	108.3	90.7* (1.95%)	118.5	121.7
6 hours	96.2* (1.79%)	114.2	112.8	94.2* (2.82%)	116.6	111.1	97.0 (2.03%)	109.1	107.2	94.7* (3.72%)	124.6	126.2
24 hour	100.2 (1.91%)	120.1	116.4	99.4 (3.42%)	120.3	120.0	99.6 (1.52%)	109.8	109.8	98.9 (1.66%)	131.7	130.2

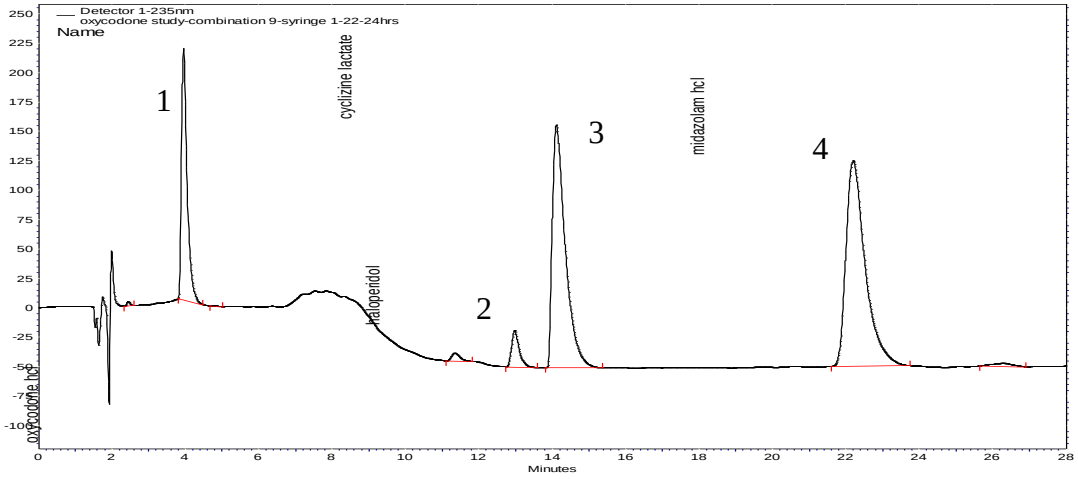
* denotes where a significant difference was observed (one-way ANOVA, sample size n=4)

Figure 3.37. Representative chromatograms for combination 9 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2, 3 and 4 represent oxycodone hcl (retention time 3.9min), haloperidol (retention time 12.8min), cyclizine lactate (retention time 14.0min) and midazolam hcl (retention time 21.9min) respectively

(A)



(B)



Overall, the supportive drug combinations tested with hydromorphone, 1-4, 6, 7 and 9, showed compatibility over the 24 hour study period. Unfortunately, combination 5 could not be included due to the results obtained.

3.3.5. Alfentanil Combinations

Alfentanil is related to fentanyl. Stability of fentanyl admixtures:
(1) fentanyl 1000µg, hyoscine N-butyl bromide 30mg and midazolam 15mg, and
(2) fentanyl 1000µg, metoclopramide hydrochloride 20mg and midazolam 15mg,
in polypropylene syringes has been demonstrated for storage in the dark below 32°C for up to 1 week *(Peterson et al, 1998).*

Combination 1

Table 3.41 contains the results for combination 1 and figure 3.38 shows the chromatography.

Combination 2

Refer to table 3.42 for the results for this combination and figure 3.39 for the associated chromatography. Statistical tests indicated significant difference in the midazolam results. This was suspected to be caused by the decrease in concentration at the 3 hour time point. A real drop in concentration was not suspected because the initial and final time point results were consistent with each other.

Combination 3

The results and chromatography for combination 3 are tabulated in table 3.43 and figure 3.40.

Combination 4

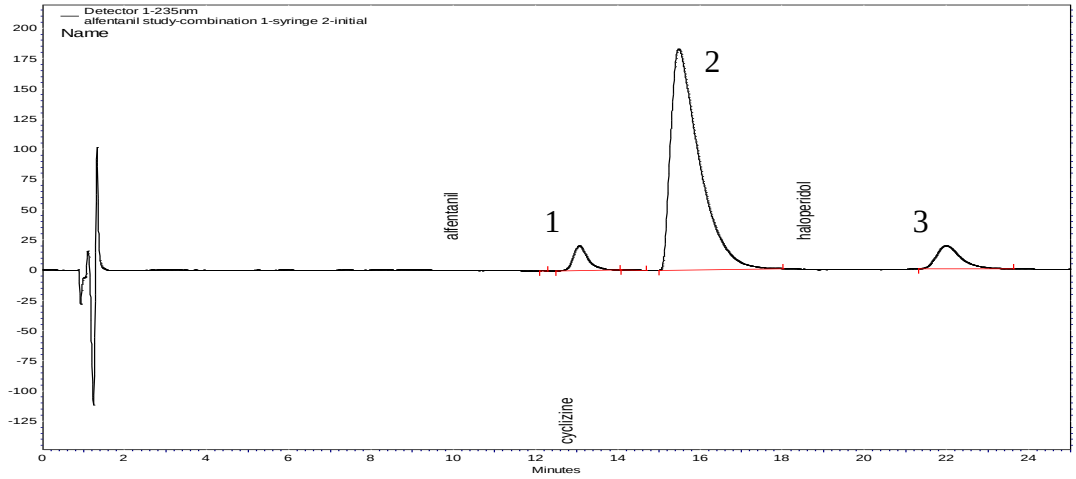
Table 3.44 tabulates the results and figure 3.41 shows the chromatography for this combination. The decrease at the 3 hour and 6 hour time points for midazolam was suspected to be the cause of the significant difference indicated by statistical tests. This decrease was not deemed significant because the results at these time points were lower than those obtained at the initial and final time points.

Table 3.41. Average results of the HPLC assay for alfentanil combination 1

	Alfentanil % initial			Cyclizine % initial			Haloperidol % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (1.97%)	112.3	108.8	100.0 (1.28%)	104.4	102.3	100.0 (2.93%)	116.2	110.6
3 hours	103.8 (2.93%)	113.3	116.2	101.3 (1.69%)	106.1	103.3	100.6 (3.69%)	117.9	116.1
6 hours	102.7 (2.91%)	110.7	116.3	101.2 (1.49%)	105.9	103.4	96.8 (4.33%)	120.1	115.0
24 hours	102.9 (1.82%)	113.3	114.2	100.8 (0.32%)	104.4	103.9	101.0 (4.14%)	119.3	115.6

Figure 3.38. Representative chromatograms for combination 1 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent alfentanil (retention time 13.0min), cyclizine (retention time 15.5min) and haloperidol (retention time 22.0min) respectively

(A)



(B)

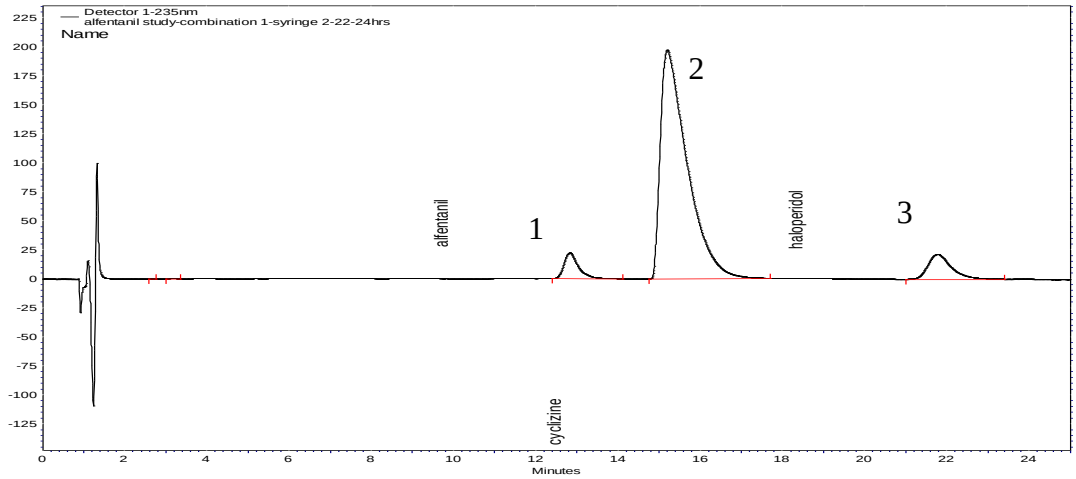


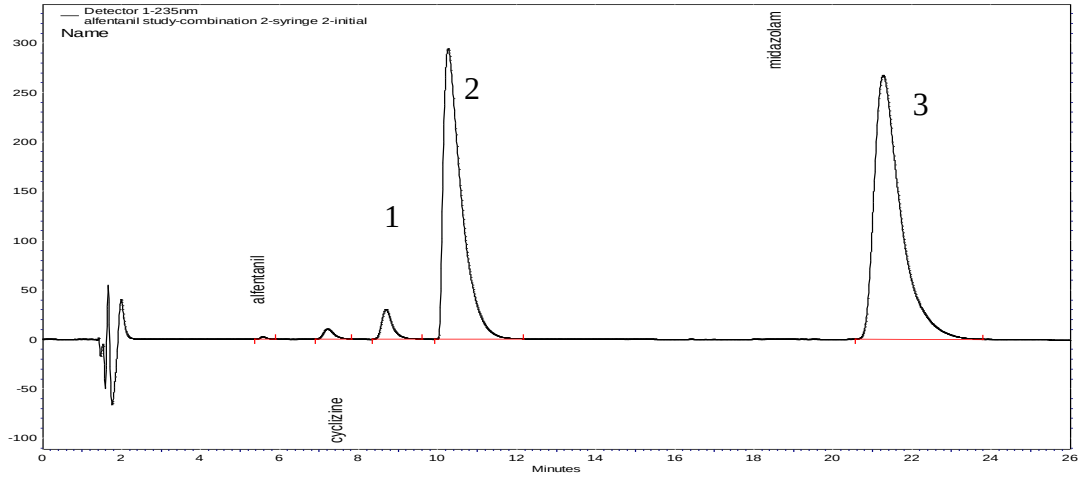
Table 3.42. Average results of the HPLC assay for alfentanil combination 2

	Alfentanil % initial			Cyclizine % initial			Midazolam % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (0.97%)	114.6	113.0	100.0 (3.18%)	106.4	100.7	100.0 (2.34%)	120.2	116.1
3 hours	101.5 (2.15%)	117.1	113.9	100.2 (3.12%)	106.6	101.0	95.2* (1.67%)	113.8	111.1
6 hours	99.2 (2.11%)	114.0	111.8	100.0 (2.90%)	106.1	100.9	97.5 (1.95%)	116.7	113.7
24 hours	100.9 (3.40%)	118.2	111.6	101.1 (4.06%)	108.4	101.0	100.7 (3.76%)	122.8	115.2

* denotes where a significant difference was observed (one-way ANOVA, sample size n=4)

Figure 3.39. Representative chromatograms for combination 2 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent alfentanil (retention time 8.7min), cyclizine (retention time 10.3min) and midazolam (retention time 21.3min) respectively

(A)



(B)

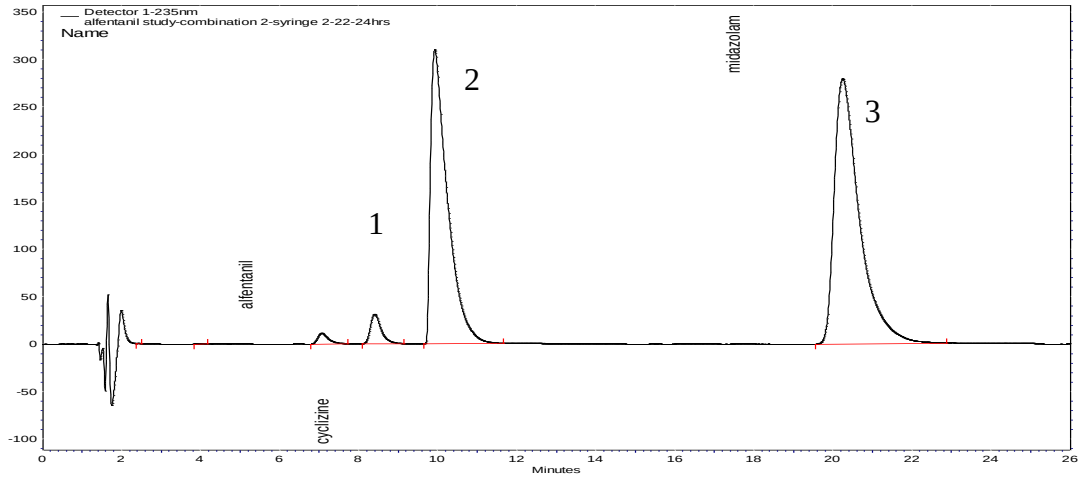
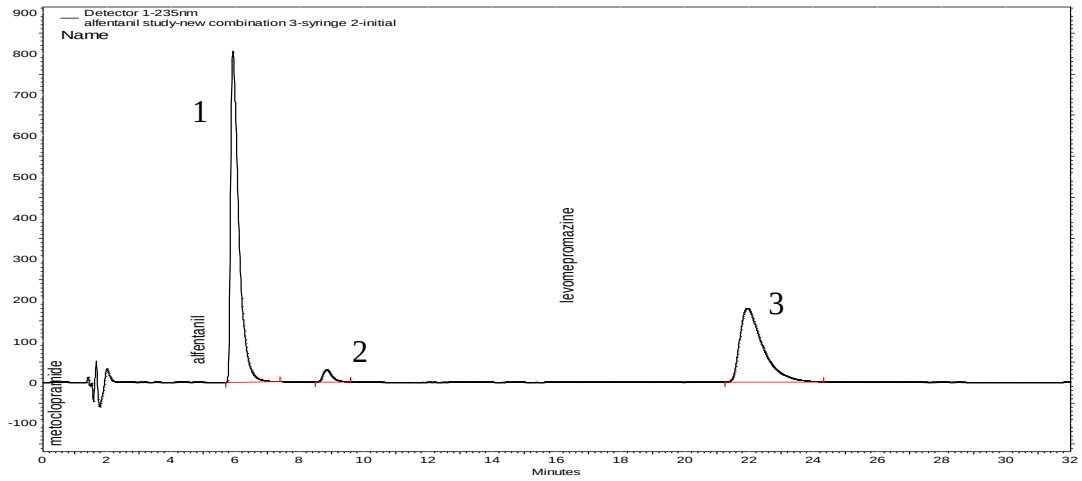


Table 3.43. Average results of the HPLC assay for alfentanil combination 3

	Alfentanil % initial			Metoclopramide % initial			Levomepromazine % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (0.41%)	116.9	116.1	100.0 (0.33%)	115.6	115.8	100.0 (2.31%)	131.3	126.3
3 hours	100.0 (0.33%)	116.8	116.1	98.2 (0.50%)	113.1	114.1	97.1 (0.92%)	125.9	124.2
6 hours	99.2 (1.28%)	115.0	116.2	98.8 (0.25%)	114.2	114.4	98.1 (0.99%)	127.3	125.4
24 hours	98.8 (1.03%)	115.1	115.1	99.6 (0.37%)	115.2	115.1	98.4 (0.62%)	127.3	126.2

Figure 3.40. Representative chromatograms for combination 3 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent metoclopramide (retention time 6.0min), alfentanil (retention time 8.9min) and levomepromazine (retention time 22.1min) respectively

(A)



(B)

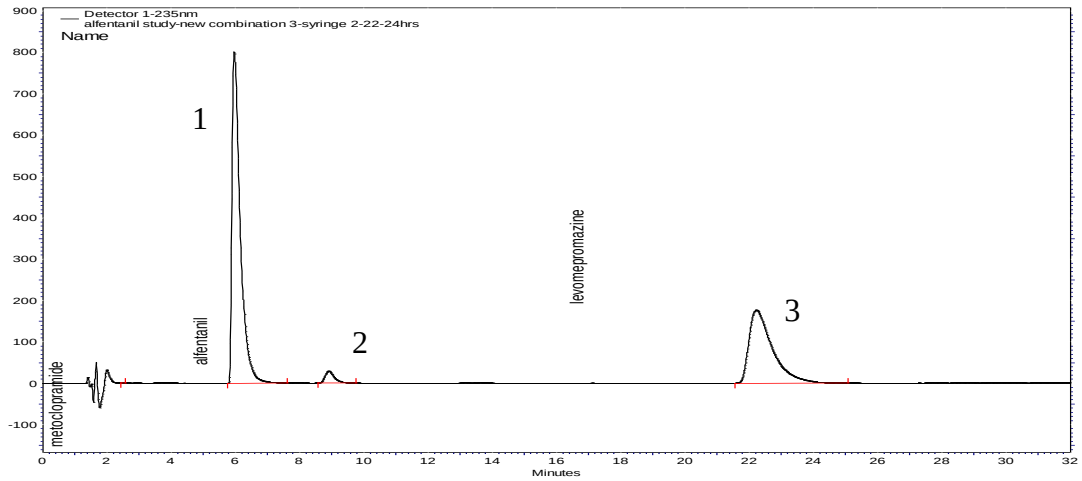


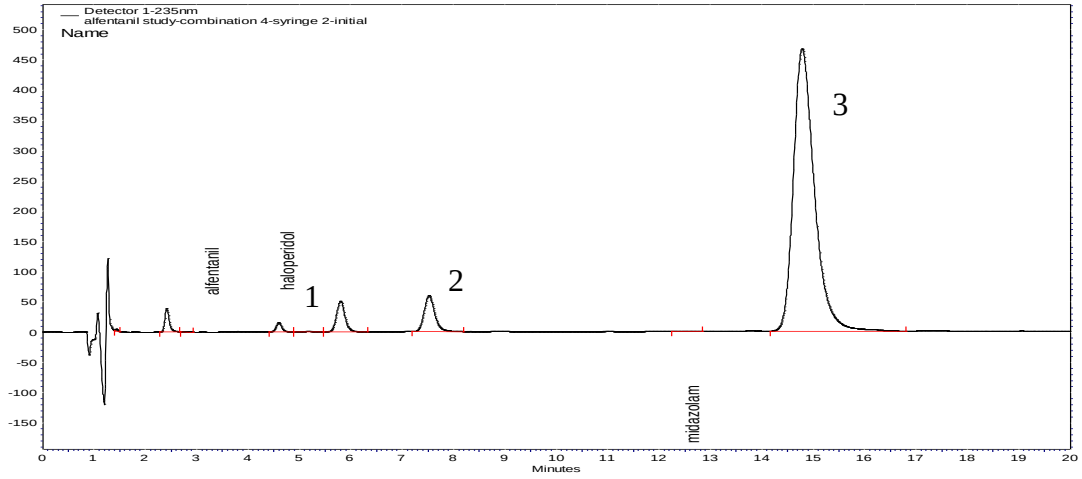
Table 3.44. Average results of the HPLC assay for alfentanil combination 4

	Alfentanil % initial			Haloperidol % initial			Midazolam % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (1.20%)	116.7	118.5	100.0 (1.79%)	121.1	117.7	100.0 (2.27%)	120.0	123.6
3 hours	100.5 (1.72%)	118.8	116.4	97.3 (2.03%)	116.4	115.9	93.6* (4.28%)	111.1	117.0
6 hours	98.1 (1.80%)	116.8	113.9	97.4 (1.73%)	117.4	115.4	94.3* (4.47%)	111.6	118.1
24 hours	98.5 (0.72%)	115.3	116.2	99.6 (1.08%)	119.6	118.2	99.5 (3.07%)	118.9	123.6

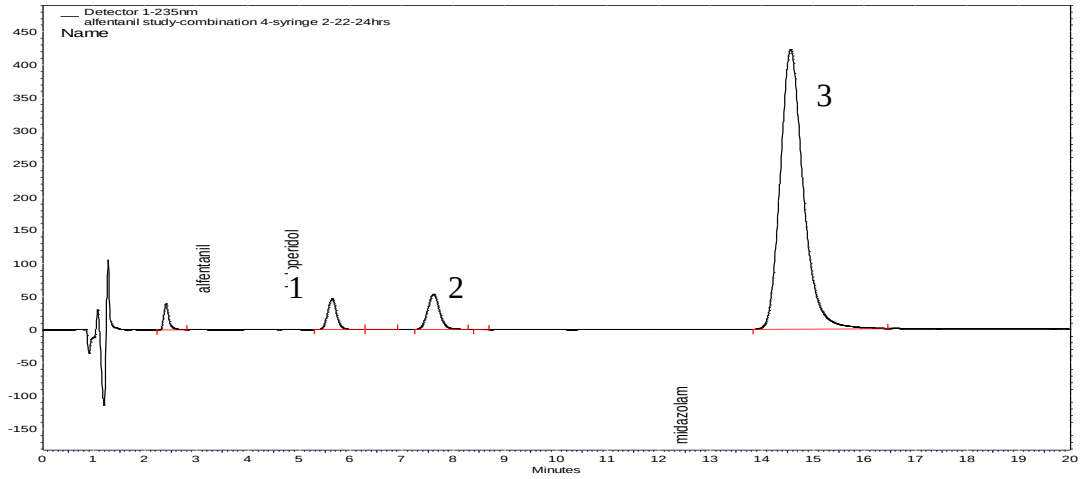
* denotes where a significant difference was observed (one-way ANOVA, sample size n=4)

Figure 3.41. Representative chromatograms for combination 4 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent alfentanil (retention time 5.8min), haloperidol (retention time 7.5min) and midazolam (retention time 14.7min) respectively

(A)



(B)



Combination 5

Table 3.45 contains the results for combination 5 and figure 3.42 shows the associated chromatography. Extremely high RSD values have been obtained for the levomepromazine results. This was because the results for one syringe preparation were considerably lower than the other syringe preparation at each time point. A difference in the levomepromazine syringe preparations has been seen previously for this combination, and again was suspected to be due to the syringe preparation process. The data from this combination was not used. However, the data for each syringe preparation is consistent if considered separately, but for this research a confirmation of results was required by the second preparation, which has not occurred.

Combination 6

Refer to table 3.46 for the results and figure 3.43 for the chromatography for combination 6.

Combination 7

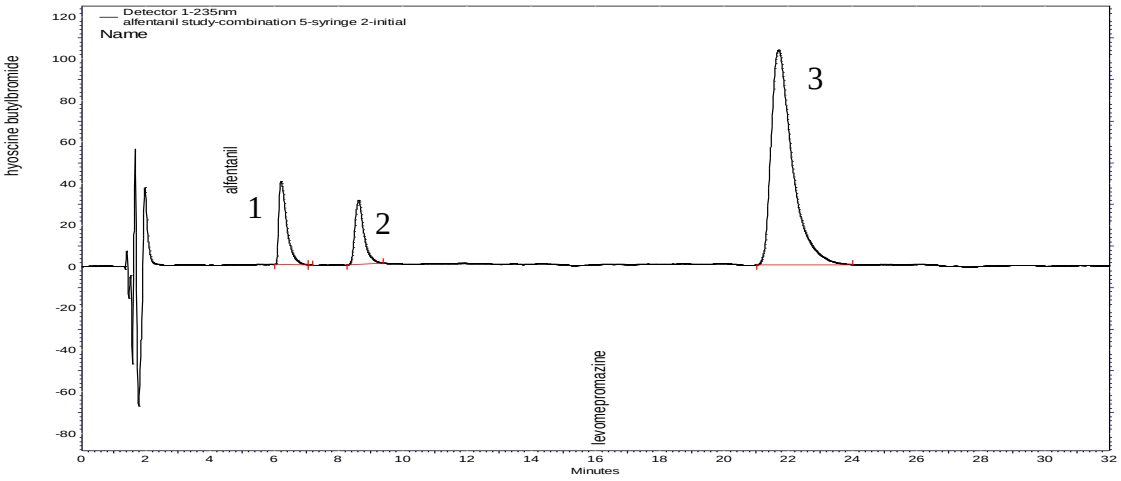
The results and the chromatography are shown in table 3.47 and figure 3.44 respectively. Statistical tests indicated significant difference in the midazolam results, which was attributed to the decrease at the 3 hour and 6 hour time points. A real drop in concentration has not been considered as the 0 hour and 24 hour time points have consistent results.

