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Determination of sodium artesunate in plasma using ion-pairing high-performance liquid chromatography

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Abstract

A chromatographic method is described for the determination of sodium artesunate in plasma. This includes cetyltrimethylammonium bromide as a cationic pairing ion in a reversed-phase system using an octadecylsilica 100×4.6 mm I.D. 3 μm analytical column with a mobile phase of acetonitrile/acetate buffer at pH7. Column switching incorporating a 5 μm octadecylsilica 100×4.6 mm I.D. precolumn is used in addition to off-line solid-phase extraction for pretreatment of plasma samples in order to eliminate interference from endogenous components. Detection is by post-column derivatisation with 1.0 *M* methanolic KOH followed by UV detection at 289 nm. Calibration is linear over the range 100–1600 ng ml^{-1} and the limit of detection is estimated as 20 ng ml^{-1}. Illustrative results are shown of the artesunate plasma levels determined by the proposed method following the administration of artesunate as tablets and as suppositories to healthy volunteers.

Keywords: Sodium artesunate

1. Introduction

Sodium artesunate, the structure of which is shown in Fig. 1, is one form of the antimalarial chemical derived from the traditional Chinese herb Qinghaosu or artemisinin [1]. Following administration, sodium artesunate is rapidly metabolised to the active metabolite dihydroartemisinin which exists as two isomers α and β. It has been reported that artesunate is detectable in plasma for a relatively short time compared to dihydroartemisinin [2,3]. Limited data, using small numbers of individual

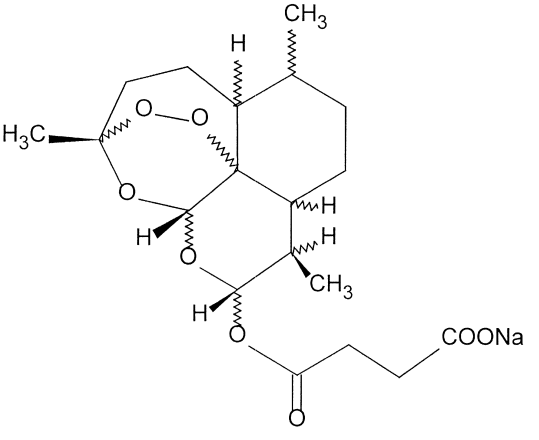

Fig. 1. Structure of sodium artesunate.

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subjects, on the disposition of these compounds in the body have been obtained by previous studies [2–7]. There is a need for additional, well-validated, analytical and pharmacokinetic data on these compounds. Due to the low intrinsic molar absorptivity in the ultraviolet region of these compounds, the main methods for quantification used in these studies have been electrochemical detection [3–6] or post-column derivatisation with methanolic alkali and subsequent ultraviolet detection in the region 289–290 nm [2,8]. It has been reported in the literature that the determination of these compounds in plasma represents a challenging problem [9].

As part of a more extensive pharmacokinetic study, an assay procedure for the determination of artesunate and dihydroartemisinin using the post-column derivatisation detection method was validated using bulk plasma. When this procedure was applied to subject samples obtained from Sudan it was found that the dihydroartemisinin could be quantified as anticipated. Peaks due to endogenous compounds, however, in almost all the particular subject samples, consistently obscured the artesunate peak. This rendered the determination of artesunate impractical due to the magnitude and variability of the endogenous peaks.

The present paper describes how the selectivity of the chromatographic system was manipulated to allow the separate determination of artesunate alone. The method is an application of previously established ion pairing liquid chromatography [10]. It will have general applicability in drug assay situations where a particular matrix contains unforeseen endogenous components. This problem of variable matrix interference is general and will be most significant when the responses of these components to the detection system used are comparable to that of the analyte. Thus the selectivity of the assay becomes poorer as the analyte concentration approaches the limit of quantification.

