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Automated solid-phase extraction method for the determination of piperaquine in capillary blood applied onto sampling paper by liquid chromatography

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Abstract

A bioanalytical method for the determination of piperaquine in 100 μL blood applied onto sampling paper, by solid-phase extraction and liquid chromatography, has been developed and validated. Blood spots were cut into small pieces prior to addition of 0.3 M perchloric acid, acetonitrile and phosphate buffer containing an internal standard. The liquid phase was loaded onto a mixed phase cation-exchange (MPC) solid-phase extraction column. Piperaquine and the internal standard were analysed by liquid chromatography and separated on a Chromolith Performance (100 mm × 4.6 mm) column with acetonitrile:phosphate buffer pH 2.5, $I = 0.1$ (8:92, v/v) at the flow of 3.5 mL/min. The UV detection was performed at 345 nm. The intra-assay precision was 12.0% at 0.150 μM, 7.3% at 1.25 μM and 7.3% at 2.25 μM. The inter-assay precision was 1.8% at 0.150 μM, 5.2% at 1.25 μM and 2.8% at 2.25 μM. The lower limit of quantification (LLOQ) was determined to 0.050 μM where the precision was 14.7%.

Keywords: Piperaquine

1. Introduction

Malaria causes over 170 million clinical cases per year and among these over a million people die [1]. Protozoa of the genus *Plasmodium* cause the infection. Of the four different species that affects humans, *Plasmodium falciparum* is most lethal [2]. Malaria is found in over 100 countries, but is most prevalent in the African countries, where over 90% of the deaths from falciparum malaria occur [3]. In 1966, the Shanghai Research Institute of Pharmaceutical Industry synthesised a new antimalarial drug called piperaquine (PQ) and in 1978 PQ was registered in China [4,5]. Clinical studies have shown that PQ is effective in the treatment of chloroquine-resistant falciparum malaria [4,6]. In order to delay the development of piperaquine-resistant falciparum malaria, theraphy is essential [5,7,8]. Combinations of PQ and highly effective artemisinin derivates are recommended by the WHO. Artekin® (piperaquine-dihydroartemisinin) is the simplest and least expensive of the recommended combinations [9]. Further pharmacokinetic studies and evaluation of efficacy and toxicity are needed for the combinations, although some are known for the individual components [7,9].

1.1. Advantages of the presented method

A bioanalytical method for the determination of PQ in 100 μL capillary blood applied onto sampling paper, using solid-phase extraction (SPE) and liquid chromatography, has been developed and validated. The assay has been validated with respect to accuracy, precision, linearity, selectivity, stability and extraction recovery according to published guidelines [10]. Methods for the determination of PQ in plasma [11,12] and in whole blood [13] have recently been published. Those methods do not allow determination of piperaquine on sampling paper. The method presented in

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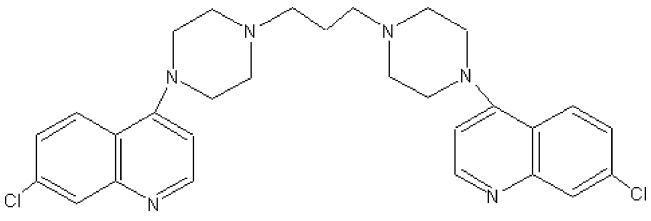
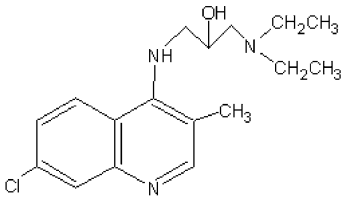


PQ



IS

Fig. 1. Structures of piperaquine (PQ) and the internal standard (IS).

this article has several advantages. Previous SPE methods utilised a pure cation exchanger, this method introduces a mixed phase combination of both cation exchanger and a C8 phase. This will ensure reproducible SPE even though ionic strength after paper extraction is relatively high. Storage and transportation are easier to handle. The need for immediate freezing is eliminated, thus facilitating clinical studies in remote areas. Samples can be sent to the laboratory by ordinary mail. The small sample volume needed and the use of capillary blood instead of venous blood is of great importance when sampling children. The use of capillary blood applied on sampling paper also reduces the risk of HIV and other pathogens that makes handling the samples safer for the laboratory personnel [14]. To the best of our knowledge, there are no published methods for the quantification of PQ in blood applied onto sampling paper.

