A review on the latest advances in extraction and analysis of artemisinin

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Abstract

Introduction:

Artemisinin (**1**), a well-known natural antimalarial drug, is a sesquiterpene lactone that contains a unique peroxide bridge. Since its discovery, the amount of research into the analysis of artemisinin has increased considerably, and it has been further intensified since the Noble Prize win by Tu Youyou in the year 2015 for the discovery of artemisinin.

Objective:

To review published literature on the extraction and analysis of artemisinin, published during 2017-present, and to present an appraisal of those methods.

Methodology:

Extensive literature search was carried out which involved, but not limited to, the use of, various databases, like Web of Knowledge, PubMed and Google Scholar, and relevant published materials including published books. The keywords used, in various combinations, with artemisinin being present in all combinations, in the search were artemisinin, *Artemisia annua*, analysis, extraction, quantitative, qualitative and quality control.

Results:

During the period covered in this review, there are several methods of analysis of artemisinin have been reported, the most of which were LC-based methods. However, the use of new methods like near infrared analysis, fluorometirc analysis and molecular imprinting, and a significant increase in the use of computational tools have been observed. Mainly several methods involving supercritical fluid extraction and ultrasound-assisted extraction of artemisinin have dominated the extraction area.

Conclusions:

Newer analytical tools, as well as improved protocols for the known analytical tools, for qualitative and quantitative determination of artemisinin (**1**), have been made available by various researchers during the period covered by this review. Supercritical fluid extraction and ultrasound-assisted extraction are still the methods of choice for extraction of artemisinin.

Keywords

Artemisia annua; analysis; artemisinin; extraction; supercritical fluid extraction; ultrasound-assited extraction; HPLC; LC-MS; Nobel Prize; Tu Youyou

1 INTRODUCTION

Artemisinin (**1**) (Figure 1), a well-known natural antimalarial drug, is a sesquiterpene lactone that contains a unique peroxide bridge, also known as endoperoxy (1,2,4-trioxane ring) functionality, which is believed to be responsible for how this compounds acts against malarial infection[1-3]. Recently, anticancer potential of artemisinin (**1**) and its analogues has been documented in a number of publications[4]. Since its discovery from the Chinese medicinal plant, *Artemisia annua* L. (family: Asteraceae), common name, "qinghao", in 1972 by Tu Youyou, the winner of the 2015 Noble Prize in Medicine or Physiology[2], there has been a large body of research carried out on various aspects of artemisinin (**1**), covering its production, biosynthesis, extraction, analysis/assay and bioactivity, most of which, until the end of 2016, have been captured in different chapters of the book written by Tu Youyou, published in 2017[5]. In fact, research into artemisisin (**1**) has been further intensified after Tu's wining of the Nobel Prize, and consequently, during the last couple of years, a good number of publications on the extraction and analysis of artemisinin (**1**) have become available. This review focuses on and appraises the scientific papers that report on various extraction methods and analytical/assay methods for artemisinin (**1**) that have been published since 2017 until to date.

2 EXTRACTION

Artemisinin (**1**) is distributed within the range of 0.01-1.4% in various parts of *A. annua*, including leaves, flowers and buds[6]. The traditional way of extracting artemisinin (**1**) from this plant is rather time consuming, and usually involves steeping or stirring the leaves in the extraction solvent (generally *n*-hexane or ethanol) for several days. Whilst *n*-hexane is the most common solvent used in this method of extraction, other solvents like ethanol, toluene and petroleum ether are also used. It is also quite common to use elevated temperature and refluxing (*e.g.*, at 40^{o}C) of plant material in a chosen solvent over 10-48 hours to increase the extraction yield of artemisinin (**1**). In fact, as the amount of artemisinin (**1**) present in *A. annua* is extremely low, continuous efforts were devoted to employ more effective and environmentally friendly extraction methods, *e.g.*, Soxhlet, ultrasound and microwave-assited extraction, and supercritical fluid extraction (often supercritical CO_2 extraction) to increase extraction yield of this compound, and these efforts have also

continued to date (Table 1). A clear shift towards predicting the best extraction parameters for a certain type of extraction method, *e.g.,* ultrasound-assited extraction, utilizing various computational tools and mathematical modelling, has been observed in recent years. During the period from 2017 to present, mainly several protocols of ultrasound-assisted extraction and supercritical fluid extraction of artemisinin (**1**) have been reported (Table 1)[7-21], which will be discussed briefly in the following subsections.

2.1 Supercritical fluid extraction (SFE)

Supercritical fluid extraction (SFE) is an extraction method that utilizes a supercritical fluid, *e.g*., supercritical CO_2, as the extracting solvent to extract usually from a solid matrix[22,23]. Sometimes, the main supercritical fluid is modified by the addition of small volume of co-solvents such as ethanol or methanol. This extraction method is particularly useful for relatively nonpolar thermolabile materials. Artemisinin (**1**) is a nonpolar sesquiterpene, and can be extracted successfully from *A. annua* by SFE[12-15]. In fact, artemisinin's solubility in supercritical CO_2, in terms of mole fraction, was found to be within the range of 10^{-4} to 10^{-3}, which is higher than typical solubilities of many biomolecules.

Martinez-Correa et al.[15] reported an integrated SFE method for the extraction of artemisinin (**1**) from the leaves of *A. annua*, and the method had two steps: the first one involved supercritical CO_2 as the solvent, and the second step utilized ethanol or water to extract the solid residue obtained from the first step. The second step only extracted polar compounds, *e.g.,* phenolics, but no artemisinin (**1**). As a result, after the ethanol or water extraction, the initial SFE solid residue mainly contained or was rich in artemisinin (**1**), and free from other polar impurities. Another recently published SFE method for artemisinin (**1**) from *A. annua* established that the best extraction yield could be achieved using 100 bar pressure and 40^{o}C temperature[14]. Based on this optimization, later, the scale up and economic analysis of SFE for artemisisin (**1**) has been reported[24].

Negi et al.[13] reported a supercritical CO_2 method for the extraction of artemisinin (**1**) from *A. annua* in temperature and pressure ranges of 313.1-333.1 K and 15-25 MPa. In this work, artemisinin (**1**) global yield isotherms were established producing a maximum yield of around 4%. Artemisinin (**1**) was also Soxhlet-extracted with *n*-hexane, and the resulting crude extract was subjected to SFE using supercritical CO_2 after adsorption on silica gel, yielding much purer artemisinin (**1**). The SFE was carried out in the LAB SFE 100 mL (Separex),

composed of a CO_2 heater, an extractor with extraction cells of varied volumes, a temperature and pressure control system and an extract collector. The extraction unit could handle up to 50 MPa and 423.1 K. The flow rate of CO_2 was maintained constant at 25 g/min, and the samples were collected at different intervals from 10 to 90 min. A similar work was carried out by Rodrigues et al.[12], where the SFE operating conditions, *e.g.,* temperature, pressure and co-solvent use to obtain artemisinin-enriched extract were optimized using a fully randomized factorial design comprising three levels of temperatures, pressures and co-solvent contents, and the economic evaluation of the SFE compared to conventional ethanol extraction was reported. It was found that the artemisinin extraction by conventional ethanol extraction offered higher global yield (about 12 g/100 g of plant material) than that from SFE (max. 9.5 g/100 g of plant material), but with lower artemisinin (**1**) content. SFE was more specific for extracting artemisinin (1) than the conventional ethanol extraction.

