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PAPER

Automatic miniaturized flow methodology with in-line solid-phase extraction for quinine determination in biological samples

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The present work describes an analytical platform based on a multipumping flow injection analysis (MPFS) technique combined with in-line solid-phase extraction (SPE). The flow network has been tested with the determination of quinine in biological samples using fluorometry as the detection technique. Amberlite XAD-4 resin has been used as a solid phase and the implementation of a pH control strategy resulted in a simple and environmental approach for the preconcentration of quinine. Two solenoid valves allowed the deviation of the flow towards the resin column to carry out SPE procedures. The influence of parameters such as concentration, flow rate and volume of the different solutions on the sensitivity and performance of the MPFS was studied. Dynamic calibration ranges (0.78–150 ng mL^{-1}) for quinine determination were applied by using a variable sample volume (120–1000 μL). The developed methodology provided high relative extraction recoveries from human urine samples (85–115%). The proposed automatic methodology turns out to be very efficient and sustainable compared to the available procedures and it could prove to be an attractive alternative tool to perform in-line sample pre-treatment and subsequent direct determination of relevant organic compounds in pharmaceutical and clinical analyses.

1. Introduction

Flow techniques in general are recognized nowadays as powerful and useful methodologies for the automation of analytical procedures in many areas. The inherent advantages of flow techniques such as versatility, simplicity and low cost make these procedures a valuable choice for the analysis of complex matrixes, coupled to sample pre-treatment devices and different detection systems.[1]

Multi-pumping flow systems (MPFSs) were recently proposed by Lapa *et al.*[2] and are based on the use of a series of individual small size solenoid-actuated micro-pumps that are controlled by a computer, strategically positioned in the flow manifold, which are accountable for sample–reagent insertion, propelling and commutation, conditioning the establishment and subsequent detection of the reaction zone. Being the only active components of the flow manifold, they enable miniaturized and compact flow systems to be developed, with a higher degree of simplicity and improved operational characteristics and a straightforward run-time control of important analytical variables.

In clinical and pharmaceutical analyses, it is almost imperative to carry out sample manipulation and pre-treatment stages before the measurement step. Furthermore, a direct in-line coupling of the sample preparation procedure to the analytical system minimizes sample handling and thereby the risk of contamination or loss of analyte, making the automation of the whole process possible.

One of the most widely used sample pre-treatment procedures is automated solid-phase extraction (SPE),[3] which employs an appropriate solid- or liquid-extraction material attached on a suitable support for which MPFS is ideally suited. SPE has been widely used in flow analysis since it allows trace enrichment (concentration), matrix simplification and medium exchange.[3–5] In spite of the new trends in SPE, the use of packed columns with conventional resin, a scarcely exploited feature in MPFSs, should be pointed out. In fact, the mechanical action of pulsed flows in these systems enabled a chaotic pattern that maintains the resin particles in circulating motion.[6] This way it was possible to minimise some of the drawbacks usually associated with the utilisation of packed columns, such as backpressure effects and preferential pathways.

In the present work, the MPFS-SPE assembly was applied to the determination of quinine in biological samples. Quinine is a natural occurring cinchona alkaloid that has been widely used for the treatment of the parasitical disease spread by mosquitoes

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called malaria.[7] It is also present in other pharmaceutical formulations due to its antipyretic, analgesic and anti-inflammatory properties. In the food industry, quinine is also utilized as a flavouring agent in soft drinks such as tonic water due to its bitter taste. However, quinine is a potentially toxic drug. The typical syndrome of quinine side effects is called cinchonism that is mild in usual therapeutic dosage or severe in larger doses. Quinine side effects include thrombocytopenia, visual, auditory, gastrointestinal, cardiovascular and neurological effects, such as nausea, headache and fever, disturbed vision, renal failure, chest pain and asthma.[8] Its determination is therefore of utmost importance in clinical chemistry, in different body fluids, for proper treatment of malaria cases. In this sense, several methods have been reported for the determination of quinine in human biological fluids (Table 1). These methods include high-performance liquid chromatography (HPLC) procedures using normal[9] or reversed-phase columns after liquid–liquid/solid extraction of the drug and either ultraviolet[10] or fluorescence detection.[11,12] Chemiluminometric,[13,14] capillary electrophoresis,[15] gas chromatography[16] and thin layer chromatography[17] methods have also been developed. However, some procedures involve high-cost equipment and are time consuming with low sampling throughput, requiring sample pre-treatment (including most of the time extraction with organic solvents). Indeed, although sample pre-treatment is an important stage in most methodologies it is completely separated from the quantification procedure (Table 1). Consequently, the determination of quinine in biological fluids could clearly benefit from a simple, rapid, methodology with an in-line sample pre-treatment step with exact time control of reaction conditions.