Table 3.45. Average results of the HPLC assay for alfentanil combination 5

	Alfentanil % initial			Hyoscine butylbromide % initial			Levomepromazine % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (1.30%)	117.5	119.2	100.0 (1.06%)	109.9	111.2	100.0 (14.14%)	117.6	150.4
3 hours	100.5 (3.94%)	115.6	122.3	99.7 (1.92%)	108.6	112.0	95.6 (13.29%)	113.4	142.8
6 hours	99.1 (2.77%)	114.9	119.7	99.1 (1.37%)	108.6	110.5	95.6 (15.02%)	111.5	144.8
24 hours	98.8 (1.44%)	115.7	118.0	100.0 (2.07%)	109.5	111.8	95.9 (13.72%)	113.3	143.8

Figure 3.42. Representative chromatograms for combination 5 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent hyoscine butylbromide (retention time 6.2min), alfentanil (retention time 8.6min) and levomepromazine (retention time 21.9min) respectively

(A)



(B)

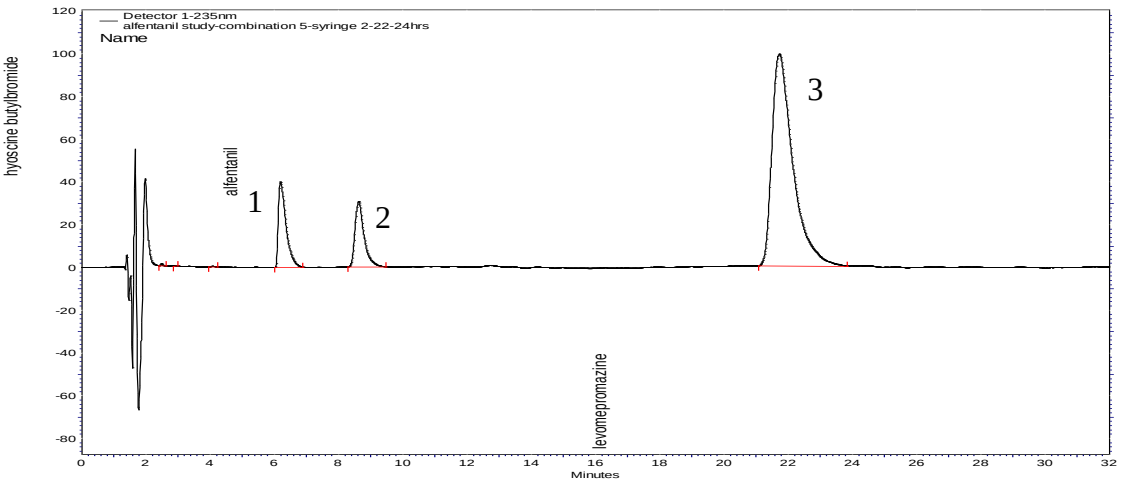
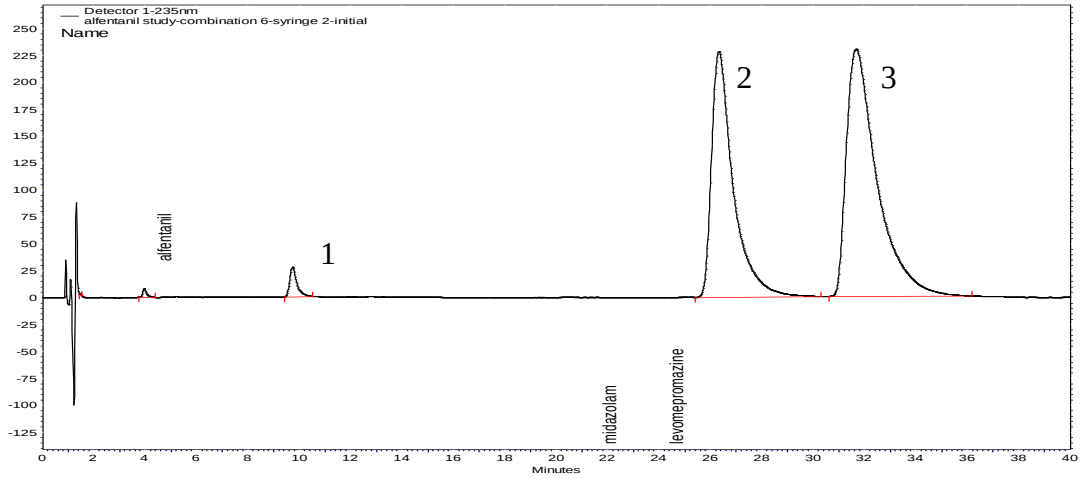


Table 3.46. Average results of the HPLC assay for alfentanil combination 6

	Alfentanil % initial			Levomepromazine % initial			Midazolam % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (0.85%)	114.6	113.0	100.0 (1.39%)	126.2	128.8	100.0 (1.88%)	124.3	127.2
3 hours	99.3 (0.84%)	113.4	112.8	98.7 (1.08%)	124.6	127.0	95.1 (2.21%)	118.7	120.5
6 hours	99.8 (2.87%)	114.7	112.4	99.8 (1.21%)	126.0	128.6	97.3 (3.25%)	119.9	124.9
24 hours	100.0 (2.82%)	111.8	116.3	99.6 (0.30%)	126.8	127.2	97.7 (2.36%)	121.8	123.9

Figure 3.43. Representative chromatograms for combination 6 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent alfentanil (retention time 9.7min), midazolam (retention time 26.3min) and levomepromazine (retention time 31.8min) respectively

(A)



(B)

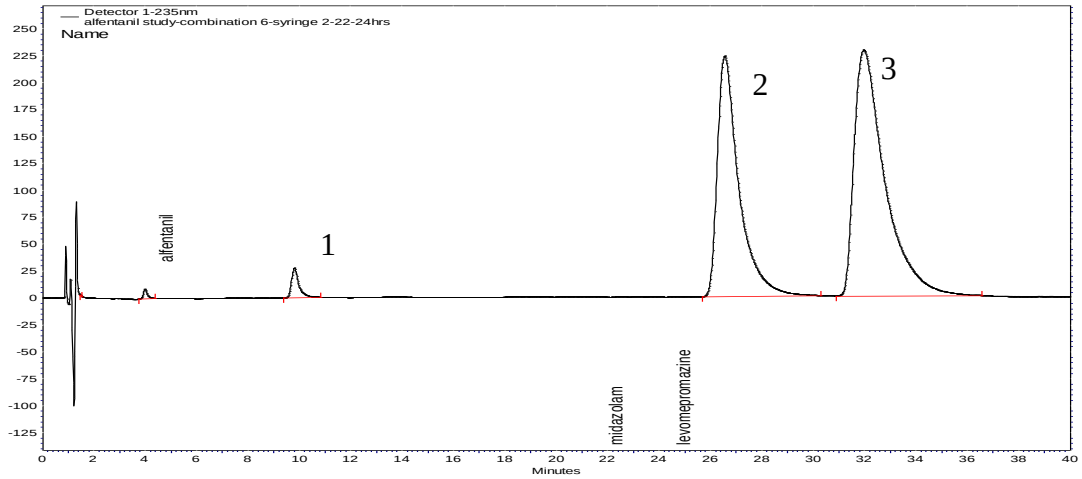


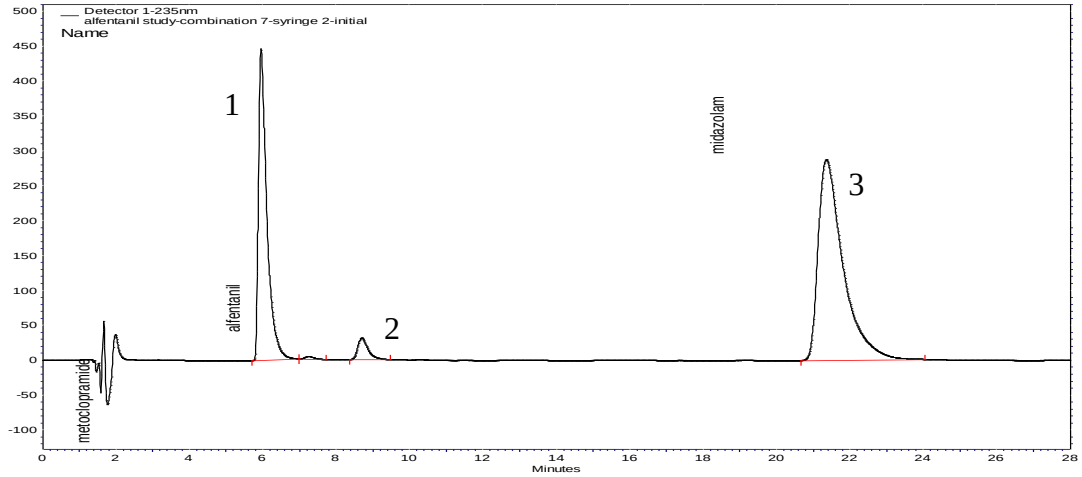
Table 3.47. Average results of the HPLC assay for alfentanil combination 7

	Alfentanil % initial			Metoclopramide % initial			Midazolam % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (2.15%)	113.4	114.0	100.0 (0.25%)	116.9	116.7	100.0 (2.49%)	121.1	124.7
3 hours	100.8 (2.66%)	112.8	116.2	99.2 (0.40%)	115.9	115.7	96.0* (0.91%)	117.2	118.7
6 hours	98.9 (1.94%)	110.8	114.2	98.7 (0.64%)	114.7	115.7	96.8* (3.07%)	116.8	121.2
24 hours	96.8 (1.03%)	110.4	109.6	101.3 (0.78%)	119.0	117.5	100.6 (1.44%)	123.5	123.6

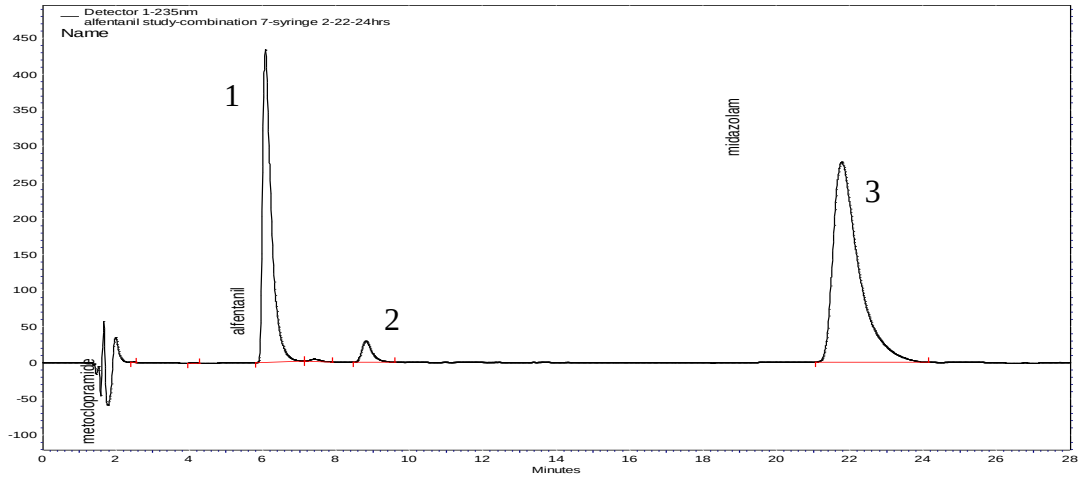
* denotes where a significant difference was observed (one-way ANOVA, sample size n=4)

Figure 3.44. Representative chromatograms for combination 7 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2 and 3 represent metoclopramide (retention time 6.0min), alfentanil (retention time 8.7min) and midazolam (retention time 21.5min) respectively

(A)



(B)



Combination 9

Table 3.48 contains the results for this combination and figure 3.45 depicts the chromatography. The decrease at the 3 hour time point for midazolam was suspected to be the cause of the significant difference indicated by the statistical tests. A real drop in concentration was not suspected because the results at the other time points were consistent with each other.

Dickman *et al* reported that alfentanil 0.24mg/ml, cyclizine 7.14mg/ml, haloperidol 0.48mg/ml and midazolam 1.9mg/ml are physically compatible in WFI for 24 hours based on clinical observation (*Dickman et al*, 2005). The data is based on 21ml being the volume in the syringe. The results presented for this combination can further support physical compatibility for 24 hours in WFI.

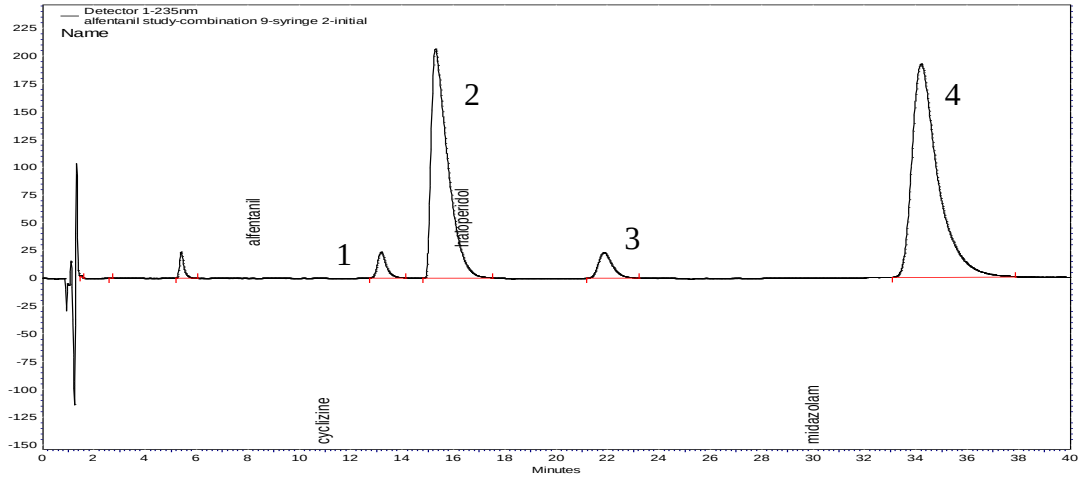
Table 3.48. Average results for the HPLC assay for alfentanil combination 9

	Alfentanil % initial			Haloperidol % initial			Cyclizine % initial			Midazolam % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (2.06%)	119.1	116.3	100.0 (1.38%)	120.5	122.9	100.0 (0.84%)	110.7	109.2	100.0 (0.58%)	125.3	126.3
3 hours	100.4 (1.42%)	119.3	117.0	96.3 (1.56%)	116.0	118.5	99.0 (0.86%)	109.7	108.1	94.8* (1.76%)	118.7	117.5
6 hours	100.3 (2.34%)	117.7	118.3	97.2 (2.68%)	118.4	118.2	100.0 (0.74%)	110.6	109.3	100.0 (1.82%)	121.9	124.1
24 hour	100.2 (1.08%)	118.5	117.3	97.9 (1.86%)	118.4	119.9	98.2 (2.15%)	110.0	106.0	102.8 (0.43%)	125.6	125.6

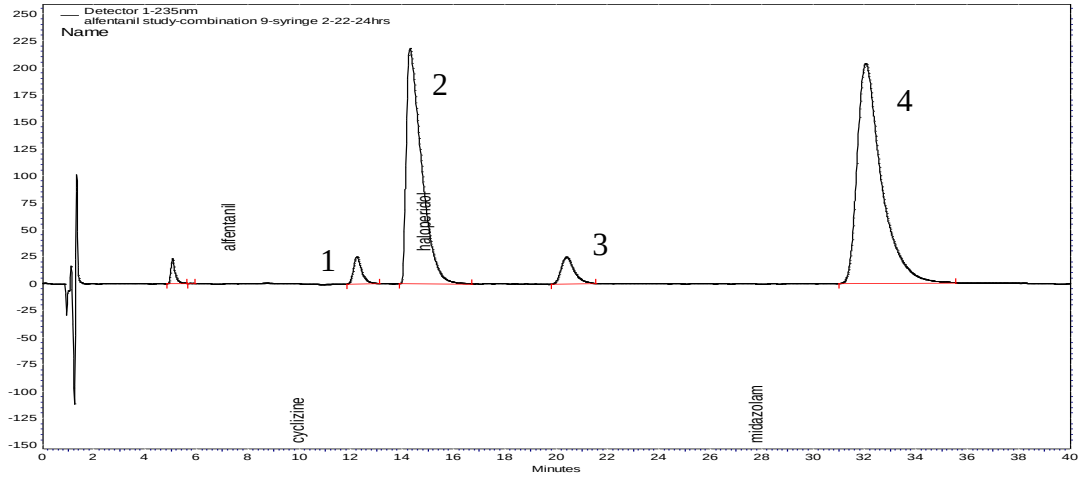
* denotes where a significant difference was observed (one-way ANOVA, sample size n=4)

Figure 3.45. Representative chromatograms for combination 9 at (A) 0hr and (B) 24hr. Peaks labelled 1, 2, 3 and 4 represent alfentanil (retention time 13.1min), cyclizine (retention time 15.3min), haloperidol (retention time 21.7min) and midazolam (retention time 34.1min) respectively

(A)



(B)



As with the oxycodone combinations, combination 5 was not included in this compatibility assessment. The combinations 1-4, 7 and 9 tested with alfentanil showed compatibility over the 24 hour infusion period, with the appearance and pH results confirming this.

3.4. LCMS/MS Assay

In the method development stage of LCMS/MS each drug combination was fragmented in order to identify the fragment ions of the drug component. This involved the parent ion (MH+) of the drug components being isolated and then fragmented. This process generated spectra showing all the fragment ions detected and their associated m/z value. The m/z value with the largest intensity was equivalent to the most abundant fragment of the parent ion component and this m/z value was chosen as the fragment that was detected in order to quantify the drug component in the combination. In order to quantify the drug component a standard for each drug component was prepared and its fragmentation spectra integrated and the results used to generate a calibration curve. The combination results for each drug component were then calculated from the calibration curve.

Analysis using LCMS/MS was carried out on the combinations containing glycopyrronium i.e. combinations 8 and 10, because testing by HPLC highlighted chromatographic problems with regard to the smaller peak size of glycopyrronium compared to the other drug components in the combination. The criteria associated with the data generated by LCMS/MS are different from that for HPLC. The accuracy associated with the calibration graph is deemed acceptable with values between 80% and 120% (the default criteria for the instrument). The calibration curve should be forced through zero where appropriate and the correlation coefficient (R^2) should be greater than 0.99. The above criteria for the glycopyrronium data in both morphine combinations 8 and 10 complied, as did hydromorphone combination 10. For hydromorphone combination 8 and diamorphine combination 8, all the criteria was met apart from the accuracy for the hydromorphone and cyclizine calibration graphs respectively. The calibration graph was generated from the five levels of standard that were prepared for each drug component.

The fragmentation m/z spectra for each combination tested (figure 3.46 to 3.50) has been included, along with the fragmentation pathway for the drug components in combinations 8 and 10 (figure 3.51 to 3.56).

3.4.1. Morphine Combinations 8 and 10

For these two combinations, only the concentration of the glycopyrronium component has been obtained by LCMS/MS. Each combination was prepared in duplicate. At each time point a sample from each of the two syringes was analysed by the LCMS/MS method for that combination. Each preparation was injected twice, generating four results for each time point. Refer to tables A43 and A44 in section 6.6 for the full set of results for these combinations.

Combination 8

Table 3.49 tabulates the results for combination 8 and figure 3.46 shows its associated m/z fragmentation spectra. The high RSD value at the 24 hour time point was due to one syringe preparation having two variable results compared to the other syringe preparation.

Combination 10

The results for combination 10 are in table 3.50 and figure 3.47 depicts the fragmentation spectra for this combination. Only one set of results was obtained at the 3 hour time point due to the contents of one of the sampling vessels not being diluted appropriately, resulting in just an injection of water being performed.

Table 3.49. Average results for the LCMS/MS assay for morphine combination 8

	Glycopyrronium % initial		
	Mean (±RSD)	S1	S2
0 hours	100.0 (2.13%)	113.8	110.2
3 hours	96.0 (1.88%)	106.5	108.4
6 hours	96.0 (1.44%)	108.5	106.6
24 hours	95.7 (6.74%)	107.5	106.8

Figure 3.46. m/z fragmentation spectra for morphine combination 8

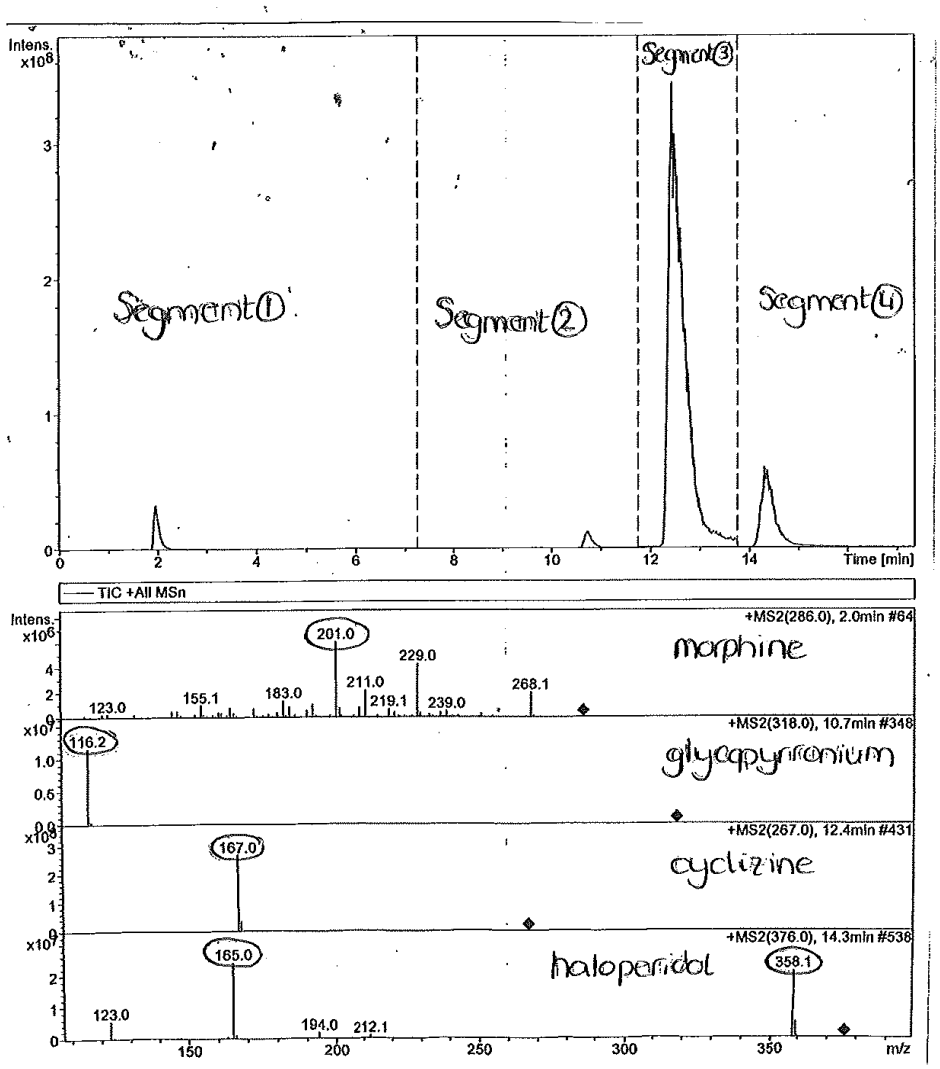
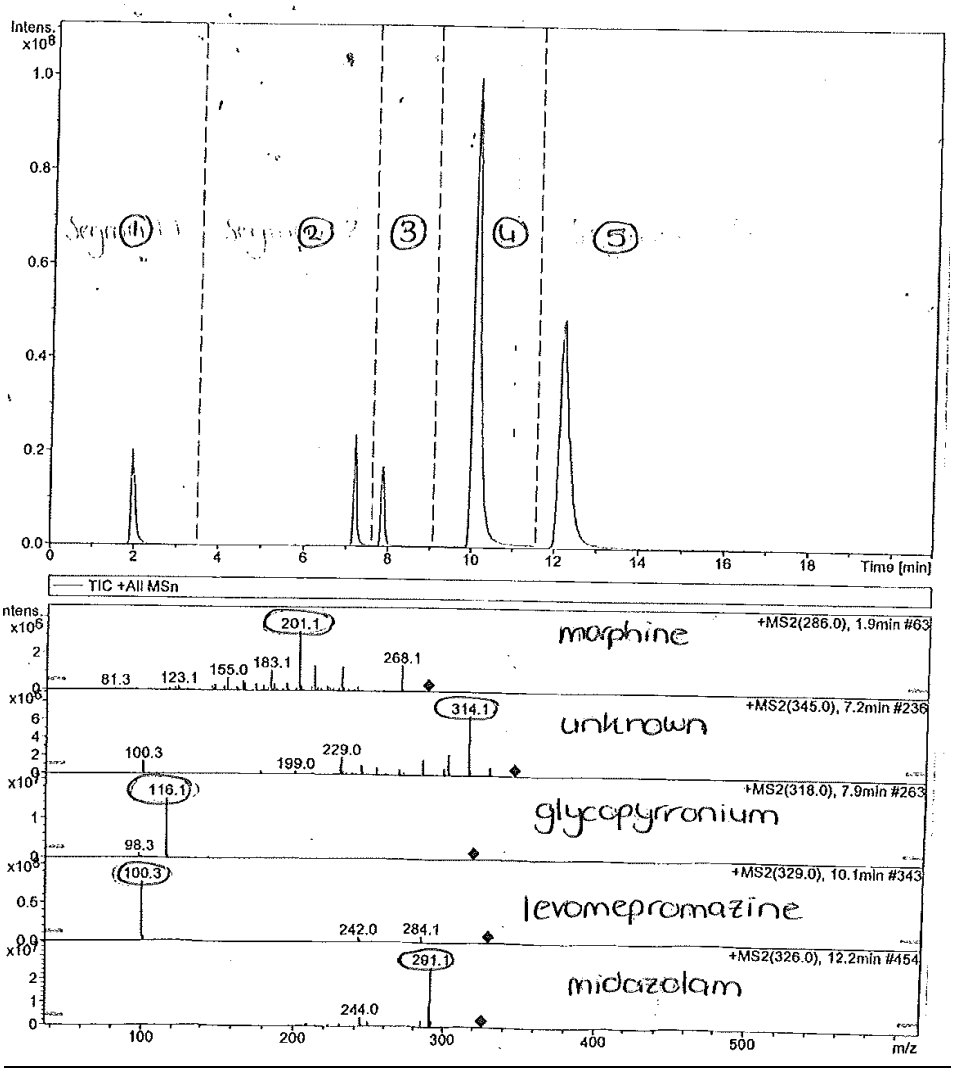


Table 3.50. Average results for the LCMS/MS assay for morphine combination 10

	Glycopyrronium % initial		
	Mean (±RSD)	S1	S2
0 hours	100.0 (2.53%)	107.6	107.1
3 hours	99.5 (2.73%)	106.8	-
6 hours	100.5 (1.86%)	107.7	108.2
24 hours	97.8 (3.62%)	103.4	106.7

Figure 3.47. m/z fragmentation spectra for morphine combination 10



In both combinations considered here, no significant change had occurred for glycopyrronium over the 24 hour period. The compatibility of these combinations was determined from the data of both the HPLC analysis and the LCMS analysis. Both combinations were deemed compatible.