In recent years, the use of computational tools and mathematical models has increased significantly in all types of phytochemical methods, particularly in the optimization of extraction parameters in various extraction techniques[25]. A supercritical CO_2 extraction protocol for artemisinin (**1**), modified with ethanol, based on response surface methodology developed from central composite rotatable design that helped optimize extraction pressure, temperature, and co-solvent use, has recently been reported[16]. It can be noted that the response surface methodology explores the interplay between several explanatory variables and one or more response variables, and can be used to maximize the production of a special substance by optimization of operational factors/variables. In this study, various extraction conditions, *e.g*., pressure (9.9-30 MPa), temperature (33-67°C), and co-solvent (ethanol, 0-12.6%) were explored. The optimum SFE extraction conditions were established at 30 MPa and 33°C without ethanol, yielding 1.09% of artemisinin (**1**). It was also reported that the Soxhlet extraction with *n*-hexane produced higher artemisinin (**1**) yields and no significant difference could be detected in the purity of the SFE extracts and Soxhlet extracts.

A strategy for increasing artemisinin yield by combining a traditional maceration and SFE was reported by Baldino & Reverchon[7]. The aerial parts of *A. annua* was macerated with *n*-hexane to obtain a crude extract, which was further extracted with supercritical CO_2 extraction to obtain artemisinin-enriched extract[7]. This hybrid approach produced the final product that contained 71% of active compounds, which was more than twice the

concentration that can be obtained from optimized direct SFE, and 2.6 times richer in active principles than the traditional solvent extraction could produce.

2.2 Ultrasound-assisted extraction

As the name implies, ultrasound-assisted extraction, utilizes ultrasound or ultrasonic agitation to enhance extraction yield from a solid matrix using a solvent or solvents mixture[26,27]. The use of this technique for the isolation of artemisinin (**1**) from *A. annua* is not new, and there are several publications on this topic, especially using organic solvents[6]. However, there have been further reports on ultrasound extraction of artemisinin (**1**) in recent years, particularly since 2017. Cai et al.[21] reported an ultrasound-assited extraction method for artemisisin (**1**) from the leaves of *A. annua* using a deep eutectic solvent, where they demonstrated the tunability of hydrophobic deep eutectic solvents as designer solvents for efficient extraction of bioactive compounds, *e.g*., artemisinin, (**1**) from plant materials. A hydrophobic deep eutectic solvent ($N8_1Cl$-NBA) was prepared from methyl trioctyl ammonium chloride and 1-butanol at a molar ratio of 1:4, and this solvent showed the highest extraction yield. Again, the main factors affecting the extraction efficiency were optimized by a central composite design combined with a response surface methodology. The optimized conditions were as follows: solvent-solid ration 35:2, ultrasonic power 180 W, temperature 45^{o}C, particle size 80 mesh, and extraction time 70 min. These optimized conditions afforded a higher extraction yield of about 8 mg/g than conventional organic solvent petroleum ether. The artemisinin recovery from the extraction solution was accomplished by AB-8 macroporous resin (recovery yield ~86%). This study established that it is not only a hydrophilic deep eutectic solvent, but also a hydrophobic deep eutectic solvent can be a true designer solvent for use as a green and safe extraction solvent.

Box-Behnken experimental design along with response surface methodology was employed for ultrasound-assisted extraction of artemisinin (**1**) from *A. annua* using ethanol as the solvent[20]. The Box-Behnken design is an independent quadratic design in that it does not contain an embedded factorial or fractional factorial design, where the treatment combinations are at the midpoints of edges of the process space and at the centre[25]. In the study carried out by Silva et al.[20], the effects of the extraction parameters, *i.e.,* ethanol graduation, previous shaking time in an ultrasound bath, and drug/solvent ratio, on the artemisinin extraction efficiency were evaluated.

Zhang et al.[19] reported an ultrasound extraction protocol for artemisinin (**1**) using mono-ether based solvents, *e.g.*, propylene glycol methyl ether. Compared with conventional extraction, this reported method had a higher extraction efficiency (13.79 mg/g vs. 13.29 mg/g) and much shorter extraction time (0.5 h vs 8 h) at a relatively low temperature. Moreover, propylene glycol methyl ether had low toxicity and volatility, which made the extraction process safer and more reliable. An ultrasonic bath (KQ-250DB, Kunshan, Jiangsu, China) was used as the ultrasonic source. The unit was a rectangular container (23.5 × 13.3 × 10.2 cm), in which 20 kHz transducers were annealed at the bottom. An ultrasound-assisted extraction of artemisinin (**1**) using *n*-hexane has recently been reported[18], where artemisinin (**1**) contents in selected *Artemisia* species from Tajikistan (Central Asia) were determined. The extraction protocol was rather simple; the extraction process of dried aerial parts of *Artemisia* plants involved the following steps: 10 g of plant materials were crushed into smaller pieces, *n*-hexane (150 mL) was added at room temperature, and sonicated in an ultrasonic bath at a frequency of 35 kHz for 15 min at room temperature. Plant mixtures were allowed to stand for 12 h at room temperature, then filtered through Whatman filter paper and used for the designed chemical analysis.

Ruan et al.[17] reported a ultrasound extraction method for artemisinin (**1**), where an orthogonal experiment was used. The ratio of material to liquid, extraction time and number of extractions were selected as the investigation factors. The content of five compounds in samples of *A. annua* was used as the index, and the experimental optimal extraction process conditions were established as follows: extraction with methanol by ultrasonic extraction, comprising two cycles of 0.25 h each, and a ratio of material to liquid of 1:20 (g/mL). Single factor experiments were performed to optimize the extraction process.

2.3 Miscellaneous extraction

Artemisinin (**1**) extraction from *A. annua* based on systematic and model-assited process design, which was established from lab-scale experiments and miniplant-scale pilot, were reported[8,9]. Artemisinin (**1**) was extracted from *A. annua* by conventional percolation using acetone, and pressurized hot water extraction was performed at 80^0C to extract artemisinin (**1**)[9]. In both cases, a systematic and model-assisted process design was used aiming at defining resource-efficient and economic processes. Fresh solvent was pumped through a column filled with plant material, which allowed maintenance of a concentration

gradient between the solvent and the plant material, making a complete leaching possible. The study demonstrated a systematic and model-based comparison of two different ways of extracting artemisinin (**1**), where high productivities and yields were observed.