2. Experimental

2.1. Chemicals

PQ, 1,3-bis-(4-[7-chloroquinolyl-4]-piperazinyl-1)-propane, was obtained from Guangzhou University of Traditional Chinese Medicine (Guangzhou, China). The internal standard (IS), 3-methyl-4-(3-hydroxy-4-dimethylaminopropyl)-7-chloroquine, was obtained from Glaxo Wellcome (Hertfordshire, UK). The structures are shown in Fig. 1. Acetonitrile (Chromasolv, for chromatography) and methanol (Chromasolv, for chromatography) were obtained from Riedel-de Haën (Seelze, Germany). Triethylamine (for synthesis), *ortho*-phosphoric acid (pro analysi), sodium hydroxide (pro analysi) and perchloric acid (pro analysi) were obtained from VWR International (Darmstadt, Germany). The phosphate buffer solutions were prepared by mixing sodium hydroxide, *ortho*-phosphoric acid and Milli-Q deionised water. Deionised water was prepared by a Milli-Q deionised water system (Millipore, Bedford, USA). A concentrated stock solution (0.61 M) of trichloroacetic acid (Sigma, St. Louis, USA) was prepared in Milli-Q deionised water. Sampling paper 31ETCHR was from Whatman (Whatman International, Maidstone, UK). Drug-free venous blood was obtained from the Department of Blood Transfusion, Falun Central Hospital, Sweden.

2.2. Instrumentation and chromatographic conditions

The LC system consisted of a Degasys DG-1310 degasser (Uniflows, Tokyo, Japan), a Spectraphysics SP 8800 ternary HPLC pump (Spectraphysics, CA, USA), a Gilson 234 autoinjector (Gilson, Middleton, WI, USA) and a Spectra 100 UV-detector (Thermo Separation Products, San Jose, CA, USA). The detector was set at 345 nm. Data acquisition was performed using chromatography station for Windows 32 1.2.4 (DataApex Ltd., Prague, The Czech Republic). The mobile phase was acetonitrile:phosphate buffer pH 2.5, $I = 0.1$ (8:92, v/v) with a flow rate of 3.5 mL/min. The chromatographic separation was achieved on a Chromolith Performance column (100 mm × 4.6 mm I.D.) (VWR International, Darmstadt, Germany), protected by a guard column SecurityGuard C18, 5 μm (Phenomenex, Torrance, CA, USA).

Solid-phase extraction was performed using mixed phase cation-exchange (MPC, strong cation-exchange and C8) columns (4 mm, 1 mL, 3M Empore, 3M Centre, St. Paul, USA) on an automated SPE system, ASPEC XL (Gilson, Middleton, WI, USA). The ASPEC system uses a positive air pressure instead of vacuum in order to push the fluids through the column.

2.3. Preparation of calibration standards and quality control samples

Stock solutions of PQ (500 μM) and IS (500 μM) were prepared in methanol:hydrochloric acid (99:1, v/v). PQ stock solution was used to prepare PQ working solutions (100, 50, 10 and 5 μM). IS stock solution was used to prepare an IS working solution (100 μM).Working solutions were prepared in methanol. All solutions were stored dark at +4 °C. PQ working solutions were added to blank venous heparin blood to obtain calibration standards (0–2.50 μM), all together seven different concentrations including a zero sample. Quality control (QC) samples were also prepared in this manner, used for the determination of accuracy, precision and recovery (0.150, 1.25 and 2.25 μM). All spiked samples contained less than 2% working solution (v/v). One hundred microlitres venous blood were applied onto sampling paper and kept over night at room temperature to dry. The samples were stored at −86 °C until analysis. The Whatman 31ETCHR sampling paper was used for all parts of

Table 1
ASPEC SPE procedure for the extraction of PQ in venous blood applied onto sampling paper

SPE-step	Liquid dispensed	Dispensing volume (μL)	Dispensing flow rate (μL/min)	Pressuring air volume (μL)
Conditioning	Methanol	500	3000	200
	Phosphate buffer pH 2.5, $I = 0.05$	300	3000	700
Sample loading		8000[a]	1500	1000
Washing	Milli-Q deionized water	300	1500	1200
	Methanol:phosphate buffer pH 2.5, $I = 0.05$ (80:20, v/v)	500	6000	1500
Elution	Methanol:triethylamine (98:2, v/v)	700	1500	700

[a] The volume after centrifugation is less than the volume noted here. This is the volume that is in the programme for the ASPEC XL. Therefore, the whole liquid phase is loaded on the SPE column.

this paper, except in Section 2.5.1. See Section 3 for further explanation.

