



'APAAN in the neck' - A reflection on some novel impurities found in seized materials containing amphetamine in Ireland during routine forensic analysis.

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Keywords:	amphetamine, APAAN, synthesis, by-products, importations, adulterants
Abstract:	none, as this is a perspective article.

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Components selected for EU Harmonized Amphetamine Profiling	
4-Methyl-5-phenylpyrimidine	
4-Benzylpyrimidine	
<i>N,N</i> -Di-(β -phenylisopropyl) amine	
<i>N,N</i> -Di-(β -phenylisopropyl)formamide	
<i>N</i> -(β -Phenyl-isopropyl)benzyl methyl ketimine	
<i>N</i> -Benzoylamphetamine	
1-Phenyl-2-propanol	
2-Oxo-1-phenyl-(β -phenylisopropylamino)propane	
<i>N</i> -(β -Phenylisopropyl)cathinone	
Benzyl methyl ketoximine	
2-Methyl-3-phenylaziridine	
1,3-Diphenyl-2-propylamine	
<i>N</i> -Formylamphetamine	
<i>N,N</i> -Di-(β -phenylisopropyl)methylamine	
2,4-Dimethyl-3,5-diphenyl-pyridine	
<i>N</i> -Acetylamphetamine	
<i>N</i> -Benzylamphetamine	
1-Oxo-1-phenyl-2-(β -phenylisopropylamino)propane	
<i>N</i> , β -Hydroxy- <i>N,N</i> -di-(β -phenylisopropyl)amine	
2-Nitro-1-phenyl-propene	
<i>N</i> -(β -Phenyl-isopropyl)benzalimine	

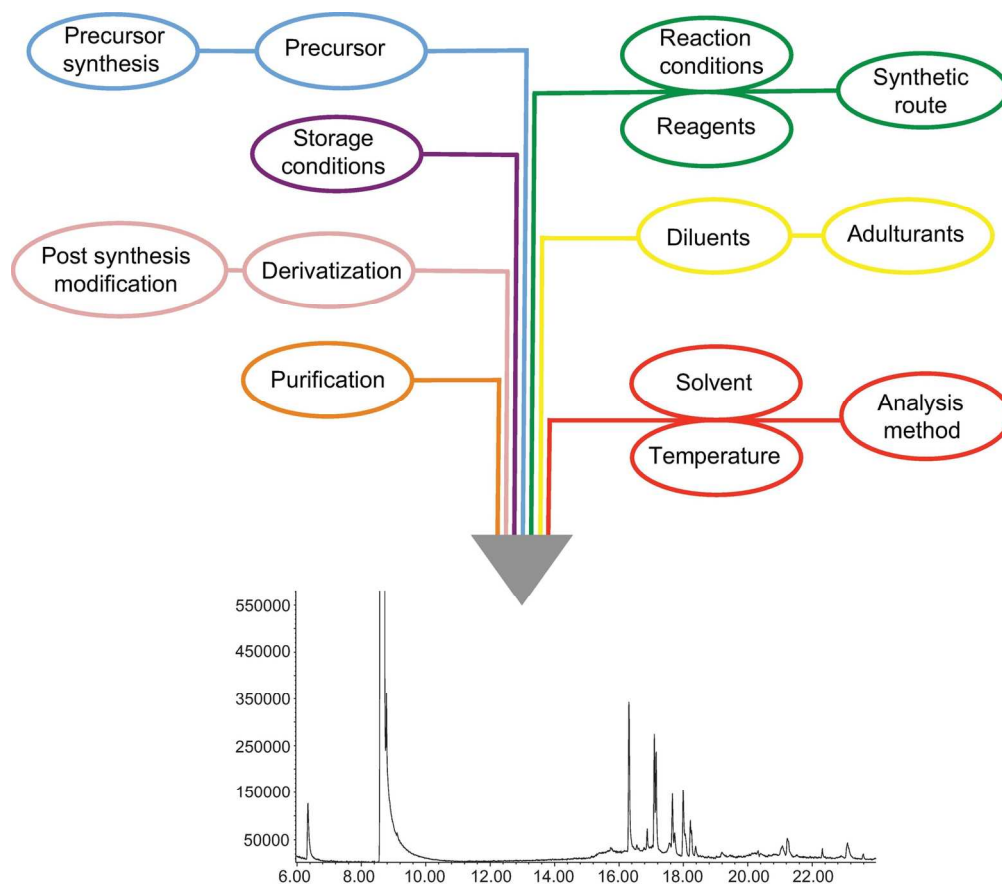


Figure 1. A general scheme for the origin of the different components found in gas chromatograms of seized materials.

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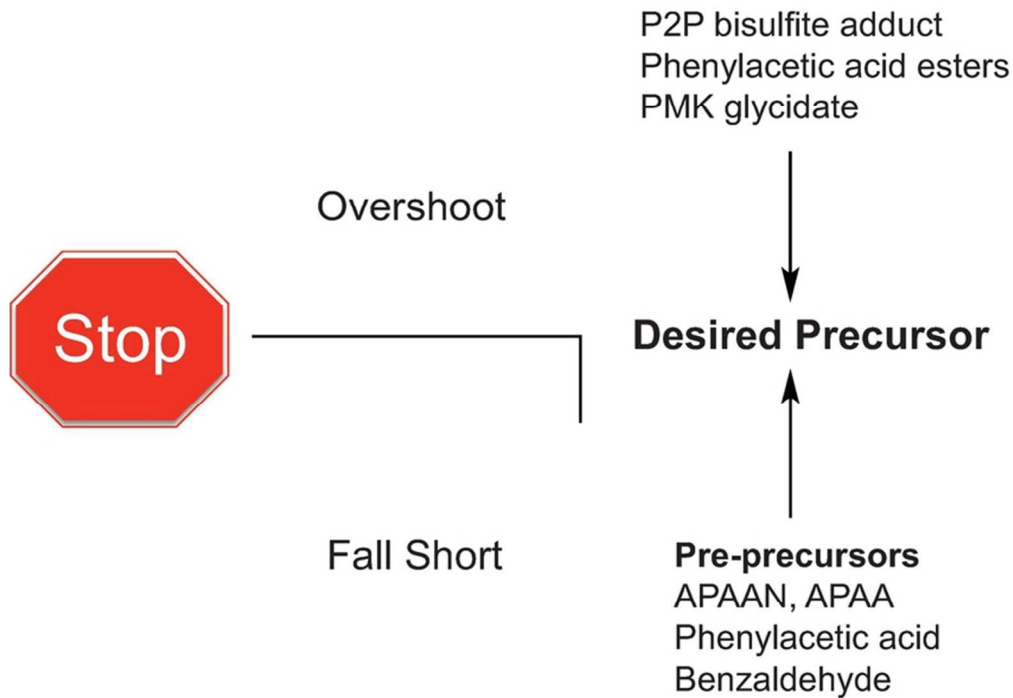
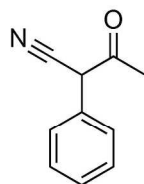
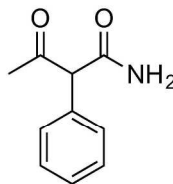


Figure 2. Possible changes in precursor supply methods. The aim of illicit suppliers of substances is to exploit any weakness in the multitude of legal or trade control measures to deliver the required precursors or product to the desired destination. [46,47]

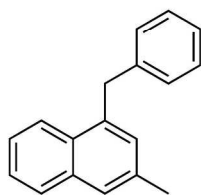
71x49mm (300 x 300 DPI)



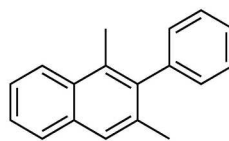
alpha-Phenylacetoacetonitrile
(APAAN)



3-oxo-2-Phenylbutanamide
(APAA)



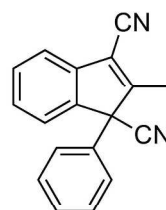
1-Benzyl-3-methylnaphthalene



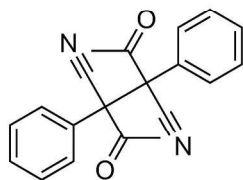
1,3-Dimethyl-2-phenylnaphthalene



4,6-Dimethyl-3,5-diphenylpyridin-2(1H)-one



2-Methyl-1-phenyl-1H-indene-
1,3-dicarbonitrile



2,3-Diacetyl-2,3-diphenylsuccinonitrile

Figure 3. Amphetamine impurities reported by the authors previously. [50,53,54]

