

MINI REVIEW

Analysis of Artemether and Dihydroartemisinin by high performance high liquid chromatography in biological fluids-issues and solutions

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Abstract: Artemether-Lumefantrine is the most widely recommended antimalarial combination used to treat millions of patients suffering from malaria. Artemether undergoes rapid metabolism and gets converted to its active metabolite dihydroartemisinin. Drug analysis is a vital aspect to evaluate drugs in research. There are a number of methods available for the determination of artemether in biological fluids. These methods include HPLC based UV detection, GS-MS, HPLC-ECD and HPLC-MS/MS. This article reviews different methods for the determination of artemether in the biological fluids. Among the available methods HPLC-MS/MS proves to be the most accurate and reliable one for analysis. This has the advantage of improved sensitivity and selectivity with smaller sample volume.

Keywords: Malaria, Artemether, Dihydroartemisinin, HPLC, Analytical methods, HPLC MS/MS.

INTRODUCTION

The malaria is a serious tropical disease and poses a major health problem in most parts of the world especially in South East Asia and Africa. Drug resistance has emerged as an alarming and big challenge in the treatment of malaria (WHO, 2008). The use of conventional classical antimalarial drugs in *Plasmodium falciparum* has caused a considerable rise and increase in the development of resistance which has initiated the discovery and development of new and alternative antimalarial drugs (Price, 2000). Scientists at the Academy of Military Medical Sciences in Beijing, People's Republic of China synthesized and developed Co-artemether which is a combination of newly discovered artemisinin derivative, Artemether (ARM) and a longer acting, arylalcohol, Lumefantrine an antimalarial drug (van Agtmael *et al.*, 1999). This fixed dose oral artemisinin combination therapy (ACT) preparation is highly recommended and is used to treat millions of patients each year (Rehwagen, 2006). After administration Artemether undergoes extensive and quick metabolism and is converted to the active metabolite Dihydroartemisinin (DHA) in the human tissues (van Agtmael *et al.*, 1998) which is said to be more active antimalarial than the parent drug (White, 2008).

Analysis of drugs is an extremely important aspect of pharmacological as well as biological research. For the development of effective and new antimalarial combinations, it is utmost necessary to perform pharmacokinetic studies. The evaluation and development

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of new methods for the detection of the drugs in biological fluids is a prerequisite to these studies. Analysis and determination of drug plasma levels is a very crucial step in order to find out the cause of treatment failure and to investigate if resistance could be the cause.

Although ACT are extensively used still there is relatively limited data reported on the pharmacokinetics of artemisinin derivatives (White *et al.*, 1999). Due to this reason it is very difficult to measure artemether and dihydroartemisinin in the body fluids (White, 1993). A researcher faces major problems in the discovery and validation of selective methods for detection and analysis of artemisinin and its derivatives since the drugs are sensitive to high temperature and due to the absence of functional groups the reliable derivatisation becomes very difficult (Teja-Isavadharm *et al.*, 2004). Furthermore, the activity and analysis of these drugs is inadequate as it requires assays which are sensitive enough to detect these compounds in the low concentration range i.e. nanogram per millilitre (Edwards, 1994).

Analytical methods

For the determination and quantification of artemether and dihydroartemisinin, High performance liquid chromatography (HPLC) proves to be the most popular method. Before injecting samples into chromatographic system, purification steps have been used as liquid-liquid extraction, solid-phase extraction and derivatization procedures. All artemisinin derivatives are heat labile and for the enhancement of their stability low temperatures are required (Lindergardh *et al.*, 2009). Several High