2. Experimental

2.1. Equipment

The chromatographic arrangement is shown in Fig. 2. The equipment used comprised a Jasco (Essex, UK) PU-880 isocratic chromatography pump coupled to a Rheodyne (Cotati, CA, USA) 7125 six port injection valve and a 100×4.6 mm stainless steel precolumn slurry packed in the laboratory with 5 μm Hypersil ODS (HETP, Macclesfield, UK). The eluent of acetonitrile–0.1 *M* acetate buffer, pH 7, containing 60 m*M* cetyltrimethylammonium bromide (60:40, v/v) at a flow-rate of 0.8 ml min^{-1} was then passed to a Rheodyne 7000 switching valve. A second Jasco PU-980 pump delivering the same solvent at a flow-rate of 0.7 ml min^{-1} was connected to this valve. A 0.5 ml sample was injected into the

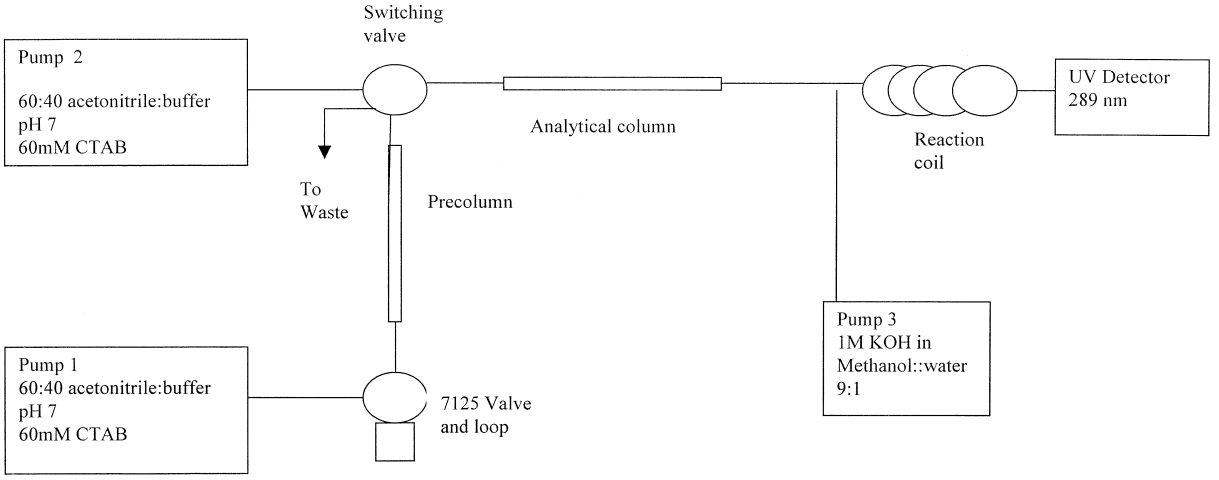

Fig. 2. Diagram of chromatographic system.

loop of the 7125 Rheodyne valve. Three min after transferring the sample to the precolumn the Rheodyne 7000 switching valve was operated and the eluent from the precolumn diverted from waste to the analytical column. This arrangement allowed additional clean up of the sample after off-line pretreatment by rejecting the initial rapidly eluting components (0–3 min.) to waste. It also maintained steady flow conditions through the 100×4.6 mm I.D. analytical column which was similarly packed with 3 μm Hypersil ODS. A zero dead volume tee piece inserted after the analytical column allowed the addition of 1 *M* potassium hydroxide in methanol–water (9:1, v/v) by a an additional Jasco PU-980 pump at a flow-rate of 0.3 ml min^{-1}. After passing through a PTFE reaction coil (3 m×0.5 mm I.D.) immersed in a water bath thermostatted at 70°C detection was at 289 nm in a Jasco UV-975 detector. Chromatograms were recorded on a standard potentiometric recorder. Retention times were recorded from the time of switching the solvent containing the appropriate analytes to the analytical column. Standards of sodium artesunate, artemisinin and dihydroartemisinin were kindly donated by Mepha Ltd., Aesch-Basel, Switzerland. All other chemicals used were of analytical grade.

2.2. Sample pretreatment

2-Naphthoic acid was selected as the internal standard since it behaved similarly to sodium artesunate in the chromatography system developed. Acetate buffer (0.5 ml) and 100 μl of 1 μg ml^{-1} aqueous solution of 2-naphthoic acid were added to a 0.5 ml plasma sample containing sodium artesunate. After vortex mixing and centrifugation at 13 000 *g* and off-line solid-phase extraction using Phenyl Bondelut cartridges [2] the analytes were reconstituted in 0.3 ml 50% aqueous methanol. Immediately before injection an additional 0.2 ml water were added. The total sample solution was injected into the chromatograph.