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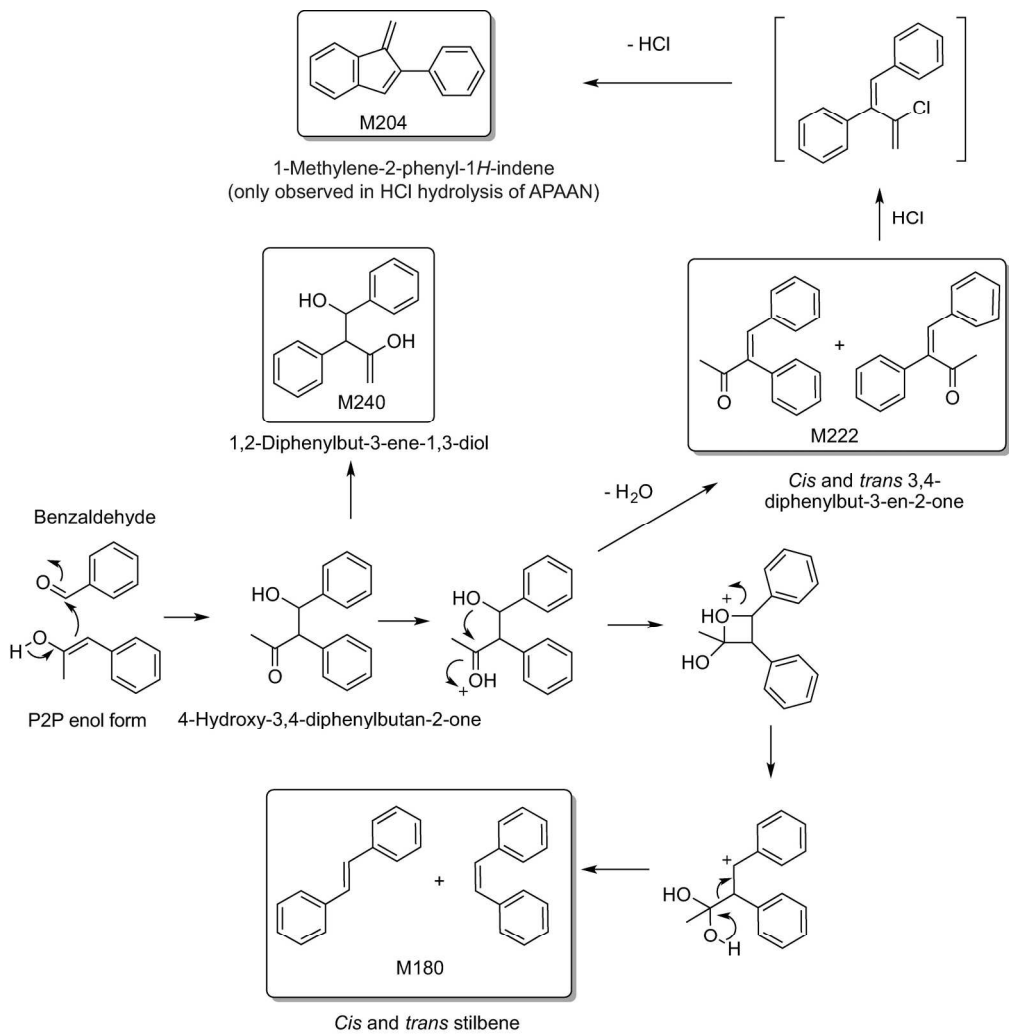


Figure 4. Schematic showing examples of by-products formed during the acidic hydrolysis of APAAN. By-products derived from benzaldehyde and P2P dimerization. The amount of stilbenes (M180) found was greatly dependent on the choice acid with H₂SO₄ >> H₃PO₄ >> HCl.

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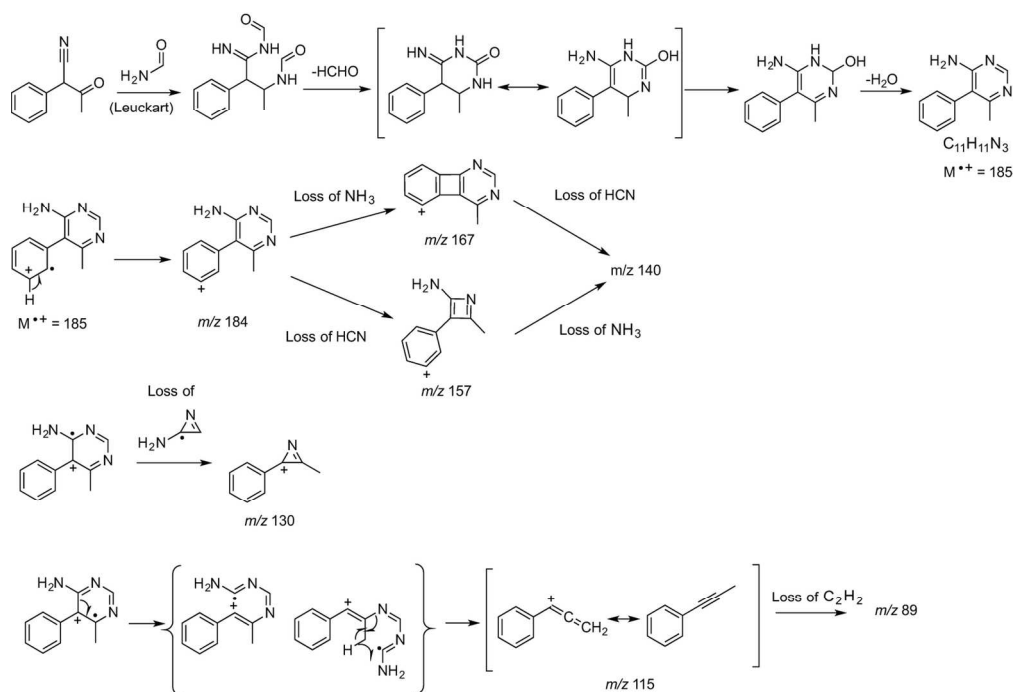


Figure 5. Proposed mechanism of 6-methyl-5-phenylpyrimidin-4-amine formation during Leuckart reaction conditions and mass spectral data.

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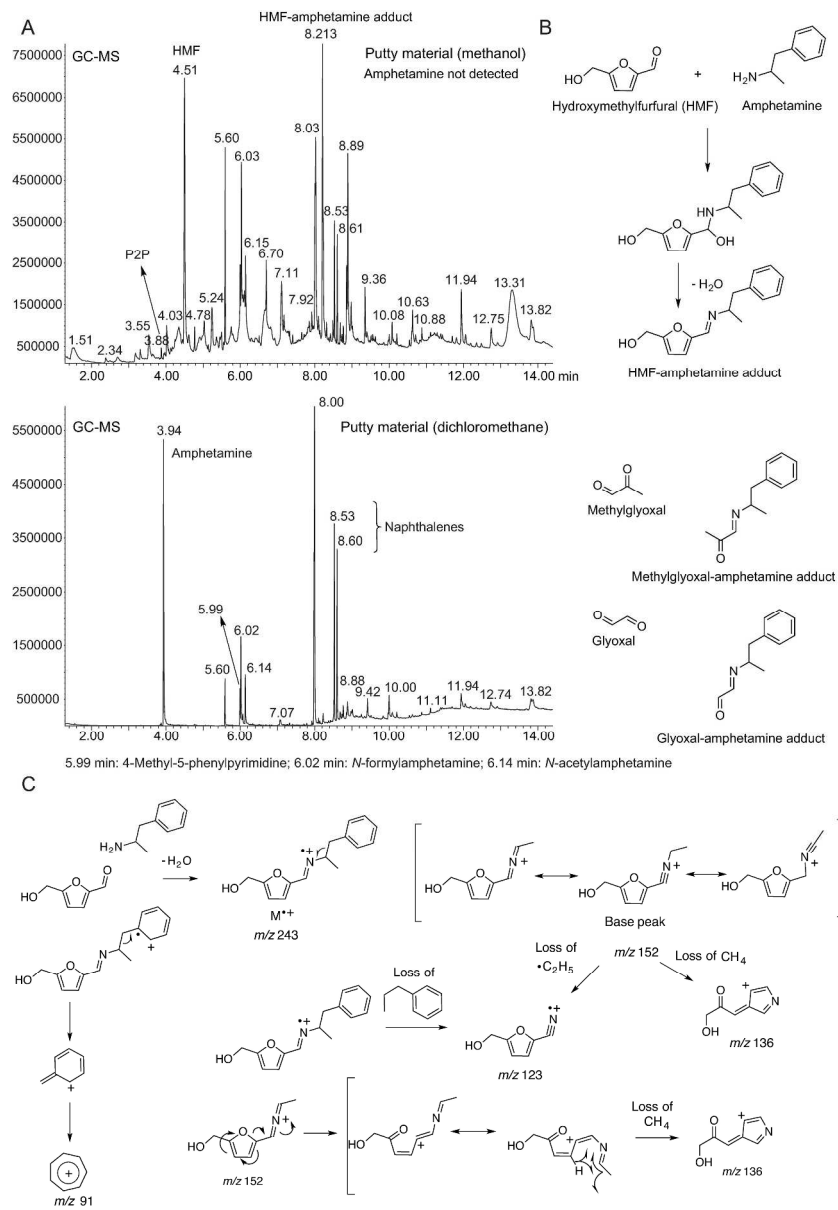