2.4. Sample preparation of dried blood spots

Dried blood spots were cut into small pieces and placed in polypropylene tubes. Two thousand microlitres 0.3 M perchloric acid, 1000 μL acetonitrile and 5000 μL phosphate buffer pH 2.5, $I = 0.05$ containing the IS (0.100 μM) were added (the order as presented). The tubes were shaken on a mechanical shaker for 60 min and then centrifuged at 3030 × g for 7 min. The liquid phase was decanted into new polypropylene tubes and then loaded onto the SPE columns. The extraction procedure for the ASPEC XL is shown in Table 1. The eluates were evaporated at 70 °C under a stream of air and then reconstituted in 250 μL acetonitrile:Milli-Q water:trichloroacetic acid 0.61 M (5:93:2, v/v/v). One hundred microlitres of the reconstituted samples were injected into the LC system.

2.5. Method development

2.5.1. Analysis of extraction recovery

A small, preliminary study of the reasons for loss of PQ in the sample preparation was performed by comparing extraction recoveries of:

1. 700 μL spiked methanol:triethylamine (98:2, v/v) that was evaporated and then reconstituted in 250 μL acetonitrile:Milli-Q water:trichloroacetic acid 0.61 M (5:93:2, v/v/v). One hundred microlitres of the reconstituted sample were injected into the LC system;
2. 5000 μL spiked phosphate buffer pH 2.5, $I = 0.05$, to which was added 2000 μL 0.3 M perchloric acid and 1000 μL acetonitrile and then loaded onto SPE columns, evaporated, reconstituted and injected on the LC system (see Section 2.4 for details);
3. 100 μL spiked plasma;
4. 100 μL spiked plasma dried onto sampling paper;
5. 100 μL spiked venous blood;
6. 100 μL spiked venous blood dried onto sampling paper.

All samples were spiked at a concentration corresponding to 100 μL of 1.0 μM PQ in venous blood dried onto sampling paper, in the sample preparation procedure. Samples 3–6 were submitted to the full sample preparation procedure.

Sampling papers were wetted in acetonitrile, methanol or Milli-Q water for 60 s prior to the application of 100 μL spiked blood (0.150 and 2.25 μM). Sampling papers were also wetted with 10 mM dodecylethyldimethylammonium bromide (DOD) (Sigma, St. Louis, USA) for 60 s, left in room temperature for 24 h to dry and then 100 μL spiked blood was added to the sampling paper (0.150 and 2.25 μM). The samples were stored at −86 °C until analysis. Two alternative sampling papers were also tested (903, Schleicher & Schuell Bioscience, Dassel, Germany and 3MM, Whatman International, Maidstone, UK) under the same conditions as the Whatman 31ETCHR. The extraction recoveries were then compared with ANOVA in order to quantitate the difference between the mean values.

Extraction recoveries were determined by comparing the PQ peak areas to direct injections at the same nominal concentrations as after reconstitution. The direct injections were prepared in acetonitrile:Milli-Q water:trichloroacetic acid 0.61 M (5:93:2, v/v/v) and stored at −86 °C until the day of analysis. One hundred microlitres were injected into the LC system.

2.6. Method validation

2.6.1. Accuracy, precision and lower limit of quantification

The accuracy and precision of the method were determined by analysis of 100 μL spiked venous blood applied onto sampling paper at three different concentrations (0.150, 1.25 and 2.25 μM) in triplicate for 5 days. Concentrations were determined by calibration curves prepared on the day of analysis and the intra-, inter-, and total-precisions were calculated. The lower limit of quantification (LLOQ) was set as the concentration where the analyte response of a spiked sample was five times the response of a blank sample and where the analyte response had a precision of 20% and accuracy of 80–120% [10].

2.6.2. Linearity

Calibration graphs were constructed using seven standards (0–2.50 μM) including a zero sample. The ratio of the PQ peak area and the IS peak area at each concentration was

plotted as a function of PQ concentration and non-weighted linear regression analysis was performed.

2.6.3. Extraction recovery

Three concentrations of PQ (0.150, 1.25 and 2.25 μM) in triplicate were analysed for 5 days. The PQ peak areas and the IS peak areas were compared to direct injections at the same nominal concentrations as after reconstitution. The direct injections were prepared in acetonitrile:Milli-Q water:trichloroacetic acid 0.61 M (5:93:2, v/v/v) and stored at −86 °C until the day of analysis. One hundred microlitres were injected into the LC system.