Performance Liquid Chromatography analytical methods have been developed including HPLC with Ultraviolet (UV) detection (Edlund *et al.*, 1984; Idowu *et al.*, 1989; Thomas *et al.*, 1992; Muhia *et al.*, 1994; Batty *et al.*, 1996; Cesar *et al.*, 2008), bioassay (Teja-Isavadharm *et al.*, 2004) HPLC with electrochemical detection (ECD) (Zhou *et al.*, 1987; Melendez *et al.*, 1991; Navaratnam *et al.*, 1995; Teja-Isavadharm *et al.*, 1996; Sandrenan *et al.*, 1997; Karbwang *et al.*, 1997; Navaratnam *et al.*, 1997; van Agtmael *et al.*, 1998) and liquid chromatography with mass spectrometry (LC-MS) (Na-Bangchang *et al.*, 1998; Souppart *et al.*, 2002; Sabrinath *et al.*, 2003; Peys *et al.*, 2005; Yu *et al.*, 2006; Xing *et al.*, 2006; Shi *et al.*, 2006; Hanpithakpong *et al.*, 2008; Huang *et al.*, 2009; Lindergardh *et al.*, 2009). Following is the summary of different analytical methods developed for the analysis of artemether and dihydroartemisinin in biological fluids.

HPLC-UV

It is a strong, workhorse method excellent for the analysis of drugs in formulations where there is no interference from the excipients. HPLC-UV method has turned out to be a cost effective and easy to use with good precision for making quantitative measurements of drugs in formulations (Thomas *et al.*, 1992). The loopholes in the basic method can be overcome by the use of derivative spectra. This method is only moderately selective. Due to the lack of chromophore moieties in the structure of artemisinin, the selectivity of the method becomes very difficult (White *et al.*, 1999). This method cannot be applied for the analysis of mixtures.

This method has been shown to be less sensitive for the detection of artemether and arteether due to lack of UV or fluorescent chromophore moieties in their structure while highly sensitive for artemisinin, artesunate and dihydroartemisinin (Teja-Isavadharm *et al.*, 1996; Souppart *et al.*, 2002). Several HPLC methods for the determination of ARM and DHA have been developed using UV detector but there exists certain limitations. Some of the methods adopted for determination of artemether and its active metabolite Dihydroartemisinin by UV detection are discussed below:

One of the methods introduced by Thomas and Edward (1992) for the quantification of artemether and its active metabolite DHA is by HPLC with UV detector. This method yields to produce UV-absorbing product, an alpha, beta unsaturated decatone, by extraction of plasma with dichloromethane, solid phase separation of the two analytes and acid decomposition with hydrochloric acid followed by chromatography on a C18 column. The mobile phase used was acetonitrile: Water (50:50, v/v) while the internal standard was progesterone with a run time of 30 min. Detection was done at 254nm while the retention time was 10.5 and 17min for ARM and DHA respectively.

Muhia *et al* (1994) suggested that although absorption of the artemisinin compounds in the UV region is very poor but still can be readily reduced to alpha, beta unsaturated decatone and alpha, beta aldehydes which can be detected at 254nm. The basis of the separation was shown to be pH dependent with differential extraction of the drugs from plasma. The mobile phase used was water-acetonitrile (40:60) and derivatized extracts were chromatographed on a 5- μ m ODS column. The internal standard used was progesterone. The limit of detection was 10ng/ml. Previously expensive absorption cartridges (BondElut) were used but this developed method excluded the need for these cartridges and shorter elution time was observed in comparison with previous methods. LLOQ was 10ng/ml in 0.5ml sample. Similarly, Edlund *et al* (1984) reported LOD at 19 and 14ug/l for ARTS and DHA respectively.

Another method was developed by Batty *et al* (1996) using UV detector. This method was used for artesunate and alpha- and beta-DHA, using post alkali decomposition. Analysis was carried by C8 column and detected at 290nm while extraction was performed with solid phase extraction cartridges. The mobile phase was 50% acetonitrile in 0.1M acetate buffer (pH 4.8). The LOD was 20ug/l for DHA.

A recently introduced method by César *et al* (2008) using HPLC-UV for the simultaneous determination of artemether and DHA with a diode array detector (DAD) at 210nm, turned out to be highly sensitive and reliable one. The columns selected were Zorbax SB-Ciano (150 \times 4.6 mm i.d.; 5 μ m particle size) and Symmetry C₁₈. The volume used was 20 μ l. Acetonitrile and 0.05% trifluoroacetic acid (60:40, v/v), an isocratic mobile phase was used. LOD was 5 μ g/ml whereas LOQ of artemether was 15 μ g/ml.