Figure 6.
A: GC-MS of putty sample containing amphetamine in methanol and dichloromethane.
B: HMF-amphetamine imine: (E)-(5-(((1-Phenylpropan-2-yl)imino)methyl)furan-2-yl)methanol;
methylglyoxal-amphetamine imine: (E)-1-((1-Phenylpropan-2-yl)imino)propan-2-one; glyoxal-amphetamine
imine: (E)-2-((1-Phenylpropan-2-yl)imino)acetaldehyde.
C: Proposed amphetamine-HMF imine e.i. fragmentation pattern.

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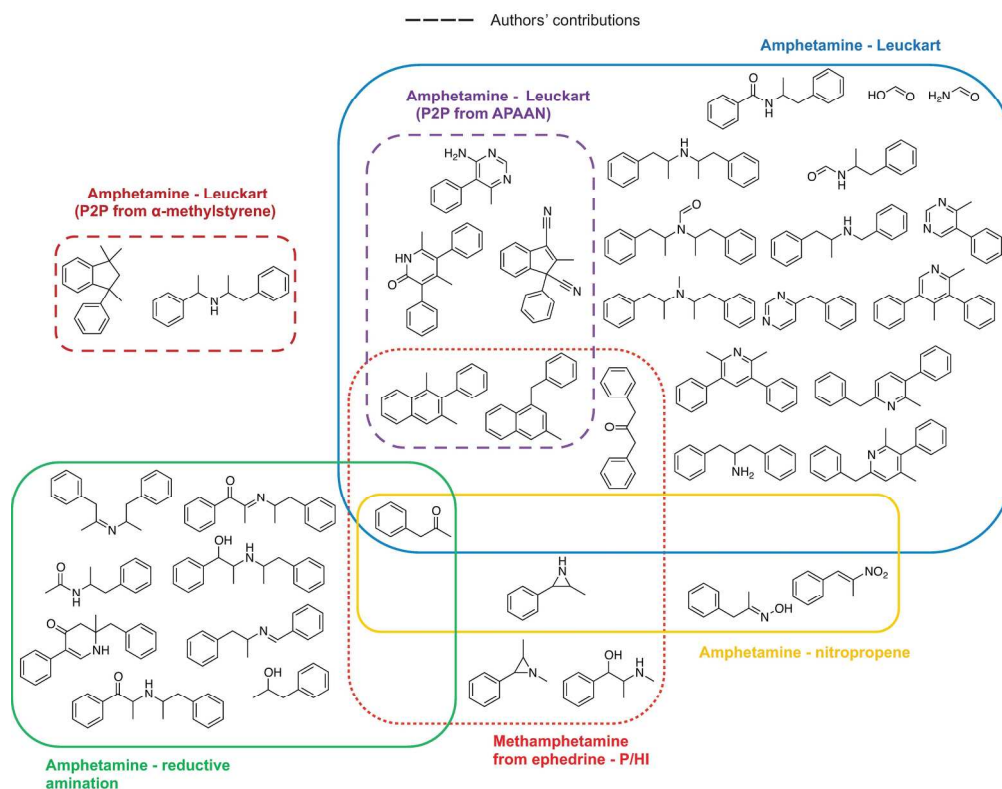


Figure 7. Relationship of the compounds discussed in this perspective to synthesis routes.

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‘APAAN in the neck’ - A reflection on some novel impurities found in seized materials containing amphetamine in Ireland during routine forensic analysis.

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Keywords: Amphetamine, APAAN, synthesis, by-products, importations, adulterants.

Running head: Novel impurities in amphetamine imported to Ireland.

Introduction

The Republic of Ireland has a population of approximately 4.7 million citizens.^[1] Illicit drug misuse is tackled by legislative control mechanisms and the sole national forensic laboratory (Forensic Science Ireland) is tasked with detailing any controlled drugs present in seized materials by issuing a 'certificate of analysis' which is utilized for court purposes.^[2]

This perspective confines itself to exploring changes observed in the analysis of seized amphetamine importations to Ireland and relates these observations to changes in illicit amphetamine production. For any particular drug, the observation of changing chromatographic profiles recorded during routine forensic analyses can be indicative that something different has occurred during the production or distribution processes. These changes may occur, for example, when restrictions are placed on traditional precursors used by illicit amphetamine producers so that alternatives precursors are sought after.

Amphetamine is typically imported into the Republic of Ireland from mainland European countries and no evidence exists for any domestic large-scale illicit synthesis. This paper reports on the newly identified impurities detected in Irish amphetamine importation seizures, some of which have been published previously, others presented for the first time. Reagent purity and synthesis conditions have been shown to affect the components observed during analysis of seizures. Post synthesis additions (adulteration) and storage conditions may also have a profound effect on the analytical profiles obtained from seized items. The finding of new impurities, their abundances, the use of reagents that contain or form known existing impurities and post synthesis additions all have the potential to adversely affect existing profiling methodologies, which aim to link different seizures to a source.

The suggestion is made that the remit of forensic drug laboratories, which is presently centered on uniquely identifying the presence of controlled substances in submitted samples for law enforcement purposes, could be expanded to encompass other objectives. For example, the data generated during routine forensic analysis could be made available, in secure formats, to non-forensic researchers. This would allow those interested in different aspects of substance misuse, to explore and base their findings on the most recent seizure data available. Other researchers might be interested in exploring raw analytical data to generate information on trends observed with respect to the controlled drug reported and / or what else was present (which may not have been recorded in the certificate of analysis issued for law enforcement purposes). Forensic drug laboratories typically store electronic analytical data for long periods of time, so retrospective examination of previously generated data is possible. Those forensic institutes that utilize profiling methodologies for intelligence led policing need up to date information on any new trends observed in illicit amphetamine production.

The forensic analysis of drug seizures

Forensic drug laboratories analyze substances seized by law enforcement officers that are suspected of containing a controlled drug. From a regulatory perspective, forensic laboratory personnel are under pressure from law enforcement agencies to issue the results of laboratory tests on the contents of exhibits submitted in a timely manner. This is to ensure that any charges that may have to be processed through the legal system are initiated within a reasonable timeframe. When forensic laboratory personnel are dealing with drugs seizures, the main objective of their analyses is to identify and confirm the presence of a legally controlled substance present in the materials submitted. Analytical strategies and methods are devised to allow for typical seizures to be examined in a routine and efficient manner. These routine analytical methods are normally qualitative in nature, robust, capable of detecting a large range of compounds and dealing with large variations in drug concentrations. The routine analytical methods employed should identify both known and generally encountered controlled drugs and also indicate the presence of newly encountered substances.^[2-4]

Both liquid chromatography mass spectrometry (LC-MS) and gas chromatography mass spectrometry (GC-MS) methods are typically employed in the routine forensic analysis of controlled substances.^[5-7] A distinct advantage GC-MS analysis has in routine analysis is that the mass spectral data generated is typically generated using standard ionization conditions and is generally unique to a particular compound or a class of compounds. Therefore, the mass spectral data generated from GC-MS instruments lend themselves to the use of searchable libraries, which greatly aid in the identification of substances. Many forensic laboratories share mass spectral library data on newly encountered substances *via* assorted international organizations.^[8,9] There are some limiting factors in the use of GC-MS analysis for routine analyses, the sample components taken from the seizure must be soluble in a suitable solvent and also be sufficiently volatile. Thermally labile components can suffer from degradation, which may result in the chromatogram reflecting artificially induced pyrolysis products rather than the components actually present in the sample. In an attempt to increase volatility and enhance chromatographic properties, many laboratories use assorted derivatization methods.^[10,11]

The recent emergence of new psychoactive substances (NPS) (560 of which were notified to the European Monitoring Centre for Drugs and Drug Addiction since 2008) can pose analytical challenges associated with their unique identifications.^[12-14] Assorted ring-substitutions and additions to the nitrogen and to the side-chain of phenethylamines and β -keto-phenethylamines have been encountered amongst emerging NPS.^[13-17] Many forensic laboratories have to use analytical techniques not previously routinely utilized in their respective laboratories to uniquely identify compounds. In the case of many forensic laboratories, including our own, this has resulted in increased interactions with non-forensic institutions and researchers who have access to the required instruments.