2.6.4. Selectivity

Blank venous heparin blood from six healthy volunteers were prepared and analysed by the procedure described in this paper, in order to investigate endogenous compounds that could interfere with the quantification of PQ and the IS. A number of common antimalarials and some of their metabolites (amodiaquine, monodesethylamodiaquine, atovaquone, chloroquine, desethylchloroquine, mefloquine, proguanile, 4-chlorophenylbiguanide, pyronaridine, sulfadoxine, trimethoprim, primaquine) were injected into the LC-system for evaluation of chromatographic interferences. The injected concentrations were 5 and 50 μM.

2.6.5. Cross-validation

Each cross-validation set consisted of seven calibration standards ranging between 0 and 2.50 μM (including a zero sample) and nine samples, in triplicate, at different concentrations. The samples were prepared at Dalarna University College and stored at −86 °C. One set of samples was sent to Mahidol University, Bangkok, Thailand, and the other set was from this time stored at room temperature, at Dalarna University College, until the day of analysis. The samples were prepared and analysed in both laboratories at the same day. Sample concentrations determined at each laboratory were compared using non-weighted linear regression analysis. A two-sided *t*-test was performed to test for significant correlation.

2.7. Stability

The stabilities of PQ working solutions (100, 50, 10 and 5 μM) and the IS working solution (100 μM) were tested at $t = 0$ and 6 h. The stock solutions were diluted to 1.00 μM (PQ) and 2.00 μM (IS) with acetonitrile:Milli-Q water:trichloroacetic acid 0.61 M (5:93:2, v/v/v) prior to analysis. The mean value of $t = 6$ was compared to $t = 0$ in order to quantitate differences, by calculating the statistic *t*.

Stability of PQ and the IS in the liquids used in the analytical procedure was also evaluated. Two concentrations of PQ (0.150 and 2.25 μM) were analysed. Samples were prepared and kept at room temperature for $t = 0$, 24 and 48 h in each fluid (i.e. extraction, elution and reconstitution, see Section 2.4 for details). The extraction recovery was determined (see Section 2.6.3 for details). The mean values of $t = 0$, $t = 24$ and $t = 48$ were then compared using ANOVA to quantitate differences.

The stability of PQ in dried blood was evaluated. Spiked venous blood with PQ concentrations of 0.150 and 2.25 μM was applied onto sampling paper in 100 μL aliquots. These dried blood spots were stored at −86, +4, +23 °C (room temperature) and +37 °C (tropical temperature). All samples were analysed in triplicate on day 1, 5, 15 and 30. The stability was determined by studying the extraction recoveries (see Section 2.6.3 for details). The mean ± 1 S.D. of determined extraction recovery (see Section 3.2.3 for details) was set as limits for stability.

2.8. Biological application/clinical drug study

Patients received three tablets of Artekin® (40 mg dihydroartemisinin and 320 mg piperaquine per tablet) once daily at 0, 24 and 48 h. Capillary blood was collected at random times and applied to sampling paper. The samples were sent to our laboratory for analysis.

3. Results and discussion

3.1. Method development

3.1.1. Analysis of extraction recovery

There was very little loss in the SPE procedure, evaporation and reconstitution (extraction recovery was 86.7 ± 2.6%, $n = 3$) and only a small part might be due to evaporation and reconstitution (97.7 ± 3.0%, $n = 3$). No indication of PQ still binding to plasma proteins after sample preparation was found (90.0 ± 1.0%, $n = 3$). Since the spiked plasma dried onto sampling paper had lower extraction recovery (60.9 ± 2.8%, $n = 3$), this indicates that some PQ is lost due to binding to the sampling paper. For the spiked venous blood samples, extraction recoveries appeared the same as for the blood dried onto sampling paper (49.7 ± 18.1% and 52.0 ± 2.0%, $n = 3$). These results might be due to unbroken bonds to blood cells. However, since the S.D. of the venous blood is much greater than for the dried blood, no conclusions can be drawn. The method presented in this paper is developed for the analysis of blood dried onto sampling paper, and not for fresh blood. Therefore, an alternative approach was made in the attempt to determine whether the loss of PQ in the sample preparation was due to binding to the sampling paper and/or binding to blood cells. This was made by evaluating effects of pre-treating the sampling paper. Two alternative sampling papers were also tested (see below).