Recoveries were calculated by comparison of the peak heights obtained by applying the above pretreatment to a known (spiked) concentration of analyte with that obtained by injection of the same concentration of aqueous solution.

The solvent system used was established by determining the retention times of artesunate, artemisinin and dihydroartemisinin in a selection of mobile phases (acetonitrile-buffer pH7 (60:40, v/v) containing increasing concentrations of CTAB (10–100 m*M*). Plasma samples containing the interfering endogenous components were spiked with artesunate and dihydroartemisinin and subjected to solid-phase extraction. The resulting extracts were chromatographed using a mobile phase containing the optimum concentration of CTAB. Recovery was determined as follows. Peak heights produced following extraction of plasma samples spiked with artesunate at 100, 200, 400, 800 and 1000 ng ml^{-1} were measured. These were compared with those obtained following direct injection of the same concentration prepared in the solvent used for extract reconstitution.

2.3. Quantitation

Using the optimised solvent system the linearity of calibration was established by measuring the peak height ratio artesunate/internal standard as a function of artesunate concentration. Five sodium artesunate concentrations covering the range 100–1600 ng ml^{-1} were used. The internal standard 2-naphthoic acid was incorporated in each at a fixed concentration of 100 ng ml^{-1}.

To determine the accuracy of the method plasma was spiked with specific concentrations of artesunate. These concentrations covered the calibration range. The percentage difference between the mean concentration value determined from the calibration line and the concentration of sodium artesunate spiked was calculated.

Within day precision was determined by injection of 6 extracts of plasma spiked with sodium artesunate at 100 and 800 ng ml^{-1} and 7 replicates of extracts of plasma spiked with sodium artesunate at 20 and 40 ng ml^{-1}. Day to day precision was determined by injecting all standards in the range 100–1600 ng ml^{-1} on four successive days.

The limit of detection was established by injecting plasma extracts containing successively smaller concentrations of artesunate and measuring the signal-to-noise ratio.

3. Results and discussion

3.1. Pretreatment

The mean recovery for the artesunate species was determined as 95.7±4.3% (n=7). This figure is higher than previously reported for this compound and may be a result of the addition of acetate buffer prior to solid-phase extraction. It is comparable with the recovery of 96.7±2.4 found for dihydroartemisinin using this extraction procedure. The mean recovery of the 2-naphthoic acid internal standard from plasma was 69±2% (n=4).

3.2. Separation

Fig. 3 shows the variation of the retention times of artesunate and related compounds as a function of CTAB concentration. The retention of the artesunate anion initially increases, goes through a maximum and subsequently decreases as CTAB concentration increases. The retention times of the neutral analytes α and β-dihydroartemisinin and artemisinin decrease at varying rates under these conditions until at a CTAB concentration of 40 mM they co-elute at a retention time of 5 min. Fig. 4 shows a chromatogram of blank plasma run with a mobile phase containing 60 mM CTAB as pairing ion and shows no peaks after approximately 8 min. The addition of CTAB has thus reduced the retention times of these endogenous peaks. Fig. 4b is a representative chromatogram of plasma spiked with 100 ng ml^{-1} of the internal standard 2-naphthoic acid. Fig. 4c is a representative chromatogram of plasma spiked with artesunate at a concentration of 800 ng ml^{-1} and 2-naphthoic acid at a concentration of 100 ng ml^{-1}. Fig. 4 serves to establish peak identity for the assay. The related compounds dihydroartemisinin and artemisinin are essentially unretained in this solvent system as shown in Fig. 3.