Post column derivatisation methods in which the conversion of artemether and dihydroartemisinin to UV-detectable analytes has established different results, while the reproducibility is very poor too. In addition, haemolysis of blood samples greatly affected these methods (Muhia *et al.*, 1994; Batty *et al.*, 1996). Moreover they are sensitive to acid and base treatments (Idowu *et al.*, 1989). In these methods sensitivity has still been limited (Limit of detection around 20-30 ng/ml) (Edlund *et al.*, 1984).

HPLC-ECD

This technique is highly recommended for the simultaneous detection of artemisinin derivatives in biological fluids because of its precision, specificity and linearity to analyze the parent drug (Batty *et al.*, 1996; Karbwang *et al.*, 1997; van Agtmael *et al.*, 1998) artemether, arteether or artemisinin and a major metabolite dihydroartemisinin simultaneously (Zhou *et al.*, 1987).

Table: Comparison of different analytical methods for the determination of ARM and DHA by HPLC techniques.

S#	Technique	Detector	Columns	Mobile phase	Sensitivity and productivity
1	César et al	Diode array detector DAD	Zorbax SB-Ciano (150×4.6mm, 5µm)	Acetonitrile and 0.05% trifluoroacetic acid (60:40, v/v)	LOD was 5 µg/ml LOQ of ARM was 15 µg/ml
2	Ali et al	LCMSMS	Hypersil Gold C18 (100×2.1mm, 5 µm)	Methanol-ammonium acetate 10mM pH 3.5 (70:30, v/v)	LOD of ARM was 0.5 ng/ml, LLOQ: 1.4 ng/ml
3	Sanderan et al	ECD	Nova-PakCN,4m column (150mm x 3.9 mm I.D.)	Acetate/acetonitrile (85:15, v/v) pH:5	LOQ: ARM: 10.9ng/ml DHA 11.2 ng/ml
4	Niklas et al	LC-MS/MS	Hypersil Gold C18 (100 mm × 2.1 mm, 5µm)	Acetonitrile/ammonium acetate 10mM pH 3.5 (50:50, v/v)	LOD: ARM: 0.257 ng/ml
5	Navaratnam et al	ECD	100 CN stainless steel column 250x4.0mm, 5µm	Acetonitrile-0.05M acetic acid, (15:85 v/v), pH 5.0	LOQ : ARM: 2.5ng/ml DHA: 1.25ng/ml
6	Muhia et al	UV	5µm ODS column(10mmx 4.6mm)	Water/acetonitrile (40/60, v/v)	LOD: ARM+DHA: 10ng/ml
7	Souppart et al	LC-MS	C18 RP column	Acetonitrile- glacial acetic acid 0.1% (66.34)	LOQ: ARM+DHA: 5ng/ml
8	Karbwang et al	ECD	Waters Bondapak CN column (15cmx3.9mm)	Acetonitrile/water (20:80,v/v)	LOD: ARM: 5ng/ml DHA: 3ng/ml

Zhou *et al* (1987) developed a novel and sensitive method using electrochemical detector for the analysis of the antimalarial drugs artesunic acid (ARTS) and dihydroqinghaosu (DQHS) in blood. The limit of detection is 4µg/ml for ARTS and 200ng/ml for DQHS.

Similarly a selective reproducible high-performance liquid chromatographic assay using electrochemical detection in the reductive mode was introduced by Navaratnam *et al* (1997). Simultaneous quantitation of artesunic acid (ARS) and dihydroartemisinin (DQHS) was done using Artemisinin (QHS) as an internal standard. The column selected for the analysis of ARS and DQHS was Econosil C8 with artemisinin as an internal standard and acetonitrile-0.05M acetic acid (42:58, v/v) adjusted to pH 5.0. used as a mobile phase. The LOD in plasma was 4.0 ng/ml for both the compounds. The LOD was 2.5 and 1.25mg/ml for ARS and DHA respectively.