Profiling – redundant or effective in a changing precursor environment?

The comparison of data generated from different seizures forms a central part in profiling activities. The central objective of any profiling exercise is to examine data and to propose linkages of different seizures to a common source.^[18-22] The data compared can be chemical, such as residual solvents or impurities, or be physical, such as size, shape, logo impressions or markings. Profiling methodologies require that data be obtained in a consistent manner and for data on different seizures to be collated in computerized data analysis systems. Analytical profiling methods normally focus in on the minor components (impurities) rather than major components in seizures. An impurity may originate from many sources including; a synthesis side reaction, a diluent added later to bulk up the end product, an adulterant added to mimic some physical property of the desired compound or substances added to allow the product to be tableted or flow freely as a powder.

Illicit drug productions are typically done in batch type processes and inter and intra batch variations may occur. Inter-batch variations may occur due to any differences or changes made in the production process from run to run. Intra- batch variation may occur for a number of reasons including inhomogeneity of reagents or the presence of hot spots (temperature spikes) in synthesis equipment that may produce random side reaction products. For a profiling method to be successful, intra-batch variations should ideally be comparatively smaller than inter-batch variations for the particular components or impurities selected for comparison.^[20,23,24] Generally, the degree of relationship between samples from seizures is measured by the assigning a score, utilizing comparison metrics like scatter plot data, pearson correlation, modified square cosine and euclidean distance.^[20,22,25-28]

Profiling is a very time consuming exercise and, in general, once a large amount of population data is collected, it may become difficult to change data collection parameters. The analytical run times employed for GC-MS profiling tend to be much longer than those employed in routine analysis. This allows for the resolution of any co-eluting components that may be present during shorter routine analysis. Whatever the choice of analytical profiling methodology employed, the components selected for comparison and how they are to be measured have to be decided from the outset and applied consistently so that population data can be collected.^[20,29-31]

When changes occur in the synthesis of the precursors required for conventional illicit production methods, this can add an additional layer of complexity to the interpretation of the significance of specific by-products present in any final product. Information on the by-products arising from any new precursor production methods encountered needs to be borne in mind as to its possible effects on any by-products measured as part of an established harmonized profiling methodologies.

Discriminatory aspects of impurities

With profiling, the components selected for quantification and subsequent comparisons should be discriminating to some extent. An example of 21 substances chosen for comparison is included in Table 1 for a European harmonized method designed for the profiling of amphetamine. These 21 by- products were selected as typical, for at least one of the three most common amphetamine synthesis approaches employed in Europe. Two of these routes employ phenyl- 2-propanone (P2P), namely the Leuckart route and the reductive amination route and finally, the nitrostyrene (oxime) route.^[32]

Table 1. Substances associated with specific synthesis routes and chosen for comparison in a European amphetamine profiling study. [18,32]

What is actually seen and recorded in the chromatogram of a seized sample is dependent on a number of factors that include: the synthesis methods employed, technical sophistication of the apparatus, size of production, sample preparation and the response of the analytical instrument to particular components present in the sample. A general scheme for the origin of the different components found in the chromatograms of seized material is outlined in Figure 1. Gaining an understanding of all aspects of production and at what stage in the production or post-production processes particular substances might arise is critical to making informed decisions about the stability and the discriminating nature of particular components found in the seized material. As time passes, new reagents or new illicit production synthesis routes may be employed that alter the discriminatory power of particular components or a highly discriminating new by-product may be identified. Sharing findings about unusual or novel impurities found during routine examination of seized items with others forensic laboratories (especially those using profiling methods) and with the wider scientific community is important and should be valued as part of a forensic laboratories remit.

Figure 1. A general scheme for the origin of the different components found in gas chromatograms of seized material.

Amphetamine production in Europe

Amphetamine has a long history of use and misuse and it has been extensively studied.[33] It was first synthesized in Germany in 1887[34,35] and is a synthetic derivative of phenethylamine (2-phenylethan-1-amine) and is chiral in nature.[36] Derivatives of amphetamine comprise a large number of substances, with varying substituents attached at the phenyl ring, α -carbon or nitrogen.[12,37] The synthesis routes to both amphetamine and methamphetamine overlap significantly. Some synthesis impurities have been reported to be 'route specific' to a particular synthesis route and as such may be selected as targeted components in profiling methodologies as highly discriminating substances (see Table 1)[20,23,32, 38-40]. Some researchers have demonstrated that when the synthesis procedures were performed in a controlled environment, e.g. using the same reagents, experimental vessels, production method and operator, some components were observed in varying amounts following multiple repeats.[41] Illicit amphetamine manufacture is generally performed outside controlled environments by untrained chemists, thus, potentially leading to considerable variation in synthesis conditions employed for particular production batches.

In Europe, the use of P2P as a starting material for the Leuckart reaction remains the most dominant route to amphetamine production. Europol reported that in 2013-2014, of the 62 amphetamine production sites of known synthesis routes, 48 used P2P as the precursor and at 42 of these 48 sites, the Leuckart synthesis route was used.[38,42] Large amphetamine production facilities have been found for amphetamine in the Netherlands, Belgium, Poland, The Baltic States, Bulgaria, Germany and Turkey. In 2014, the total reported seizure data for amphetamine in Europe was 7.1 tons.[42]

Ephedrine can easily be converted to methamphetamine. Ephedrine is generally employed in larger scale illicit productions, especially when nearby legitimate manufacturing and some diversion of ephedrine stock occurs. More typically, ephedrine (obtained from over the counter medications) may be found in smaller scale domestic type productions. Methamphetamine destined for the European market is predominately synthesized in the Czech Republic, although production facilities were also discovered in Germany, Austria and Bulgaria during 2014.^[12,42] Research into amphetamine synthesis in recent years has moved from classical methods to developing methods that target specific chiral versions of ATS. It remains true, however, that most illicit ATS production facilities detected still employ classic synthesis routes.^[35]

The legitimate sale and distribution of both P2P and ephedrine are internationally monitored and subject to control measures. The availability of starting materials to illicit producers supplied by criminal organizations also appears to be tightly controlled. It has been reported that, ordinarily, no excess starting materials were found at numerous raids on European illicit production sites, therefore, it could be surmised that exact volumes of P2P are provided 'on demand' for each production batch.^[43] In addition, clandestine laboratory investigators have found precursor production is taking place at production sites separate from those where the desired drugs are being prepared. There is further evidence to suggest that the distinct production stages in the illicit manufacture of drugs are spreading to different locations, possibly in attempts to evade detection. For example, in 2015 several European countries reported that amphetamine base oil from the Netherlands was converted to amphetamine sulfate on their territories.^[42,43] The increase in the number of locations where production is in progress increases the number of sites where chemical waste is generated. The amount of waste generated at a Leuckart amphetamine production facility has been estimated to range from 18 to 24 kilograms of chemicals for every kilogram of amphetamine produced.^[36] The disposal of such an amount of waste is an obvious environmental concern. The Netherlands reported that 157 illicit chemical dumping sites were found in 2014 with an average of 800 kilograms of waste per site.^[42,44] Whatever the size of an illicit production facility, the dumping of waste from these sites poses a major health risk to the general public and first responders at such dump locations. From a forensic evidence viewpoint, any assistance in linking components found in waste to seizures or production sites would be beneficial.

Alternatives options when restrictions apply to traditional starting materials for amphetamine production

When restrictions are placed on the sale or international trade of existing precursors, alternative sources of supply are needed for illicit producers to circumvent these restrictions:

- A. Diversion of starting materials from manufacturers who have regulated access to and large stocks of the required reagents;
- B. Falsely declared or concealed contents of imported reagents with the hope that the shipment gets through international custom controls;
- C. Bulk purchases and storage of desired reagents prior to them becoming internationally monitored;
- D. Identification of an alternative route to obtaining the desired starting materials using as yet freely available, unmonitored reagents;
- E. International suppliers may be asked to supply a compound using nomenclature that does not make the supplier aware that it is supplying a substance that is in fact controlled in the destination country;
- F. The required chemical supplies and equipment might be obtained from specialized criminal organizations.