Previous chromatographic systems for the determination of artesunate and its metabolite dihydroartemisinin [2–6] have employed reversed-phase systems using octadecylsilica stationary phase with a buffered aqueous mobile phase containing about 38–50% v/v acetonitrile as organic modifier. Such systems achieved resolution of these compounds and also separation of artemisinin used as an internal standard. The separation depends upon the varying hydrophobicities of the individual species since in this system at pH 4.8–5.0 the ionisation of the artesunate is suppressed. In the present method

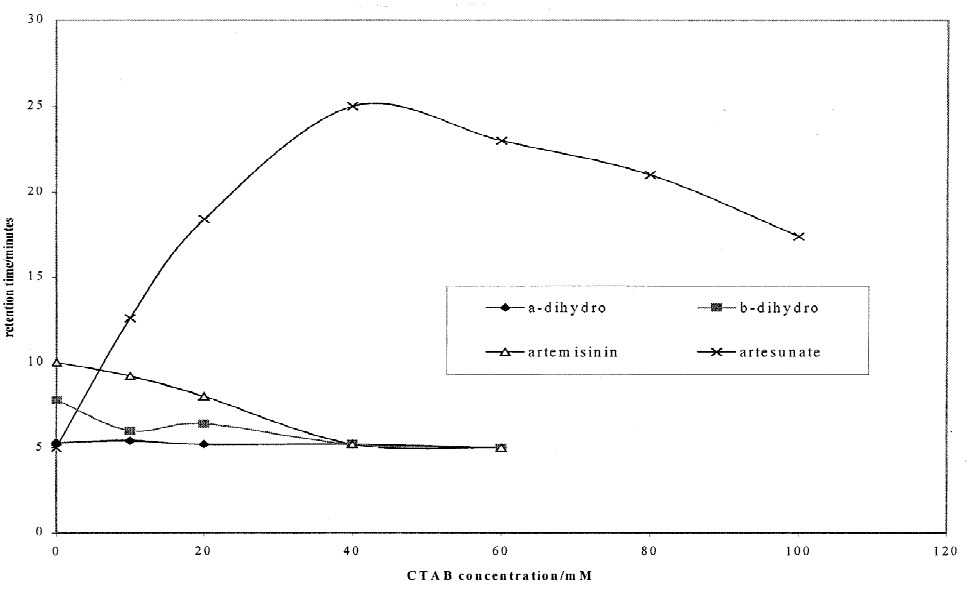

Fig. 3. Variation of retention times of artesunate and related compounds with CTAB concentration in a mobile phase of acetonitrile–pH 7 buffer (60:40, v/v). Compound identification as indicated in figure.