Karbwang *et al* (1997) described an accurate and reproducible method using electrochemical detection in the reductive mode. This method was found to be a reliable one the determination of artemether (ARM) and dihydroartemisinin (DHA: alpha and beta isomers) in plasma. The method involved the extraction of ART, DHA with dichloromethane-tert.-methylbutyl ether (1:1, v/v) or n-butyl chloride-ethyl acetate (9:1, v/v). Chromatographic separation was performed with a mobile phase of acetonitrile-water (20:80, v/v) containing 0.1 M acetic

acid pH 5.0, running through a micro Bondapak CN column. The internal standard was artemisinin. The retention times of alpha-DHA, beta-DHA, ARN and ART were 4.6, 5.9, 7.9 and 9.6 min, respectively. LOD for ARM and alpha-DHA were 5 and 3ng/ml, respectively.

A method using reductive electrochemical detection (ED) was set up with some important modifications as compared to previously published assays by van Agtmael *et al* (1998). A different technique of deoxygenating resulted in a factor 2-3 lower background current. A Spectroflow 400 liquid chromatograph in combination with a Triathlon auto injector coupled to a Decade electrochemical detector in the reductive mode was used as a closed system under chromatography grade helium to exclude any access of oxygen. It was possible to determine the various derivatives in the same chromatogram by increasing acetonitril or lowering the pH of the mobile phase. The concentration range was 5 to 220 ng/ml.

In the same manner, Sanderan *et al* (1997) described a method with electrochemical detection in the reductive mode at a potential of -1.0 V. Extraction of ARM, DHA and artemisinin, the internal standard from 1 ml plasma was done with 1-chlorobutane-isooctane (55:45, v/v). The analytes were dried by evaporation under nitrogen with subsequent addition of 600 microliters of water-ethyl alcohol (50:50, v/v) to dissolve the reconstituted samples.

The mobile phase consisted of pH 5 acetate-acetonitrile (85:15, v/v). Chromatography was performed on a Nova-Pak CN, 4 microns analytical column (150mm x 3.9mm I.D.) at 35 degrees C. For ARM, LOQ was 10.9ng/ml and for DHA, the LOQ was 11.2ng/ml

Although ECD in the reductive mode using the endoperoxide configuration of the compounds has enabled better sensitivity (LOQ 5-20ng/ml) but this method requires rigorous procedures, difficult extractions, expensive instrumentation, definite stability and high profile techniques (Zhou *et al.*, 1987; Na-Bangchang *et al.*, 1998). The method employed cannot be utilized on daily basis as complications occur due to the entry of dissolved oxygen into the flow cell which causes noise due to the high potentials used and baseline drift. These results in low number of samples being analyzed per day with LOD 5-10 µg/L (Karbwang *et al.*, 1997).

HPLC-MS

Detection of the drugs can be accurately achieved by means of Mass spectrometry. This method was found to be reliable and highly selective for quantification and confirming the structure of drugs and raw materials used in their manufacturing along with their individualization (Xing *et al.*, 2006). Liquid chromatography (LC) coupled with mass spectrometry (MS) is said to be a gold method used with great success and has a potential for the precise validation of artemisinin derivatives in the biological fluids (Sabarinath *et al.*, 2003). Furthermore HPLC-MS/MS has proven to be a basic tool for the analysis and identification of impurities and other excipients (Souppart *et al.*, 2002). Liquid chromatography coupled with mass spectrometry has during the last couple of years been used with great potential and success for quantification of artemisinin derivatives in biological fluids with improved sensitivity (Sabarinath *et al.*, 2003). This method is a preferred one as it has improved sensitivity for pharmacokinetic analysis of artemether and its active metabolite dihydroartemisinin. A very small plasma volume of 0.5ml is used for validation in the range of 5-200ng/ml (Hanpithakpong *et al.*, 2008). This method is considered as a preferred choice.