Laboukhi-Khorsi et al.[10] reported the extraction method using Hansen solubility parameters in practice approach, where isopropanol, a green alternative to *n*-hexane, was used for artemisinin (**1**) extraction from *A. annua* with the yield of 65%. It can be noted that Hansen solubility parameters are another potentially useful concept for determination of the degree of compatibility and incompatibility of two materials.

A high-throughput analytical method employing deep eutectic solvents, which are generally formed from a eutectic mixture of Lewis or Brønsted acids and bases that can contain several anionic and cationic species, for mechanochemical extraction combined with direct analysis in real time mass spectrometry (DART-MS), was developed by Wang et al.[11] to quantify thermolabile artemisinin (**1**) and related components of *A. annua*. Mechanochemical extraction was performed at room temperature, and the target analytes were released into deep eutectic solvents within seconds, demonstrating multiple advantages over traditional extraction methods using organic solvents. This study displayed a method combining high-efficiency sample pretreatment and rapid chemical analysis from complex matrices, by eliminating time-consuming separation procedure, and avoiding the use of toxic organic solvents needed for extraction and analysis of artemisinin (**1**).

3 ANALYSIS

The analysis or assay of artemisinin (**1**), either in the source plant, *i.e., A. annua*, or in other biological matrices, *e.g.,* plasma, generally involves various analytical tools and/or chemical means. The qualitative and quantitative determination of artemisinin (**1**) are two major objectives usually the researchers want to achieve from any particular analytical protocol. Since the discovery of artemisinin (**1**) almost half a century ago, the chromatographic methods have evolved considerably, especially with the introduction of various hyphenated techniques[26] and the remarkable advancements in computational methods[25] leading to automation and precision of any analytical method. In the earlier years of artemisinin development, qualitative and quantitative determination of artemisinin (**1**) often involved thin layer chromatography, and then high performance thin layer chromatography. However, nowadays most of such analyses are carried out using modern

hyphenated techniques, *e.g.,* HPLC-UV, HPLC-PDA or HPLC-MS. Most of these analytical techniques or assays for artemisinin (**1**) reported until the end of year 2016 have been well documented and appraised in the book published by Tu[5]. However, since then, several modified, and somewhat more precise and fast analytical protocols (Table 2)[28-41], mainly involving various HPLC systems, have been reported in the literature. The following subsections will summarize and appraise those new published protocols.

3.1 Computer-assisted structure analysis

Artemisinin (**1**), albeit has only 15 carbon atoms, is a complex molecule (Figure 1) in terms of structure elucidation, especially concerning relative and absolute stereochemistry. Various 1D and 2D NMR techniques have previously been used to deduce the structure of this unique sesquiterpene[5]. However, attempts to improve the structure elucidation process involving modern computational aids, particularly to define the 3D structure of artemisinin (**1**) have continued. As a result of such an effort, Navarro-Vazquez et al.[28] have recently reported a computer-assisted 3D structural elucidation (CASE-3D) method based on the use of isotropic and/or anisotropic NMR data was established to elucidate relative configuration and preferred conformation in complex natural products like artemisinin (**1**). This approach incorporated the selection of conformational models through employing the Akaike Information Criterion (AIC) and scoring of the different configurations. It can be noted that AIC is an estimator of the relative quality of statistical models for a given set of data, considering the quality of each model, relative to each other models. Because of this computer-assisted 3D structural analysis, the correct configuration of the already known artemisinin (**1**) could be confirmed. The method described here was an extension of existing CASE-3D protocol using other NMR observables, mainly ^{13}C NMR chemical shifts, either alone or in conjunction with ^{1}H NMR chemical shifts, $^{1}D_{CH}$ RDCs, and $^{3}J_{HH}$ couplings.

In this study, all 1D and 2D NMR data were obtained on a commercially available sample of artemisinin (**1**), which were then fed into the MNova structural elucidation software[42]. After the ^{13}C NMR empirical chemical shift prediction, the first scored structure, as shown in Figure 1, was obtained. The next step involved feeding of 2D SDF file into the previously published Python programme[43], which encapsulated the diastereoisomer generation and conformational search steps, followed by the development of new scripts to automatically launch the DFT chemical shift computations. Because of the geometrical

hindrance created by the peroxide ring, only 32 diastereoisomers from possible 64 could be obtained. A list of ^{13}C NMR chemical shifts sourced from the automated peak picking in Mnova was fed into StereoFitter, which could be run with assigned or unassigned data sets. When the latter were used, it produced moderate discrimination of the relative configuration of artemisinin (**1**), with AIC differences ΔAIC = AIC – AIC_{min} of 13-14 over the closest scoring forms. A higher discrimination (ΔAIC > 25, relative probability $<2 \times 10^{-4}$ %) was obtained using the assigned data.

Navarro-Vazquez et al.[28] highlighted that $^1D_{CH}$ RDCs could not be sufficient to ascertain the relative configuration of artemisinin (1); RDC-only fitting could only result in the correct structure *S,R,S,R,S,R,R* and its isomer *R,R,S,R,S,S,R,* where C-1 and C-10 positions were epimerized with very similar AIC values (the correct configuration had a probability of 0.4 over the incorrect form). The probable reason behind this was explained with the fact that inversion at carbons C-1 and C-10 just could invert 180^o the CH vectors at that position with respect to a common molecular frame.

3.2 Cyclic voltametric analysis

Cyclic voltammetry is a potentiodynamic electrochemical measurement, where the working electrode potential is ramped linearly versus time[44]. After the set potential is reached in this experiment, the working electrode's potential is ramped in the opposite direction to return to the initial potential. A combination of cyclic voltammetry and amperometry was applied for the determination of electrolytic products of artemisinin (**1**)[29]. Although this was not exactly the method for quantitative or qualitative analysis of artemisisin (**1**), it was a method for analysing artemisinin derivatives.

In this experiment, amperometric and cyclic voltametric techniques were performed with a computer controlled electrochemical workstation (CHI 660c, USA) with 98 % ohmic drop compensation. A three-electrode electrochemical cell was used for all electrochemical experiments. Glassy carbon (area, 0.017 cm_2) was employed as the working electrode, and a platinum wire as the counter electrode. Artemisinin (**1**) was reduced on an electrode surface by cyclic voltammetry as well as amperometry to generate one major peak wave at -1.0 V and a minor one at -0.3 V vs Ag/AgCl reference electrode. The bulk electrolysis of artemisinin on a carbon electrode resulted in two other irreversible peak waves at around -0.7 and -0.1 V. An LC-MS method

was used to analyze the electrolytic products of artemisinin (**1**). Dihydroartemisinin was detected as the main reduction product, which produced further reduction products.