International criminal organizations change their supply routes to all levels of the illicit market over time. In recent years, they became distributors rather than manufacturers of 'product', supplying on-line and head-shop retailers, as well as their traditional illicit drug market vendors.^[14,42] Obtaining product and remaining 'legal' (and thus beyond control mechanisms) may provide information about what appears to be the possible start of a new trend in the supply of precursors for illicit production facilities. Rather than providing the final desired product, which may or may not be nationally or internationally controlled, a compound may be supplied that structurally falls just short of the desired substance but which can be easily manipulated to the desired substance. Alternatively, some moiety may be added to the desired synthesized substance that can subsequently be easily removed. For example, some on-line drug forums are discussing claims made by an Israeli chemist that he can supply a 'meth-expresso' type apparatus, which is stated to allow a purchaser to convert a currently legal substance into a controlled substance in the privacy of their own home.^[45] Alternatively, derivative versions of controlled substances or pro-drugs might be supplied. This potential change in precursor supply scenario is represented in Figure 2, where the desired product is represented as a stop line at a junction, which is deliberately over or under shot, making the supplied product have a status of not monitored or controlled. There are seizures reported where derivativized drugs rather than the drugs themselves have been detected.^[46,47] The illicit precursor suppliers exploit the fact that in many countries (including Ireland), no legal sanctions apply to substances that are not specifically listed either internationally or in their respective national legislation as precursors.^[48]

Figure 2. Possible changes in precursor supply methods. The aim of illicit suppliers of substances is to exploit any weaknesses in the multitude of legal or trade control measures to deliver the required precursors or product to the desired destination. ^[46,47]

Analysis of Irish amphetamine importation seizures

Ireland is not a major producer of illicit substances including ATS. Law enforcement seizure of substances suspected of containing controlled materials, tend to be street level quantities, local dealer distribution quantities or importations from European criminal organizations with links to Irish criminal gangs. Importations may be destined for the domestic market or in transit on route to another country. A few small-scale 'kitchen type' production facilities in domestic settings reducing pseudoephedrine, obtained from over the counter medications to methamphetamine have been found and dismantled in recent years. There is evidence of some coordinated buying of ephedrine-based medications from pharmacies and increased regulation is currently under consideration with respect to the sale of these products.^[49]

In 2013, an Irish customs amphetamine importation seizure was analysed, 1-benzyl-3-methylnaphthalene and 1,3-dimethyl-2-phenyl-naphthalene were found as impurities in the seizure. This pair of isomeric naphthalenes was previously reported as specific to amphetamine synthesized from ephedrine, however, it also contained P2P Leuckart route specific components ^[50] (Figure 3). Personal communications from Dutch colleagues indicated that α -phenylacetoacetonitrile (APAAN) was found at some illicit production sites in 2012/2013 and it was suggested that APAAN acidic hydrolysis was a newly employed route to P2P production.^[51] In 2013, no information was available in the literature that specifically examined the impurities that might be found in P2P derived from the acid hydrolysis of APAAN. In-house experiments were conducted involving the sulfuric acidic hydrolysis of APAAN to yield P2P. This in-house P2P was subsequently used to synthesize amphetamine, *via* the Leuckart method. On analysis of the in-house generated amphetamine, similar GC-MS chromatographic profiles to those acquired for the 2013 customs importation seizure were obtained. This 'new' route to P2P contained impurities previously associated with an ephedrine based amphetamine synthesis route.

Figure 3. Amphetamine impurities reported by the authors previously. ^[50,53,54]

In Europe, the reported seizures of P2P had rapidly declined in the years 2009 to 2013, although there was no corresponding decrease in the supply of amphetamine to the wider European market.^[52] Shipments of APAAN to Europe were increasing with little known legitimate commercial use for this pre-precursor. In 2013, 36 tons of APAAN were seized in the Netherlands and 5.4 tons were seized in Belgium.^[42] Separate dedicated APAAN to P2P production facilities were discovered in Poland and subsequently linked to Dutch illicit drug producers. APAAN was scheduled in the European Union (EU) as a precursor in December 2013 and became an internationally monitored chemical after a UNODC Commission on Narcotic drugs decision 57/1 in March 2014.^[43] The ingenuity and chemical know how of those behind supplying illicit producers was demonstrated with the finding of 3-oxo-2-phenylbutanamide also known as α -phenylacetoacetamide (APAA), a substance that can also easily be converted to P2P, at a Dutch amphetamine production site in 2013.^[12] This might indicate that illicit producers had already investigated ways to circumvent the APAAN control measures prior to its introduction.

In 2015, over 600 kg of APAA was seized in Poland and Germany.^[42] Worldwide seizures of P2P derivatives, phenylacetic acid and benzaldehyde have increased in recent years, indicating a shift to P2P as the main precursor used in both North and Central America, as well as in Europe, in the production of both amphetamine and methamphetamine.^[12,13,15]

In some in-house experiments, which involved the sulfuric acid hydrolysis of APAAN, a large amount of a white substance precipitated from solution, which was isolated and identified as 4,6-dimethyl-3,5-diphenylpyridin-2-one^[53] (Figure 3). The provision of information about new impurities arising from new methods of production is important for clandestine laboratory investigators. These investigators often examine sites where active production has ceased. The amount of impurities present in waste materials may be greater than quantities observed in the final production product. Consequently, the ability to link impurities from samples taken at various stages in production to a possible synthesis route is beneficial. The detection of 4,6-dimethyl-3,5-diphenylpyridin-2-one in amphetamine seizures suggested that the P2P used in the amphetamine synthesis might have derived from APAAN.

APAAN hydrolysis experiments

APAAN, when subjected to acidic medium forms P2P and benzaldehyde along with other by-products. Dimerization and subsequent condensation reactions between these by-products and APAAN account for many of the impurities detected in the final products of the acidic hydrolysis experiments. Experiments on the acidic hydrolysis of APAAN continued in the authors' laboratories with variations of some of the conditions that might occur at an illicit production site, such as varying reaction time, acidic strength and acid employed. It was hoped that the experiments might identify potential impurities that would be indicative of the production process and specific acid used for hydrolysis. Hydrochloric acid, sulfuric acid and phosphoric acid have been reported as used in discovered APAAN conversion facilities.

The major and consistently observed feature of the acid hydrolysis of APAAN was that both *cis* and *trans* stilbenes (M180) were found with all three acids used in the hydrolysis experiments but in vastly different quantities. Stilbene abundance was greatest with sulfuric acid as the hydrolyzing agent, much less was detected with phosphoric acid and only minor trace quantities with hydrochloric acid. Stilbene formation was reversible and essentially occurred from the dimerization of P2P and benzaldehyde (M222) and subsequent condensations. When investigating the ratio of *cis* to *trans* stilbene, it was noted that under strong acidic conditions *cis*-stilbene converted to *trans*-stilbene but not vice versa. In hydrochloric acid experiments on the by-product M222, 1-methylene-2-phenyl-1*H*-indene (M204) was predominately formed in preference to stilbene formation (Figure 4).

Figure 4. Schematic showing examples of by-products formed during the acidic hydrolysis of APAAN. By-products derived from benzaldehyde and P2P dimerization. The amount of stilbenes (M180) found was greatly dependent on the choice acid with $H_2SO_4 \gg H_3PO_4 \gg HCl$.

The isomers 1-benzyl-3-methylnaphthalene and 1,3-dimethyl-2-phenylnaphthalene were found in all APAAN acid hydrolysis experiments to varying degrees. Under the conditions studied, acid-specific by-products could not be detected. One noteworthy impurity found in the APAAN hydrolysis experiments was 3-oxo-2-phenylbutanamide (APAA), a substance found at some clandestine amphetamine production sites, which as previously mentioned in this paper, can also be used to synthesize P2P under similar conditions used for APAAN hydrolysis. The knowledge that at least one APAAN hydrolysis impurity was itself now used as a starting reagent in the manufacture of P2P revealed the increasing complexity in trying to identify discriminating components linkable to a particular hydrolyzing acid. Recognizing that investigators would be looking at specific impurities present in any final amphetamine product, and that the experiments to date showed great variation in what was actually observed, further hydrolysis experiments were not continued.