2. Materials and methods

2.1. Reagents and solutions

Water from a Milli-Q system was used for the preparation of all solutions, and all chemicals were of analytical reagent grade.

The stock quinine hydrochloride dihydrate solution containing 100 mg L^{-1} was obtained by accurately weighing the appropriate mass and dissolving it in 100 mL. An intermediate stock solution containing 10 mg L^{-1} of quinine and 0.10 mol L^{-1} NaOH was also prepared. The working standards were prepared daily by careful dilution of the previous solution in 0.10 mol L^{-1} NaOH. In the MPFS, the carrier was 0.05 mol L^{-1} H_2SO_4 solution. Amberlite XAD-4 resin was purchased from Fluka.

Urine samples were obtained from healthy adult volunteers. The urine samples were filtered (Corning® filters, 0.20 μm), the pH was adjusted to 13.0 with NaOH and diluted 1 : 20 with 0.1 M NaOH before insertion in the multipumping flow system.

2.2. Extraction column preparation

Initially, 50 mg of Amberlite XAD-4 resin was suspended in water and some drops of ethanol. After that, this suspension was packed in an acrylic column (4 cm length; 3 mm inner diameter). Two filters of 35 μm pore size (Mobicol M 1002, MoBiTec) were placed at both ends of the column to entrap the resin. The resin particles were then sequentially washed using water : methanol (2 : 1 v/v), methanol, water, 0.01 mol L^{-1} H_2SO_4, 0.1 mol L^{-1} NaOH and water, being inserted into the flow system.

2.3. Apparatus

The flow manifold comprised four solenoid micropumps (Ref. 090SP Bio-Chem Valve Inc.), which were fixed displacement diaphragm pumps, dispensing a volume of 10 μL per stroke, and three solenoid commutation valves (NResearch 161 T031) to that allows the access to two different pathways. These micropumps (approximately 5 cm in length) are diaphragm pumps, operated by a solenoid, in which the diaphragm is maintained, closed by means of an inner spring mechanism. When voltage is applied, the solenoid coil is activated in order to open the diaphragm. This opening action permits the fluid to be drawn into the pump chamber. The fluid is dispensed from the pump by dropping the applied voltage, thus de-energizing the solenoid coil; the spring then forces the diaphragm back to the closed status.

A LabAlliance fluorescence detector LC 305 equipped with an 8 μL flow-cell was used as the detection system and the excitation and emission monitoring wavelengths were set at 250 and

Table 1 Comparison between different methodologies for quinine assay in biological samples

Methodology	Detection	Biological samples	Extraction step	Linear range	Detection limit	Time analysis	Ref.
HPLC	Spectrophotometry (ultraviolet)	Plasma, cerebrospinal fluid and perilymph	Off-line organic solvent extraction	100–10 000 ng mL^{-1}	10 ng mL^{-1}	Run time >7 min	10
HPLC	Fluorometry	Serum and urine	Off-line organic solvent extraction	1–670 ng mL^{-1}	0.3 ng	Run time >5 min	11
Flow-injection analysis	Chemiluminometry	Plasma and urine	Off-line organic solvent extraction	15–10 000 ng mL^{-1}	1.5 ng mL^{-1}	—	13
Flow-injection analysis	Chemiluminometry	Urine and serum	—	100–80 000 ng mL^{-1}	32 ng mL^{-1}	—	14
Capillary electrophoresis	Spectrophotometry (ultraviolet)	Blood and urine	Off-line organic solvent extraction	60–5000 ng mL^{-1} (blood) 150–10 000 ng mL^{-1} (urine)	30 ng mL^{-1} (blood) 20 ng mL^{-1} (urine)	Run time <20 min	15
Gas chromatography	Mass spectrometry	Plasma	Off-line organic solvent extraction	Up to 10 000 mg mL^{-1}	12.2 mg mL^{-1}	Run time <13 min	16
Thin layer chromatography (TLC)	TLC ultraviolet/ visible spots	Urine	—	Up to 10 mg mL^{-1}	250 ng mL^{-1}	—	17

450 nm, respectively. Analytical signals were recorded on a strip chart recorder (Linseis, L250E) or acquired.