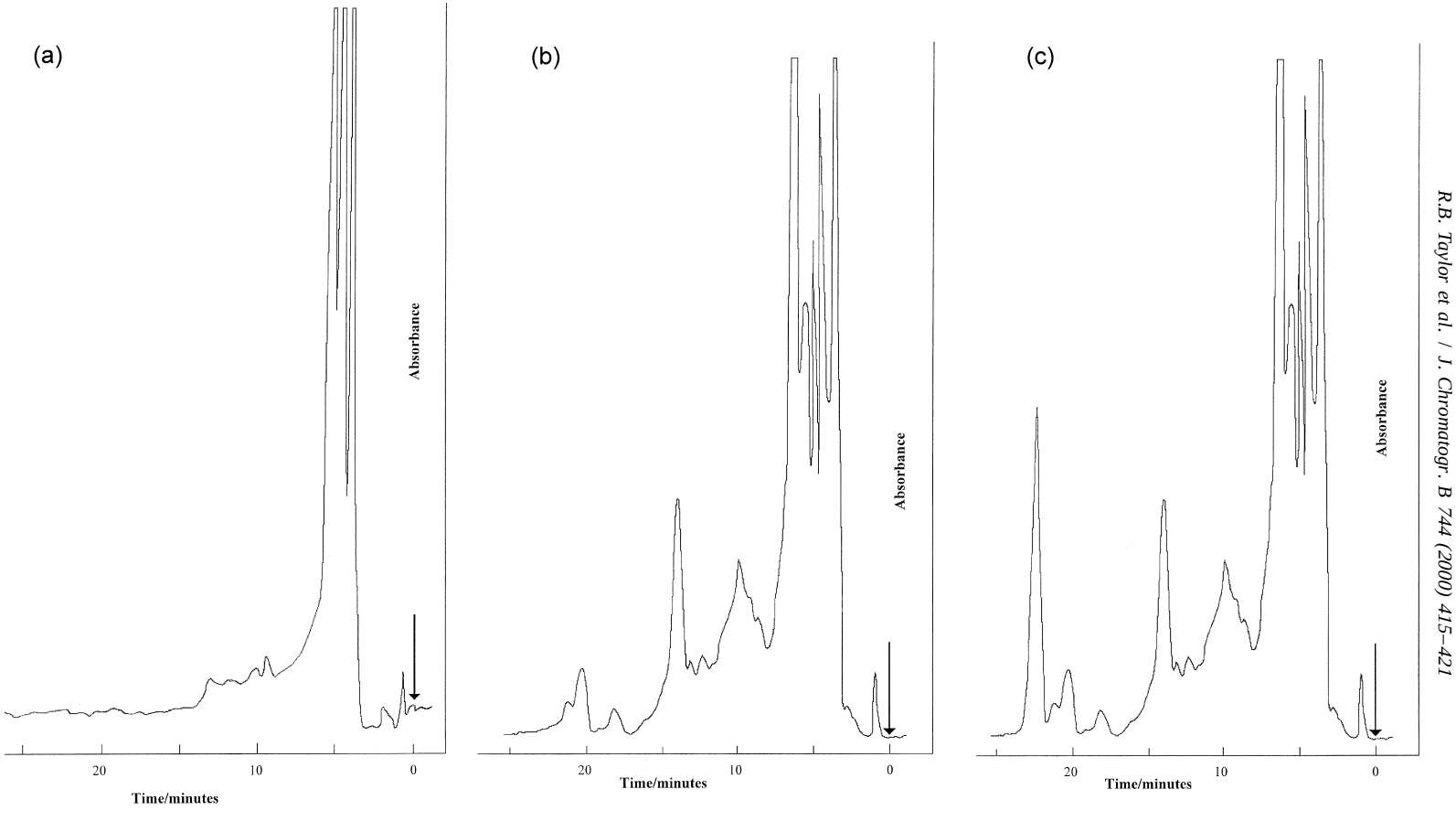

Fig. 4. Representative chromatograms using a mobile phase of acetonitrile–pH 7 buffer (60:40, v/v) containing 60 m*M* CTAB of (a) blank plasma, (b) plasma spiked with 2-naphthoic acid (100 ng ml^{-1}), (c) plasma spiked with 2-naphthoic acid (100 ng ml^{-1}) and sodium artesunate (800 ng ml^{-1}).

CTAB is added to the mobile phase and the pH adjusted to 7. The consequent adsorption of the cationic surfactant on to the octadecylsilica surface dramatically alters the selectivity for this set of artemisinin related analytes. At this pH, the artesunate is appreciably ionised and its retention is increased as a result of coulombic interaction with adsorbed CTAB. The partial coverage of the octadecylsilica surface by the CTAB reduces the availability of surface for hydrophobic desolvation of analytes which are electrically neutral with consequent reduction in their retention times. Likewise the retention of endogenous species, if they are uncharged at this pH, should be reduced. In addition, anionic solutes should be more readily separable from the anionic artesunate in such ion pairing systems on the basis of their generally increased capacity factors.

3.2.1. Method validation

3.2.1.1. Linearity of calibration. The variation of peak height ratio with concentration for artesunate was linear over the concentration range studied with r^2 values from repeated calibrations being in the range 0.997–0.999. A representative linear regression equation for the calibration was Peak Height Ratio=2.20×10^{-3} ($\pm4.5\times10^{-5}$) artesunate concn.+ 3.45×10^{-2} ($\pm3.39\times10^{-2}$). The intercept represents <2% of the maximum peak height ratio used in the calibration series.

3.2.1.2. Accuracy. The % bias, determined as described above, ranged from 3.15% at the concentration of the smallest standard (100 ng ml^{-1}) to 0.45% at the highest standard (1600 ng ml^{-1}).