3.3 Fluorometric analysis

Fluorometry is superior to conventional spectrometry in terms of sensitivity and specificity. In fact, the sensitivity of fluorescence is 10-1000-fold greater in comparison to absorbance measurements. It is somewhat a bit difficult to design specific fluorescent probes for artemisinin (1) because this sesquiterpene does not have any groups for binding to excepting for the peroxide functionality (Figure 1). However, there are some probes available for now for this compound[30,31]. While a sensitive, rapid and simple fluorometric analytical method for the analysis of artemisinin (**1**) in a concentration range 0.1-7 μM using microperoxidase-11 as a peroxidase biomimetic has been reported recently[30], Zou et al.[31] published another fluorometric method for the determination of artemisinin (**1**) in tablets and dried leaf samples of *A. annua*. In the method presented by Muginova et al.[30], the determination of artemisinin (**1**) in a dietary supplement was based on the fluorescence quenching of the cationic xanthene dye pyronin B in the presence of microperoxidase-11; the correctness and reliability of the results were confirmed by HPLC-MS analysis. It was observed that the use of oligopeptide microperoxidase-11 in the place of haeme-containing proteins, *e.g.,* haemoglobin, could shorten the duration of artemisinin determination by a factor of two with the retention sensitivity and selectivity.

Zou et al.[31] reported that alkaline-hydrolyzed artemisinin (**1**) could specifically recognize and react with vitamin B1 to produce fluorometrically detectable thiochromes. This method could work at a low artemisinin (**1**) concentration in a Tris–HCl buffer solution (pH 7.5) at room temperature, resulting in a >260-fold enhancement in the blue emission at 442 nm. It was observed that the fluorescence intensity of the vitamin B1-based probes could linearly increase with increasing artemisinin (**1**) concentration range 1-230 μM /mL, and a detection limit as low as 11.5 nM/ mL could be achieved. Thus, this method was found to be more sensitive than other reported UV-vis absorption and electrochemical methods. Additionally, this method was proved to have better selectivity over other ions and biomolecules, did not require the preparation of fluorescent probes, and all the detection processes could be accomplished within 20 min. In this experiment, all fluorescence measurements were recorded using a Hitachi F-7000 Fluorescence Spectrophotometer

(Tokyo, Japan) with the excitation slit set at 5 nm band pass and emission at 2.5 nm band pass in 1 cm x 1 cm quartz cells, the UV-vis spectra were recorded on a Shimadzu UV-1750 spectrophotometer (Tokyo, Japan), and the fourier transform infrared (FTIR) spectra were recorded on a Shimadzu IR Prestige-21 spectrometer (Tokyo, Japan).

Earlier, Muginova et al.[38] demonstrated the utilization of a cellulose hydrogel film reconstituted from ionic liquid, 1-butyl-3-methylimidazolium chloride, for the fluorescent determination of artemisinin (**1**). Very recently, Zhu et al.[37] have published a method for the determination of artemisinin (**1**) by using graphene quantum dots as the fluorescent probes.

3.4 HPLC and UPLC methods

Nowadays, HPLC is one of the most popular analytical tools for the analysis of a variety of materials including phytochemicals, both qualitatively and quantitatively (Sarker and Nahar, 2012)[26]. HPLC can be coupled to various detection technologies, of which, a simple UV-Vis spectrometer is the most widely used one. UV-Vis detection can work with compounds that possess chromophores to absorb UV-Vis light. Various HPLC methods were previously applied for the qualitative and quantitative determination of artemisinin (**1**) in various matrices[45,46], and the use of simple HPLC-UV-Vis method for artemisinin (**1**) analysis has continued in recent years[18,32]. Some of the popular HPLC columns and solvent systems used in the analysis of artemisinin are presented in Table 3.

A simple HPLC-UV method for the determination of artemisinin (**1**) in the *n*-hexane extract of *A. annua* to determine the extraction yield has recently been published[18]. The HPLC analysis was carried out by the HPLC UltiMate 3000 system (Thermo Fisher Scientific, USA) coupled with a PDA detector, using Waters columns and gradient mobile phase comprising acetonitrile in water (Table 3). The artemisinin (**1**) content per dry weight of *Artemisia* species ranged from 0.07% to 0.45%, and the highest content of **1** was found in *A. annua* (0.45%), followed by *A. vachanica* (0.34%). The lowest artemisinin content was detected in *A. dracunculus* (0.07%). Guo et al.[32] reported a simple HPLC method for the quality testing of artemisinin (**1**)-based antimalarial medications prescribed in Myanmar.

Similarly, simple HPLC methods, but using different types of detectors, *e.g.,* refractive index and electrochemical detectors, have been outlined in two other recent studies[12,13], where HPLC analyses were employed to monitor the extraction yield of artemisinin (**1**). Rodriguez et al.[12] analyzed the artemisinin content of the *A. annua* extracts by using a Waters

HPLC system coupled with a refractive index detector (IR-Waters, 2414, Pittsburg, USA), employing the methanol-water solvent system and the column outlined in Table 3. The HPLC runs were performed at a slightly elevated temperature of 35°C. Negi et al.[13] reported a similar HPLC method, but using a reversed-phase C_{18} column (Table 3), for the quantification of artemisinin (**1**) to determine extraction efficiency; they also used a Waters HPLC system comprising a Spectra System P2000 pump with an SCM 100 vacuum membrane degasser and an electrochemical detector 2465 with glassy carbon electrode (3 mm in diameter), potential 180 mV. 200 nA.

When hyphenation is established between HPLC and mass spectrometry, the technique becomes significantly powerful, and provides rich structural information that facilitates identification of separated compounds from an HPLC run[26]. In recent years, LC-MS and/or LC-MS/MS (also known as LC-tandem MS) methods have been reported for the analysis of artemisinin (**1**)[11,33,34]. For example, an LC-MS/MS method for the determination of artemisinin (**1**) in rat blood sample for pharmacokinetic study[33], an LC-MS/MS method for simultaneous determination of artemisinin (**1**) and six synergistic components in *A. annua*, with a run time of 6 min[34], and an LC method, coupled with real time mass spectrometry (DART-MS) to quantify artemisinin (**1**) and related components of *A. annua*[11].