3.4.2. Diamorphine Combination 8 & Hydromorphone Combinations 8 and 10

All the drug components in these combinations were assessed by LCMS/MS. This was so the analysis did not have to be performed twice, once by HPLC for three of the drug components and then again by LCMS/MS for the glycopyrronium component. Tables A45, A46 and A47 show the full set of results for these combinations in sections 6.7, 6.8 and 6.9. The results showed significant variation and have not been used to draw any conclusions. The variation in results obtained were attributed to the integration of the resulting mass spectra for the fragment ion.

Diamorphine Combination 8

Table 3.51 and figure 3.48 show the results and m/z fragmentation spectra for this combination. Dickman *et al* reported physical compatibility, based on clinical observations, for diamorphine 200mg, cyclizine 150mg, glycopyrronium 0.8mg and haloperidol 5mg for 24 hours in WFI (*Dickman et al*, 2005). The data is based on 17ml being the volume in the syringe.

Hydromorphone Combination 8

The results and m/z fragmentation spectra for this combination can be seen in table 3.52 and figure 3.49 respectively.

Hydromorphone Combination 10

Table 3.53 and figure 3.50 show the results and m/z fragmentation spectra for this combination.

Table 3.51. Average results for the LCMS/MS assay for diamorphine combination 8

	Diamorphine % initial			Cyclizine % initial			Glycopyrronium % initial			Haloperidol % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (5.83)	107.3	97.1	100.0 (3.58)	126.5	119.5	100.0 (4.69)	108.1	100.3	100.0 (1.46)	125.2	127.8
3 hours	101.3 (3.02)	105.2	101.8	96.3 (1.99)	120.5	116.5	98.3 (1.96)	103.7	100.9	101.6 (1.70)	129.4	127.6
6 hours	105.0 (4.21)	104.7	109.9	103.3 (6.84)	119.6	134.5	107.9 (3.57)	109.3	115.4	105.2 (5.19)	127.6	138.7
24 hour	86.2 (2.97)	90.3	85.9	85.7 (4.62)	102.2	108.6	90.5 (2.05)	95.2	93.4	91.8 (1.12)	115.4	116.7

Figure 3.48. m/z fragmentation spectra for diamorphine combination 8

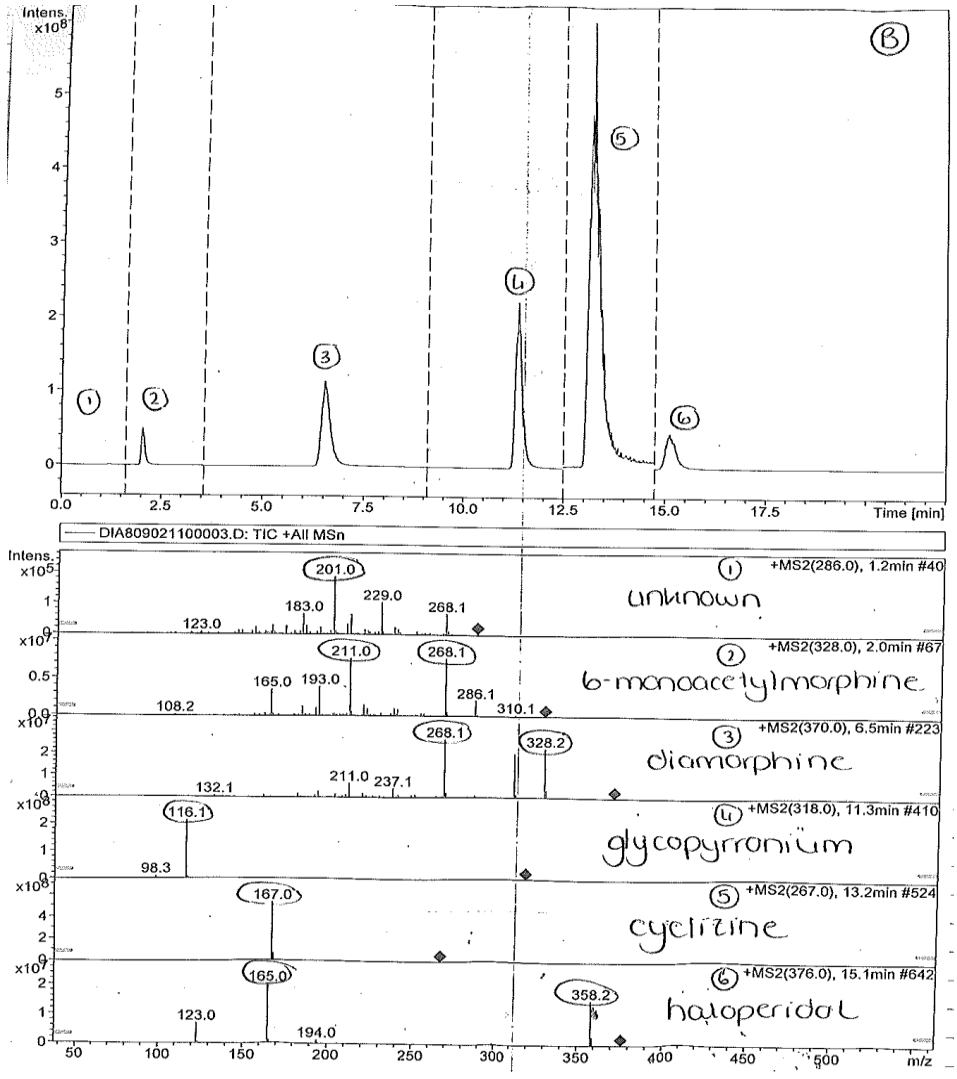


Table 3.52. Average results for the LCMS/MS assay for hydromorphone combination 8

	Hydromorphone % initial			Cyclizine % initial			Glycopyrronium % initial			Haloperidol % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (0.34)	105.7	105.2	100.0 (2.21)	122.7	120.1	100.0 (1.83)	105.4	105.5	100.0 (9.47)	121.3	135.1
3 hours	123.3 (7.85)	137.9	122.3	109.4 (3.59)	136.2	129.4	125.4 (10.43)	144.0	120.3	122.2 (13.00)	174.1	139.1
6 hours	116.5 (8.17)	130.8	115.0	103.4 (3.58)	129.2	121.7	111.3 (4.45)	120.7	113.9	96.7 (16.67)	122.6	125.4
24 hour	102.1 (4.45)	107.5	107.8	89.5 (4.61)	108.9	108.5	101.3 (1.61)	106.3	107.2	91.1 (10.22)	109.3	124.1

Figure 3.49. m/z fragmentation spectra for hydromorphone combination 8

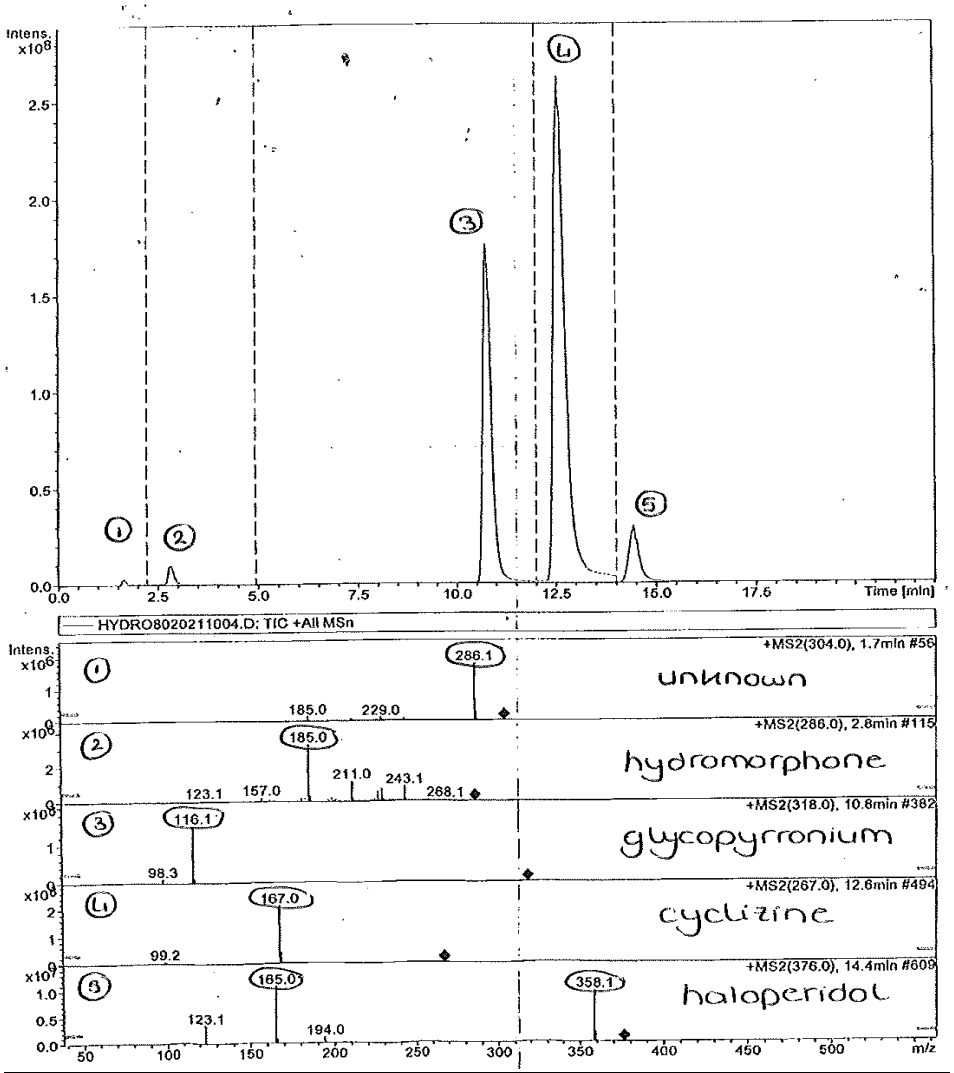
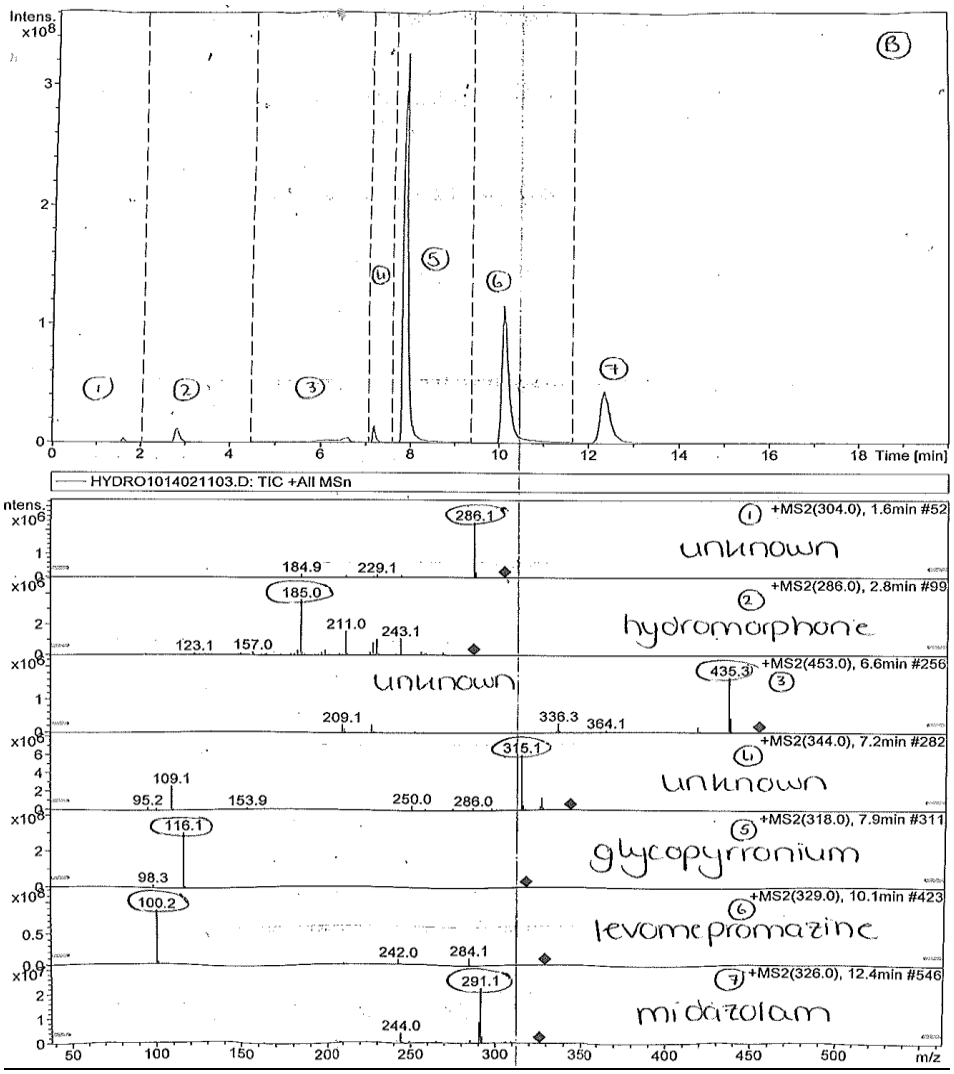


Table 3.53. Average results for the LCMS/MS assay for hydromorphone combination 10

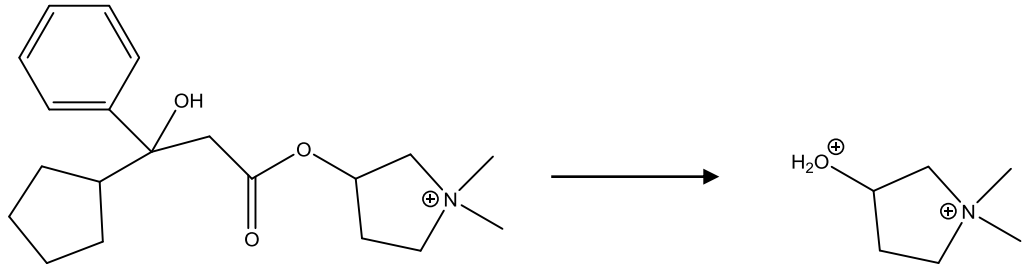
	Hydromorphone % initial			Glycopyrronium % initial			Levomepromazine % initial			Midazolam % initial		
	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2	Mean (±RSD)	S1	S2
0 hours	100.0 (3.73)	112.5	117.1	100.0 (1.88)	109.3	108.9	100.0 (2.68)	131.8	132.1	100.0 (0.95)	131.3	131.1
3 hours	103.4 (6.22)	122.6	114.8	97.1 (2.32)	105.9	106.0	99.1 (1.65)	129.0	132.3	94.6 (2.71)	121.5	126.8
6 hours	101.9 (3.17)	116.1	117.9	97.9 (1.04)	106.8	106.8	98.5 (2.04)	131.5	128.2	95.0 (4.07)	121.3	128.0
24 hour	86.8 (2.53)	101.3	97.9	93.5 (4.07)	104.0	100.0	86.7 (2.36)	116.5	112.3	90.9 (2.09)	119.5	119.0

Figure 3.50. m/z fragementation spectra for hydromorphone combination 10



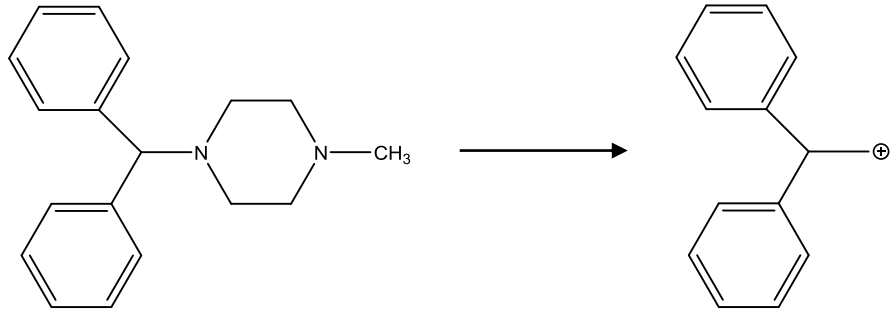
The m/z fragementation spectra for the above combinations show the fragment ion that has been detected for each of the drug components and the following figures (3.51 to 3.56) show the fragmentation pathway for these drug components.

Figure 3.51. glycopyrronium fragmentation



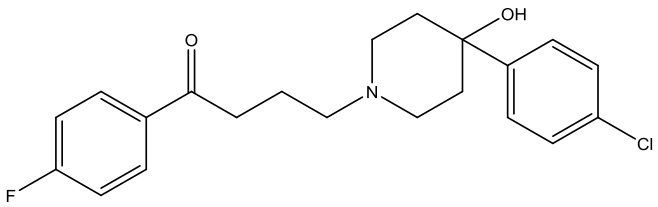
m/z 318 (parent ion) m/z 116 (fragment ion)

Figure 3.52. cyclizine fragmentation

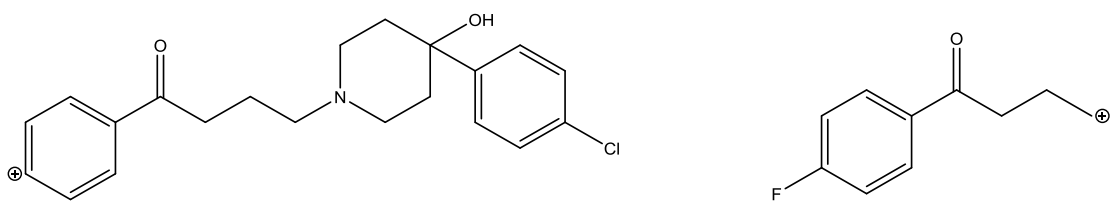


m/z 287 (parent ion) m/z 167 (fragment ion)

Figure 3.53. haloperidol fragmentation

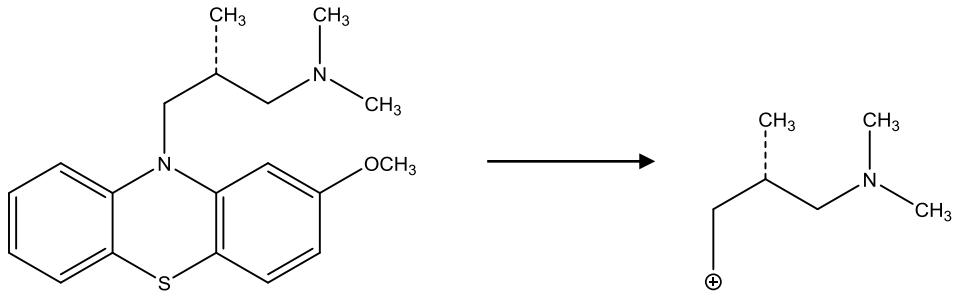


m/z 376 (parent ion)



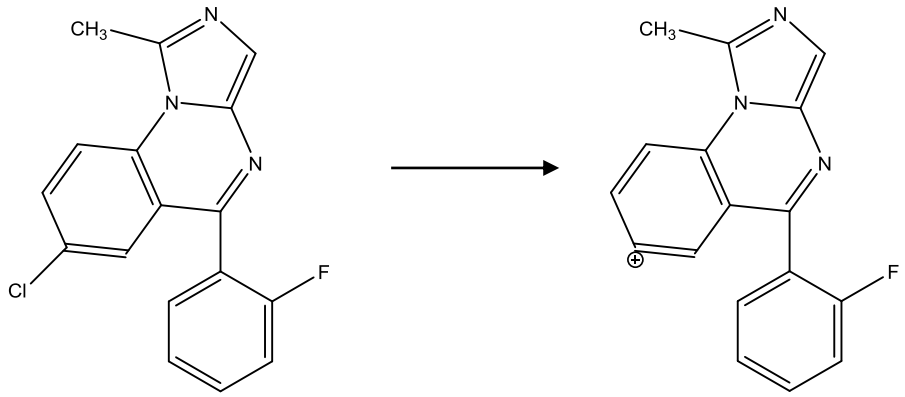
m/z 358 (fragment ion) m/z 165 (fragment ion)

Figure 3.54. levomepromazine fragmentation



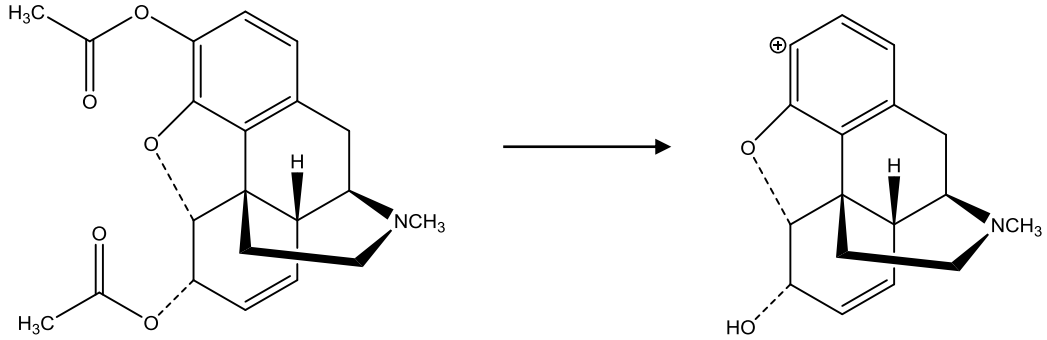
m/z 329 (parent ion) m/z 100 (fragment ion)

Figure 3.55. midazolam fragmentation



m/z 326 (parent ion) m/z 291 (fragment ion)

Figure 3.56. diamorphine fragmentation



m/z 370 (parent ion) m/z 268 (fragment ion)

3.4.3 Remaining Combinations

Time constraints and the availability of the LCMS instrumentation did not allow for the remaining combinations containing glycopyrronium to be completed; diamorphine combination 10, oxycodone combinations 8 and 10, along with alfentanil combinations 8 and 10.