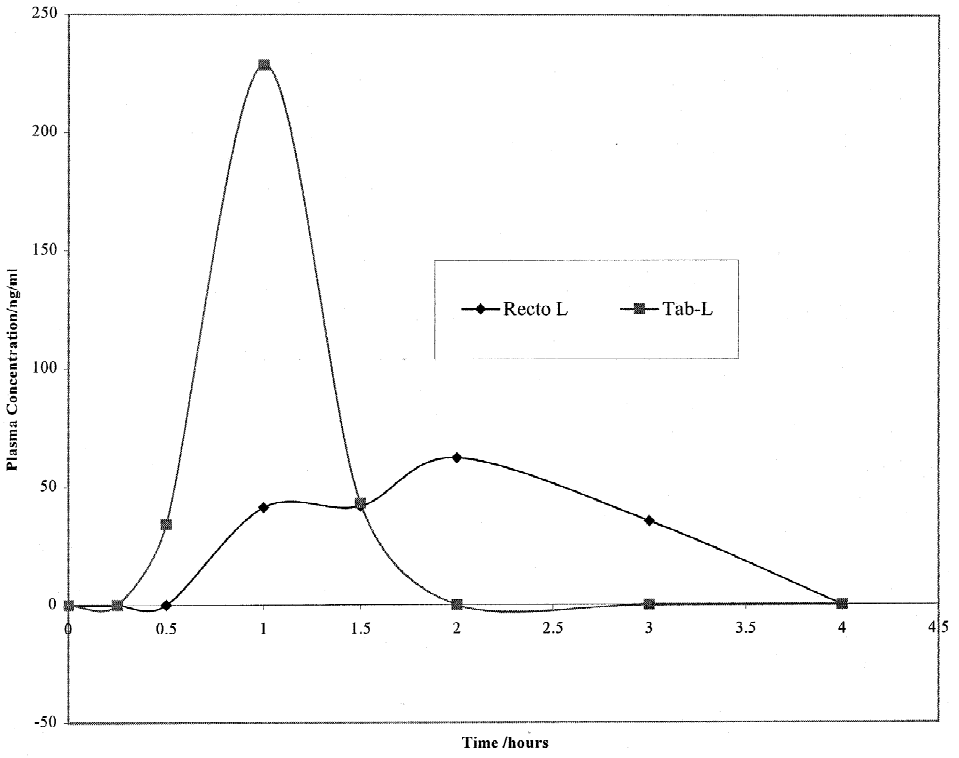

Fig. 5. Representative plasma concentration–time graphs for artesunate in a healthy volunteer following single dose administration of 200 mg as tablets and suppositories.

3.2.1.3. Precision. The within day precision of replicate injections ($n=6$) gave relative standard deviations of 4.6 and 6.6% at the 100 and 800 ng ml^{-1} levels, respectively. Concentration levels of 20 and 40 ng ml^{-1} yielded within day relative standard deviations of 13.4 and 9.6% ($n=7$), respectively.

The day to day relative standard deviations for the standards 100, 200, 400, 800, and 1600 ng ml^{-1} were determined as 2.3, 7.1, 1.1, 8.6 and 7.9%, respectively.

3.2.1.4. Limits of detection and quantification. The LOD ($S/N=3$) was determined as 20 ng ml^{-1} and is comparable with those quoted in the literature determined by non-ion pairing chromatographic methods [2,8]. The limit of quantification is estimated as 40 ng ml^{-1} with 102±12% ($n=7$) recovery from plasma and a within day RSD of 9.6%.

Fig. 5 shows representative plasma profiles in a single subject for artesunate with time following oral and rectal administration (200 mg). The analysis of subject plasma samples by the present method gave variable results among different subjects. Also the variation of concentration of artesunate with time in some individual subjects showed more than one maximum. These effects were general both for tablets and suppository administration of the drug and are consistent with the findings of previous workers [4]. In some subjects no artesunate could be detected which further indicates the rapid conversion of artesunate to the primary metabolite.

4. Conclusion

The proposed method thus appears capable of determining the parent artesunate species in the presence of metabolites and represents an alternative approach to the chromatography of these compounds for pharmacokinetic studies. The addition of CTAB as a cationic pairing ion has, in this situation, the general effect previously described [10]. It has reduced the retention time of neutral components and increased the retention time of the ionised artesunate and allows selective determination of artesunate in the presence of related compounds and endogenous components.

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