For the pharmacokinetic study with artemisinin (**1**), Dai et al.[33] performed the LC-MS/MS analysis on a Shiseido NANOSPACE 1312 HPLC system (Tokyo, Japan) coupled with an AB Sciex 4000 Q Trap (Ontario, Canada), using Analyst 1.5 (Applied Biosystems, USA) for data acquisition and quantification. A reversed-phase gradient elution comprising acetonitrile and water was used (Table 3). The retention time for artemisinin (**1**) was 2.3 min. In this experiment, the optimized MS/MS conditions were: source temperature 25◦C, ion spray voltage 5500 V, curtain gas 10 psi, nebulizing gas, 50 psi, turbo ion spray gas 50 psi, entrance voltage 4 V and dwell time 150 ms. Qiu et al.[34] reported a quality evaluation method based on an LC-MS/MS protocol and hierarchical cluster analysis for simultaneous quantification of artemisinin (**1**) and six synergistic components in *A. annua*. This accurate and rapid high-performance liquid chromatography tandem mass spectrometric (HPLC–MS/MS) assay was performed on an HPLC system comprising an LC-20AD pump, DGU-20 A3 degasser, SIL-20AC autosampler, and CTO-20A column oven (Shimadzu, Japan). In this study, like the previous one, a reversed-phase linear gradient elution comprising acetonitrile and water was used (Table 3). The LC system was hyphenated to an API 4000 Q Trap MS detector (Applied

Biosystems, Ontario, Canada) via a Turbo IonSpray ionization interface. The optimized MS operating conditions were quite similar to those mentioned earlier. Another LC method, coupled with real time mass spectrometry (DART-MS) to quantify artemisinin (**1**) and related components of *A. annua* has been reported recently[11].

Ultra Performance Liquid Chromatography (UPLC) is an advanced liquid chromatographic technique that offers a significantly short analysis time and small amount of solvent(s) as a mobile phase[47]. Although this technique has been around for over 15 years, only in recent years, its use in the analytical area has become popular because of affordability of the commercially available UPLC units. This system utilizes a special column packed with much smaller particles (typically 1.5-1.7 mm), instead of 3 or 5 mm particles used routinely in column packing for phytochemical analysis. The application of UPLC methods in the analysis of artemisinin (**1**) has also become more frequent recently[17,19,39]. Ruan et al.[17] have recently reported a simple UPLC-PDA method for the analysis of artemisinin (**1**), where they used An ACQUITY UPLC BEH C_{18} column (100 x 2.1 mm, 1.7 μm), and an isocratic elution with the mobile phase comprising 0.1% formic acid aqueous solution and acetonitrile (40:60). The injection volume was 1 μL, and the data were recorded at 191 nm. An Agilent 1290 UPLC system (Agilent, USA), comprising a pump, degasser, column oven, autosampler and PDA detector was used. A similar UPLC method has also been reported by Zhang et al.[19] for the quantification of artemisinin (**1**) to determine extraction efficiency from an ultrasonic extraction of *A. annua*, where they used a Waters UPLC system equipped with Empower2 software, a Waters autosampler, a UV detector, and an ACQUITY UPLC® BEH C_{18} column (1.7 μm, 2.1 mm × 50 mm; Waters, Ireland). It can be noted that although the column diameter and particle size were exactly the same as those used in the method presented by Ruan, the column length was just 50 mm as opposed to 100 mm. In addition, instead of a mobile phase comprising acetonitrile in water, in this study, they used methanol in sodium acetate aqueous solution, and instead of an isocratic elution, a gradient elution was employed. Raju et al.[39] used a UPLC system with a HSS Cyano column (100 x 2.1 mm, particle size: 1.8 μm) and the mobile phase consisting of 20 mM ammonium formate buffer (pH 6.5) and 0.04% formic acid in methanol in a gradient elution programme, which was developed using the systematic trials suggested by the software using quality by design (QbD), which is rather a new approach in analytical chemistry, particularly where chromatographic methods are used[48].

3.5 Molecular imprinting method

Molecular imprinting technology is a method of making a molecular lock to match a molecular key to create molecularly imprinted polymers with tailor-made binding sites complementary to the template molecules in shape, size and functional groups. This technique has recently been used in the analysis of artemisinin (**1**)[40,41]. For example, molecularly imprinted membranes with multifunctional layers for the separation and purification of artemisinin (**1**) were constructed on porous chitosan membranes[40], and a biomimetic electrochemical sensor for the determination of artemisinin (**1**) in plant extracts and for pharmacokinetic studies was introduced where a novel molecularly imprinted polymer-based electrochemical sensor was developed by electropolymerization of *o*-phenylenediamine in the presence of artemisinin (**1**) on gold wire surface[41]. Zhang et al.[40] fabricated the molecularly imprinted layer through an in situ activator generated by electron transfer-atom transfer radical polymerization method by using artemisinin (**1**) as the template molecule. It was demonstrated that this type of molecularly imprinted membrane could have desirable adsorption ability for artemisinin (about 19 mg/g). This method could offer an environmentally friendly and sustainable technology for separation and purification of artemisinin (**1**).

Waffo et al.[41] reported a novel biomimetic electrochemical sensor was developed for rapid, sensitive and specific determination of artemisinin (**1**) using a simple and cost-effective approach. The formation of both non-imprinted polymer and molecularly imprinted polymer layers could be qualitatively characterized spectrophotometrically. Thus, marker bands distinctive for the artemisinin, the o-PD monomer as well as the electrochemically formed polymer could be identified and were used for monitoring the entire underlying process. In such way, polymer synthesis, template removal and rebinding of artemisinin to the polymer-coated electrode could be confirmed. It was suggested that the assay protocol developed in their work could offer the least complex approach for artemisinin detection to date. It was also mentioned that the developed molecularly imprinted polymers could enable measurement of artemisinin (**1**) in an artificial plant matrix, containing five different substances found in *A. annua*, with high selectivity and sensitivity.

3.6 Miscellaneous

Raman spectroscopy is based on inelastic scattering of monochromatic light, usually from a laser source. Inelastic scattering means that the frequency of photons in monochromatic light changes upon interaction with a sample. Raman spectroscopy has recently been used to analyse artemisinin (**1**)[36]. In fact, Kong et al.[36] reported the qualitative and quantitative analysis of artemisinin (**1**) using Raman spectroscopy, where they used the spectral range of 100~3500 cm^{-1}. It was demonstrated that the phonon mode at 724 cm^{-1} could be directly correlated with a representative vibrational mode of the ring containing the endoperoxy bridge, and could be utilized for Raman detection of this bridge in artemisinin (**1**), making it a artemisinin-specific detection technique. Similarly, the phonon mode at 734 cm^{-1} could be implicated to the vibrational mode of the lactone ring, and could be used for further identification of artemisinin using Raman spectroscopy. By studying the relative intensity ratio of these two phonon modes, the Raman method could be applied for quantitative analysis of artemisinin purity. It was suggested that this method was much more powerful, faster, more convenient, more accurate than HPLC-based methods, and could be applied for the analysis of homogeneity of purity of artemisinin samples offering a practical quality control measure for the Chinese medicines based on *A. annua*.