Earlier it was Souppart *et al* (2002) who employed an accurate and selective method by using LC-MS in a single ion monitoring mode using atmospheric pressure chemical ionization (APCI) as an interface for the determination of ARM and DHA in human plasma. The plasma samples (0.5ml per assay) underwent liquid-liquid extraction followed by dryness of the supernatant by means of evaporation. Artemisinin was used as an internal standard. Acetonitrile-glacial acetic acid 0.1% (66:34) was used as a mobile phase while chromatography was performed on a C (18) reversed-phase column (Zhou *et al.*, 1987). Validation of the method was carried out over a

concentration range of 5-200ng/ml. The lower limit of quantification was set at 5ng/ml for both the compounds. Sabarinath *et al* (2003) developed a method with good linearity and analysis of alpha-,beta-arteether (alpha-,beta-AE) and its metabolite alpha-dihydroartemisinin (DHA) in monkey plasma by mass spectrometry in the multiple reaction monitoring mode. The internal standard was propyl ether analogue of beta-arteether (PE). Liquid-liquid extraction with hexane was carried out and C (18) reversed-phase chromatographic column was selected for the separation of the analytes by isocratic elution with methanol-ammonium acetate buffer (pH 4) (92: 8, v/v) Run time of the chromatography was 7 min. The limit of detection and lower limit of quantification in monkey plasma were 0.39 and 0.78 ng ml(-1) respectively for all the analytes.

A highly selective LC-MS/MS method was described by Huang *et al* (2009) validated and introduced an accurate LC-MS/MS method for the detection of artemether and its metabolite dihydroartemisinin in human plasma using electrospray ionization (ESI) as the ionization mode. The separation and extraction of 0.5ml of human plasma was done with solid phase extraction technique. C₁₈ LC column (Waters, Symmetry[®], 150 mm × 4.6 mm, 5 µm) was selected and the elutes were analyzed by injecting onto it. Artemisinin was used as an internal standard. In order to improve ionization (M+NH₄⁺) ammonium formate was added to the LC mobile phase keeping in view the low ionization capacity of artemether. The LLOQ was (2ng/mL).

Peys *et al* (2005) also described a method for the quantification of β-artemether (AM) and its metabolite dihydroartemisinin (DHA) in human plasma and urine by liquid chromatography mass spectrometry (LC-MS) using positive electrospray ionisation (ESI). Liquid-liquid extraction was carried out using 2,2,4-trimethylpentane - ethyl acetate (7:3 v/v) The supernatant obtained was evaporated to dryness and analyzed. Artemisinin was used as internal standard. The reconstituted samples were run through a C18 reversed-phase column using a gradient of acetonitrile- ammonium acetate 10mM, glacial acetic acid 0.1% as a mobile phase. The improved method is sensitive and reliable.

A more accurate and sensitive LC/MS/MS method was developed by Xing *et al* (2006) to monitor artemisinin, artemether and the internal standard with an electrospray ionization (ESI) TurboIonSpray inlet in the positive ion multiple reaction monitoring (MRM) mode. It followed with a simple liquid-liquid extraction of the samples with ether. The method had a lower limit of quantification of 1.0ng/mL for artemisinin in 100µl of plasma.

Shi *et al* (2006) obtained very good results with high-performance liquid-chromatographic-tandem mass

spectrometric (MS) method using atmospheric-pressure chemical-ionization (APCI) interface. Artemisinin was used as an internal standard while the mobile phase was acetonitrile-0.1% formic acid solution, 80:20 (v/v). LLOQ of 5ng mL⁻¹ were achieved for ARM and DHA

Hanpithak *et al* (2008) introduced a LC-MS/MS method coupled to positive ion mode tandem mass spectroscopy which proved to be a reliable and sensitive one for the determination of artemether and dihydroartemisinin in human plasma. Extraction was done in the 96-well plate format. An improved sensitivity and detection was observed by using a lower plasma volume with LOD at 0.36 ng/ml while the LOD was 5-10ng/ml in the previous developed methods. This improved method has a wide range application in the clinical setup especially in pediatric cases where very low plasma volume posse's limitations.