The recovery of PQ was not improved when the two alternative sampling papers not treated with DOD were used. The recovery of PQ was however different when DOD-treated sampling paper was used. The results are shown in Table 2. These results were verified by a *t*-test for

Table 2
Analysis of extraction recovery

Concentrations (μM)	W31 (mean ± S.D.) (%)	W3MM (mean ± S.D.) (%)	S&S903 (mean ± S.D.) (%)	*F* calculated (*F* critical 5.14)
Extraction recovery of PQ in spiked venous blood applied onto Whatman 31ETCHR (W31), Whatman 3 MM (W3MM) and Schleicher & Schuell 903 (S&S903)				
0.150	81.0 ± 1.0	93.2 ± 9.3	75.4 ± 3.0	7.75
2.25	55.7 ± 2.8	53.0 ± 6.6	62.0 ± 7.2	1.89

Concentrations (μM)	W31 (mean ± S.D.) (%)	W31DOD (mean ± S.D.) (%)	W3MMDOD (mean ± S.D.) (%)	S&S903DOD (mean ± S.D.) (%)	*F* calculated (*F* critical 4.07)
Extraction recovery of PQ in spiked venous blood applied onto Whatman 31ETCHR (W31DOD), Whatman 3 MM (W3MMDOD) and Schleicher & Schuell 903 (S&S903DOD) when treated with DOD and onto untreated Whatman 31ETCHR (W31)					
0.150	81.0 ± 1.0	97.7 ± 1.0	101.5 ± 1.4	87.9 ± 0.3	268.53
2.25	55.7 ± 2.8	59.0 ± 5.3	66.5 ± 2.2	64.9 ± 0.5	7.59

Concentrations (μM)	W31 (mean ± S.D.) (%)	W31ACN (mean ± S.D.) (%)	W31MetOH (mean ± S.D.) (%)	W31mQ (mean ± S.D.) (%)	*F* calculated (*F* critical 4.07)
Extraction recovery of PQ in spiked venous blood applied onto soaked sampling paper (acetonitrile (ACN), methanol (MetOH), Milli-Q water (mQ)) compared to spiked venous blood (W31), applied onto dry sampling paper. The Whatman 31ETCHR is used					
0.150	71.0 ± 15.1	57.4 ± 3.4	54.3 ± 1.7	63.7 ± 6.7	7.54
2.25	46.3 ± 4.2	37.8 ± 9.2	43.8 ± 5.4	32.9 ± 7.0	2.43

comparison of means (results are not shown) that indicated that the increase for PQ was valid only for the DOD-treated 3MM sampling paper compared to the untreated Whatman 31ETCHR. While comparing the extraction means of untreated sampling paper and DOD-treated sampling paper of the same brand, by a *t*-test for comparison of means (results are not shown), no increase was found for the Whatman 31ETCHR. When blood was added to the sampling paper (Whatman 31ETCHR) wetted with acetonitrile, methanol or Milli-Q water, the extraction recoveries were not improved. The results described in this section indicate that PQ might be bound to the sampling paper as well as blood cells. Extraction recovery was no different for the DOD treatment, or when achieving ruptured blood cells, on Whatman 31ETCHR. Therefore, no conclusions of loss due to binding to sampling paper and/or to blood cells can be drawn.

To enhance the extraction recovery of PQ, DOD-treated 3MM sampling paper could be used, but if so, that needs to be more thoroughly examined. Also, the applicability for field conditions must be taken under consideration. Bergqvist et al. [15] have described the deactivation of the charged cellulose matrix of the Whatman 31ETCHR sampling paper with DOD. There are no published methods using DOD-treated sampling paper, although the improvement of extraction recoveries were stated by Bergqvist et al. [15]. A method without the need of pre-treatment of the sampling paper is a much better alternative. Therefore, the DOD treatment was not further evaluated in this paper.

3.2. Method validation

The IS has a contamination that results in a peak eluting near the PQ peak that appears on using trichloroacetic acid solution, but not without it. Trichloroacetic acid is used in order to enhance efficacy [13]. When analysing low concentrations, the PQ peak is separated from this impurity but not when analysing higher concentrations. The integration of the PQ peak was found to be easier when adding the peak area of this impurity at all concentrations. When referring to the PQ area in this paper, the area of this impurity is also added.