A microsensor array coupled with electrochemiluminescence imaging technique, which is a widely used analytical technique with the advantages of high sensitivity and low background signal, was used for the detection of artemisinin (**1**) in human serum and *A. annua*[35]. The microsensor array was constructed by integrating a patterned indium tin oxide glass plate with two perforated hydrophobic paper covers. This method could show a good selectivity and stability towards artemisinin detection.

4. CONCLUSIONS

Supercritical fluid extraction and ultrasound-assisted extraction are still the methods of choice for extraction of artemisinin (**1**). Newer analytical tools, as well as improved protocols for known analytical tools, for the qualitative and quantitative determination of artemisinin (**1**) have been made available by various researchers during the period covered by this review. Although LC-based assays for artemisinin have dominated this period, a clear shit from conventional HPLC- to UPLC-based methods has been noted.

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REFERENCES

1. Boden R. Editorial: On artemisinin, avermectins and Nobel Prizes. *FEMS Microbiol Letts.* 2015; 362(23): Article number fnv 199.
2. White NJ, Hien TT, Nosten FH. A brief history of Qinghaosu. *Trends Parasitol.* 2015; 31(12): 607-610.
3. Wang JG, Xu CC, Wong YK, et al. Artemisinin, the magic drug discovered from Traditional Chinese Medicine. *Engineering* 2019; 5(1): 32-39.
4. Wong YK, Xu CC, Kalesh KA, et al. Artemisinin as an anticancer drug: Recent advances in target profiling and mechanisms of action. *Medicinal Res Rev.* 2017; 37(6): 1492-1517.
5. Tu Y. From *Artemisia annua* L. to artemisinins: The discovery and development of artemisinins and antimalarial agents. 1st Edition, Academic Press, USA, 2017.
6. Briars R, Paniwnyk L. Examining the extraction of artemisinin from *Artemisia annua* using ultrasound. In B.B.J. Linde, J. Pączkowski & N. Ponikwicki (Eds). *International Congress on Ultrasonics: Gdańsk* 2011; pp 581-585. American Institute of Physics.
7. Baldino L, Reverchon E, *Artemisia annua* organic solvent extract, processed by supercritical CO_2. *J Chem Technol Biotechnol.* 2018; 93(11): 3171-3175.
8. Sixt M, Schmidt A, Mestmacker F, et al. Systematic and model-assited process design for the extraction and purification of artemisinin from *Artemisia annua* L. Part I: Conceptual process design and cost estimation. *Processes* 2018; 6(9): Article number: 161.

9. Sixt M, Strube J. Systematic and model-assited evaluation of solvent based – or pressurized hot water extraction for the extraction of artemisinin from *Artemisia annua* L. *Processes* 2017; 5(4): Article number 86.
10. Laboukhi-Khorsi S, Daoud K, Chemat S. Efficient solvent selection approach for high solubility of active phytochemicals: application for the extraction of an antimalarial compound from medicinal plants. *ACS Sustainable Chem Eng.* 2017; 5(5): 4332-4339.
11. Wang JQ, Zhou YY, Wang M, et al. High-throughput analysis for artemisinins with deep eutectic solvents mechanochemical extraction and direct analysis in real time mass spectrometry. *Anal Chem.* 2018; 90(5): 3109-3117.
12. Rodrigues MFF, Sousa IMO, Vardanega R, et al. Techno-economic evaluation of artemisinin extraction from *Artemisia annua* L. using supercritical carbon dioxide. *Industrial Crops & Products* 2019; 132: 336-343.
13. Negi AS, Cortesi A, Kikic I, et al. Desorption of artemisinin extracts of CIM-Arogya by supercritical carbon dioxide. *J Supercritical Fruids* 2018; 133: 42-48.
14. Baldino L, Revenchon E, Della Porta G. An optimized process for SC-CO_2 extraction of antimalarial compounds from *Artemisia annua* L. *J Supercritical Fluids* 2017; 128: 89-93.
15. Martinez-Correa HA, Bitencourt RG, Kayano ACAV, et al. 2017; Integrated extraction process to obtain bioactive extracts of *Artemisia annua* L. leaves using supercritical CO_2, ethanol and water. *Industrial Crops & Products* 2017; 95: 535-542.
16. Ciftci ON, Cahyadi J, Guigard SE, et al. Optimization of artemisinin extraction from *Artemisia annua* L. with supercritical carbon dioxide plus ethanol using response surface methodology. *Electrophoresis* 2018; 39(15): 1926-1933.
17. Ruan JQ, Liu ZY, Qiu F, et al. Simultaneous quantification of five sesquiterpene components after ultrasound extraction in *Artemisia annua* L. by an accurate and rapid UPLC-PDA assay. *Molecules* 2019; 24(8): article number 1530.
18. Numonov S, Sharopov F, Salimov A, et al. Assessment of artemisinin contents in selected *Artemisia* species from Tajikistan (Central Asia). *Medicines* 2019; 6(1): DOI:10.3390/medicines6010023.
19. Zhang YQ, Prawang P, Li CS, et al. Ultrasonic assisted extraction of artemisinin from *Artemisia annua* L. using monoether-based solvents. *Green Chem.* 2018; 20(3): 713-723

20. Silva ED, Borges LL, da Conceicao EC, et al. Box-Behnken experimental design for extraction of artemisinin from *Artemisia annua* and validation of the assay method. *Braz J Pharmacog* 2017; 27(4): 519-524.
21. Cao J, Yang M, Cao FL, et al. Well-designed hydrophobic deep eutectic solvents as green and efficient media for the extraction of artemisinin from *Artemisia annua* leaves. *ACS Sustainable Chem Eng.* 2017; 5(4): 3270-3278.
22. Nahar L, Sarker SD. Supercritical fluid extraction in natural products analysis. In "Natural Products Isolation" (Editors: Sarker, S. D. and Nahar, L.), 3rd Edition, Humana Press/Springer-Verlag, New Jersey, pp 43-74; 2012.
23. Manjare SD, Dhingra K. Supercritical fluids in separation and purification: A review. *Materials Science for Energy Technologies* 2019; 2: 463-484.
24. Baldino L, De Luca R, Revenchon E. Scale-up and economic analysis of a supercritical CO_2 plant for antimalarial active compounds extraction. *Chem Eng Trans.* 2018; 64: 1-6.
25. Sarker SD, Nahar L. Computational Phytochemistry, 1st Edition, Elsevier Limited, London, 2018.
26. Sarker SD, Nahar L. Natural Products Isolation, 3rd Edition, Humana Press – springer Verlag, New Jersey, 2012.
27. Chemat F, Rombaut N, Sicaire A-G, Meullemiestre A, Fabiano-Tixier A-S, Albert-Vian M. Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols, and applications. A review. *Ultrasonics Sonochemistry* 2017; 34: 540-560.
28. Navarro-Vazquez A, Gil RR, Blinov K. Computer-assisted 3D structure elucidation (CASE-3D) of natural products combining isotropic and anisotropic NMR parameters. *J Nat Prods.* 2018; 81(1): 203-210.
29. Obwayo F, Mazzochette Z, Mugweru A. Electrochemical reduction of artemisinin: chromatographic identification of bulk electrolysis products. *J Electrochemical Sci Eng.* 2019; 9(3): 165-174.
30. Muginova SV, Vakhraneva ES, Myasnikova DA, et al. Fluorometric determination of artemisinin using the pyronin B-microperoxidase-11 system. *J Anal Chem.* 2019; 74(2): 100-107.