Some of these combinations have been reported in literature but not at the concentrations presented in this work. Dickman *et al* has reported physical compatibility, based on clinical observations, for:

- differing concentrations of diamorphine, glycopyrronium, levomepromazine and midazolam in both 0.9% NaCl and WFI over 24 hours
- alfentanil 2mg, cyclizine 150mg, glycopyrronium 0.8 mg and haloperidol 10mg in WFI over 24 hours, and
- differing concentrations of alfentanil, glycopyrronium, levomepromazine and midazolam in both 0.9% NaCl and WFI over 12 hours and over 24 hours *(Dickman et al, 2005)*.

3.5. Overall Comments on HPLC and LCMS

In summary, for the HPLC testing of all the combinations, statistical tests revealed significant difference for some of the results obtained. On assessment of the data, this difference was generally attributed to the results at a particular time point being inconsistent with the results at the other time points. These inconsistencies could have been caused by an error in the dilution process or a change in the chromatographic integration. Also, some of the data showed high RSD values. As mentioned for the individual combinations this was usually due to differences in the results between the two syringe preparations or due to variation in the set of results for that particular time point. Drug concentration variation picked up in the analyses could also be attributed to the actual preparation of the syringes, however this represents what would be occurring in the clinical setting, where only one syringe would be prepared to be administered to the patient. The variations between syringe preparations have been attributed to the fact that the drug component ampoules being used will have overages and limits associated with them and this research has used the stated concentrations on the labels. It is therefore the inherent accuracy of the chosen methodology of assessment for this study that may not be entirely suitable for purpose. The results from S1 and S2 are not dissimilar for the majority of combinations but there are instances where there is a large difference between

them. These differences have been attributed to the variation in the individual drug component ampoules, along with possible chromatographic differences. In order to improve the consistency between syringes an improvement would be to combine sufficient drug component ampoules so there is a homogeneous solution and draw the exact volume from this solution for each syringe, therefore reducing the potential for variation in results. However, there would be limitations to doing this in clinical practice due to availability of equipment and facilities in a healthcare setting, along with the cost.

Assessment of the chromatography also revealed additional peaks in both the standard and sample chromatograms. They occurred at all time points but in most instances these peaks did not increase in size over the study period and could be attributed to either a standard chromatogram or one of the diluents used in the study. It was apparent in some studies that a peak occurring in the sample chromatogram was larger than the corresponding peak in the standard chromatograms. This was thought to be because it occurred in more than one of the standard solutions thus the observed increase is due to the cumulative concentration.

Other analytical techniques were considered. Assay by titration was not considered viable due to the volume of sample that would have been required (this can be up to 20ml) and the fact that more than one component required assaying. Ebeshi *et al* (2009) used visual titration with an indicator to assay Ibuprofen in tablets *(Ebeshi et al, 2009).* Given the complex composition of the samples assessed in this work, assay by titration was not considered viable. Thin Layer Chromatography (TLC) could have been employed to identify some of the potential drug degradants. Darwish *et al* (2008) have used TLC to show that Ribvarin and its degradation products are well resolved from each other with significantly different R_f values *(Darwish et al, 2008)*. For the purpose of this research TLC would not have been viable as it would not be able to quantify drug component concentrations. This technique was therefore also deemed inappropriate. Gas Liquid Chromatography (GLC) was also considered. Olajos and Sztaniszláv developed a gas chromatographic method for the determination of a piperazine derivative and its major metabolites in biological fluids *(Olajos and Sztaniszláv, 1986)*. It was decided not to use this technique as it would not normally be the technique of choice, so it did not make sense to develop methods with no potential further application. HPLC was the preferred technique, although it was not without limitations. The developed methods had relatively long run times which caused extremely long analysis times, in order to complete all the standard and sample injections, which impacted on the processing of the data to generate

the results. The mobile phase was continually running through the HPLC system and, hence, through the column for over 24 hours. This could have potentially been modifying the column, for example shifting retention times or baseline fluctuations, therefore, impacting on the chromatographic integration. At times the HPLC system was waiting for the next time point to be sampled before continuing with the analysis (for example between the 6h and 24h samples), which at times caused the peaks from the first injection of the set to be lower than the others. For future work there is the possibility of using UHPLC (Ultra HPLC), which could drastically reduce the time taken for development and for analysis. UHPLC uses the same separation methodology as conventional HPLC, the technique used in this research; however, it has the capability of operating at much higher pressures and faster flow rates whilst using shorter columns with a particle size of less than 2µm whilst providing better separation and faster analysis. Oláh *et al* (2012) converted a known HPLC method for the determination of levetiracetam in plasma samples to uHPLC, which resulted in reduced analysis time *(Oláh et al, 2012)*.

4. CONCLUSION

The aim of this research was to assess a set of drug combinations for both chemical and physical compatibility. During the work forty combinations have been successfully assessed. The testing of the combinations was achieved through the objectives defined in section 1.8. HPLC methods were developed that allowed the separation of the drug components in the combination and these methods were then used to determine the concentration of each drug component. The combinations were prepared as per the clinical setting and infused over a 24 hour time period using a syringe driver. The prepared syringes were assessed by visual observations for changes in appearance upon preparation and over the infusion period, along with recording the pH values.

Based on the visual observations, pH and concentration of the drug components, all forty combinations were deemed compatible. These are as follows:

- supportive drug combinations 1-10 with morphine
- supportive drug combinations 1-7 and 9 with diamorphine and hydromorphone
- supportive drug combinations 1-4, 6-7 and 9 with oxycodone and alfentanil

(refer to table 1.1. for supportive drug combination numbers)

The combinations assessed in this study have been considered compatible if there was less than a 10% loss in concentration of the drug components and there was no evidence of precipitation. Compatibility is also based on the combinations being used immediately after preparation with no prior storage. So, even though this research has indicated the compatibility of a number of drug combinations, the data are specific to the conditions mentioned in this research; the brand of syringe, the diluents used, the infusion duration and the concentration of the drugs being combined. Changes in any of these conditions could potentially result in compatibility problems. The data is specific to the CME T34 Syringe Pump with a 100-172S administration set, with the drugs in the combination having been combined in the order that they have been written in and the temperature being maintained at ambient (18-25^{o}C). It is still essential that the syringe contents and administration line are regularly monitored over the infusion period in case of any change in physical compatibility. This research has tried to replicate clinical practice as far as practically possible; however, the work presented is evidence of drug compatibility at ambient temperature before administration into a patient. The research concentrated on testing the combinations as they exited the administration line but in a

clinical setting the combination would pass through a cannula first and then into the patient.

From data obtained for the combinations tested there is no data that suggests incompatibility. However, when preparing and administering the syringes containing combinations of drugs the healthcare staff should still be vigilant and look for any visual changes. Some of the combinations showed drug concentrations above that expected however some of these excesses were significant and although they did not have an effect on the chemical compatibility of the combinations there may be therapeutic or clinical effects. In the instances where the drug concentrations were above 100% this was attributed to the manufacturing process of the drug ampoules where they can have an overage of between 5-10%. However, this is variable and may not always occur. Healthcare staff should extract the required volume and not necessarily the whole contents of the vial to draw up into the syringe. Even though some of the concentrations are in excess of what is expected they are still considered safe to administer.

It is known that many combinations of drugs, which have been combined in the same syringe, have been used successfully within a clinical framework without supporting laboratory data being available about them. However, stability and compatibility confirmation is always best obtained after analytical laboratory testing. The testing of combinations can involve a number of different factors: the concentration of the drugs, the diluent being used to obtain the fill volume of the syringe, the mixture of drugs being used and the temperature. The important point to remember is that it is the concentration of the drug in the solution not the dose that needs considering when comparing data for combinations because it is known that drug combinations are compatible at certain concentrations but not at others (*CPPE Hospital Pharmacy Learning Programme*).

On assessment of the data it was noted that the sample chromatograms contained additional chromatographic peaks to the analyte peaks. In most instances these additional peaks occurred in one of the standard solution chromatograms indicating it was related to that particular drug component in the combination. Diluent peaks were also apparent which were expected due to the standard and sample solutions being diluted in the same diluent. The additional peaks were not deemed to be significant.

HPLC was the principal technique employed, which was deemed the most appropriate for the testing carried out. However, due to advances in technology the use of

uHPLC would now be a more efficient way of testing a large number of combinations due to the shorter analysis times this technique can achieve. The time taken to develop the methods to analyse the combinations would be significantly reduced, allowing more extensive validation work to be performed. With regard to the HPLC analysis, an additional time point between 6 and 24 hours would be recommended due to the large gap in time between these time points, however, due to restrictions in the laboratory opening times this was not possible.

This research has only touched on a small number of combinations that are used in end of life care but new evidence now exists about the chemical and physical compatibility of drug combinations prepared for continuous subcutaneous infusion. This is a start in helping make sure clinical staff has access to laboratory tested compatibility information on the drugs they are combining for patient care. There is potential for this research to be extended further, whether through completing the combinations that had not been tested or identifying further combinations for laboratory testing. As with most industries, there is continual advancement in the healthcare setting in the drugs and technology being used, so further work would have to take this into account. This could involve reviewing the most commonly used syringe driver in the healthcare setting, along with a fresh assessment of the most commonly combined drugs used in CSCI. For the combinations that have been tested, further work could involve assessing the drug combinations at higher and lower concentrations so a range of concentrations for the combination could be provided. This work has only touched on chemical compatibility but consideration of biological compatibility is needed because once the combination has been administered into the body each drug component has its own pharmacokinetic profile and will have different effects on the body.

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6. APPENDICES

6.1. Morphine Combinations

Table A1. Full set of HPLC assay and pH results for morphine combination 1

Time point	pH	Morphine (6mg/ml)		Cyclizine (7.5mg/ml)		Haloperidol (0.25mg/ml)	
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal
Initial	3.68	6.5230	108.7	0.2553	102.1	8.0734	107.6
		6.5039	108.4	0.2587	103.5	8.0492	107.3
	3.71	6.4678	107.8	0.2543	101.7	8.0173	106.9
		6.5064	108.4	0.2573	102.9	7.8777	105.0
Mean (n=4)		**6.5003**	**108.3**	**0.2564**	**102.6**	**8.0044**	**106.7**
3hrs	3.71	6.5623	109.4	0.2728	109.1	8.1271	108.4
		6.5687	109.5	0.2660	106.4	8.1575	108.8
	3.80	6.5036	108.4	0.2614	104.6	7.9510	106.0
		6.5364	108.9	0.2593	103.7	7.9932	106.6
Mean (n=4)		**6.5428**	**109.1**	**0.2649**	**106.0**	**8.0572**	**107.5**
6hrs	3.85	6.4751	107.9	0.2658	106.3	8.0166	106.9
		6.4839	108.1	0.2590	103.6	8.0822	107.8
	3.81	6.4901	108.2	0.2524	101.0	7.9184	105.6
		6.4778	108.0	0.2735	109.4	7.9693	106.3
Mean (n=4)		**6.4817**	**108.1**	**0.2627**	**105.1**	**7.9966**	**106.7**
24hrs	3.65	6.4427	107.4	0.2688	107.5	7.9812	106.4
		6.5027	108.4	0.2695	107.8	8.1263	108.4
	3.62	6.7786	113.0	0.2706	108.2	8.0160	106.9
		6.7272	112.1	0.2547	101.9	8.0212	106.9
Mean (n=4)		**6.6128**	**110.2**	**0.2659**	**106.4**	**8.0362**	**107.2**

Table A2. Full set of HPLC assay and pH results for morphine combination 2

Time point	pH	Morphine (6mg/ml)		Cyclizine (7.5mg/ml)		Midazolam (1.5mg/ml)	
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal
Initial	3.77	6.4634	107.7	7.7693	103.6	1.9069	127.1
		6.4867	108.1	7.8369	104.5	1.9727	131.5
	3.77	6.6650	111.1	8.0657	107.5	1.9988	133.3
		6.6922	111.5	8.0054	106.7	1.9998	133.3
Mean (n=4)		**6.5768**	**109.6**	**7.9193**	**105.6**	**1.9696**	**131.3**
3hrs	3.75	6.4420	107.4	7.8611	104.8	1.8180	121.2
		6.5089	108.5	7.8863	105.2	1.8352	122.3
	3.75	6.6152	110.3	8.0335	107.1	1.8211	121.4
		6.6305	110.5	7.9899	106.5	1.8058	120.4
Mean (n=4)		**6.5492**	**109.2**	**7.9427**	**105.9**	**1.8200**	**121.3**
6hrs	3.75	6.3478	105.8	7.7783	103.7	1.8454	123.0
		6.3935	106.6	7.8152	104.2	1.8930	126.2
	3.74	6.6380	110.6	8.0332	107.1	1.9300	128.7
		6.6650	111.1	8.0092	106.8	1.9387	129.2
Mean (n=4)		**6.5111**	**108.5**	**7.9090**	**105.5**	**1.9018**	**126.8**
24hrs	3.75	6.3385	105.6	7.6938	102.6	1.8948	126.3
		6.2789	104.6	7.7164	102.9	1.9137	127.6
	3.71	6.6629	111.0	8.1254	108.3	1.9856	132.4
		6.5740	109.6	8.0223	107.0	1.9646	131.0
Mean (n=4)		**6.4636**	**107.7**	**7.8895**	**105.2**	**1.9397**	**129.3**

Table A3. Full set of HPLC assay and pH results for morphine combination 3

Time point	pH	Morphine (6mg/ml)		Metoclopramide (3mg/ml)		Levomepromazine (1.25mg/ml)	
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal
Initial	4.13	6.6091	110.2	3.5523	118.4	1.6743	133.9
		6.4681	107.8	3.5035	116.8	1.6537	132.3
	4.19	6.3990	106.7	3.4839	116.1	1.6298	130.4
		6.3265	105.4	3.4197	114.0	1.6031	128.2
Mean (n=4)		**6.4507**	**107.5**	**3.4899**	**116.3**	**1.6402**	**131.2**
3hrs	4.49	6.4584	107.6	3.5617	118.7	1.7153	137.2
		6.5264	108.8	3.5335	117.8	1.6524	132.2
	4.85	6.6272	110.5	3.5992	120.0	1.6754	134.0
		6.6033	110.1	3.5902	119.7	1.6729	133.8
Mean (n=4)		**6.5538**	**109.3**	**3.5712**	**119.1**	**1.6790**	**134.3**
6hrs	4.16	6.5680	109.5	3.6392	121.3	1.8179	145.4
		6.5883	109.8	3.5741	119.1	1.7186	137.5
	4.48	6.5074	108.5	3.5572	118.6	1.6741	133.9
		6.5934	109.9	3.5883	119.6	1.6837	134.7
Mean (n=4)		**6.5643**	**109.4**	**3.5897**	**120.0**	**1.7236**	**137.9**
24hrs	4.38	6.9046	115.1	3.7066	123.6	1.7145	137.2
		6.8554	114.3	3.6756	122.5	1.6956	135.6
	4.46	6.7274	112.1	3.6406	121.4	1.6680	133.4
		6.6732	111.2	3.6149	120.5	1.6689	133.5
Mean (n=4)		**6.7902**	**113.2**	**3.6594**	**122.0**	**1.6868**	**134.9**

Table A4. Full set of HPLC assay and pH results for morphine combination 4

Time point	pH	Morphine (6mg/ml)		Haloperidol (0.25mg/ml)		Midazolam (1.5mg/ml)	
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal
Initial	3.49	6.4695	107.8	0.2662	106.5	1.8230	121.5
		6.4916	108.2	0.2666	106.6	1.8992	126.6
	3.48	6.3104	105.2	0.2582	103.3	1.8776	125.2
		6.3323	105.5	0.2583	103.3	1.8853	125.7
Mean (n=4)		**6.4010**	**106.7**	**0.2623**	**104.9**	**1.8713**	**124.8**
3hrs	3.47	6.5842	109.7	0.2702	108.1	1.7839	118.9
		6.5982	110.0	0.2703	108.1	1.8622	124.1
	3.50	6.3470	105.8	0.2593	103.7	1.8060	120.4
		6.4102	106.8	0.2605	104.2	1.8275	121.8
Mean (n=4)		**6.4849**	**108.1**	**0.2651**	**106.0**	**1.8199**	**121.3**
6hrs	3.50	6.5894	109.8	0.2709	108.4	1.8262	121.7
		6.6025	110.0	0.2713	108.5	1.9040	126.9
	3.42	6.1991	103.3	0.2529	101.2	1.8147	121.0
		6.2892	104.8	0.2562	102.5	1.8442	122.9
Mean (n=4)		**6.4201**	**107.0**	**0.2628**	**105.2**	**1.8473**	**123.1**
24hrs	3.54	6.5829	109.7	0.2699	108.0	1.8565	123.8
		6.5841	109.7	0.2703	108.1	1.9133	127.6
	3.53	6.4110	106.9	0.2621	104.8	1.8892	125.9
		6.4156	106.9	0.2626	105.0	1.8961	126.4
Mean (n=4)		**6.4984**	**108.3**	**0.2662**	**106.5**	**1.8888**	**125.9**

Table A5. Full set of HPLC assay and pH results for morphine combination 5

Time point	pH	Morphine (6mg/ml)		Hyoscine butylbromide (6mg/ml)		Levomepromazine (0.625mg/ml)	
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal
Initial	4.46	6.3790	106.3	6.2621	104.4	0.7239	115.8
		6.1640	102.7	6.3369	105.6	0.7262	116.2
	4.21	6.1294	102.2	6.2662	104.4	0.6476	103.6
		6.0300	100.5	6.3181	105.3	0.6494	103.9
Mean (n=4)		**6.1756**	**102.9**	**6.2958**	**104.9**	**0.6868**	**109.9**
3hrs	4.63	6.0026	100.0	6.2805	104.7	0.6949	111.2
		5.9805	99.7	6.2119	103.5	0.6917	110.7
	4.50	5.9419	99.0	6.0359	100.6	0.6197	99.2
		5.9621	99.4	6.1972	103.3	0.6189	99.0
Mean (n=4)		**5.9718**	**99.5**	**6.1814**	**103.0**	**0.6563**	**105.0**
6hrs	5.44	6.0998	101.7	6.2139	103.6	0.7287	116.6
		6.1098	101.8	6.3457	105.8	0.7113	113.8
	4.48	6.1231	102.1	6.4082	106.8	0.6438	103.0
		6.2103	103.5	6.3082	105.1	0.6279	100.5
Mean (n=4)		**6.1358**	**102.3**	**6.3190**	**105.3**	**0.6779**	**108.5**
24hrs	4.89	6.6798	111.3	6.5253	108.8	0.7406	118.5
		6.4130	106.9	6.5728	109.5	0.7197	115.2
	4.55	6.2613	104.4	6.5071	108.5	0.6327	101.2
		6.1674	102.8	6.5574	109.3	0.6256	100.1
Mean (n=4)		**6.3804**	**106.4**	**6.5407**	**109.0**	**0.6797**	**108.8**

Table A6. Full set of HPLC assay and pH results for morphine combination 6

Time point	pH	Morphine (6mg/ml)		Levomepromazine (2.5mg/ml)		Midazolam (1.5mg/ml)	
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal
Initial	3.61	7.0620	117.7	2.8407	113.6	1.7139	114.3
		7.1149	118.6	2.8402	113.6	1.7328	115.5
	3.53	6.7084	111.8	3.0509	122.0	1.8720	124.8
		6.7202	112.0	3.0503	122.0	1.9002	126.7
Mean (n=4)		**6.9014**	**115.0**	**2.9455**	**117.8**	**1.8047**	**120.3**
3hrs	3.74	6.7829	113.0	3.1004	124.0	1.8242	121.6
		6.7807	113.0	3.0676	122.7	1.7805	118.7
	3.88	6.8497	114.2	3.0657	122.6	1.8119	120.8
		6.8335	113.9	3.0653	122.6	1.8297	122.0
Mean (n=4)		**6.8117**	**113.5**	**3.0748**	**123.0**	**1.8116**	**120.8**
6hrs	3.81	6.6651	111.1	3.0878	123.5	1.8477	123.2
		6.7319	112.2	3.0584	122.3	1.8336	122.2
	3.66	6.8087	113.5	3.0787	123.1	1.8659	124.4
		6.8339	113.9	3.0878	123.5	1.8917	126.1
Mean (n=4)		**6.7599**	**112.7**	**3.0782**	**123.1**	**1.8597**	**124.0**
24hrs	3.72	6.7434	112.4	3.1573	126.3	1.8476	123.2
		6.7842	113.1	3.1264	125.1	1.8681	124.5
	3.80	6.8746	114.6	3.1292	125.2	1.9133	127.6
		6.9116	115.2	3.1201	124.8	1.9349	129.0
Mean (n=4)		**6.8285**	**113.8**	**3.1333**	**125.4**	**1.8910**	**126.1**

Table A7. Full set of HPLC assay and pH results for morphine combination 7

Time point	pH	Morphine (6mg/ml)		Metoclopramide (1.5mg/ml)		Midazolam (1.5mg/ml)	
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal
Initial	3.84	6.6489	110.8	1.8587	123.9	1.7644	117.6
		6.6284	110.5	1.8699	124.7	1.8261	121.7
	3.67	6.5832	109.7	1.8713	124.8	1.9134	127.6
		6.5019	108.4	1.8585	123.9	1.8919	126.1
Mean (n=4)		**6.5906**	**109.9**	**1.8646**	**124.3**	**1.8490**	**123.3**
3hrs	3.82	6.5061	108.4	1.8424	122.8	1.7058	113.7
		6.5983	110.0	1.8662	124.4	1.7458	116.4
	3.66	6.5581	109.3	1.8780	125.2	1.8300	122.0
		6.5838	109.7	1.8754	125.0	1.8376	122.5
Mean (n=4)		**6.5616**	**109.4**	**1.8655**	**124.4**	**1.7798**	**118.7**
6hrs	3.80	6.5134	108.6	1.8430	122.9	1.7647	117.6
		6.5774	109.6	1.8647	124.3	1.8226	121.5
	3.70	6.4948	108.2	1.8609	124.1	1.8636	124.2
		6.5328	108.9	1.8584	123.9	1.8874	125.8
Mean (n=4)		**6.5296**	**108.8**	**1.8568**	**123.8**	**1.8346**	**122.3**
24hrs	3.67	6.1756	102.9	1.8216	121.4	1.7846	119.0
		6.4124	106.9	1.8435	122.9	1.8429	122.9
	3.70	6.3327	105.5	1.8417	122.8	1.8364	122.4
		6.3115	105.2	1.8404	122.7	1.8272	121.8
Mean (n=4)		**6.3081**	**105.1**	**1.8368**	**122.5**	**1.8228**	**121.5**