Recently Niklas *et al* (2009) described a reliable LC-MS/MS method with high sensitivity and specification. In order to facilitate a rapid extraction of a large number of samples a day i.e 192 in this case, Oasis HLB™ μ -elution solid phase extraction 96-well plates were used. For the analysis of artemisinin and artesunate (internal standard) liquid chromatography and MS/MS detection on a Hypersil Gold C18 (100 mm \times 2.1 mm, 5 μ m) column was carried out. Mobile phase used was acetonitrile-ammonium acetate 10mM pH 3.5 (50:50, v/v) at a flow rate of 0.5mL/min. A very low plasma volume was used (50 μ L). The limit of detection was 0.257 ng/mL for artemisinin.

Ali *et al* (2010) similarly used a low plasma volume (50 μ l) by developing and validating a sensitive lower limit of quantification robust high throughput LC-MS/MS method. Analysis of the compounds was done on a Hypersil Gold C18 (100 \times 2.1 mm, 5 μ m) column protected by a security guard column with a Hypersil Gold C18 (10 \times 2.1 mm, 3 μ m) guard cartridge under isocratic conditions using a mobile phase containing methanol-ammonium acetate 10 mM pH 3.5 (70:30, v/v) at a flow rate of 500 μ L/min with a wash out gradient. The LOD was 0.5 ng/mL and LLOQ was 1.43ng/ml

CONCLUSION

Analytical methods holds an important application in the development of the new drugs, evaluation of their pharmacokinetic profile and assessing their clinical efficiency. This review shows that most methods allow quantitative determination of artemether and dihydroartemisinin in biological fluids but the most favorite method for the analysis of artemether and dihydroartemisinin has changed to LC-MS. The advantages are improved sensitivity and selectivity with

smaller sample volume as low as 50 μ L. (table given below)

REFERENCES

- World Health Organization (2008). World Malaria Report Price RN (2000). Artemisinin drugs: novel antimalarial agents. *Expert Opin. Investig. Drugs*, **8**: 1815-27.
- Van Agtmael MA, Eggelte TA and van Boxtel CJ (1999). Artemisinin drugs in the treatment of malaria: from medicinal herb to registered medication. *Trends Pharmacol. Sci.*, **5**: 199-205.
- Rehwagen C (2006). WHO Ultimatum on artemisinin monotherapy is showing results. *BMJ.*, **332**: 1176.
- Van Agtmael MA, Van Der Graaf CA, Dien TK, Koopmans RP and van Boxtel CJ (1998). The contribution of the enzymes CYP2D6 and CYP2C19 in the demethylation of artemether in healthy subjects. *Eur. J. Drug Metab. Pharmacokinet.*, **3**: 429-436.
- White NJ (2008). Qinghaosu (artemisinin): The price of success. *Science*, **320**: 330-334.
- White NJ, van Vugt M and Ezzet F (1999). Clinical pharmacokinetics and pharmacodynamics and pharmacodynamics of artemether-lumefantrine. *Clin Pharmacokinet.*, **37**(2): 105-25.
- White NJ (1993). Clinical pharmacokinetics and pharmacodynamics of artemisinin and derivatives. *Trans. R. Soc. Trop. Med. Hyg.*, **88**: S41-S43.
- Teja-Isavadharm P, Peggins JO, Brewer TG, White NJ, Webster HK and Kyl DE (2004). Plasmodium falciparum-based bioassay for measurement of artemisinin derivatives in plasma or serum. *Antimicrob Agents Chemother.*, **48**(3): 954-960.
- Edwards G (1994). Measurement of artemisinin and its derivatives in biological fluids. *Trans R Soc Trop Med Hyg.*, **88**: Suppl S37-9.
- Lindegardh N, Hanpithakpong W, Kamanikom B, Singhasivanon P, Socheat D, Yi P, Dondorp AM, McGready R, Nosten F, White NJ and Day B NPJ (2009). Major pitfalls in the measurement of artemisinin derivatives in plasma in clinical studies. *J. Pharm. Biomed. Anal.*, **49**(3): 768-773.
- Edlund PO, Westerlund D, Carlqvist J, Bo-Liang W and Yun-Hua J (1984). Determination of artesunate and dihydroartemisinin in plasma by liquid chromatography with post-column derivatization and UV-detection. *Acta. Pharm. Suec.*, **21**: 223-234.
- Idowu OR, Edwards G, Ward S A, Orme ML and Breckenridge AM (1989). Determination of arteether in blood plasma by high-performance liquid chromatography with ultraviolet detection after hydrolysis acid. *J. Chromatogr.*, **493**: 125-136.
- Thomas CG, Ward SA and Edwards G (1992). Selective determination, in plasma, of artemether and its major metabolite, dihydroartemisinin, by high-performance liquid chromatography with ultraviolet detection. *J. Chromatogr.*, **583**(1): 131-136.