Reagent purity, post synthesis additions or distillations and analysis conditions may all affect the impurities observed

It is useful to examine the purity of reagents used when conducting synthesis experiments as some impurities present may remain detectable in the final synthesis product or contribute to the formation of other impurities. For example, APAAN was observed to contain the impurity 2,3-diacetyl-2,3-diphenylsuccinonitrile that, in the heat of a GC injection port, converted to 2-methyl-1-phenyl-1H-indene-1,3-dicarbonitrile (Figure 3).^[54] The detection of this indene pyrolysis product suggested that it might be an analytical marker for the involvement of APAAN in the amphetamine production process, at least when employing GC-based analysis.

In a 2016, a customs importation seizure was submitted for analysis. This seizure consisted of a bi-layered liquid with a lower colorless layer and top layer of what appeared to be a red oily liquid. Both layers were examined by GC-MS and both were found to contain amphetamine. The red liquid showed a concentration of amphetamine free base at 40%(w/w), whereas the colorless liquid revealed an amphetamine concentration of 2%(w/w). The importation of this liquid was speculated to represent an example of the diversification of the final amphetamine production stage to different locations. Because the importation consisted only of 5 liters of liquid, it may have been a trial or test importation to check custom controls or to check the customer's view of the product in that physical form. The GC-MS chromatogram of the red oil showed a profile typical for APAAN to P2P Leuckart generated amphetamine as observed in our laboratory with the exception of one relatively large peak. This peak had a mass spectrum base peak at m/z 185 and a second peak about 80% of the base peak at m/z 184.

It was speculated that something concerning the reagents and or production conditions had changed to a significant extent so that the abundance of this impurity had significantly increased. Experiments were conducted that examined the range of compounds known to be present at APAAN to amphetamine production facilities. One set of experiments were designed to investigate the reaction products found when APAAN was subjected to Leuckart reaction conditions, which included the extension of reaction times (APAAN, ammonium formate and formic acid at 130° Celsius for 14 h). The resulting product was first acid extracted and subsequently base extracted. A peak at the same retention time and with the same mass spectral fragmentation pattern was observed as the major component in the base extract and suspected to represent 6-methyl-5-phenylpyrimidin-4-amine. This compound was subsequently synthesized and NMR analysis conducted which confirmed the substitution arrangement of the pyrimidine. The conclusion drawn was that the presence of APAAN in any batch production of P2P, followed by Leuckart reaction conditions, would result in the detection of 6-methyl-5-phenylpyrimidin-4-amine in the amphetamine product. It is suggested that 6-methyl-5-phenylpyrimidin-4-amine is more likely to be observed, when the Leuckart reaction times and/or temperatures are extended beyond what is typically employed during this synthesis. A proposed mechanism of formation of 6-methyl-5-phenylpyrimidin-4-amine and the corresponding mass spectral data are shown in Figure 5. It is suggested that formaldehyde reacts with the nitrile (addition reaction) and with the carbonyl carbon to form a Schiff base). This might be followed by loss of CO and ring closure and subsequent loss of H₂O from the tautomer to yield 6-methyl-5-phenylpyrimidin-4-amine. The presence of pyridines and pyrimidines has long been associated with the Leuckart synthesis of P2P.^[40,55,56] To the authors' knowledge, the formation and detection of 6-methyl-5-phenylpyrimidin-4-amine in association with amphetamine manufacturing has not yet been reported. A sample from the 2013 amphetamine paste importation was re-analyzed, which confirmed the detection of this impurity, thus, adding to the suggestion that the P2P used in the seized material from 2013 was prepared from APAAN.

Figure 5. Proposed mechanism of 6-methyl-5-phenylpyrimidin-4-amine formation during Leuckart reaction conditions and mass spectral data.

Post synthesis additions

The post synthesis addition of substances can also significantly affect the impurity profiles observed during analysis. Some of these effects were evident during routine GC-MS analysis of seized brown putty-like materials submitted as street deals suspected to contain and sold as amphetamine in early 2016. When dissolving some of the putty-like material in methanol in preparation for routine GC-MS analysis, a significant amount of white powder (greater than 80%, w/w) precipitated from solution and analysis by infrared (IR) and by thin layer chromatography (TLC) showed this powder to be a mixture of sugars,

predominantly consisting of lactose. The soluble putty material underwent routine GC-MS analysis, and initially, no amphetamine was detected in the resulting chromatogram. However, many of the components commonly observed in a APAAN to amphetamine conversion were detected, such as, P2P, pyrimidines, *N*-formylamphetamine, *N*-acetylamphetamine and a pair of naphthalenes. Surprisingly, when the same sample analyzed by TLC, a strong amphetamine spot was observed, which suggested that complete degradation of amphetamine must have occurred during GC-MS analysis.

One of the peaks present in the GC-MS profile of the methanolic extract obtained from the putty-like material was attributed to hydroxymethylfurfural (HMF). Assorted sugars including lactose are commonly found in seizures of powders suspected of containing amphetamine and HMF can be obtained from the dehydration of certain sugars. Fermentation of sugars can produce 3-hydroxybutanone (acetoin) and butanedione (diacetyl). Methylglyoxal and glyoxal are other known dicarbonyls produced when sugars are subjected to heat and humidity.^[56-59] As a consequence, these sugar by-products can impact on the quality of amphetamine during storage or during chemical analysis, for example, by forming assorted imines (Schiff bases). When an amphetamine standard in methanol was injected along with glyoxal, methylglyoxal or HMF into the GC-MS, an almost complete disappearance of amphetamine was observed, and imines were detected as analyses by-products. The choice of GC-MS solvent influenced the formation of the imines. When mixtures of the putty amphetamine sample with glyoxal, methylglyoxal or HMF were analyzed by GC-MS using dichloromethane, ethylacetate or toluene as the solvent, amphetamine could be observed in the chromatograms (Figure 6A). Consequently, these results challenged the robustness of the routine qualitative GC-MS method for the detection of amphetamine in the presence of sugars. The presence of HMF and amphetamine in the heat of the GC injection port led to the formation of an amphetamine-HMF imine, suggested to be ((5-(((1-phenylpropan-2-yl)imino)methyl)furan-2-yl)methanol and similar imines were formed with methylglyoxal and glyoxal (Figure 6B). An added complication for the determination of the compounds formed when sugars are also present is that imine formation is pH dependent and that hydrolysis can reverse this process. A electron ionization mass spectra fragmentation pathway for the HMF-amphetamine imine (*m/z* 243, 152(base peak), 136, 123, 91) is proposed.(Figure 6 C).

HMF itself can degrade and form formic acid. When an amphetamine standard was analyzed by our routine GC-MS qualitative analysis method in the presence of HMF and methanol, *N*-formyl-amphetamine was detected along with the amphetamine-HMF imine. *N*-Formyl-amphetamine has previously been thought of as a specific marker for the Leuckart synthesis route and the addition of sugars post synthesis has been shown to have a considerable influence on the components detected in an amphetamine product with consequent implications for synthesis route determinations and profiling methodologies.

Figure 6.

A: GC-MS of putty sample containing amphetamine in methanol and dichloromethane.

B: HMF-amphetamine imine: (E)-((5-(((1-Phenylpropan-2-yl)imino)methyl)furan-2-yl)methanol; methylglyoxal-amphetamine imine: (E)-1-((1-Phenylpropan-2-yl)imino)propan-2-one; glyoxal-amphetamine imine: (E)-2-((1-Phenylpropan-2-yl)imino)acetaldehyde.

C: Proposed amphetamine-HMF imine e.i. fragmentation pattern.

The presence of sugars in illicit preparations could also account for the browning sometimes observed over time in some amphetamine powder cases due to Maillard-type reactions.^[60] The extent of these Maillard reactions depends on variations in pH, humidity and temperature, which are factors that may change significantly during storage and transport of the illicit products before reaching the drug users. This phenomenon is reminiscent of the browning that can be observed in tablets containing assorted amphetamine-type stimulants that are formulated with sugars and cellulose, both of which can lead to HMF formation and may be responsible for flecks of brown appearing or browning of these tablets. The conclusion drawn from these observations was that alcohols are poor GC-MS solvents to use when investigating seizures suspected of containing ATS that may also contain sugars. Amphetamine is a primary amine and other primary amines such as procaine and benzocaine may be present as adulterants along with sugars in illicit seizures of street level amphetamines. In these situations, the use of alcohols as a GC-MS solvent might therefore also have implications on the analysis of these compounds.^[61]

Conclusion

For forensic laboratories involved in seeking to enforce controlled drug legislation, analytical challenges will be continuously present as long as new and untested substances continue to be made available. It is desirable that timely information is made available to forensic drug analysts on how substances are synthesized, new trends in illicit manufacture and what other substances may be present in seized materials. Links to academic institutions or industry should be encouraged so as to foster research as Government funded enforcement laboratories have limited time to research the materials presented to them. If benefits are to accrue from increased co-operation at a national or international level, then, a value needs to be placed on this type of information exchange and time allocated to these activities within national forensic institutions.