Table A8. Full set of HPLC assay and pH results for morphine combination 8

Time point	pH	Morphine (6mg/ml)		Glycoprronium (0.06mg/ml)		Cyclizine (7.5mg/ml)		Haloperidol (0.25mg/ml)	
		mg/ml	% nom	mg/ml	% nom	mg/ml	% nom	mg/ml	% nom
Initial	3.64	6.5394	109.0	0.0705	117.5	8.1907	109.2	0.2710	108.4
		6.4696	107.8	0.0662	110.3	8.2058	109.4	0.2657	106.3
	3.66	6.2323	103.9	0.0702	117.0	8.0066	106.8	0.2488	99.5
		6.2771	104.6	0.0652	108.7	8.0715	107.6	0.2544	101.8
Mean (n=4)		**6.3796**	**106.3**	**0.0680**	**113.4**	**8.1187**	**108.3**	**0.2600**	**104.0**
3hrs	3.60	6.3734	106.2	0.0678	113.0	8.1493	108.7	0.2635	105.4
		6.3764	106.3	0.0681	113.5	8.2602	110.1	0.2668	106.7
	3.59	6.2201	103.7	0.0649	108.2	8.1476	108.6	0.2524	101.0
		6.3022	105.0	0.0736	122.7	8.1553	108.7	0.2484	99.4
Mean (n=4)		**6.3180**	**105.3**	**0.0686**	**114.4**	**8.1781**	**109.0**	**0.2578**	**103.1**
6hrs	3.65	6.2209	103.7	0.0709	118.2	8.0441	107.3	0.2582	103.3
		6.2883	104.8	0.0811	135.2	8.1106	108.1	0.2591	103.6
	3.65	6.2308	103.8	0.0701	116.8	8.1049	108.1	0.2521	100.8
		6.2846	104.7	0.0664	110.7	8.0643	107.5	0.2503	100.1
Mean (n=4)		**6.2562**	**104.3**	**0.0721**	**120.2**	**8.0810**	**107.8**	**0.2549**	**102.0**
24hrs	3.59	6.8893	114.8	0.0635	105.8	8.3059	110.7	0.2689	107.6
		6.8606	114.3	0.0740	123.3	8.2935	110.6	0.2728	109.1
	3.59	6.6228	110.4	0.0679	113.2	8.1441	108.6	0.2532	101.3
		6.5159	108.6	0.0737	122.8	8.1365	108.5	0.2564	102.6
Mean (n=4)		**6.7222**	**112.0**	**0.0698**	**116.3**	**8.2200**	**109.6**	**0.2628**	**105.2**

Table A9. Full set of HPLC assay and pH results for morphine combination 9

Time point	pH	Morphine (6mg/ml)		Haloperidol (0.25mg/ml)		Cyclizine (7.5mg/ml)		Midazolam (1.5mg/ml)	
		mg/ml	% nom	mg/ml	% nom	mg/ml	% nom	mg/ml	% nom
Initial	3.65	6.4952	108.3	0.2472	98.9	7.9707	106.3	1.9267	128.4
		6.4219	107.0	0.2481	99.2	7.8814	105.1	1.9402	129.3
	3.72	6.3511	105.9	0.2523	100.9	7.7953	103.9	1.9046	127.0
		6.3533	105.9	0.2467	98.7	7.7679	103.6	1.9160	127.7
Mean (n=4)		**6.4054**	**106.8**	**0.2486**	**99.4**	**7.8538**	**104.7**	**1.9219**	**128.1**
3hrs	3.64	6.3352	105.6	0.2381	95.2	7.7429	103.2	1.8085	120.6
		6.3673	106.1	0.2448	97.9	7.9341	105.8	1.7934	119.6
	3.78	6.4131	106.9	0.2563	102.5	7.9202	105.6	1.8473	123.2
		6.4309	107.2	0.2589	103.6	7.9015	105.4	1.8403	122.7
Mean (n=4)		**6.3866**	**106.5**	**0.2495**	**99.8**	**7.8747**	**105.0**	**1.8224**	**121.5**
6hrs	3.69	6.4690	107.8	0.2579	103.2	8.0871	107.8	1.9040	126.9
		6.5567	109.3	0.2553	102.1	8.0785	107.7	1.9043	127.0
	3.65	6.5467	109.1	0.2614	104.6	8.1209	108.3	1.9286	128.6
		6.5443	109.1	0.2564	102.6	8.0600	107.5	1.9533	130.2
Mean (n=4)		**6.5292**	**108.8**	**0.2578**	**103.1**	**8.0866**	**107.8**	**1.9226**	**128.2**
24hrs	3.74	6.7959	113.3	0.2569	102.8	8.0972	108.0	1.9530	130.2
		6.7936	113.2	0.2564	102.6	8.1102	108.1	1.9512	130.1
	3.74	6.8178	113.6	0.2697	107.9	8.2540	110.1	1.9910	132.7
		6.8544	114.2	0.2563	102.5	8.2525	110.0	2.0074	133.8
Mean (n=4)		**6.8154**	**113.6**	**0.2598**	**104.0**	**8.1785**	**109.1**	**1.9757**	**131.7**

Table A10. Full set of HPLC assay and pH results for morphine combination 10

Time point	pH	Morphine (5mg/ml)		Glycopyrronium (0.1mg/ml)		Levo (2.0829mg/ml)		Midazolam (1.2504mg/ml)	
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal
Initial	3.43	4.9920	99.8	0.1062	106.2	2.7004	129.6	1.3455	107.6
		4.9343	98.7	0.0984	98.4	2.6733	128.3	1.4278	114.2
	3.32	5.0144	100.3	0.1080	108.0	2.6886	129.1	1.4722	117.8
		5.0982	102.0	0.1138	113.8	2.6508	127.3	1.4886	119.1
Mean (n=4)		**5.0097**	**100.2**	**0.1066**	**106.6**	**2.6783**	**128.6**	**1.4335**	**114.7**
3hrs	3.46	5.0398	100.8	0.1246	124.6	2.7159	130.4	1.3273	106.2
		5.1420	102.8	0.0979	97.9	2.7398	131.5	1.4486	115.9
	3.24	5.1283	102.6	0.1114	111.4	2.7083	130.0	1.4753	118.0
		5.1693	103.4	0.1128	112.8	2.7116	130.2	1.5015	120.1
Mean (n=4)		**5.1199**	**102.4**	**0.1117**	**111.7**	**2.7189**	**130.5**	**1.4382**	**115.1**
6hrs	3.42	4.8934	97.9	0.1163	116.3	2.6059	125.1	1.2420	99.4
		4.9043	98.1	0.0990	99.0	2.6179	125.7	1.3547	108.4
	3.27	4.9124	98.2	0.0984	98.4	2.6492	127.2	1.4034	112.3
		4.9667	99.3	0.1166	116.6	2.6201	125.8	1.4231	113.8
Mean (n=4)		**4.9192**	**98.4**	**0.1076**	**107.6**	**2.6233**	**126.0**	**1.3558**	**108.5**
24hrs	3.23	5.2255	104.5	0.1221	122.1	2.6868	129.0	1.3661	109.3
		5.2089	104.2	0.1252	125.2	2.6635	127.9	1.4162	113.3
	3.11	5.1899	103.8	0.1200	120.0	2.6830	128.8	1.4620	117.0
		5.1712	103.4	0.1103	110.3	2.6612	127.8	1.4702	117.6
Mean (n=4)		**5.1989**	**104.0**	**0.1194**	**119.4**	**2.6736**	**128.4**	**1.4286**	**114.3**

6.2. Diamorphine Combinations

Note: '6-mono' in the tables denotes the diamorphine metabolite 6-monoacetylmorphine.

Table A11. Full set of HPLC assay and pH results for diamorphine combination 1

Time point	pH	Diamorphine (5mg/ml)		Cyclizine (7.5mg/ml)		Haloperidol (0.25mg/ml)		6-mono
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal	area %
Initial	3.67	5.2658	105.3	8.1140	108.2	0.2595	103.8	1.21
		5.3362	106.7	8.0870	107.8	0.2636	105.4	1.15
	3.73	5.1101	102.2	8.0206	106.9	0.2587	103.5	1.17
		5.1301	102.6	8.0548	107.4	0.2535	101.4	1.16
Mean (n=4)		**5.2106**	**104.2**	**8.0691**	**107.6**	**0.2588**	**103.5**	**1.18**
3hrs	3.71	5.1855	103.7	8.0026	106.7	0.2582	103.3	1.25
		5.2377	104.8	8.0139	106.9	0.2566	102.6	1.19
	3.72	5.1075	102.2	7.9792	106.4	0.2505	100.2	1.32
		5.0636	101.3	7.9989	106.7	0.2506	100.2	1.28
Mean (n=4)		**5.1486**	**103.0**	**7.9987**	**106.7**	**0.2540**	**101.6**	**1.26**
6hrs	3.74	5.2481	105.0	8.0859	107.8	0.2652	106.1	1.31
		5.2877	105.8	8.1354	108.5	0.2635	105.4	1.24
	3.74	5.1010	102.0	8.0117	106.8	0.2476	99.0	1.23
		5.0990	102.0	8.0113	106.8	0.2535	101.4	1.27
Mean (n=4)		**5.1840**	**103.7**	**8.0611**	**107.5**	**0.2575**	**103.0**	**1.27**
24hrs	3.69	5.2735	105.5	8.1391	108.5	0.2674	107.0	1.51
		5.2435	104.9	8.0904	107.9	0.2574	103.0	1.79
	3.69	5.1259	102.5	8.0875	107.8	0.2512	100.5	1.55
		5.0735	101.5	8.0024	106.7	0.2490	99.6	1.49
Mean (n=4)		**5.1791**	**103.6**	**8.0799**	**107.7**	**0.2563**	**102.5**	**1.59**

Table A12. Full set of HPLC assay and pH results for diamorphine combination 2

Time point	pH	Diamorphine (5mg/ml)		Cyclizine (7.5mg/ml)		Midazolam (1.5mg/ml)		6-mono
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal	area %
Initial	3.73	5.0412	100.8	8.3154	110.9	1.9079	127.2	1.12
		5.0678	101.4	8.2671	110.2	1.9438	129.6	1.14
	4.68	5.3862	107.7	8.3061	110.7	1.9501	130.0	1.19
		5.3830	107.7	8.2436	109.9	1.9382	129.2	1.13
Mean (n=4)		**5.2196**	**104.4**	**8.2831**	**110.4**	**1.9350**	**129.0**	**1.15**
3hrs	3.79	5.0199	100.4	8.2325	109.8	1.8201	121.3	1.21
		4.9930	99.9	8.2469	110.0	1.8788	125.3	1.20
	3.75	5.3604	107.2	8.2608	110.1	1.8425	122.8	1.26
		5.4200	108.4	8.2546	110.1	1.8526	123.5	1.40
Mean (n=4)		**5.1983**	**104.0**	**8.2487**	**110.0**	**1.8485**	**123.2**	**1.27**
6hrs	3.92	4.9694	99.4	8.1448	108.6	1.8393	122.6	1.40
		4.9988	100.0	8.2648	110.2	1.8900	126.0	1.37
	3.68	5.4165	108.3	8.3252	111.0	1.9108	127.4	1.20
		5.3994	108.0	8.3439	111.3	1.9128	127.5	1.34
Mean (n=4)		**5.1960**	**103.9**	**8.2697**	**110.3**	**1.8882**	**125.9**	**1.33**
24hrs	3.70	5.1230	102.5	8.3536	111.4	1.9324	128.8	1.48
		5.0926	101.9	8.3313	111.1	1.9686	131.2	1.49
	3.68	5.4069	108.1	8.1798	109.1	1.9289	128.6	1.49
		5.3350	106.7	8.1360	108.5	1.9185	127.9	1.54
Mean (n=4)		**5.2394**	**104.8**	**8.2502**	**110.0**	**1.9371**	**129.1**	**1.50**

Table A13. Full set of HPLC assay and pH results for diamorphine combination 3

Time point	pH	Diamorphine (5mg/ml)		Levomepromazine (1.25mg/ml)		Metoclopramide (3mg/ml)		6-mono
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal	area %
Initial	4.38	4.6962	93.9	1.5633	125.1	3.4462	114.9	2.77
		4.7540	95.1	1.5860	126.9	3.4185	114.0	2.78
	4.41	4.6847	93.7	1.5911	127.3	3.3526	111.8	2.87
		4.7308	94.6	1.6143	129.1	3.3572	111.9	2.90
Mean (n=4)		**4.7164**	**94.3**	**1.5887**	**127.1**	**3.3936**	**113.2**	**2.83**
3hrs	4.64	4.7239	94.5	1.4683	117.5	3.2965	109.9	3.24
		4.7344	94.7	1.4615	116.9	3.3012	110.0	3.50
	4.35	4.6139	92.3	1.4949	119.6	3.2431	108.1	3.57
		4.6053	92.1	1.4812	118.5	3.2341	107.8	3.79
Mean (n=4)		**4.6694**	**93.4**	**1.4765**	**118.1**	**3.2687**	**109.0**	**3.53**
6hrs	4.35	4.7979	96.0	1.5899	127.2	3.3073	110.2	3.43
		4.8537	97.1	1.5972	127.8	3.3316	111.1	3.48
	4.37	4.6105	92.2	1.5550	124.4	3.2337	107.8	3.60
		4.6366	92.7	1.5567	124.5	3.2519	108.4	3.64
Mean (n=4)		**4.7247**	**94.5**	**1.5747**	**126.0**	**3.2811**	**109.4**	**3.54**
24hrs	4.64	4.6973	93.9	1.5130	121.0	3.5880	119.6	5.68
		4.6422	92.8	1.5102	120.8	3.4784	115.9	6.00
	4.49	4.6506	93.0	1.5996	128.0	3.4112	113.7	5.58
		4.6409	92.8	1.5938	127.5	3.3545	111.8	5.61
Mean (n=4)		**4.6578**	**93.1**	**1.5542**	**124.3**	**3.4580**	**115.3**	**5.72**

Table A14. Full set of HPLC assay and pH results for diamorphine combination 4

Time point	pH	Diamorphine (5mg/ml)		Haloperidol (0.25mg/ml)		Midazolam (1.5mg/ml)		6-mono
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal	area %
Initial	3.41	4.7550	95.1	0.2824	113.0	1.5844	105.6	2.64
		4.7509	95.0	0.2790	111.6	1.7558	117.1	3.11
	3.14	4.9240	98.5	0.2847	113.9	1.8227	121.5	2.78
		4.9092	98.2	0.2911	116.4	1.8505	123.4	2.71
Mean (n=4)		**4.8348**	**96.7**	**0.2843**	**113.7**	**1.7534**	**116.9**	**2.81**
3hrs	3.37	4.8337	96.7	0.2852	114.1	1.5737	104.9	2.88
		4.9086	98.2	0.2855	114.2	1.7611	117.4	2.77
	3.33	5.0330	100.7	0.2954	118.2	1.8113	120.8	2.90
		5.0501	101.0	0.2929	117.2	1.8297	122.0	3.04
Mean (n=4)		**4.9564**	**99.2**	**0.2898**	**115.9**	**1.7440**	**116.3**	**2.90**
6hrs	3.41	4.8735	97.5	0.2756	110.2	1.5892	105.9	2.88
		4.8984	98.0	0.2771	110.8	1.7809	118.7	2.83
	3.34	4.9209	98.4	0.2851	114.0	1.7990	119.9	2.96
		4.9176	98.4	0.2892	115.7	1.8116	120.8	2.81
Mean (n=4)		**4.9026**	**98.1**	**0.2818**	**112.7**	**1.7452**	**116.3**	**2.87**
24hrs	3.33	5.0468	100.9	0.2925	117.0	1.7199	114.7	3.17
		5.0078	100.2	0.2942	117.7	1.8404	122.7	3.18
	3.31	5.0565	101.1	0.2956	118.2	1.8628	124.2	3.15
		5.0747	101.5	0.2885	115.4	1.8609	124.1	3.25
Mean (n=4)		**5.0465**	**100.9**	**0.2927**	**117.1**	**1.8210**	**121.4**	**3.19**

Table A15. Full set of HPLC assay and pH results for diamorphine combination 5

Time point	pH	Diamorphine (5mg/ml)		Hyoscine butylbromide (6mg/ml)		Levomepromazine (0.625mg/ml)		6-mono
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal	area %
Initial	5.49	4.5975	92.0	6.6070	110.1	0.6130	98.1	2.99
		4.6472	92.9	6.4589	107.6	0.6114	97.8	2.81
	4.66	4.4806	89.6	6.2002	103.3	0.6316	101.1	2.76
		4.4066	88.1	6.0101	100.2	0.6279	100.5	2.85
Mean (n=4)		**4.5330**	**90.7**	**6.3191**	**105.3**	**0.6210**	**99.4**	**2.85**
3hrs	4.65	4.6180	92.4	6.2634	104.4	0.5990	95.8	2.87
		4.5959	91.9	6.5225	108.7	0.6040	96.6	2.97
	4.42	4.5057	90.1	6.1227	102.0	0.6280	100.5	2.76
		4.4681	89.4	6.3725	106.2	0.6159	98.5	2.90
Mean (n=4)		**4.5469**	**91.0**	**6.3203**	**105.3**	**0.6117**	**97.9**	**2.88**
6hrs	4.80	4.5999	92.0	6.5088	108.5	0.6112	97.8	2.95
		4.5848	91.7	6.3409	105.7	0.6147	98.4	2.96
	4.54	4.4692	89.4	6.2089	103.5	0.6338	101.4	2.90
		4.4717	89.4	6.3965	106.6	0.6234	99.7	2.85
Mean (n=4)		**4.5314**	**90.6**	**6.3638**	**106.1**	**0.6208**	**99.3**	**2.92**
24hrs	4.57	4.5195	90.4	6.2323	103.9	0.6106	97.7	3.37
		4.5349	90.7	6.3759	106.3	0.6134	98.1	3.44
	4.60	4.4190	88.4	6.2181	103.6	0.6227	99.6	3.34
		4.4307	88.6	6.4688	107.8	0.6248	100.0	3.41
Mean (n=4)		**4.4760**	**89.5**	**6.3238**	**105.4**	**0.6179**	**98.9**	**3.39**

Table A16. Full set of HPLC assay and pH results for diamorphine combination 6

Time point	pH	Diamorphine (5mg/ml)		Levomepromazine (2.5mg/ml)		Midazolam (1.5mg/ml)		6-mono
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal	area %
Initial	3.99	5.2041	104.1	3.3538	134.2	1.7906	119.4	2.59
		5.2357	104.7	3.3599	134.4	1.8860	125.7	2.82
	3.77	4.8851	97.7	3.1562	126.2	1.8505	123.4	2.58
		4.8729	97.5	3.1257	125.0	1.8689	124.6	2.51
Mean (n=4)		**5.0495**	**101.0**	**3.2489**	**130.0**	**1.8490**	**123.3**	**2.62**
3hrs	3.68	5.0634	101.3	3.1327	125.3	1.6887	112.6	2.61
		5.0791	101.6	3.1494	126.0	1.7274	115.2	2.76
	3.58	4.8986	98.0	3.1852	127.4	1.8043	120.3	3.13
		4.9156	98.3	3.1664	126.7	1.8122	120.8	2.68
Mean (n=4)		**4.9892**	**99.8**	**3.1584**	**126.4**	**1.7582**	**117.2**	**2.80**
6hrs	3.64	5.1261	102.5	3.1408	125.6	1.7340	115.6	2.96
		5.0776	101.6	3.1574	126.3	1.7774	118.5	3.18
	3.65	4.8807	97.6	3.1973	127.9	1.8284	121.9	2.94
		4.9104	98.2	3.1787	127.1	1.8295	122.0	2.85
Mean (n=4)		**4.9987**	**100.0**	**3.1686**	**126.7**	**1.7923**	**119.5**	**2.98**
24hrs	3.70	5.1682	103.4	3.3451	133.8	1.7939	119.6	3.18
		5.2296	104.6	3.3298	133.2	1.8565	123.8	3.22
	3.71	4.9809	99.6	3.2965	131.9	1.8732	124.9	3.30
		4.9723	99.4	3.2740	131.0	1.8910	126.1	3.23
Mean (n=4)		**5.0878**	**101.8**	**3.3114**	**132.5**	**1.8537**	**123.6**	**3.24**

Table A17. Full set of HPLC assay and pH results for diamorphine combination 7

Time point	pH	Diamorphine (5mg/ml)		Metoclopramide (1.5mg/ml)		Midazolam (1.5mg/ml)		6-mono
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal	area %
Initial	3.66	4.6065	92.1	1.8427	122.8	1.6772	111.8	2.67
		4.6122	92.2	1.8503	123.4	1.8370	122.5	2.66
	3.58	4.8911	97.8	1.8452	123.0	1.8753	125.0	2.65
		4.9107	98.2	1.8739	124.9	1.9168	127.8	2.73
Mean (n=4)		**4.7551**	**95.1**	**1.8530**	**123.5**	**1.8266**	**121.8**	**2.68**
3hrs	3.57	4.6558	93.1	1.8452	123.0	1.6622	110.8	2.74
		4.6639	93.3	1.8526	123.5	1.7933	119.6	2.78
	3.56	4.8861	97.7	1.8393	122.6	1.8458	123.1	2.75
		4.8763	97.5	1.8410	122.7	1.8492	123.3	2.79
Mean (n=4)		**4.7705**	**95.4**	**1.8445**	**123.0**	**1.7876**	**119.2**	**2.77**
6hrs	3.65	4.6273	92.5	1.8186	121.2	1.7587	117.2	2.84
		4.6199	92.4	1.7989	119.9	1.7814	118.8	2.82
	3.90	4.9169	98.3	1.8165	121.1	1.8443	123.0	2.81
		4.8716	97.4	1.8247	121.6	1.8472	123.1	2.89
Mean (n=4)		**4.7589**	**95.2**	**1.8147**	**121.0**	**1.8079**	**120.5**	**2.84**
24hrs	3.62	4.6928	93.9	1.8490	123.3	1.7076	113.8	3.28
		4.6561	93.1	1.8447	123.0	1.8081	120.5	3.31
	-	4.8465	96.9	1.8388	122.6	1.8525	123.5	3.37
		4.7597	95.2	1.7973	119.8	1.9193	128.0	3.36
Mean (n=4)		**4.7388**	**94.8**	**1.8325**	**122.2**	**1.8219**	**121.5**	**3.33**

Table A18. Full set of HPLC assay and pH results for diamorphine combination 9

Time point	pH	Diamorphine (5mg/ml)		Cyclizine (7.5mg/ml)		Haloperidol (0.25mg/ml)		Midazolam (1.5mg/ml)		6-mono
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal	area %
Initial	3.62	4.8286	96.6	8.1607	108.8	0.3124	121.5	1.8224	125.0	2.73
		4.6852	93.7	8.0554	107.4	0.3013	121.1	1.8165	120.5	2.71
	3.61	4.7047	94.1	8.0481	107.3	0.3088	120.9	1.8136	123.5	2.74
		4.6712	93.4	8.0654	107.5	0.3123	121.8	1.8264	124.9	2.71
Mean (n=4)		**4.7224**	**94.5**	**8.0824**	**107.8**	**0.3087**	**123.5**	**1.8197**	**121.3**	**2.72**
3hrs	3.52	4.6294	92.6	7.8313	104.4	0.2975	111.6	1.6741	119.0	2.76
		4.7175	94.4	8.0016	106.7	0.3007	117.1	1.7561	120.3	2.73
	3.40	4.6648	93.3	8.0149	106.9	0.3058	116.1	1.7413	122.3	2.79
		4.6610	93.2	7.9662	106.2	0.3096	116.0	1.7407	123.8	2.76
Mean (n=4)		**4.6682**	**93.4**	**7.9535**	**106.1**	**0.3034**	**121.4**	**1.7281**	**115.2**	**2.76**
6hrs	3.57	4.6691	93.4	7.9992	106.7	0.3005	114.8	1.7224	120.2	3.14
		4.6557	93.1	7.9956	106.6	0.3054	117.2	1.7582	122.2	2.98
	3.61	4.6394	92.8	7.9623	106.2	0.3054	117.2	1.7580	122.2	2.79
		4.6415	92.8	7.9979	106.6	0.3070	118.0	1.7694	122.8	2.79
Mean (n=4)		**4.6514**	**93.0**	**7.9888**	**106.5**	**0.3046**	**121.9**	**1.7520**	**116.8**	**2.93**
24hrs	3.81	4.8164	96.3	8.2249	109.7	0.3129	121.9	1.8290	125.2	3.27
		4.8514	97.0	8.2070	109.4	0.3103	122.2	1.8326	124.1	3.21
	3.82	4.7223	94.4	8.1374	108.5	0.3100	121.2	1.8178	124.0	3.30
		4.6941	93.9	8.0812	107.7	0.3082	121.2	1.8175	123.3	3.27
Mean (n=4)		**4.7711**	**95.4**	**8.1626**	**108.8**	**0.3104**	**124.2**	**1.8242**	**121.6**	**3.26**