- Muhia DK, Mberu EK and Watkins WM (1994). Differential extraction of artemether and its metabolite dihydroartemisinin from plasma and determination by high-performance liquid chromatography. *J. Chromatogr. B. Biomed. Appl.*, **660**(1): 196-199.
- Batty KT, Davis TM, Thu LT, Binh TQ, Anh TK and Ilett KF (1996). Selective high-performance liquid chromatographic determination of artesunate and α - and β -dihydroartemisinin in patients with falciparum malaria. *Journal of Chromatography B., Biomedical Sciences and Applications*, **677**(2): 345-350.
- César I da C, Nogueira FHA and Pianetti GA (2008). Simultaneous determination of artemether and lumefantrine in fixed dose combination tablets by HPLC with UV detection. *J. Pharm. Biomed. Anal.*, **48**(3): 951-954.
- Zhou Z M, Anders J C, Chung H and Theoharides AD (1987). Analysis of artesunic acid and dihydroqinghaosu in blood by high-performance liquid chromatography with reductive electrochemical detection. *J. Chromatogr.*, **414**: 77-90.
- Melendez V, Peggins JO, Brewer TG and Theoharides AD (1991). Determination of the antimalarial arteether and its deethylated metabolite dihydroartemisinin in plasma by high-performance liquid chromatography with reductive electrochemical detection. *J. Pharm. Sci.*, **80**: 132-138.
- Navaratnam V, Mansor SM, Chin LK, Mordi MN, Asokan M and Nair NK (1995). Determination of artemether and dihydroartemisinin in blood plasma by high-performance liquid chromatography for application in clinical pharmacological studies. *J. Chromatogr. B. Biomed. Appl.*, **669**(2): 289-294.
- Teja-Isavadharm P, Nosten F, Kyle DE, Luxemburger C, Ter Kuile F, Peggins JO, Brewer TG and White NJ (1996). Comparative bioavailability of oral, rectal and intramuscular artemether in healthy subjects: Use of simultaneous measurement by high performance liquid chromatography and bioassay. *Br. J. Clin. Pharmacol.*, **42**(5): 599-604.
- Sandrenan N, Sioufi A, Godbillon J, Netter C, Donker M and van Valkenburg C (1997). Determination of artemether and its metabolite, dihydroartemisinin, in plasma by high-performance liquid chromatography and electrochemical detection in the reductive mode. *J. Chromatogr. B. Biomed. Sci. Appl.*, **69**(1): 145-153.
- Karbwang J, Na-Bangchang K, Molunto P, Banmairuroi V and Congpuong K(1997). Determination of artemether and its major metabolite, dihydroartemisinin, in plasma using high-performance liquid chromatography with electrochemical detection. *J. Chromatogr. B. Biomed. Sci. Appl.*, **690**(1-2): 259-265.
- Navaratnam V, Mordi MN and Mansor SM (1997). Simultaneous determination of artesunic acid and dihydroartemisinin in blood plasma by high-performance liquid chromatography for application in clinical pharmacological studies. *J. Chromatogr. B. Biomed. Sci. Appl.*, **692**(1): 157-62.25.
- van Agtmael MA, Butter JJ, Portier EJ and van Boxtel CJ (1998). Validation of an improved reversed-phase high-performance liquid chromatography assay with reductive electrochemical detection for the determination of artemisinin derivatives in man. *Ther. Drug Monit.*, **(1)**: 109-16.