31. Zou W, Gong FC, Cao Z, et al. A simple and effective strategy for detecting artemisinin based on oxidative cyclization of vitamin B-1 eliciting fluorescence turn-on. *Analytical Methods* 2019; 11(1): 88-96.
32. Guo SQ, Kyaw MP, He L, et al. Quality testing of artemisinin-based antimalarial drugs in Myanmar. *Am J Trop Med Hygiene* 2017; 97(4): 1198-1203.
33. Dai TM, Jian WF, Guo Z, et al. Comparison of *in vitro/in vivo* blood distribution and pharmacokinetics of artemisinin, artemether and dihydroartemisinin in rats. *J Pharm Biomed Anal.* 2019; 162: 140-148.
34. Qiu F, Wu S, Lu XR, et al. Quality evaluation of the artemisinin-producing plant *Artemisia annua* L. based on simultaneous quantification of artemisinin and six synergistic components and hierarchical cluster analysis. *Industrial Crops & Products* 2018; 118: 131-141.
35. Tang XC, Xiao X, Li, et al. Simple, fast and sensitive detection of artemisinin in human serum and *Artemisia annua* using microsensor array coupled with electrochemiluminescent imaging technique. *Talanta* 2019; 196: 124-130.
36. Kong MH, Wu DX, Chen XB, et al. Qualitative and quantitative studies on artemisinin with Raman spectroscopy. *Spectroscopy & Spectral Anal.* 2017; 37(3): 778-782.
37. Zhu S, Yan X, Sun J, et al. A novel and sensitive fluorescent assay for artemisinin with graphene quantum dots based on inner filter effect. *Talanta* 2019; 200: 163-168.
38. Muginova SV, Vakhraneva ES, Myasnikova DA, et al. Fluorescence-based artemisinin sensing using a pyronin B-doped cellulose film reconstituted from ionic liquid. *Anal Letts.* 2018; 51(6): 870-891.
39. Raju CHJ, Rao TB, Goud PSK, et al. Development and validation of liquid chromatography method using the principles of QbD for antimalarials used in artemisinin based combination therapy. *J Liq Chromatog Related Technol.* 2018; 41(17-18): 955-963.
40. Zhang YQ, Tan X, Liu X, et al. Fabrication of multi-layered molecularly imprinted membrane for selective recognition and separation of artemisinin. ACS Sustainable Chem Eng. 2019; 7(3): 3127-3137.
41. Waffo AFT, Yesildag C, Caserta G, et al. Fully electrochemical MIP sensor for artemisinin. *Sensors & Actuators B-Chemical* 2018; 275: 163-173.
42. MNova 12.0, MestReLab Research SL: Santiago de Compostela, 2017.

43. Torche-Pesqueira E, Anklin C, Gil RR, et al. Computer-assited 3D structure elucidation of natural products using residual dipolar couplings. *Angew Chem Int Ed Engl.* 2017; 56(13): 3660-3664.

44. Elgrishi N, Rountree KJ, McCarthy BD, et al. A practical beginner's guide to cyclic voltammetry. *J Chem Educ.* 2018; 95(2): 197-208.

45. Ferreira JFS, Gonzalez JM. Analysis of underivatized artemisinin and related sesquiterpene lactones by high-performance liquid chromatography with ultraviolet detection. *Phytochem Anal*. 2009; 20: 91–97.

46. Lapkin AA, Walker A, Sullivan N, et al. Development of HPLC analytical protocol for artemisinin quantification in plant materials and extracts. *J Pharm Biomed Anal.* 2009; 49: 908-915.

47. Nahar L, Sarker SD. Guest editorial: UPLC in phytochemical analysis. *Trends Phytochem Res.* 2019; 3: 1-2.

48. Panda SS, Beg S, Bera RKVV, et al. Implementation of quality by design approach for developing chromatographic methods with enhanced performance: A mini review. *J Anal Pharm Res.* 2016; 2(6): 1-4.

49. Chen L, Wang X, Lu W, et al. Molecular imprinting: perspectives and applications. *Chem Soc Rev.* 2016; 45: 2137-2211.

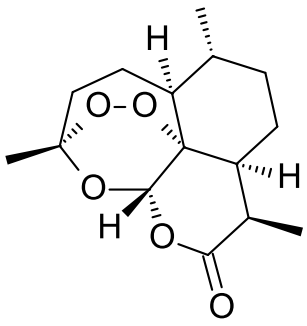

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FIGURE 1 The molecular structure of artemisinin (**1**), isolated from *Artemisia annua* L.

TABLE 1 Different extraction methods for artemisinin (**1**) reported during 2017-19

Extraction methods	**Brief details**
Combination of maceration and supercritical CO_2 extraction	The aerial parts of *A. annua* was macerated with *n*-hexane to obtain a crude extract, which was further extracted with supercritical CO_2 extraction to obtain artemisinin-enriched extract[7].
Extraction based on model-assited process design	A systematic process design, based on lab-scale experiments and miniplant-scale pilot, for the extraction and purification of artemisinin (**1**) from *A. annua*[8].
	Artemisinin (**1**) was extracted from *A. annua* by percolation using acetone[9].
	Pressurized hot water extraction was performed at 80^0C to extract artemisinin (**1**) from *A. annua*[9].
Extraction using Hansen solubility parameters in practice approach	Isopropanol was used for artemisinin (**1**) extraction from *A. annua* with the yield of 65%[10].
Mechanochemical extraction using deep eutectic solvents	A high-throughput analytical method employing deep eutectic solvents for mechanochemical extraction combined with direct analysis in real time mass spectrometry (DART-MS) was developed to quantify thermolabile artemisinin (**1**) and related components of *A. annua*[11].
Supercritical fluid extraction	Optimization of operating conditions, *e.g.,* temperature, pressure and co-solvent use to obtain artemisinin-enriched extract[12].
	A supercritical CO_2 method for the extraction of artemisinin from *A. annua* in temperature and pressure rages of 313.1-333.1 K and 15-25 MPa[13].
	Supercritical CO_2 extraction of artemisinin (**1**) from *A. annua* was optimized, and the best extraction yield was achieved using 100 bar pressure and 40^oC temperature[14].
	Supercritical CO_2 extraction, followed by extraction with ethanol and water, was used to extract artemisinin (**1**) from the leaves of *A. annua*[15].
Supercritical fluid extraction using response surface methodology	Supercritical CO_2 extraction of artemisinin (**1**), modified with ethanol, based on response surface methodology developed from central composite rotatable design, that helped optimize extraction pressure, temperature, and co-solvent use[16].
Ultrasound extraction	Ultrasound-assited extraction of the leaves of *A. annua*[17].