6.3. Hydromorphone Combinations

Table A19. Full set of HPLC assay and pH results for hydromorphone combination 1

Time point	pH	Hydromorphone (2.5mg/ml)		Cyclizine (7.5mg/ml)		Haloperidol (0.25mg/ml)	
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal
Initial	3.69	2.6993	108.8	7.8411	104.5	0.2664	106.6
		2.7107	108.4	7.6003	101.3	0.2632	105.3
	3.73	2.4769	99.1	7.1671	95.6	0.2568	102.7
		2.4688	98.8	7.1724	95.6	0.2526	101.0
Mean (n=4)		**2.5889**	**103.8**	**7.4452**	**99.3**	**0.2598**	**103.9**
3hrs	3.69	2.7221	108.9	7.5840	101.1	0.2698	107.9
		2.6834	107.3	7.7217	103.0	0.2668	106.7
	3.71	2.4117	96.5	7.2905	97.2	0.2447	97.9
		2.4576	98.3	7.2103	96.1	0.2515	100.6
Mean (n=4)		**2.5687**	**102.8**	**7.4516**	**99.4**	**0.2582**	**103.3**
6hrs	3.69	2.6977	107.9	7.6388	101.9	0.2728	109.1
		2.7018	108.1	7.8847	105.1	0.2596	103.8
	3.67	2.4709	98.8	7.1657	95.5	0.2422	96.9
		2.4688	98.8	7.2193	96.3	0.2373	94.9
Mean (n=4)		**2.5848**	**103.4**	**7.4771**	**99.7**	**0.2530**	**101.2**
24hrs	3.92	2.7356	109.4	7.6703	102.3	0.2776	111.0
		2.6934	107.7	7.7179	102.9	0.2537	101.5
	3.92	2.4729	98.9	7.1559	95.4	0.2461	98.4
		2.4135	96.5	7.1772	95.7	0.2424	97.0
Mean (n=4)		**2.5789**	**103.1**	**7.4303**	**99.1**	**0.2550**	**102.0**

Table A20. Full set of HPLC assay and pH results for hydromorphone combination 2

Time point	pH	Hydromorphone (2.5mg/ml)		Cyclizine (7.5mg/ml)		Midazolam (1.5mg/ml)	
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal
Initial	3.75	3.0339	121.4	8.1431	108.6	1.9774	131.8
		2.9510	118.0	8.1402	108.5	2.0541	136.9
	3.77	2.9106	116.4	8.1935	109.2	2.0495	136.6
		2.9600	118.4	8.1705	108.9	2.0601	137.3
Mean (n=4)		**2.9639**	**118.6**	**8.1618**	**108.8**	**2.0352**	**135.7**
3hrs	3.73	3.0261	121.0	8.1552	108.7	1.9416	129.4
		2.9575	118.3	8.1001	108.0	1.9961	133.1
	3.73	2.9799	119.2	8.1603	108.8	1.9435	129.6
		2.9339	117.4	8.1520	108.7	1.9409	129.4
Mean (n=4)		**2.9744**	**119.0**	**8.1419**	**108.6**	**1.9555**	**130.4**
6hrs	3.69	2.9861	119.4	8.0801	107.7	1.9851	132.3
		2.9341	117.4	8.0800	107.7	2.0008	133.4
	3.72	2.9127	116.5	8.1950	109.3	2.0010	133.4
		2.9481	117.9	8.1586	108.8	2.0155	134.4
Mean (n=4)		**2.9743**	**117.8**	**8.1284**	**108.4**	**2.0006**	**133.4**
24hrs	3.76	2.9975	119.9	8.1553	108.7	2.0275	135.2
		2.9487	117.9	8.1607	108.8	2.0497	136.6
	3.79	2.9681	118.7	8.2412	109.9	2.0419	136.1
		2.9583	118.3	8.2407	109.9	2.0239	134.9
Mean (n=4)		**2.9682**	**118.7**	**8.1995**	**109.3**	**2.0358**	**135.7**

Table A21. Full set of HPLC Assay and pH results for hydromorphone combination 3

Time point	pH	Hydromorphone (2.5mg/ml)		Metoclopramide (3mg/ml)		Levomepromazine (1.25mg/ml)	
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal
Initial	4.23	2.8560	114.2	3.5468	118.2	1.6332	130.7
		2.8873	115.5	3.5684	118.9	1.6402	131.2
	4.25	2.8223	112.9	3.5267	117.6	1.6885	135.1
		2.8000	112.0	3.5152	117.2	1.6327	130.6
Mean (n=4)		**2.8414**	**113.7**	**3.5393**	**118.0**	**1.6487**	**131.9**
3hrs	4.25	2.8519	114.1	3.5533	118.4	1.6397	131.2
		2.8707	114.8	3.5308	117.7	1.6113	128.9
	4.20	2.8037	112.1	3.4362	114.5	1.5999	128.0
		2.7762	111.0	3.4089	113.6	1.6347	130.8
Mean (n=4)		**2.8256**	**113.0**	**3.4823**	**116.1**	**1.6214**	**129.7**
6hrs	4.16	2.8027	112.1	3.4878	116.3	1.6131	129.0
		2.8046	112.2	3.4858	116.2	1.6430	131.4
	4.11	2.8025	112.1	3.4577	115.3	1.6675	133.4
		2.7838	111.4	3.4304	114.3	1.6160	129.3
Mean (n=4)		**2.7984**	**112.0**	**3.4654**	**115.5**	**1.6349**	**130.8**
24hrs	4.18	2.8733	114.9	3.5300	117.7	1.6095	128.8
		2.8540	114.2	3.5401	118.0	1.6089	128.7
	4.20	2.7915	111.7	3.5048	116.8	1.6353	130.8
		2.8325	113.3	3.4896	116.3	1.6472	131.8
Mean (n=4)		**2.8378**	**113.5**	**3.5161**	**117.2**	**1.6252**	**130.0**

Table A22. Full set of HPLC assay and pH results for hydromorphone combination 4

Time point	pH	Hydromorphone (2.5mg/ml)		Haloperidol (0.25mg/ml)		Midazolam (1.5mg/ml)	
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal
Initial	3.58	2.9085	116.3	0.2619	104.8	1.9072	127.1
		2.8843	115.4	0.2679	107.2	2.0152	134.3
	3.62	2.9006	116.0	0.2502	100.1	2.0213	134.8
		2.8971	115.9	0.2515	100.6	2.0167	134.4
Mean (n=4)		**2.8976**	**115.9**	**0.2579**	**103.2**	**1.9901**	**132.7**
3hrs	3.58	2.9859	119.4	0.2770	110.8	1.8305	122.0
		2.9408	117.6	0.2560	102.4	1.9395	129.3
	3.52	2.9442	117.8	0.2612	104.5	1.9801	132.0
		2.9453	117.8	0.2603	104.1	2.0050	133.7
Mean (n=4)		**2.9541**	**118.2**	**0.2636**	**105.5**	**1.9388**	**129.3**
6hrs	3.59	2.9469	117.9	0.2778	111.1	1.8429	122.9
		2.8829	115.3	0.2636	105.4	1.9767	131.8
	3.57	2.9739	119.0	0.2622	104.9	2.0357	135.7
		2.9772	119.1	0.2544	101.8	2.0486	136.6
Mean (n=4)		**2.9452**	**117.8**	**0.2645**	**105.8**	**1.9760**	**131.8**
24hrs	3.58	2.9679	118.7	0.2566	102.6	1.8663	124.4
		2.9268	117.1	0.2446	97.8	1.9576	130.5
	3.55	2.9373	117.5	0.2619	104.8	2.0065	133.8
		2.8852	115.4	0.2455	98.2	1.9682	131.2
Mean (n=4)		**2.9293**	**117.2**	**0.2522**	**100.9**	**1.9497**	**130.0**

Table A23. Full set of HPLC assay and pH results for hydromorphone combination 5

Time point	pH	Hydromorphone (2.5mg/ml)		Hyoscine butylbromide (6mg/ml)		Levomepromazine (0.625mg/ml)	
		mg/ml	% nominal	Mg/ml	% nominal	mg/ml	% nominal
Initial	4.32	3.1705	126.8	7.0350	117.3	0.8610	137.8
		3.1254	125.0	6.5524	109.2	0.8316	133.1
	4.38	3.0585	122.3	6.9147	115.2	0.8200	131.2
		3.0654	122.6	6.7414	112.4	0.8157	130.5
Mean (n=4)		**3.1050**	**124.2**	**6.8109**	**113.5**	**0.8321**	**133.2**
3hrs	4.36	-	-	-	-	-	-
		3.1017	124.1	6.4379	107.3	0.7882	126.1
	4.33	3.0623	122.5	6.9211	115.4	0.7827	125.2
		3.0610	122.4	7.2543	120.9	0.8220	131.5
Mean (n=3)		**3.0750**	**123.0**	**6.8711**	**114.5**	**0.7976**	**127.6**
6hrs	4.32	3.1388	125.6	7.2200	120.3	0.8636	138.2
		3.0710	122.8	7.4909	124.8	0.8167	130.7
	4.33	2.9768	119.1	6.9203	115.3	0.8225	131.6
		3.0333	121.3	7.0631	117.7	0.7431	118.9
Mean (n=4)		**3.0550**	**122.2**	**7.1736**	**119.5**	**0.8115**	**130.0**
24hrs	4.39	3.0709	122.8	6.4022	106.7	0.8416	134.7
		3.0327	121.3	6.9016	115.0	0.8071	129.1
	4.36	3.0505	122.0	6.6928	111.5	0.8505	136.1
		2.9774	119.1	6.8822	114.7	0.8527	136.4
Mean (n=4)		**3.0329**	**121.3**	**6.7197**	**112.0**	**0.8380**	**134.1**

Table A24. Full set of HPLC assay and pH results for hydromorphone combination 6

Time point	pH	Hydromorphone (2.5mg/ml)		Levomepromazine (2.5mg/ml)		Midazolam (1.5mg/ml)	
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal
Initial	3.88	2.7825	111.3	3.2537	130.1	1.8426	122.8
		2.6960	107.8	3.2398	129.6	1.8681	124.5
	3.78	2.7840	111.4	3.2718	130.9	1.9271	128.5
		2.7762	111.0	3.2404	129.6	1.9259	128.4
Mean (n=4)		**2.7597**	**110.4**	**3.2514**	**130.1**	**1.8909**	**126.1**
3hrs	3.76	2.7962	111.8	3.1581	126.3	1.7364	115.8
		2.7625	110.5	3.2448	129.8	1.7638	117.6
	3.71	2.8040	112.2	3.1462	125.8	1.7739	118.3
		2.8314	113.3	3.1292	125.2	1.7724	118.2
Mean (n=4)		**2.7985**	**112.0**	**3.1696**	**126.8**	**1.7616**	**117.5**
6hrs	3.82	2.7097	108.4	3.0777	123.1	1.7446	116.3
		2.6890	107.6	3.1418	125.7	1.7699	118.0
	3.79	2.6986	107.9	3.1235	124.9	1.8113	120.8
		2.7538	110.2	3.1485	125.9	1.8422	122.8
Mean (n=4)		**2.7128**	**108.5**	**3.1229**	**124.9**	**1.7920**	**119.5**
24hrs	3.81	2.7645	110.6	3.3205	132.8	1.8301	122.0
		2.7917	111.7	3.2930	131.7	1.8816	125.4
	3.84	2.7934	111.7	3.2943	131.8	1.9066	127.1
		2.7859	111.4	3.2692	130.8	1.9086	127.2
Mean (n=4)		**2.7839**	**111.4**	**3.2943**	**131.8**	**1.8817**	**125.4**

Table A25. Full set of HPLC assay and pH results for hydromorphone combination 7

Time point	pH	Hydromorphone (2.5mg/ml)		Metoclopramide (1.5mg/ml)		Midazolam (1.5mg/ml)	
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal
Initial	3.86	2.9335	117.3	1.9279	128.5	1.8612	124.1
		2.9050	116.2	1.9181	127.9	1.9153	127.7
	3.85	2.9389	117.6	1.9045	127.0	1.9960	133.1
		2.9267	117.1	1.9123	127.5	2.0608	137.4
Mean (n=4)		**2.9260**	**117.1**	**1.9157**	**127.7**	**1.9583**	**130.6**
3hrs	3.94	2.7973	111.9	1.8794	125.3	1.8341	122.3
		2.8145	112.6	1.8893	126.0	1.7440	116.3
	3.68	2.8743	115.0	1.9197	128.0	1.8764	125.1
		2.8761	115.0	1.8613	124.1	1.9078	127.2
Mean (n=4)		**2.8406**	**113.6**	**1.8874**	**125.9**	**1.8406**	**122.7**
6hrs	3.80	2.8623	114.5	1.9025	126.8	1.8742	124.9
		2.8782	115.1	1.8988	126.6	1.9477	129.8
	3.79	2.9177	116.7	1.9050	127.0	1.9205	128.0
		2.9013	116.1	1.8852	125.7	1.9615	130.8
Mean (n=4)		**2.8899**	**115.6**	**1.8979**	**126.5**	**1.9260**	**128.4**
24hrs	3.79	2.9432	117.7	1.8960	126.4	1.8032	120.2
		2.8597	114.4	1.9047	127.0	1.9539	130.3
	3.87	2.8957	115.8	1.8732	124.9	1.9423	129.5
		2.8918	115.7	1.8684	124.6	1.9368	129.1
Mean (n=4)		**2.8976**	**115.9**	**1.8856**	**125.7**	**1.9091**	**127.3**

Table A26. Full set of HPLC assay and pH results for hydromorphone combination 9

Time point	pH	Hydromorphone (2.5mg/ml)		Haloperidol (0.25mg/ml)		Cyclizine (7.5mg/ml)		Midazolam (1.5mg/ml)	
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal
Initial	3.67	2.8957	115.8	0.3124	125.0	8.0387	107.2	2.0629	137.5
		2.8673	114.7	0.3286	131.4	8.0637	107.5	2.0357	135.7
	3.71	2.8755	115.0	0.2984	119.4	8.0345	107.1	2.0203	134.7
		2.8638	114.6	0.3133	125.3	8.0187	106.9	1.9823	132.2
Mean (n=4)		**2.8756**	**115.0**	**0.3132**	**125.3**	**8.0389**	**107.2**	**2.0253**	**135.0**
3hrs	3.63	2.8578	114.3	0.3064	122.6	8.0695	107.6	1.9535	130.2
		2.8588	114.4	0.3149	126.0	8.0102	106.8	1.9473	129.8
	3.63	2.9189	116.8	0.3105	124.2	8.1652	108.9	1.9737	131.6
		2.9245	117.0	0.3038	121.5	8.0561	107.4	1.9649	131.0
Mean (n=4)		**2.8900**	**115.6**	**0.3089**	**123.6**	**8.0753**	**107.7**	**1.9599**	**130.7**
6hrs	3.68	2.9056	116.2	0.3171	126.8	8.1436	108.6	1.9959	133.1
		2.9140	116.6	0.3062	122.5	8.0770	107.7	2.0268	135.1
	3.68	2.9184	116.7	0.3092	123.7	8.0397	107.2	1.9782	131.9
		2.9052	116.2	0.3039	121.6	8.0579	107.4	2.0122	134.1
Mean (n=4)		**2.9108**	**116.4**	**0.3091**	**123.7**	**8.0796**	**107.7**	**2.0033**	**133.6**
24hrs	3.73	2.9354	117.4	0.3092	123.7	8.0429	107.2	1.9749	131.7
		2.9040	116.2	0.3183	127.3	8.1001	108.0	2.0201	134.7
	3.72	2.8755	115.0	0.3111	124.4	8.0335	107.1	1.9920	132.8
		2.8780	115.1	0.3057	122.3	7.9339	105.8	1.9697	131.3
Mean (n=4)		**2.8982**	**115.9**	**0.3111**	**124.4**	**8.0276**	**107.0**	**1.9892**	**132.6**

6.4. Oxycodone Combinations

Table A27. Full set of HPLC assay and pH results for oxycodone combination 1

Time point	pH	Oxycodone (2.5mg/ml)		Cyclizine (7.5mg/ml)		Haloperidol (0.25mg/ml)	
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal
Initial	3.78	3.0857	123.4	8.2477	110.0	0.2942	117.7
		3.0400	121.6	8.3687	111.6	0.2994	119.8
	3.79	2.8636	114.5	7.9791	106.4	0.3013	120.5
		2.8583	114.3	7.9702	106.3	0.2963	118.5
Mean (n=4)		**2.9619**	**118.5**	**8.1414**	**108.6**	**0.2978**	**119.1**
3hrs	3.78	3.0393	121.6	8.2131	109.5	0.2973	118.9
		2.9960	119.8	8.4060	112.1	0.2954	118.2
	3.78	2.8549	114.2	8.0776	107.7	0.3103	124.1
		2.8845	115.4	7.9931	106.6	0.2948	117.9
Mean (n=4)		**2.9437**	**117.8**	**8.1725**	**109.0**	**0.2995**	**119.8**
6hrs	3.80	2.9786	119.1	8.1914	109.2	0.2882	115.3
		2.9693	118.8	8.1824	109.1	0.2992	119.7
	3.85	2.8326	113.3	7.9088	105.5	0.2781	111.2
		2.8457	113.8	7.8776	105.0	0.2877	115.1
Mean (n=4)		**2.9066**	**116.3**	**8.0401**	**107.2**	**0.2883**	**115.3**
24hrs	3.80	3.0178	120.7	8.2898	110.5	0.3084	123.4
		3.0407	121.6	8.2899	110.5	0.3001	120.0
	3.82	2.8508	114.0	7.9963	106.6	0.2998	119.9
		2.8537	114.1	8.0150	106.9	0.2944	117.8
Mean (n=4)		**2.9408**	**117.6**	**8.1478**	**108.6**	**0.3007**	**120.3**

Table A28. Full set of HPLC assay and pH results for oxycodone combination 2

Time point	pH	Oxycodone (2.5mg/ml)		Cyclizine (7.5mg/ml)		Midazolam (1.5mg/ml)	
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal
Initial	3.69	2.9048	116.2	8.1623	108.8	1.8701	124.7
		3.0094	120.4	8.3937	111.9	1.9502	130.0
	3.72	3.0146	120.6	8.4114	112.2	1.9224	128.2
		3.0146	120.6	8.4454	112.6	1.9257	128.4
Mean (n=4)		**2.9859**	**119.5**	**8.3532**	**111.4**	**1.9171**	**127.8**
3hrs	3.81	2.9776	119.1	8.4574	112.8	1.8309	122.1
		2.9595	118.4	8.4476	112.6	1.8453	123.0
	3.82	2.9574	118.3	8.2836	110.4	1.8005	120.0
		2.9545	118.2	8.3070	110.8	1.7995	120.0
Mean (n=4)		**2.9623**	**118.5**	**8.3739**	**111.7**	**1.8191**	**121.3**
6hrs	3.83	3.0107	120.4	8.5577	114.1	1.9213	128.1
		3.0495	122.0	8.4901	113.2	1.9159	127.7
	3.86	3.0609	122.4	8.4969	113.3	1.9327	128.8
		3.0258	121.0	8.3715	111.6	1.9143	127.6
Mean (n=4)		**3.0367**	**121.5**	**8.4791**	**113.1**	**1.9211**	**128.1**
24hrs	3.71	2.9960	119.8	8.3697	111.6	1.8639	124.3
		2.9792	119.2	8.5037	113.4	1.9033	126.9
	3.80	3.0613	122.5	8.4656	112.9	1.8769	125.1
		2.9950	119.8	8.3794	111.7	1.8882	125.9
Mean (n=4)		**3.0079**	**120.3**	**8.4296**	**112.4**	**1.8831**	**125.6**

Table A29. Full set of HPLC assay and pH results for oxycodone combination 3

Time point	pH	Oxycodone (2.5mg/ml)		Metoclopramide (3mg/ml)		Levomepromazine (1.25mg/ml)	
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal
Initial	4.75	2.4579	98.3	3.5932	119.8	1.6279	130.2
		2.4542	98.2	3.6533	121.8	1.6492	131.9
	4.66	2.5830	103.3	3.6803	122.7	1.6346	130.8
		2.5977	103.9	3.6820	122.7	1.6238	129.9
Mean (n=4)		**2.5232**	**100.9**	**3.6522**	**121.8**	**1.6339**	**130.7**
3hrs	4.59	2.5243	101.0	3.6427	121.4	1.5867	126.9
		2.4790	99.2	3.6324	121.1	1.5727	125.8
	4.47	2.5725	102.9	3.6637	122.1	1.5477	123.8
		2.5783	103.1	3.6852	122.8	1.5313	122.5
Mean (n=4)		**2.5385**	**101.6**	**3.6560**	**121.9**	**1.5596**	**124.8**
6hrs	4.75	2.4257	97.0	3.6117	120.4	1.6021	128.2
		2.4861	99.4	3.6624	122.1	1.6345	130.8
	4.59	2.5855	103.4	3.6588	122.0	1.5823	126.6
		2.5536	102.1	3.6869	122.9	1.5923	127.4
Mean (n=4)		**2.5127**	**100.5**	**3.6550**	**121.9**	**1.6028**	**128.3**
24hrs	4.73	2.4778	99.1	3.6751	122.5	1.6487	131.9
		2.4900	99.6	3.6786	122.6	1.6008	128.1
	5.15	2.6452	105.8	3.8221	127.4	1.6777	134.2
		2.6162	104.6	3.7805	126.0	1.6515	132.1
Mean (n=4)		**2.5573**	**102.3**	**3.7391**	**124.6**	**1.6447**	**131.6**

Table A30. Full set of HPLC assay and pH results for oxycodone combination 4

Time point	pH	Oxycodone (2.5mg/ml)		Haloperidol (0.25mg/ml)		Midazolam (1.5mg/ml)	
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal
Initial	3.37	2.9038	116.2	0.2821	112.8	1.6431	109.5
		2.8904	115.6	0.2752	110.1	1.8582	123.9
	3.36	2.8811	115.2	0.2661	106.4	1.8916	126.1
		2.8267	113.1	0.2638	105.5	1.8668	124.5
Mean (n=4)		**2.8755**	**115.0**	**0.2718**	**108.7**	**1.8149**	**121.0**
3hrs	3.26	2.9301	117.2	0.2836	113.4	1.6204	108.0
		2.9073	116.3	0.2665	106.6	1.7972	119.8
	3.29	2.8675	114.7	0.2678	107.1	1.8153	121.0
		2.8611	114.4	0.2662	106.5	1.8502	123.3
Mean (n=4)		**2.8915**	**115.7**	**0.2710**	**108.4**	**1.7708**	**118.0**
6hrs	3.28	2.8729	114.9	0.2610	104.4	1.6146	107.6
		2.8914	115.7	0.2495	99.8	1.8057	120.4
	3.27	2.8605	114.4	0.2544	101.8	1.8308	122.1
		2.8672	114.7	0.2580	103.2	1.8481	123.2
Mean (n=4)		**2.8730**	**114.9**	**0.2557**	**102.3**	**1.7748**	**118.3**
24hrs	3.29	2.8968	115.9	0.2699	108.0	1.8809	125.4
		2.8940	115.8	0.2707	108.3	1.9137	127.6
	3.31	2.8397	113.6	0.2582	103.3	1.8723	124.8
		2.8320	113.3	0.2534	101.4	1.8528	123.5
Mean (n=4)		**2.8656**	**114.7**	**0.2631**	**105.3**	**1.8799**	**125.3**