- Na-Bangchang K, Congpuong K, Hung LN, Molunto P and Karbwang J (1998). Simple high-performance liquid chromatographic method with electrochemical detection for the simultaneous determination of artesunate and dihydroartemisinin in biological fluids. *J. Chromatogr. B. Biomed. Sci. Appl.*, **708**: 201-207.
- Souppart C, Gauducheau N, Sandrenan N and Richard F (2002). Development and validation of a high-performance liquid chromatography-mass spectrometry assay for the determination of artemether and its metabolite dihydroartemisinin in human plasma. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.*, **774**(2): 195-203.
- Sabarinath S, Rajanikanth M, Madhusudanan KP and Gupta RC (2003). A sensitive and selective liquid chromatographic/electrospray ionization tandem mass spectrometric assay for the simultaneous quantification of alpha-,beta-artether and its metabolite dihydroartemisinin in plasma, useful for pharmacokinetic studies. *Journal of mass spectrometry.*, **38**(7): 732-42.
- Peys E, Vandekerckhove J, Hemel VJ and Sas B (2005). Simultaneous Determination of β -Artemether and its Metabolite Dihydroartemisinin in Human Plasma and Urine by a High-Performance Liquid Chromatography-Mass Spectrometry Assay Using Electrospray Ionisation. *Chromatographia.*, **61**: 11-12.
- Yu Y, Li Z, Zhang L, Zhong Y, Su S and Liang S (2006). Quantitative Analysis of Artemether and its Metabolite Dihydroartemisinin in Human Plasma by LC with Tandem Mass Spectrometry Bin Shi. *Chromatographia.*, **64**: 9-10.
- Xing J, Yan H, Zhang S, Ren G and Gao Y (2006). A high-performance liquid chromatography/tandem mass spectrometry method for the determination of artemisinin in rat plasma. *Rapid Commun. Mass Spectrom.*, **20**(9): 1463-1468.
- Shi B, Yu Y, Li Z, Zhang L, Zhong Y, Su S and Liang S (2006). Quantitative Analysis of Artemether and its Metabolite Dihydroartemisinin in Human Plasma by LC with Tandem Mass Spectrometry. *Chromatographia.*, **64**: 9-10.
- Hanpithakpong W, Kamanikom B, Singhasivanon B, White NJ, Day NPJ and Lindgardh N (2008). A liquid chromatographic-tandem mass spectrometric method for determination of artemether and its metabolite dihydroartemisinin in human plasma. *Bioanalysis.*, **1**(1): 37-46.

- Huang L, Jayewardene AL, Li X, Marzan F, Lizak PS and Aweeka FT (2009). Development and validation of a high-performance liquid chromatography/tandem mass spectrometry method for the determination of artemether and its active metabolite dihydroartemisinin in human plasma. *J. Pharm. Biomed. Anal.*, **50**(5): 959-965.
- Lindegardh N, Tarning J, Toi JVP, Hien TT, Farrar J, Singhasivanon P, White NJ, Ashton M and Day NPJ (2009). Quantification of artemisinin in human plasma using liquid chromatography coupled to tandem mass spectrometry. *J. Pharm. Biomed. Anal.*, **49**(3): 768-773.
- Ali S, Najmi MH, Tarning J and Lindegardh N (2010). Pharmacokinetics of artemether and dihydroartemisinin in healthy Pakistani male volunteers treated with artemether-lumefantrine. *Malar. J.*, **9**: 275.