The analytical methods and instruments potentially available to law enforcement laboratories are continuously improving, but it is clear that in some cases highly specialized instruments will be required to uniquely identify what is actually present in the illicit products seized. Time spent researching some Irish amphetamine importation seizures has led to an increased awareness of the complexity and range of impurities that may be present. New compounds not previously associated with amphetamine manufacture have been identified. Post synthesis additions have been shown to challenge the ability of some traditional analytical approaches to detect certain substances and these factors have implications for impurity profiling methodologies. Adulteration of products, either intentionally post synthesis or unknowingly as part of the manufacturing process, might also add an unknown health risk for the consumer. Figure 7 attempts to graphically show the complexity and relationships between some of the compounds discussed in this perspective. The reality remains that illicit amphetamine production processes will continue to change and be modified as long as it remains profitable for producers and demand for the product exists. The suitability of the instrumentation and analytical methods currently employed forensic drug testing laboratories for use in law enforcement should be periodically assessed to determine their fitness for purpose. Those laboratories engaged in forensic analysis of misused substances should consider how their analytical data on currently abused substances might be shared with other non-forensic researchers.

Figure 7. Relationship of the compounds discussed in this perspective to synthesis routes.

References

- [1] Irish population data , Census 2016. Available at; <http://www.cso.ie/en/releasesandpublications/ep/p-cpr/censusofpopulation2016-preliminaryresults/>, [2 December 2016].
- [2] Forensic Science Ireland. Available at :<http://www.forensicscience.ie/>, [2 December 2016].
- [3] Forensic drug chemistry. Available at; <http://www.forensicsciencesimplified.org/drugs/principles.html>, [2 December 2016].
- [4] N.Y. United Nations. United Nations Office on Drugs and Crime (UNODC), Recommended methods for the Identification and analysis of amphetamine, methamphetamine and their ring-substituted analogues in seized materials, United Nations, New York, **2006**.
- [5] Michael D. Cole, *The Analysis of Controlled Substances*. John Wiley & Sons, Ltd. New Jersey, **2003**.
- [6] Association of Forensic service provider, *Drug analysis*. Available at; <http://www.afsp.org.uk/sites/default/files/images/Drug Analysis.pdf>, [2 December 2016].
- [7] Scientific working group for the analysis of seized drugs (*SWGDRUG recommendations*). Available at; <http://www.swgdrug.org/Documents/SWGDRUG Recommendations Version 7-1.pdf>, [24 November 2016].
- [8] SWGDRUG, Mass Spectral Library. Available at; <http://swgdrug.org/ms.htm>, [24 November 2016].
- [9] Expansion of a cheminformatic database of spectral data for forensic chemists and toxicologists, Available at; <https://www.ncjrs.gov/pdffiles1/nij/grants/241444.pdf>, [24 November 2016].
- [10] D.L. Lin, S.M. Wang, C.H. Wu, B.G. Chen, R.H. Liu. Chemical derivatization for forensic drug analysis by GC- and LC-MS. *Forensic Sci. Rev.* **2016** *28*, 17.
- [11] M. Szkutova, M. Balikova. [Screening and identification of stimulant and psychedelic drugs as acetyl-derivatives by GC-MS]. *Soud lek.* **2006**, *51*, 55.
- [12] European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), European Drug Report Trends and Developments. Available at; <http://www.emcdda.europa.eu/system/files/publications/2637/TDAT16001ENN.pdf>, [24 November 2016].
- [13] J. Mounteney, P. Griffiths, R. Sedefov, A. Noor, J. Vicente, R. Simon. The drug situation in Europe: an overview of data available on illicit drugs and new psychoactive substances from European monitoring in 2015. **2016**, *111*, 34.
- [14] Commission on Narcotic Drugs. Fifty-ninth session, Vienna, 14-22 March 2014, New psychoactive substances: overview of trends, challenges and legal approaches UNODC. Available at; https://www.unodc.org/documents/commissions/CND/CND_Sessions/CND_59/ECN72016_CRP2_V1601405.pdf, [21 January 2017].
- [15] M. Evans-Brown, R. Sedefov. New psychoactive substances: driving greater complexity into the drug problem. *Addiction*. **2017**, *112*, 36.

- [16] S. Beharry, S. Gibbons. An overview of emerging and new psychoactive substances in the United Kingdom. *Forensic Sci. Int.* **2016**, 267, 25.
- [17] M. Evans-Brown, R. Sedefov. Responding to new psychoactive substances in Europe – The EU Early warning system and risk assessment. *Toxicol Lett.* **2015**, 238, S23.
- [18] L. Aalberg, K. Andersson, C. Bertler, H. Boren, M.D. Cole, J. Dahlen, Y. Finnon, H. Huizer, K. Jalava, E. Kaa, E. Lock, A. Lopes, A. Poortman-van der Meer, E. Sippola. Development of a harmonised method for the profiling of amphetamines - I. Synthesis of standards and compilation of analytical data. *Forensic Sci. Int.* **2005**, 149, 219.
- [19] W. Krawczyk, T. Kunda, I. Perkowska, D. Dudek. Impurity profiling/comparative analyses of samples of 1-phenyl-2-propanone. *Bull. Narc.* **2005**, 57, 33.
- [20] N. Stojanovska, S. Fu, M. Tahtouh, T. Kelly, A. Beavis, K.P. Kirkbride. A review of impurity profiling and synthetic route of manufacture of methylamphetamine, 3,4-methylenedioxymethylamphetamine, amphetamine, dimethylamphetamine and p-methoxyamphetamine. *Forensic Sci. Int.* **2013**, 224, 8.
- [21] M. Morelato, A. Beavis, M. Tahtouh, O. Ribaux, P. Kirkbride, C. Roux. The use of methylamphetamine chemical profiling in an intelligence-led perspective and the observation of inhomogeneity within seizures. *Forensic Sci. Int.* **2015**, 246, 55.
- [22] M. Morelato, A. Beavis, M. Tahtouh, O. Ribaux, P. Kirkbride, C. Roux. The use of forensic case data in intelligence-led policing: the example of drug profiling. *Forensic Sci. Int.* **2013**, 226, 55.
- [23] V. Kunalan, N.N. Daeid, W.J. Kerr, H.A.S. Buchanan, A.R. McPherson. Characterization of route specific impurities found in methamphetamine synthesized by the leuckart and reductive amination methods. *Anal. Chem.* **2009**, 81, 7342.
- [24] L. Dujourdy, V. Dufey, F. Besacier, N. Miano, R. Marquis, E. Lock, L. Aalberg, S. Dieckmann, F. Zrcek, J.S. Bozenko, Jr. Drug intelligence based on organic impurities in illicit MA samples. *Forensic Sci. Int.* **2008**, 177, 153.
- [25] H. Inoue, T. Kanamori, Y.T. Iwata, Y. Ohmae, K. Tsujikawa, S. Saitoh, T. Kishi. Methamphetamine impurity profiling using a 0.32 mm i.d. nonpolar capillary column. *Forensic Sci. Int.* **2003**, 135, 142.
- [26] R. Marquis, C. Weyermann, C. Delaporte, P. Esseiva, L. Aalberg, F. Besacier, J.S. Bozenko, Jr., R. Dahlenburg, C. Kopper, F. Zrcek. Drug intelligence based on MDMA tablets data: 2. Physical characteristics profiling. *Forensic Sci. Int.* **2008**, 178, 1, 34.
- [27] C. Weyermann, R. Marquis, C. Delaporte, P. Esseiva, E. Lock, L. Aalberg, J.S. Bozenko, Jr., S. Dieckmann, L. Dujourdy, F. Zrcek. Drug intelligence based on MDMA tablets data I. Organic impurities profiling. *Forensic Sci. Int.* **2008**, 177, 11.
- [28] J. Xing, M. Zang, H. Zhang, M. Zhu. The application of high-resolution mass spectrometry-based data-mining tools in tandem to metabolite profiling of a triple drug combination in humans. *Anal. Chim. Acta.* **2015**, 897, 34.
- [29] K. Tsujikawa, K. Kuwayama, H. Miyaguchi, T. Kanamori, Y.T. Iwata, H. Inoue. Chemical profiling of seized methamphetamine putatively synthesized from phenylacetic acid derivatives. *Forensic Sci. Int.* **2013**, 227, 42.
- [30] H. Inoue, Y.T. Iwata, K. Kuwayama. Characterization and Profiling of Methamphetamine Seizures. *J. Health Science.* **2008**, 54, 615.
- [31] K. Andersson, E. Lock, K. Jalava, H. Huizer, S. Jonson, E. Kaa, A. Lopes, A. Poortman-van der Meer, E. Sippola, L. Dujourdy, J. Dahlen. Development of a harmonised method for the profiling of amphetamines VI: Evaluation of methods for comparison of amphetamine. *Forensic Sci. Int.* **2007**, 169, 86.
- [32] L. Aalberg, K. Andersson, C. Bertler, M.D. Cole, Y. Finnon, H. Huizer, K. Jalava, E. Kaa, E. Lock, A. Lopes, A. Poortman-van der Meer, E. Sippola, J. Dahlen. Development of a harmonized method for the profiling of amphetamines II. Stability of impurities in organic solvents. *Forensic Sci. Int.* **2005**, 149, 231.