Table A31. Full set of HPLC assay and pH results for oxycodone combination 5

Time point	pH	Oxycodone (2.5mg/ml)		Hyoscine butylbromide (6mg/ml)		Levomepromazine (0.625mg/ml)	
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal
Initial	4.88	2.8186	112.7	6.7325	112.2	0.5766	92.3
		2.8311	113.2	6.1994	103.3	0.5220	83.5
	5.77	2.8889	115.6	6.7874	113.1	0.7459	119.3
		2.8606	114.4	7.1392	119.0	0.7374	118.0
Mean (n=4)		**2.8498**	**113.9**	**6.7146**	**111.9**	**0.6455**	**103.3**
3hrs	7.01	2.8041	112.2	6.6172	110.3	0.4710	75.4
		2.8181	112.7	6.5086	108.5	0.4658	74.5
	4.86	2.8741	115.0	7.0338	117.2	0.6654	106.5
		2.8561	114.2	6.6926	111.5	0.6689	107.0
Mean (n=4)		**2.8381**	**113.5**	**6.7131**	**111.9**	**0.5678**	**90.9**
6hrs	4.97	2.8118	112.5	6.4908	108.2	0.5205	83.3
		2.8384	113.5	6.9195	115.3	0.5616	89.9
	4.84	2.8224	112.9	6.6542	110.9	0.6653	106.4
		2.8590	114.4	6.8017	113.4	0.6628	106.0
Mean (n=4)		**2.8329**	**113.3**	**6.7166**	**112.0**	**0.6026**	**96.4**
24hrs	5.30	2.7989	112.0	6.0828	101.4	0.5570	89.1
		2.8122	112.5	6.5168	108.6	0.5821	93.1
	5.12	2.8688	114.8	7.0763	117.9	0.7366	117.9
		2.8476	113.9	6.9666	116.1	0.7053	112.8
Mean (n=4)		**2.8319**	**113.3**	**6.6606**	**111.0**	**0.6453**	**103.2**

Table A31a. Full set of HPLC assay and pH results for repeat oxycodone combination 5

Time point	pH	Oxycodone (2.5mg/ml)		Hyoscine butylbromide (6mg/ml)		Levomepromazine (0.625mg/ml)	
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal
Initial	5.11	2.8652	114.6	6.6587	111.0	0.5808	92.9
		2.8516	114.1	6.1522	102.5	0.5767	92.3
	5.80	2.8736	114.9	6.8793	114.7	0.8103	129.6
		2.8312	113.2	6.2902	104.8	0.7868	125.9
Mean (n=4)		**2.8554**	**114.2**	**6.4951**	**108.3**	**0.6887**	**110.2**
3hrs	4.81	2.9126	116.5	6.7619	112.7	0.5126	82.0
		2.9300	117.2	7.0071	116.8	0.5071	81.1
	4.69	2.5327	101.3	6.0436	100.7	0.5260	84.2
		2.5509	102.0	5.7081	95.1	0.5252	84.0
Mean (n=4)		**2.7316**	**109.3**	**6.3802**	**106.3**	**0.5177**	**82.8**
6hrs	4.80	2.9168	116.7	6.8750	114.6	0.5603	89.6
		2.9464	117.9	6.8163	113.6	0.5719	91.5
	5.37	2.8811	115.2	6.8980	115.0	0.5973	95.6
		2.8818	115.3	7.0949	118.2	0.5937	95.0
Mean (n=4)		**2.9065**	**116.3**	**6.9211**	**115.4**	**0.5808**	**92.9**
24hrs	5.93	2.9121	116.5	6.8330	113.9	0.5677	90.8
		2.9396	117.6	6.6697	111.2	0.5680	90.9
	4.94	2.9349	117.4	7.1566	119.3	0.6091	97.5
		2.9023	116.1	6.6402	110.7	0.6242	99.9
Mean (n=4)		**2.9222**	**116.9**	**6.8249**	**113.8**	**0.5923**	**94.8**

Table A32. Full set of HPLC assay and pH results for oxycodone combination 6

Time point	pH	Oxycodone (2.5mg/ml)		Levomepromazine (2.5mg/ml)		Midazolam (1.5mg/ml)	
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal
Initial	3.95	2.8221	112.9	3.2824	131.3	1.7292	115.3
		2.7791	111.2	3.2753	131.0	1.7683	117.9
	3.94	2.8301	113.2	3.2586	130.3	1.7741	118.3
		2.8338	113.4	3.2267	129.1	1.7864	119.1
Mean (n=4)		**2.8163**	**112.7**	**3.2608**	**130.4**	**1.7645**	**117.7**
3hrs	3.78	2.8401	113.6	3.0634	122.5	1.5836	105.6
		2.8136	112.5	3.0764	123.1	1.6690	111.3
	3.74	2.8078	112.3	3.0809	123.2	1.6686	111.2
		2.7926	111.7	3.0605	122.4	1.6728	111.5
Mean (n=4)		**2.8135**	**112.5**	**3.0703**	**122.8**	**1.6485**	**109.9**
6hrs	3.81	2.7799	111.2	2.9942	119.8	1.5888	105.9
		2.7738	111.0	3.0442	121.8	1.6929	112.9
	3.80	2.7580	110.3	3.0766	123.1	1.7059	113.7
		2.7580	110.3	3.0815	123.3	1.7259	115.1
Mean (n=4)		**2.7674**	**110.7**	**3.0491**	**122.0**	**1.6784**	**111.9**
24hrs	3.78	2.8204	112.8	3.3050	132.2	1.7556	117.0
		2.7935	111.7	3.2801	131.2	1.7742	118.3
	3.91	2.7892	111.6	3.2349	129.4	1.7634	117.6
		2.7647	110.6	3.2055	128.2	1.7609	117.4
Mean (n=4)		**2.7920**	**111.7**	**3.2564**	**130.3**	**1.7635**	**117.6**

Table A33. Full set of HPLC assay and pH results for oxycodone combination 7

Time point	pH	Oxycodone (2.5mg/ml)		Metoclopramide (1.5mg/ml)		Midazolam (1.5mg/ml)	
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal
Initial	3.58	2.7623	110.5	1.8939	126.3	1.6569	110.5
		2.7322	109.3	1.9263	128.4	1.7672	117.8
	3.56	2.6886	107.5	1.9288	128.6	1.8717	124.8
		2.6733	106.9	1.9157	127.7	1.9224	128.2
Mean (n=4)		**2.7141**	**108.6**	**1.9162**	**127.8**	**1.8046**	**120.3**
3hrs	3.51	2.6736	106.9	1.8898	126.0	1.6072	107.1
		2.6256	105.0	1.8915	126.1	1.7305	115.4
	3.61	2.5488	102.0	1.9404	129.4	1.8714	124.8
		2.5943	103.8	1.9871	132.5	1.9093	127.3
Mean (n=4)		**2.6106**	**104.4**	**1.9272**	**128.5**	**1.7796**	**118.7**
6hrs	3.54	2.6919	107.7	1.8892	125.9	1.6348	109.0
		2.6848	107.4	1.8915	126.1	1.7419	116.1
	3.64	2.6676	106.7	1.9316	128.8	1.8582	123.9
		2.6810	107.2	1.9259	128.4	1.8870	125.8
Mean (n=4)		**2.6813**	**107.3**	**1.9096**	**127.3**	**1.7805**	**118.7**
24hrs	3.59	2.7712	110.8	1.9417	129.4	1.7505	116.7
		2.7750	111.0	1.9365	129.1	1.8179	121.2
	3.61	2.5958	103.8	1.9558	130.4	1.8404	122.7
		2.6282	105.1	1.9408	129.4	1.8753	125.0
Mean (n=4)		**2.6926**	**107.7**	**1.9437**	**129.6**	**1.8210**	**121.4**

Table A34. Full set of HPLC assay and pH results for oxycodone combination 9

Time point	pH	Oxycodone (2.5mg/ml)		Haloperidol (0.25mg/ml)		Cyclizine (7.5mg/ml)		Midazolam (1.5mg/ml)	
		mg/ml	% nom	mg/ml	% nom	mg/ml	% nom	mg/ml	% nom
Initial	3.68	2.9654	118.6	0.3050	122.0	8.2548	110.1	1.9318	128.8
		2.9505	118.0	0.3015	120.6	8.4369	112.5	2.0225	134.8
	3.77	2.9357	117.4	0.3028	121.1	8.3739	111.7	2.0043	133.6
		2.9446	117.8	0.2996	119.8	8.3629	111.5	1.9864	132.4
Mean (n=4)		**2.9491**	**118.0**	**0.3022**	**120.9**	**8.3571**	**111.5**	**1.9863**	**132.4**
3hrs	3.67	2.9679	118.7	0.2939	117.6	8.4692	112.9	1.7987	119.9
		2.7724	110.9	0.2855	114.2	7.9827	106.4	1.7560	117.1
	3.66	2.8849	115.4	0.2949	118.0	8.1200	108.3	1.8412	122.7
		2.8585	114.3	0.2892	115.7	8.1144	108.2	1.8084	120.6
Mean (n=4)		**2.8709**	**114.8**	**0.2909**	**116.4**	**8.1716**	**109.0**	**1.8011**	**120.1**
6hrs	3.70	2.7976	111.9	0.2900	116.0	8.0157	106.9	1.7854	119.0
		2.9091	116.4	0.2927	117.1	8.3393	111.2	1.9518	130.1
	3.81	2.8328	113.3	0.2761	110.4	8.0983	108.0	1.9033	126.9
		2.8064	112.3	0.2795	111.8	7.9706	106.3	1.8807	125.4
Mean (n=4)		**2.8365**	**113.5**	**0.2846**	**113.8**	**8.1060**	**108.1**	**1.8803**	**125.4**
24hrs	3.74	3.0223	120.9	0.2990	119.6	8.4512	112.7	1.9384	129.2
		2.9811	119.2	0.3022	120.9	8.3832	111.8	2.0114	134.1
	3.79	2.8993	116.0	0.2875	115.0	8.1570	108.8	1.9486	129.9
		2.9196	116.8	0.3124	125.0	8.3009	110.7	1.9576	130.5
Mean (n=4)		**2.9556**	**118.2**	**0.3003**	**120.1**	**8.3231**	**111.0**	**1.9640**	**130.9**

6.5. Alfentanil Combinations

Table A35. Full set of HPLC assay and pH results for alfentanil combination 1

Time point	pH	Alfentanil (0.5mg/ml)		Cyclizine (7.5mg/ml)		Haloperidol (0.25mg/ml)	
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal
Initial	3.74	0.5656	113.1	7.7860	103.8	0.2887	115.5
		0.5571	111.4	7.8678	104.9	0.2923	116.9
	3.78	0.5459	109.2	7.7048	102.7	0.2786	111.4
		0.5416	108.3	7.6386	101.8	0.2746	109.8
Mean (n=4)		**0.5526**	**110.5**	**7.7493**	**103.3**	**0.2836**	**113.4**
3hrs	3.72	0.5702	114.0	7.8945	105.3	0.2876	115.0
		0.5629	112.6	8.0154	106.9	0.3018	120.7
	3.74	0.5634	112.7	7.7242	103.0	0.3010	120.4
		0.5985	119.7	7.7627	103.5	0.2796	111.8
Mean (n=4)		**0.5738**	**114.8**	**7.8492**	**104.7**	**0.2925**	**117.0**
6hrs	3.71	0.5520	110.4	7.8919	105.2	0.2927	117.1
		0.5548	111.0	7.9848	106.5	0.3075	123.0
	3.71	0.5769	115.4	7.7304	103.1	0.2977	119.1
		0.5856	117.1	7.7671	103.6	0.2770	110.8
Mean (n=4)		**0.5673**	**113.5**	**7.8436**	**104.6**	**0.2937**	**117.5**
24hrs	3.76	0.5711	114.2	7.8071	104.1	0.3030	121.2
		0.5621	112.4	7.8426	104.6	0.2934	117.4
	3.78	0.5822	116.4	7.8010	104.0	0.3013	120.5
		0.5593	111.9	7.7825	103.8	0.2764	110.6
Mean (n=4)		**0.5687**	**113.7**	**7.8083**	**104.1**	**0.2935**	**117.4**

Table A36. Full set of HPLC assay and pH results for alfentanil combination 2

Time point	pH	Alfentail (0.5mg/ml)		Cyclizine (7.5mg/ml)		Midazolam (1.5mg/ml)	
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal
Initial	3.77	0.5727	114.5	7.9984	106.6	1.7756	118.4
		0.5733	114.7	7.9548	106.1	1.8290	121.9
	3.77	0.5678	113.6	7.5483	100.6	1.7393	116.0
		0.5614	112.3	7.5518	100.7	1.7431	116.2
Mean (n=4)		**0.5688**	**113.8**	**7.7633**	**103.5**	**1.7718**	**118.1**
3hrs	3.73	0.5821	116.4	7.9415	105.9	1.6858	112.4
		0.5885	117.7	8.0377	107.2	1.7260	115.1
	3.74	0.5597	111.9	7.5869	101.2	1.6620	110.8
		0.5789	115.8	7.5630	100.8	1.6716	111.4
Mean (n=4)		**0.5773**	**115.5**	**7.7823**	**103.8**	**1.6864**	**112.4**
6hrs	3.72	0.5598	112.0	7.9102	105.5	1.7251	115.0
		0.5795	115.9	7.9975	106.6	1.7754	118.4
	3.76	0.5514	110.3	7.5664	100.9	1.6986	113.2
		0.5667	113.3	7.5708	100.9	1.7114	114.1
Mean (n=4)		**0.5644**	**112.9**	**7.7612**	**103.5**	**1.7276**	**115.2**
24hrs	3.78	0.5859	117.2	8.1379	108.5	1.8296	122.0
		0.5956	119.1	8.1124	108.2	1.8535	123.6
	3.76	0.5595	111.9	7.5654	100.9	1.7326	115.5
		0.5559	111.2	7.5808	101.1	1.7214	114.8
Mean (n=4)		**0.5742**	**114.9**	**7.8491**	**104.7**	**1.7843**	**119.0**

Table A37. Full set of HPLC assay and pH results for alfentanil combination 3

Time point	pH	Alfentanil (0.5mg/ml)		Metoclopramide (3mg/ml)		Levomepromazine (1.25mg/ml)	
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal
Initial	4.32	0.5838	116.8	3.4800	116.0	1.6497	132.0
		0.5848	117.0	3.4532	115.1	1.6321	130.6
	4.21	0.5797	115.9	3.4735	115.8	1.5842	126.7
		0.5811	116.2	3.4703	115.7	1.5725	125.8
Mean (n=4)		**0.5824**	**116.5**	**3.4693**	**115.7**	**1.6096**	**128.8**
3hrs	4.11	0.5833	116.7	3.3936	113.1	1.5805	126.4
		0.5844	116.9	3.3936	113.1	1.5675	125.4
	4.11	0.5807	116.1	3.4275	114.3	1.5569	124.6
		0.5806	116.1	3.4172	113.9	1.5471	123.8
Mean (n=4)		**0.5823**	**116.5**	**3.4080**	**113.6**	**1.5630**	**125.1**
6hrs	4.19	0.5736	114.7	3.4186	114.0	1.5845	126.8
		0.5763	115.3	3.4309	114.4	1.5973	127.8
	4.16	0.5889	117.8	3.4221	114.1	1.5613	124.9
		0.5731	114.6	3.4373	114.6	1.5720	125.8
Mean (n=4)		**0.5780**	**115.6**	**3.4272**	**114.3**	**1.5788**	**126.3**
24hrs	4.19	0.5751	115.0	3.4660	115.5	1.5964	127.7
		0.5756	115.1	3.4440	114.8	1.5863	126.9
	4.20	0.5681	113.6	3.4635	115.5	1.5794	126.4
		0.5826	116.5	3.4412	114.7	1.5737	125.9
Mean (n=4)		**0.5754**	**115.1**	**3.4537**	**115.1**	**1.5840**	**126.7**

Table A38. Full set of HPLC assay and pH results for alfentanil combination 4

Time point	pH	Alfentanil (0.5mg/ml)		Haloperidol (0.25mg/ml)		Midazolam (1.5mg/ml)	
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal
Initial	3.43	0.5868	117.4	0.3043	121.7	1.7675	117.8
		0.5797	115.9	0.3009	120.4	1.8334	122.2
	3.50	0.5969	119.4	0.2920	116.8	1.8622	124.1
		0.5875	117.5	0.2964	118.6	1.8456	123.0
Mean (n=4)		**0.5877**	**117.6**	**0.2984**	**119.4**	**1.8272**	**121.8**
3hrs	3.40	0.5861	117.2	0.2879	115.2	1.6024	106.8
		0.6021	120.4	0.2940	117.6	1.7314	115.4
	3.38	0.5783	115.7	0.2832	113.3	1.7485	116.6
		0.5848	117.0	0.2962	118.5	1.7603	117.4
Mean (n=4)		**0.5878**	**117.6**	**0.2903**	**116.2**	**1.7107**	**114.1**
6hrs	3.40	0.5773	115.5	0.2890	115.6	1.6092	107.3
		0.5903	118.1	0.2968	118.7	1.7389	115.9
	3.40	0.5742	114.8	0.2849	114.0	1.7679	117.9
		0.5652	113.0	0.2921	116.8	1.7739	118.3
Mean (n=4)		**0.5768**	**115.4**	**0.2907**	**116.3**	**1.7225**	**114.9**
24hrs	3.41	0.5753	115.1	0.3004	120.2	1.7370	115.8
		0.5768	115.4	0.2974	119.0	1.8287	121.9
	3.40	0.5847	116.9	0.2928	117.1	1.8546	123.6
		0.5776	115.5	0.2982	119.3	1.8546	123.6
Mean (n=4)		**0.5786**	**115.7**	**0.2972**	**118.9**	**1.8187**	**121.2**

Table A39. Full set of HPLC assay and pH results for alfentanil combination 5

Time point	pH	Alfentanil (0.5mg/ml)		Hyoscine butylbromide (6mg/ml)		Levomepromazine (0.625mg/ml)	
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal
Initial	4.76	0.5938	118.8	6.5378	109.0	0.7288	116.6
		0.5810	116.2	6.6445	110.7	0.7413	118.6
	4.74	0.5994	119.9	6.7085	111.8	0.9450	151.2
		0.5919	118.4	6.6371	110.6	0.9346	149.5
Mean (n=4)		**0.5915**	**118.3**	**6.6320**	**110.5**	**0.8374**	**134.0**
3hrs	4.75	0.5618	112.4	6.4608	107.7	0.7106	113.7
		0.5938	118.8	6.5649	109.4	0.7071	113.1
	4.52	0.6128	122.6	6.6983	111.6	0.8821	141.1
		0.6101	122.0	6.7374	112.3	0.9029	144.5
Mean (n=4)		**0.5946**	**119.0**	**6.6154**	**110.3**	**0.8007**	**128.1**
6hrs	4.80	0.5683	113.7	6.4431	107.4	0.6936	111.0
		0.5801	116.0	6.5849	109.7	0.6996	111.9
	4.71	0.6067	121.3	6.6207	110.3	0.9092	145.5
		0.5902	118.0	6.6441	110.7	0.9002	144.0
Mean (n=4)		**0.5863**	**117.3**	**6.5732**	**109.5**	**0.8006**	**128.1**
24hrs	4.70	0.5819	116.4	6.5436	109.1	0.7078	113.2
		0.5748	115.0	6.5883	109.8	0.7079	113.3
	4.55	0.5950	119.0	6.5698	109.5	0.8982	143.7
		0.5852	117.0	6.8396	114.0	0.8992	143.9
Mean (n=4)		**0.5842**	**116.9**	**6.6353**	**110.6**	**0.8033**	**128.5**

Table A40. Full set of HPLC assay and pH results for alfentanil combination 6

Time point	pH	Alfentanil (0.5mg/ml)		Levomepromazine (2.5mg/ml)		Midazolam (1.5mg/ml)	
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal
Initial	3.86	0.5746	114.9	3.1731	126.9	1.8346	122.3
		0.5716	114.3	3.1350	125.4	1.8932	126.2
	3.83	0.5649	113.0	3.1977	127.9	1.9024	126.8
		0.5650	113.0	3.2407	129.6	1.9142	127.6
Mean (n=4)		**0.5690**	**113.8**	**3.1866**	**127.5**	**1.8861**	**125.7**
3hrs	3.68	0.5719	114.4	3.1130	124.5	1.7398	116.0
		0.5613	112.3	3.1177	124.7	1.8199	121.3
	3.63	0.5648	113.0	3.1694	126.8	1.8268	121.8
		0.5625	112.5	3.1781	127.1	1.7871	119.1
Mean (n=4)		**0.5651**	**113.1**	**3.1446**	**125.8**	**1.7934**	**119.6**
6hrs	3.75	0.5648	113.0	3.1414	125.7	1.7479	116.5
		0.5818	116.4	3.1562	126.2	1.8500	123.3
	3.73	0.5458	109.2	3.2197	128.8	1.8699	124.7
		0.5782	115.6	3.2093	128.4	1.8753	125.0
Mean (n=4)		**0.5677**	**113.6**	**3.1817**	**127.3**	**1.8358**	**122.4**
24hrs	3.92	0.5434	108.7	3.1756	127.0	1.7779	118.5
		0.5741	114.8	3.1650	126.6	1.8745	125.0
	3.84	0.5825	116.5	3.1730	126.9	1.8552	123.7
		0.5807	116.1	3.1877	127.5	1.8607	124.0
Mean (n=4)		**0.5702**	**114.0**	**3.1753**	**127.0**	**1.8421**	**122.8**

Table A41. Full set of HPLC assay and pH results for alfentanil combination 7

Time point	pH	Alfentanil (0.5mg/ml)		Metoclopramide (1.5mg/ml)		Midazolam (1.5mg/ml)	
		mg/ml	% nominal	mg/ml	% nominal	mg/ml	% nominal
Initial	3.78	0.5804	116.1	1.7548	117.0	1.7748	118.3
		0.5530	110.6	1.7503	116.7	1.8571	123.8
	3.81	0.5755	115.1	1.7541	116.9	1.8659	124.4
		0.5643	112.9	1.7453	116.4	1.8741	124.9
Mean (n=4)		**0.5683**	**113.7**	**1.7511**	**116.8**	**1.8430**	**122.9**
3hrs	3.62	0.5539	110.8	1.7295	115.3	1.7521	116.8
		0.5740	114.8	1.7457	116.4	1.7626	117.5
	3.64	0.5911	118.2	1.7374	115.8	1.7696	118.0
		0.5712	114.2	1.7331	115.5	1.7903	119.4
Mean (n=4)		**0.5726**	**114.5**	**1.7364**	**115.8**	**1.7687**	**117.9**
6hrs	3.66	0.5576	111.5	1.7123	114.2	1.7036	113.6
		0.5500	110.0	1.7272	115.1	1.7993	120.0
	3.67	0.5666	113.3	1.7341	115.6	1.8150	121.0
		0.5751	115.0	1.7371	115.8	1.8206	121.4
Mean (n=4)		**0.5623**	**112.5**	**1.7277**	**115.2**	**1.7846**	**119.0**
24hrs	3.75	0.5501	110.0	1.7802	118.7	1.8198	121.3
		0.5542	110.8	1.7898	119.3	1.8850	125.7
	3.77	0.5421	108.4	1.7600	117.3	1.8518	123.5
		0.5540	110.8	1.7646	117.6	1.8561	123.7
Mean (n=4)		**0.5501**	**110.0**	**1.7737**	**118.2**	**1.8532**	**123.6**

Table A42. Full set of HPLC assay and pH results for alfentanil combination 9

Time point	pH	Alfentanil (0.5mg/ml)		Haloperidol (0.25mg/ml)		Cyclizine (7.5mg/ml)		Midazolam (1.5mg/ml)	
		mg/ml	% nom	mg/ml	% nom	mg/ml	% nom	mg/ml	% nom
Initial	3.69	0.5895	117.9	0.3004	120.2	8.3136	110.8	1.8706	124.7
		0.6010	120.2	0.3020	120.8	8.2985	110.6	1.8891	125.9
	3.73	0.5907	118.1	0.3044	121.8	8.1796	109.1	1.8943	126.3
		0.5718	114.4	0.3100	124.0	8.1957	109.3	1.8924	126.2
Mean (n=4)		**0.5883**	**117.7**	**0.3042**	**121.7**	**8.2469**	**110.0**	**1.8866**	**125.8**
3hrs	3.65	0.5912	118.2	0.2930	117.2	8.1908	109.2	1.7456	116.4
		0.6018	120.4	0.2868	114.7	8.2553	110.1	1.8147	121.0
	3.64	0.5879	117.6	0.2945	117.8	8.1109	108.1	1.7517	116.8
		0.5817	116.3	0.2977	119.1	8.1105	108.1	1.7724	118.2
Mean (n=4)		**0.5907**	**118.1**	**0.2930**	**117.2**	**8.1669**	**108.9**	**1.7711**	**118.1**
6hrs	3.67	0.6041	120.8	0.2869	114.8	8.2708	110.3	1.7941	119.6
		0.5725	114.5	0.3048	121.9	8.3169	110.9	1.8617	124.1
	3.67	0.5972	119.4	0.2991	119.6	8.2102	109.5	1.8569	123.8
		0.5859	117.2	0.2917	116.7	8.1807	109.1	1.8643	124.3
Mean (n=4)		**0.5899**	**118.0**	**0.2956**	**118.3**	**8.2447**	**110.0**	**1.8443**	**123.0**
24hrs	3.67	0.5960	119.2	0.2974	119.0	8.2299	109.7	1.8743	125.0
		0.5888	117.8	0.2943	117.7	8.2719	110.3	1.8933	126.2
	3.66	0.5916	118.3	0.3058	122.3	7.9655	106.2	1.8811	125.4
		0.5809	116.2	0.2938	117.5	7.9360	105.8	1.8875	125.8
Mean (n=4)		**0.5893**	**117.9**	**0.2978**	**119.1**	**8.1008**	**108.0**	**1.8841**	**125.6**