3.2.1. Accuracy, precision and lower limit of quantification

The precision and accuracy is shown in Table 3. According to FDA guidelines, the QC samples can deviate up to 15% [10]. The deviations found were less than this for all QC samples. The addition of the area of the IS impurity to the area of PQ when calculating the PQ area to IS area ratio can therefore be used without losing accuracy. The precision is within the acceptance limits of 15% or less for the QC samples [10]. The lower limit of quantification was 0.050 μM where the precision was 14.7% and the deviation was −0.98% ($n = 5$). Fig. 2 shows chromatograms of the low QC, middle QC and high QC.

Table 3
Accuracy, intra- and inter-assay precision for the determination of PQ in 100 μL venous blood applied onto sampling paper

	Venous blood applied onto sampling paper ($n = 15$, $k = 5$)				
	Added (μM)	Found, mean (μM)	S.D. (μM)	R.S.D. (%)	% Deviation (found vs. added)
Intra-assay	0.150	0.155	0.019	12.045	3.003
Inter-assay			0.003	1.815	
Intra-assay	1.25	1.320	0.097	7.316	5.269
Inter-assay			0.068	5.152	
Intra-assay	2.25	2.609	0.190	7.276	13.755
Inter-assay			0.074	2.842	

3.2.2. Linearity

Linear calibration graphs were obtained for PQ with correlation coefficients $r > 0.99$, e.g. 0.9979 ± 0.0011 ($n = 5$). The slope was 0.1755 ± 0.0064 ($n = 5$) and the intersection was 0.0199 ± 0.0026 ($n = 5$). The intersection is greater than zero due to the addition of the area of the IS impurity. When calculating the concentrations of the calibration standards, these did not deviate more than 10%. According to [10], the LLOQ can deviate up to 20% and the other calibration standards can deviate 15%. The addition of the area of the IS impurity to the area of PQ when calculating the PQ area to IS area ratio can therefore be used.

3.2.3. Extraction recovery

The extraction recovery of PQ were 65.4 ± 6.6% at 0.150 μM,48.9 ± 4.9% at 1.25 μM, 47.5 ± 5.2% at 2.25 μM and 67.0 ± 1.8% for the IS (mean ± total S.D., $n = 15$). The recovery does not have to be 100% as long as it is consistent, precise and reproducible for the analyte and the internal standard [10]. The extraction recovery is greater for the low QC samples than for the middle and high QC samples. This has also been reported in whole blood [13].

3.2.4. Selectivity

No interfering peaks from endogenous compounds were noticed in the blood from the six healthy volunteers. There were no interferences from the injected antimalarials or their metabolites.

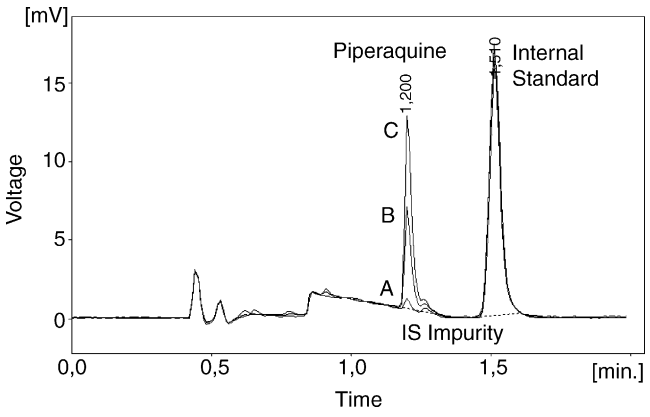


Fig. 2. (A) Chromatogram from blood spiked with 0.150 μM PQ (low QC). (B) Chromatogram from blood spiked with 1.25 μM PQ (middle QC). (C) Chromatogram from blood spiked with 2.25 μM PQ (high QC). For SPE and LC conditions see Section 2.

3.2.5. Cross-validation

The results of the cross-validation are shown in Fig. 3. The correlation coefficient and the slope were close to 1 (0.971 ± 0.059) and the intercept was close to 0 (0.0315 ± 0.0732). The two-sided *t*-test showed that there was a significant correlation between the results of each laboratory ($P = 0.05$).

3.3. Stability

The PQ working solutions and the IS working solution were found to be stable at room temperature for at least 6 h.

PQ and the IS were found to be stable in the fluids used in the analytical procedure for 48 h.