- [33] L. Laniel, *Amphetamine : a European Union perspective in the global context*, Publications Office of the European Union, Luxembourg. Available at; <http://www.emcdda.europa.eu/publications/joint-publications/amphetamine>, [21 January 2017].
- [34] N. Rasmussen. Amphetamine-Type Stimulants: The Early History of Their Medical and Non-Medical Uses. *Int. Rev. Neurobiol.* **2015**, 120, 9.
- [35] A. Allen, R. Ely, Synthesis methods for amphetamine a review. Available at; <http://www.nwafs.org/newsletters/SyntheticAmphetamine.pdf>. [21 January 2017].
- [36] European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), Europol. Amphetamine: a European Union perspective in the global context. EMCDDA/Europol, Lisbon, 2011. Available at: http://www.emcdda.europa.eu/system/files/publications/621/EMCDDA-Europol_Amphetamine-joint-publication_319089.pdf [21 January 2017].
- [37] L.A. King. New phenethylamines in Europe. *Drug Test. Anal.* **2014**, 6, 808.
- [38] V. Kunalan, W.J. Kerr, N. NicDaeid. Clarification of route specific impurities found in methylamphetamine synthesised using the Birch method. *Forensic Sci. Int.* **2012**, 223, 321.
- [39] L.A. King, K. Clarke, A. Orpet. Amphetamine profiling in the UK. *Forensic Sci. Int.* **1994**, 69, 65.
- [40] K.P. Kirkbride, A.D. Ward, N.F. Jenkins, G. Klass, J.C. Coumbaros. Synthesis of 4-methyl-5-arylpyrimidines and 4-arylpyrimidines: route specific markers for the Leuckardt preparation of amphetamine, 4-methoxyamphetamine, and 4-methylthioamphetamine. *Forensic Sci. Int.* **2001**, 115, 53.
- [41] V. Kunalan, W.J. Kerr, N. Nic Daeid. Investigation of the reaction Impurities associated with methylamphetamine synthesized using the nagai method. *Anal. Chem.* **2012**, 84, 5744.
- [42] European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), Europol. 2016 EU Drug Markets Report: In-depth Analysis EMCDDA, Europol, Lisbon, 2016. Available at: <http://www.emcdda.europa.eu/system/files/publications/2373/TD0216072ENN.PDF> [21 January 2017].
- [43] United Nations International Narcotics Control Board. Precursors and chemicals frequently used in the illicit manufacture of narcotic drugs and psychotropic substances 2015. United Nations, New York, 2016. Available at: https://www.incb.org/documents/PRECURSORS/TECHNICAL_REPORTS/2015/2015-PreAR_E.pdf [21 January 2017].
- [44] European Commission Brussels, Support to member states in improving hazardous waste management based on assessment, issued by BIPRO EU commission. Available at; http://ec.europa.eu/environment/waste/studies/pdf/hazardous%20waste%20management/SR%203%20Final%20Report%20HW%20management_FIN%20REV%204.pdf [21 January 2017]
- [45] The Guardian, Dr Zee, the godfather of legal highs: 'I test everything on myself in' (2016). Available at: <https://www.theguardian.com/tv-and-radio/2016/may/24/dr-zee-the-godfather-of-legal-highs-i-test-everything-on-myself> [29 November 2016].
- [46] M. Collins, C. Donnelly, S. Cameron, M. Tahtout, H. Salouros. Identification and characterization of N-tert-butoxycarbonyl-MDMA, a new MDMA precursor. *Drug Test. Anal.* **2017**, doi:1002/dta. 2059.
- [47] F. Westphal, U. Girreser, K. Holz, M. Erkens. Strukturaufklärung und analytische Daten eines ungewöhnlichen MDMA-Derivates. *Toxchem Krimtech* **2016**, 83, 92.

and new psychoactive Substances (NPS),
Available at; http://eurojust.europa.eu/doclibrary/Eurojust-framework/caseworkdrugtraffickingactionplan2015/Addendum%20%20-%20Judicial%20cooperation%20in%20cases%20involving%20precursors%20and%20NPS/drug-trafficking-report_addendum2-nps-precursors_2015-01-16_EN.pdf[24 February 2017].

[49] Drugs and Organised Crime Bureau, Information on amphetamine and methamphetamine use and production within the Irish state in: Personal communication to Forensic Science Ireland, not published, 2016.

[50] J.D. Power, M.G. Barry, K.R. Scott, P.V. Kavanagh. An unusual presentation of a customs importation seizure containing amphetamine, possibly synthesized by the APAAN-P2P-Leuckart route. *Forensic Sci. Int.* **2014**, 234, e10.

[51] D.F.L. (National Crime Squad), Netherlands Police Agency, National Dismantling Facility, personal communication regarding APAAN, 2013.

[52] European Drugs Markets Reoprt, A strategic Analysis. Available at ; <http://www.emcdda.europa.eu/publications/joint-publications/eu-drug-markets/2016/strategic-overview> [21 January 2017]

[53] J.D. Power, J. O'Brien, B. Talbot, M. Barry, P. Kavanagh. The identification of an impurity product, 4,6-dimethyl-3,5-diphenylpyridin-2-one in an amphetamine importation seizure, a potential route specific by-product for amphetamine synthesized by the APAAN to P2P, Leuckart route. *Forensic Sci. Int.* **2014**, 241, e13.

[54] J.D. Power, P. Kavanagh, G. McLaughlin, G. Dowling, M. Barry, J. O'Brien, B. Talbot, B. Twamley, S.D. Brandt. Forensic analysis of P2P derived amphetamine synthesis impurities: identification and characterization of indene by-products. *Drug Test. Analysis.* **2016** doi: [10.1002/dta.1944](https://doi.org/10.1002/dta.1944).

[55] T.C. Kram. Reidentification of a major impurity in illicit amphetamine. *J. Forensic Sci.* **1979**, 24, 596.

[56] M. Lambrechts, K.E. Rasmussen. Leuckart-Specific Impurities in amphetamine and methamphetamine seized in Norway. *Bull Narc.* **1984**, 36, 47.

[57] J.C. Griffin, G.S. Banker. Reactions of amine drugs with sugars I Reaction of amphetamine with aldoses, glycosylamines, and bisulfite. *J. Pharm. Sci.* **1967**, 56, 1098.

[58] J.C. Griffin, G.S. Banker. Reactions of amine drugs with sugars II Synthesis and in vivo evaluation of mannose amphetamine sulfonate and N-phenylisopropyl-d-mannamine. *J. Pharm. Sci.* **1967**, 56, 1101.

[59] Y. Wu, J. Levons, A.S. Narang, K. Raghavan, V.M. Rao. Reactive impurities in excipients: profiling, identification and mitigation of drug-excipient incompatibility. *AAPS. Pharm. Sci. Tech.* **2011**, 12, 1248.

[60] R.Castello, A.M.Mattocks, Discoloration of tablets containing amines and lactose, *J. Pharm. Sci.* 51,106.

[61] CUT, a guide to adulterants, bulking agents and contaminants found in illicit drugs. Available at; <http://www.cph.org.uk/wp-content/uploads/2012/08/cut-a-guide-to-the-adulterants-bulking-agents-and-other-contaminants-found-in-illicit-drugs.pdf>, [21 January 2017].

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