6.6. Glycopyrronium Results for Morphine Combinations 8 and 10

A43. Full set of glycopyrronium LCMS assay results for morphine combination 8

Time point	Glycopyrronium (0.06mg/ml)	
	mg/ml	% nominal
Initial	0.0681	113.5
	0.0685	114.2
	0.0670	111.7
	0.0653	108.8
Mean (n=4)	**0.0672**	**112.1**
3hrs	0.0631	105.2
	0.0647	107.8
	0.0660	109.9
	0.0641	106.8
Mean (n=4)	**0.0645**	**107.4**
6hrs	0.0647	107.9
	0.0654	109.1
	0.0632	105.4
	0.0647	107.8
Mean (n=4)	**0.0645**	**107.6**
24hrs	0.0698	116.4
	0.0592	98.7
	0.0641	106.8
	0.0641	106.8
Mean (n=4)	**0.0643**	**107.2**

A44. Full set of glycopyrronium LCMS assay results for morphine combination 10

Time point	Glycopyrronium (0.1mg/ml)	
	mg/ml	% nominal
Initial	0.1023	102.3
	0.0964	96.4
	0.0997	99.7
	0.0981	98.1
Mean (n=4)	**0.0991**	**99.1**
3hrs	0.0967	96.7
	0.1005	100.5
	0.0000	0.0
	0.0000	0.0
Mean (n=2)	**0.0986**	**98.6**
6hrs	0.0976	97.6
	0.1012	101.2
	0.1012	101.2
	0.0985	98.5
Mean (n=4)	**0.0996**	**99.6**
24hrs	0.0968	96.8
	0.0940	94.0
	0.1019	101.9
	0.0950	95.0
Mean (n=4)	**0.0969**	**96.9**

6.7. Diamorphine Combination 8

A45. Full set of LCMS assay and pH results for diamorphine combination 8

Time point	pH	Diamorphine (5mg/ml)		Cyclizine (7.5mg/ml)		Glycopyrronium (0.06mg/ml)		Haloperidol (0.25mg/ml)	
		mg/ml	% nom	mg/ml	% nom	mg/ml	% nom	mg/ml	% nom
Initial	3.72	5.3562	107.1	9.3331	124.4	0.0646	107.7	0.3126	125.1
		5.3748	107.5	9.6471	128.6	0.0651	108.6	0.3132	125.3
	3.75	4.9079	98.2	8.9080	118.8	0.0615	102.5	0.3227	129.1
		4.8000	96.0	9.0237	120.3	0.0589	98.1	0.3164	126.6
Mean (n=4)		**5.1097**	**102.2**	**9.2280**	**123.0**	**0.0625**	**104.2**	**0.3162**	**126.5**
3hrs	3.70	5.4076	108.2	9.0516	120.7	0.0623	103.8	0.3236	129.4
		5.1090	102.2	9.0207	120.3	0.0622	103.7	0.3233	129.3
	3.75	5.0870	101.7	8.7063	116.1	0.0597	99.5	0.3248	129.9
		5.0977	102.0	8.7625	116.8	0.0614	102.4	0.3129	125.2
Mean (n=4)		**5.1753**	**103.5**	**8.8853**	**118.5**	**0.0614**	**102.4**	**0.3212**	**128.5**
6hrs	3.71	5.1436	102.9	8.8471	118.0	0.0647	107.9	0.3264	130.6
		5.3222	106.4	9.0964	121.3	0.0664	110.7	0.3114	124.5
	3.74	5.6792	113.6	10.0359	133.8	0.0704	117.3	0.3473	138.9
		5.3094	106.2	10.1421	135.2	0.0681	113.5	0.3462	138.5
Mean (n=4)		**5.3636**	**107.3**	**9.5304**	**127.1**	**0.0674**	**112.4**	**0.3328**	**133.1**
24hrs	3.69	4.4950	89.9	7.4217	99.0	0.0581	96.8	0.2909	116.4
		4.5347	90.7	7.9069	105.4	0.0562	93.6	0.2861	114.5
	-	4.3162	86.3	7.9836	106.4	0.0553	92.2	0.2892	115.7
		4.2701	85.4	8.3124	110.8	0.0568	94.6	0.2941	117.6
Mean (n=4)		**4.4040**	**88.1**	**7.9062**	**105.4**	**0.0566**	**94.3**	**0.2901**	**116.1**

6.8. Hydromorphone Combination 8

A46. Full set of LCMS assay and pH results for hydromorphone combination 8

Time point	pH	Hydromorphone (2.5mg/ml)		Cyclizine (7.5mg/ml)		Glycopyrronium (0.06mg/ml)		Haloperidol (0.25mg/ml)	
		mg/ml	% nom	mg/ml	% nom	mg/ml	% nom	mg/ml	% nom
Initial	3.78	2.6435	105.7	9.3716	125.0	0.0634	105.6	0.2766	110.6
		2.6439	105.8	9.0355	120.5	0.0631	105.2	0.3302	132.1
	3.76	2.6350	105.4	9.1216	121.6	0.0619	103.1	0.3291	131.6
		2.6249	105.0	8.8978	118.6	0.0647	107.8	0.3463	138.5
Mean (n=4)		**2.6368**	**105.5**	**9.1066**	**121.4**	**0.0633**	**105.4**	**0.3206**	**128.2**
3hrs	3.74	3.5941	143.8	9.9698	132.9	0.0864	144.0	0.4284	171.4
		3.3014	132.1	10.4620	139.5	0.0865	144.1	0.4420	176.8
	3.70	3.0578	122.3	9.7585	130.1	0.0729	121.5	0.3525	141.0
		3.0577	122.3	9.6589	128.8	0.0714	119.0	0.3432	137.3
Mean (n=4)		**3.2528**	**130.1**	**9.9623**	**132.8**	**0.0793**	**132.2**	**0.3915**	**156.6**
6hrs	3.76	3.3389	133.6	9.6121	128.2	0.0699	116.5	0.3673	146.9
		3.1989	128.0	9.7723	130.3	0.0749	124.9	0.2457	98.3
	3.74	2.9836	119.3	9.0849	121.1	0.0681	113.5	0.3304	132.2
		2.7676	110.7	9.1646	122.2	0.0686	114.3	0.2964	118.6
Mean (n=4)		**3.0723**	**122.9**	**9.4085**	**125.5**	**0.0704**	**117.3**	**0.3100**	**124.0**
24hrs	3.79	2.8349	113.4	7.9793	106.4	0.0629	104.9	0.2488	99.5
		2.5421	101.7	8.3544	111.4	0.0646	107.6	0.2979	119.2
	3.84	2.7052	108.2	8.5551	114.1	0.0652	108.7	0.3037	121.5
		2.6872	107.5	7.7161	102.9	0.0635	105.8	0.3170	126.8
Mean (n=4)		**2.6924**	**107.7**	**8.1512**	**108.7**	**0.0641**	**106.8**	**0.2919**	**116.8**

6.9. Hydromorphone Combination 10

A47. Full set of LCMS assay and pH results for hydromorphone combination 10

Time point	pH	Hydromorphone (2.5mg/ml)		Glycopyrronium (0.12mg/ml)		Levomepromazine (2.5mg/ml)		Midazolam (1.5mg/ml)	
		mg/ml	% nom	mg/ml	% nom	mg/ml	% nom	mg/ml	% nom
Initial	3.46	2.6477	116.5	0.1219	111.8	3.0921	136.1	1.8003	132.0
		2.4642	108.4	0.1166	106.8	2.8975	127.5	1.7808	130.6
	3.48	2.6615	117.1	0.1187	108.8	3.0115	132.5	1.7688	129.7
		2.6628	117.2	0.1189	109.0	2.9912	131.6	1.8056	132.4
Mean (n=4)		**2.6091**	**114.8**	**0.1190**	**109.1**	**2.9981**	**131.9**	**1.7889**	**131.2**
3hrs	3.40	2.9171	128.4	0.1131	103.6	2.9142	128.2	1.6355	119.9
		2.6551	116.8	0.1180	108.1	2.9501	129.8	1.6777	123.0
	3.40	2.5132	110.6	0.1134	104.0	3.0263	133.2	1.7361	127.3
		2.7064	119.1	0.1178	108.0	2.9885	131.5	1.7228	126.3
Mean (n=4)		**2.6980**	**118.7**	**0.1156**	**105.9**	**2.9698**	**130.7**	**1.6930**	**124.1**
6hrs	3.38	2.6636	117.2	0.1163	106.6	3.0164	132.7	1.6167	118.6
		2.6123	114.9	0.1166	106.9	2.9629	130.4	1.6926	124.1
	3.40	2.5841	113.7	0.1150	105.4	2.8695	126.3	1.7058	125.1
		2.7755	122.1	0.1180	108.1	2.9555	130.0	1.7859	131.0
Mean (n=4)		**2.6589**	**117.0**	**0.1165**	**106.8**	**2.9511**	**129.9**	**1.7003**	**124.7**
24hrs	3.40	2.3273	102.4	0.1161	106.4	2.6533	116.7	1.6589	121.7
		2.2782	100.2	0.1109	101.6	2.6406	116.2	1.5988	117.2
	3.42	2.1897	96.3	0.1128	103.4	2.5870	113.8	1.5957	117.0
		2.2615	99.5	0.1053	96.5	2.5191	110.8	1.6507	121.1
Mean (n=4)		**2.2642**	**99.6**	**0.1113**	**102.0**	**2.6000**	**114.4**	**1.6260**	**119.3**

6.10. Posters

Quality Control North West

Morphine and Diamorphine Combinations in Palliative Care: A Compatibility Study

Heather Kean[1,2], Diane Rigge[2], Phil Weir[2], Andrew Evans[1], Elsie Gaskell[1], Andrew Dickman[3]

(1)School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University
(2) Quality Control North West, Stepping Hill Hospital, Stockport (3) Marie Curie Palliative Care Institute, Liverpool University

Background

The issue of Patient Safety Alert 20: Promoting safer use of injectable medicines by the National Patient Safety Agency in March 2007 includes the recommendation that healthcare staff need to have full technical information about stability in solution and compatibility information for commonly used mixtures in specialist areas only. This covers the mixing of injectable medicines in the same syringe [1].

In end of life care, injectable medicines are sometimes combined in order to treat a single symptom, or because multiple symptoms need to be treated simultaneously. It is often found that the oral route of administration is no longer available and an alternative route is required. Continuous subcutaneous infusion (CSCI) is the preferred alternative [2].

CSCI's are delivered using a syringe driver or pump. There are a number of different syringe drivers or pumps available but this research concentrates on the use of a McKinley T34 Syringe Pump, as presently it is the most popular syringe pump being adopted nationwide.

It is widely known that combining drugs for CSCI could potentially result in interactions between and amongst the different drugs. There is limited, if any, compatibility data available about them. Sources concentrate on physical compatibility data but this cannot rule out chemical incompatibilities within the syringe, which could be contributing to decreased drug concentrations.

Aim

The aim of this study was to obtain chemical compatibility information on the most commonly encountered supportive drugs in end of life care. A database search has identified these as being:

- Cyclizine and haloperidol
- Cyclizine and midazolam
- Haloperidol and midazolam
- Hyoscine butylbromide and levomepromazine
- Levomepromazine and midazolam
- Metoclopramide and midazolam
- Cyclizine, haloperidol and midazolam

The combinations are administered with an opioid via CSCI and this study concentrates on the opioids morphine sulphate and diamorphine hydrochloride.

Methodology

To achieve the aim of the study:

- Analytical methods were developed to separate the individual drug solutions in each combination using High Performance Liquid Chromatography-Diode Array Detection (HPLC-DAD).
- Individual drug solutions were heated at high temperature prior to being analysed by HPLC-DAD to identify possible degradants.
- Syringes were prepared containing the drug combination as close to clinical practice as practically possible.
- A McKinley T34 Syringe Pump was used to simulate infusion of the syringe preparation over a 24hr period.
- Samples were taken at set time points from the administration line and analysed by HPLC-DAD to obtain individual drug concentrations.
- Results assessed to determine whether drug combinations are compatible and stable.

Results

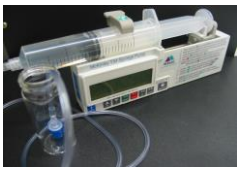
Morphine Combinations			Compatible
Cyclizine	Haloperidol	-	✓(24h)
Cyclizine	Midazolam	-	✓(24h)
Haloperidol	Midazolam	-	✓(24h)
Hyoscine butylbromide	Levomepromazine	-	✓(24h)
Metoclopramide	Midazolam	-	✓(24h)
Cyclizine	Haloperidol	Midazolam	✓(24h)

Diamorphine Combinations		Compatible
Cyclizine	Haloperidol	✓(24h)
Cyclizine	Midazolam	✓(24h)

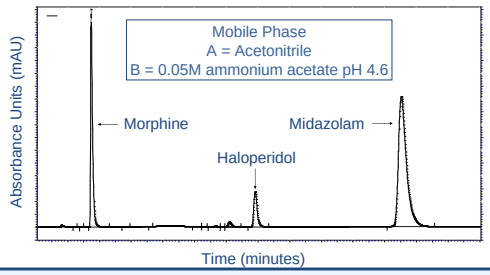
✓(24h) chemically compatible over 24hrs

The combinations remained clear and free from visible particulate matter and the pH remained constant over the 24 hour infusion.

McKinley T34 Syringe Pump



An Example Morphine Combination Chromatogram



Conclusion

The following have been identified as physically and chemically compatible and stable over 24hrs infusion:

- All seven morphine sulphate supportive drug combinations
- The first two diamorphine hydrochloride supportive drug combinations

This is the first step towards providing technical information required by healthcare staff for the mixing of injectable medicines in the same syringe, a recommendation in Patient Safety Alert 20.

Future Work

To assess the remaining five supportive drug combinations with diamorphine hydrochloride for chemical compatibility.

The potential exists to extend the study to include other opioids that are often used in end of life care.

References

[1] NHS National Patient Safety Agency (2007) Patient Safety Alert 20: Promoting safer use of injectable medicines. Ref: NPSA/2007/20
[2] Rose M., Currow D.C. (2009) The Need for Chemical Compatibility Studies of Subcutaneous Medication Combinations Used in Palliative Care. *Journal of Pain & Palliative Care Pharmacotherapy, 23 (3), 223-230*

Morphine and Diamorphine Combinations in Palliative Care: A Compatibility Study

Presented at: LJMU, Faculty of Science, Postgraduate Research Day, 20th May 2010

Morphine and Diamorphine Combinations in Palliative Care: A Compatibility Study

Heather Kean[1,2] , Andrew Dickman[3]

(1) Postgraduate Student, School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University

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CSCI's are delivered using a syringe driver or pump. There are a number of different syringe drivers or pumps available but this research concentrates on the use of a McKinley T34 Syringe Pump, as presently it is the most popular syringe pump being adopted nationwide.

It is widely known that combining drugs for CSCI could potentially result in interactions between and amongst the different drugs. There is limited, if any, compatibility data available about them. Sources concentrate on physical compatibility data but this cannot rule out chemical incompatibilities within the syringe, which could be contributing to decreased drug concentrations.

Aim

The aim of this study was to obtain chemical compatibility information on the most commonly encountered supportive drugs in end of life care. A database search has identified these as being:

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- Cyclizine and midazolam
- Haloperidol and midazolam
- Hyoscine butylbromide and levomepromazine
- Levomepromazine and midazolam
- Metoclopramide and midazolam
- Cyclizine, haloperidol and midazolam

The combinations are administered with an opioid via CSCI and this study concentrates on the opioids morphine sulphate and diamorphine hydrochloride.

Methodology

To achieve the aim of the study:

- Analytical methods were developed to separate the individual drug solutions in each combination using High Performance Liquid Chromatography-Diode Array Detection (HPLC-DAD).
- Individual drug solutions were heated at high temperature prior to being analysed by HPLC-DAD to identify possible degradants.
- Syringes were prepared containing the drug combination as close to clinical practice as practically possible.
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- Samples were taken at set time points from the administration line and analysed by HPLC-DAD to obtain individual drug concentrations.
- Results assessed to determine whether drug combinations are compatible and stable.

Results

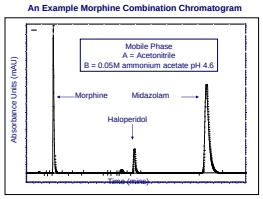
Morphine Combinations			Compatible
Cyclizine	Haloperidol	-	✓(24h)
Cyclizine	Midazolam	-	✓(24h)
Haloperidol	Midazolam	-	✓(24h)
Hyoscine butylbromide	Levomepromazine	-	✓(24h)
Metoclopramide	Midazolam	-	✓(24h)
Cyclizine	Haloperidol	Midazolam	✓(24h)

Diamorphine Combinations		Compatible
Cyclizine	Haloperidol	✓(24h)
Cyclizine	Midazolam	✓(24h)

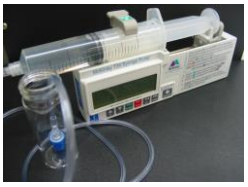
✓(24h) chemically compatible over 24hrs

The combinations remained clear and free from visible particulate matter and the pH remained constant over the 24 hour infusion.

An Example Morphine Combination Chromatogram



McKinley T34 Syringe Pump



Conclusion

The following have been identified as physically and chemically compatible and stable over 24hrs infusion:

- All seven morphine sulphate supportive drug combinations
- The first two diamorphine hydrochloride supportive drug combinations

This is the first step towards providing technical information required by healthcare staff for the mixing of injectable medicines in the same syringe, a recommendation in Patient Safety Alert 20.

IHR Network

Quality of life, living with disability or illness

The information gained from this study has the potential to be of great importance for the field of palliative medicine.

This study will help to determine whether symptom deterioration in patients is a result of disease progression rather than chemical incompatibilities in the syringe which has reduced the drug concentrations.

Future Work

To assess the remaining five supportive drug combinations with diamorphine hydrochloride for chemical compatibility.

The potential exists to extend the study to include other opioids that are often used in end of life care.

References

[1] NHS National Patient Safety Agency (2007) Patient Safety Alert 20: Promoting safer use of injectable medicines. Ref: NPSA/2007/20

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Morphine and Diamorphine Combinations in Palliative Care: A Compatibility Study

Presented at: LJMU, IHR Conference, 21st May 2010

Poster presentation achieved 3rd Prize and a separate Award for Impact

The Marie Curie Palliative Care Institute LIVERPOOL

liverpool JMU

NHS Quality Control North West

Morphine, Diamorphine and Oxycodone Combinations in Palliative Care: A Compatibility Study

Heather Kean[1,3], Diane Rigge[1], Phil Weir[1], Andrew Dickman[2], Andrew Evans[3], Elsie Gaskell[3]

(1) Quality Control North West, Stepping Hill Hospital, Stockport (2) Marie Curie Palliative Care Institute, Liverpool University
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CSCI's are delivered using a syringe driver or pump. There are a number of different syringe drivers or pumps available but this research concentrates on the use of a McKinley T34 Syringe Pump, as presently it is the most popular syringe pump being adopted nationwide.

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Aim

The aim of this study was to obtain chemical compatibility information on the most commonly encountered supportive drugs in end of life care. A database search has identified these as being:

- Cyclizine and haloperidol
- Cyclizine and midazolam
- Levomeproamzine and metoclopramide
- Haloperidol and midazolam
- Hyoscine butylbromide and levomepromazine
- Levomepromazine and midazolam
- Metoclopramide and midazolam
- Cyclizine, haloperidol and midazolam

The combinations are administered with an opioid via CSCI and this study concentrates on the opioids morphine sulphate, diamorphine hydrochloride and oxycodone hydrochloride.

Methodology

To achieve the aim of the study:

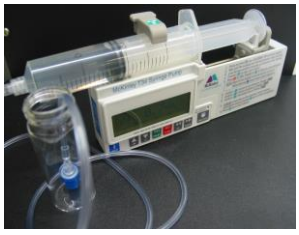
- Analytical methods were developed to separate the individual drug solutions in each combination using High Performance Liquid Chromatography-Diode Array Detection (HPLC-DAD).
- Individual drug solutions were heated at high temperature prior to being analysed by HPLC-DAD to identify possible degradants.
- Syringes were prepared containing the drug combination as close to clinical practice as practically possible.
- A McKinley T34 Syringe Pump was used to simulate infusion of the syringe preparation over a 24hr period.
- Samples were taken at set time points from the administration line and analysed by HPLC-DAD to obtain individual drug concentrations.
- Results assessed to determine whether drug combinations are compatible and stable.

Results

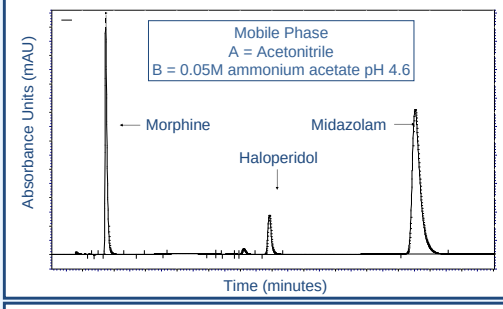
Selected Opioid			+ Supportive Drugs
Morphine 100mg	Diamorphine 100mg	Oxycodone 50mg	
✓(24h)	✓(24h)	✓(24h)	Cyclizine and Haloperidol
✓(24h)	✓(24h)	✓(24h)	Cyclizine and Midazolam
✓(24h)	To be tested	✓(24h)	Levomepromazine and Metoclopramide
✓(24h)	✓(24h)	✓(24h)	Haloperidol and Midazolam
✓(24h)	To be tested	To be tested	Hyoscine butylbromide and Levomepromazine
✓(24h)	To be tested	✓(24h)	Levomepromazine and Midazolam
✓(24h)	✓(24h)	✓(24h)	Metoclopramide and Midazolam
✓(24h)	✓(24h)	To be tested	Cyclizine, Haloperidol and Midazolam

✓(24h) chemically compatible over 24hrs
Combinations remained clear and free from visible particulate matter and pH remained constant over the 24 hour infusion.

McKinley T34 Syringe Pump



An Example Morphine Combination Chromatogram



Conclusion

The following have been identified as physically and chemically compatible and stable over 24hrs infusion:

- All eight morphine sulphate supportive drug combinations
- Five diamorphine hydrochloride and six oxycodone hydrochoride supportive drug combinations

This is the first step towards providing technical information required by healthcare staff for the mixing of injectable medicines in the same syringe, a recommendation in Patient Safety Alert 20.

Future Work

To assess the remaining supportive drug combinations with diamorphine hydrochloride and oxycodone hydrochloride for chemical compatibility.

The potential exists to extend the study to include other opioids that are often used in end of life care.

References

[1] NHS National Patient Safety Agency (2007) Patient Safety Alert 20: Promoting safer use of injectable medicines. Ref: NPSA/2007/20
[2] Rose M., Currow D.C. (2009) The Need for Chemical Compatibility Studies of Subcutaneous Medication Combinations Used in Palliative Care. *Journal of Pain & Palliative Care Pharmacotherapy, 23 (3), 223-230*

Morphine, Diamorphine and Oxycodone Combinations in Palliative Care: A Compatibility Study

Presented at: NHSQA Symposium for Technical Services 2010, September 2010

Poster presentation achieved 1st prize