PQ was shown to be stable at the three tested temperatures (+4, +23 and +37 °C) for at least 30 days. The extraction recoveries were within the mean ± 1 S.D. (65.4 ± 6.6% at 0.150 μM and 47.5 ±5.2% at 2.25 μM). The results are shown in Fig. 4. PQ has shown to be stable in whole blood at different concentrations and temperatures [13].

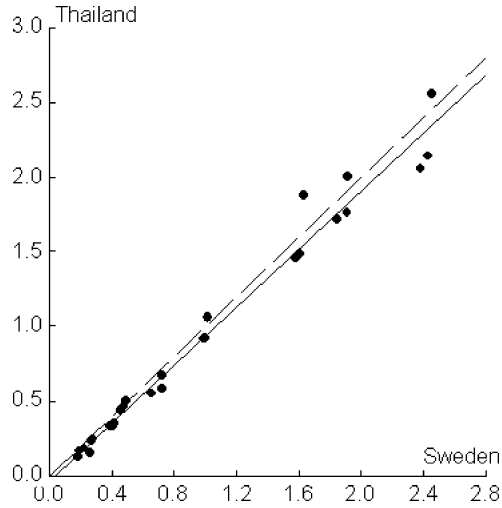


Fig. 3. Comparison between the laboratories, *y*-axis: Mahidol University, Thailand (concentration μM); *x*-axis: Dalarna University College, Sweden (concentration μM).

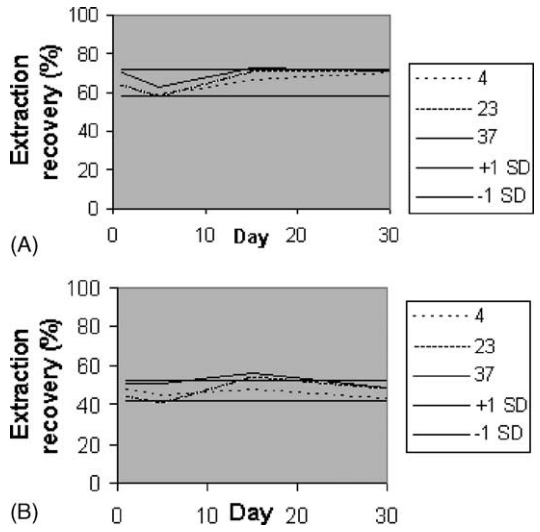


Fig. 4. Stability of PQ at the three tested temperatures for 30 days. (A) 0.150 μM and (B) 2.25 μM.

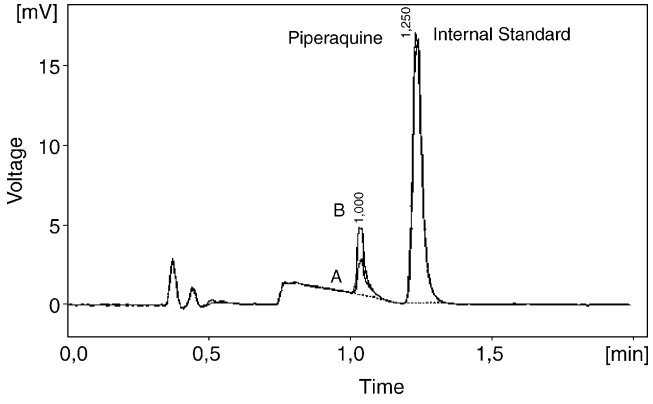


Fig. 5. Chromatogram of two samples, collected at different times, from one patient receiving three tablets of Artekin® once daily at 0, 24 and 48 h. (A) 29 h and 27 min and (B) 51 h and 59 min. For SPE and LC conditions see Section 2.

3.4. Biological application/clinical drug study

Fig. 5 shows two samples from one patient taken at 29 h and 27 min (4.27 after dose 2) and 51 h and 59 min (3.59 after dose 3). The first sample was 0.574 μM and the second 1.025 μM. The chromatogram is free of endogenous compounds.

4. Conclusion

A bioanalytical method for the determination of piperaquine in 100 μL blood applied on sampling paper, by solid-phase extraction and liquid chromatography, has been developed and validated. The method has proven to be accurate, reproducible and selective. It has been shown that this method is suitable for the determination of PQ in dried blood spots collected under field conditions and for pharmacokinetic studies in humans. By using the mixed phase cation-exchange columns a smaller volume of fluids can be used in the SPE-procedure and by the use of the Chromolith Performance LC column the time of analysis for each sample is only 2 min.

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