	Ultrasound-assisted extraction of *A. annua* aerial parts using *n*-hexane[18].
	Ultrasound extraction of artemisinin (1) using mono-ether based solvents, *e.g*., propylene glycol methyl ether[19].
	Box-Behnken experimental design was employed for ultrasound-assisted extraction of artemisinin (**1**) from *A. annua* using ethanol as the solvent[20].
	Ultrasound-assited extraction of artemisisin (**1**) from the leaves of *A. annua* was performed using a deep eutectic solvent[21].

TABLE 2 Different assays and analytical methods for artemisinin (**1**) reported during 2017-19

Assays/analytical methods	**Brief details**
Computer-assited 3D structure analysis	A computer-assisted 3D structural elucidation method based on the use of isotropic and/or anisotropic NMR data was established to elucidate relative configuration and preferred conformation in complex natural products like artemisinin (**1**)[28].
Cyclic voltametric analysis	A combination of cyclic voltammetry and amperometry for the determination of electrolytic products of artemisinin (**1**)[29].
Fluorometric analysis	A fluorometric analytical method for artemisinin (**1**) using microperoxidase-11 as a peroxidase biomimetic[30].
	For the determination of artemisinin (**1**) in tablets and dried leaf samples of *A. annua*[31].
HPLC methods	A simple HPLC-UV method for the determination of artemisinin (**1**) in the *n*-hexane extract of *A. annua*[18].
	A simple HPLC method was used for the quality testing of artemisinin (**1**)-based antimalarial medications prescribed in Myanmar[32].
	Quantification of artemisinin in the crude extract obtained from SFE [12,13].
LC-MS method	An LC-MS/MS method for the determination of artemisinin (**1**) in rat blood sample for pharmacokinetic study[33].
	An LC-MS/MS method for simultaneous determination of artemisinin and six synergistic components in *A. annua*, with a run time of 6 min[34].
	An LC method, coupled with real time mass spectrometry (DART-MS) was developed to quantify thermolabile artemisinin (**1**) and related components of *A. annua*[11].
Microsensor array coupled with electrochemiluminescent imaging	A microsensor array coupled with electrochemiluminescence imaging technique for the detection of artemisinin (**1**) in human serum and *A. annua*[35].
Raman spectroscopic analysis	Qualitative and quantitative analysis of artemisinin (**1**) was performed using Raman spectroscopy[36].
Sensitive fluorescent assay or fluorescence-based sensing	A method for the determination of artemisinin (**1**) by using graphene quantum dots as the fluorescent probes[37].

	The utilization of a cellulose hydrogel film reconstituted from ionic liquid, 1-butyl-3-methylimidazolium chloride, for the fluorescent determination of artemisinin (**1**)[38].
UPLC-PDA assay	An ACQUITY UPLC BEH C_{18} column was used and an isocratic elution with the mobile phase comprising 0.1% formic acid aqueous solution and acetonitrile (40:60)[17].
UPLC method utilizing the principles of QbD	A UPLC system using a HSS Cyano column (100 x 2.1 mm, particle size: 1.8 μm) and the mobile phase consisting of 20 mM ammonium formate buffer (pH 6.5) and 0.04% formic acid in methanol in a gradient elution programme was developed using the systematic trials suggested by the software using QbD[39].
	Quantification of artemisinin (**1**) to determine extraction efficiency[19].
Molecular imprinting method	Molecularly imprinted membranes with multifunctional layers for the separation and purification of artemisinin (**1**) were constructed on porous chitosan membranes[40].
	A biomimetic electrochemical sensor for the determination of artemisinin (**1**) in plant extracts and for pharmacokinetic studies. A novel molecularly imprinted polymer-based electrochemical sensor was developed by electropolymerization of *o*-phenylenediamine in the presence of artemisinin (**1**) on gold wire surface[41].

TABLE 3 Some most popular and effective HPLC columns and solvent systems for artemisinin (**1**) analysis

HPLC columns	**Solvent systems**
Luna 5 μm C_{18}, 250 x 4.6 mm, Phenomenex, USA	Isocratic elution with 65% acetonitrile in water with a flow rate of 1 mL/min, monitored at 192 nm[46,47].
Betasil C_{18} 5 μm, 250 x 4.6 mm, Thermo Fisher Scientific, USA	
Waters Bridge C_{18} 5 μm, 250 x 4.6 mm, Waters, USA	Gradient elution: 0–7 min, hold 60% of acetonitrile in water; 17–30 min, 60–100% of acetonitrile in water; 30–35 min, 100% of acetonitrile. The detection wavelengths were 192, 210, 254, and 320 nm, the flow rate was 1 mL/min, the injection volume was 5 mL, and the oven temperature was set 30°C[18].
XSelect CSH C_{18} 5 μm, 250 x 4.6 mm, Waters, USA	
Cyan column, Luna CN 5 μm, 250 x 4.6 mm, Phenomenex, USA	Isocratic elution with 50% methanol in water with a flow rate of 1 mL/min, at 35°C[12], using a refractive index detector
Discovery RP-C_{18}, 5 μm, 250 x 4.6 mm, Supelco, USA.	Isocratic elution with 50% methanol in water with a flow rate of 1 mL/min for 15 min, maintaining the column temperature at 303.1K, using a electrochemical detector[13].
LUBEX Ecosil ODS-3 column, 50 x 2.1 mm, 5 μm, Guangzhou, China	The mobile phase A = 5% acetonitrile in water, and B = 95% acetonitrile in water; both phases had 10 mM ammonium acetate. In the LC gradient profile, the mobile phase B was 20% for 0.4 min and linearly increased to 100% from 0.4 to 0.6 to 0.6 min, maintained at this composition from 0.6 to 2.6 min, and returned to 20% from 2.8 to 3.0 min. The total running time was 4.0 min[33]. Flow rate was 0.25 mL/min.
Zorbax XDB C18 3.5 μm, 50 x 2.1 mm, Agilent, UK	The mobile phase was composed of 0.1% formic in water (A) and 0.1% formic acid in acetonitrile (B)[34]. A linear gradient was used at a flow rate of 0.50 mL/min as follows: 2% B at 0–0.50 min, 2–98% B at 0.50–3.00 min, 98% B at 3.00–4.50 min, 98–2% B at 4.50–4.51 min, and 2% B at 4.51–6.00 min. The separation was carried out at 20°C.