Automatic control of the analytical system was accomplished by means of a Pentium-based microcomputer and software, developed using Microsoft QuickBasic 4.5. A lab-made electronic interface using a CoolDrive™ power drive board (NResearch Inc.) was used to activate the solenoid of micropumps through the LPT1 computer port.

2.4. Manifold and flow procedure

All connections were made of Omnifit (Cambridge, UK) PTFE tubing with 0.8 mm i.d. unless stated otherwise. Gilson (Villiers-le-Bel, France) end-fittings, connectors and confluences were also used.

Lab-made acrylic extraction columns of different lengths and diameters were tested.[18] The system components were arranged as shown schematically in Fig. 1.

The micropump MP_1 was responsible for insertion and propelling of the sulfuric acid solution, which was the eluent; MP_2 was used for inserting the hydroxide sodium solution, which was the regenerating solution; MP_3 and MP_4 were used for inserting the standard or sample and cleaning water solutions, respectively.

The analytical cycle (Table 2) started by actuating MP_2, inserting 2 mL of hydroxide sodium solution in the analytical path, passing through the column to regenerate it, at a fixed pulse time of 0.3 s (0.15 s activation + 0.15 s deactivation), which corresponds to a pulse frequency of 200 Hz, giving a flow rate equal to 2.00 mL min^{-1} (Step 1). Thereafter, the micropump MP_2 was switched off, and, by actuation of the micropump MP_3 at a fixed pulse time of 2 s (1 s activation + 1 s deactivation), the sample solution was inserted into the column filled with Amberlite XAD-4 resin (Step 2). Then, the micropump MP_4 was activated, inserting 2 mL of water solution to wash the column and then removing matrix remains, at a pulse time of 0.3 s (Step 3). The last step is the elution, by actuating the micropump MP_1 that propelled 3 mL of sulfuric acid solution that carried the analyte retained towards the detector, at 2.00 mL min^{-1}, after which the content of the flow cell was washed out, and thus establishing the baseline (Step 4).

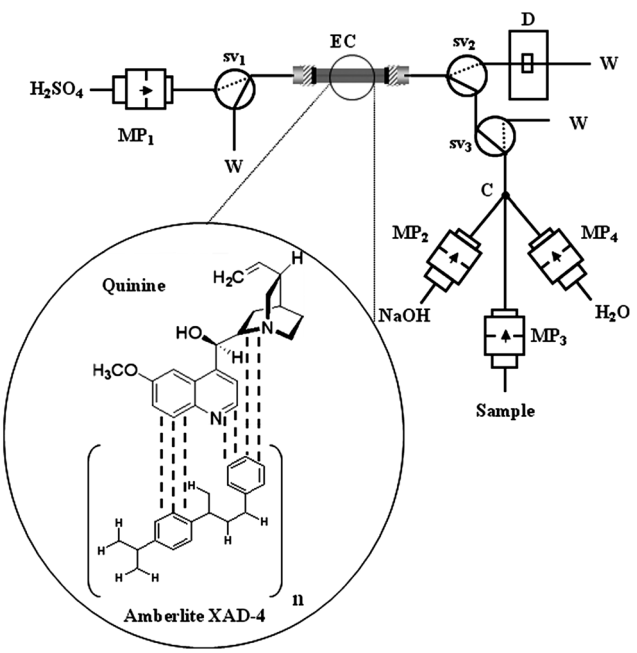

Fig. 1 Schematic depiction of the proposed automatic setup for determination of quinine in biological samples: MP_i – 10 μL solenoid micropumps; C – confluence point; D – fluorometric detector ($\lambda_{excitation}$ = 250 nm; $\lambda_{emission}$ = 450 nm); sv_i – solenoid commutation valves; EC – extraction column; W – waste; eluent solution (0.05 mol L^{-1} H_2SO_4); regeneration solution (0.1 mol L^{-1} NaOH); and cleaning solution (H_2O).

3. Results and discussion

3.1. Design and optimization of the flow system for determination of quinine

The optimum conditions of the MPFS for the determination of quinine in human urine samples were studied by the univariate approach with the influence of several parameters on its working characteristics being evaluated. The studied range of parameters as well as the final conditions selected are summarised in Table 3.

In the present paper, we aimed to determine quinine in biological samples, namely in human urine samples. As quinine exhibits strong native fluorescence, it could be detected without derivatization directly by fluorometry. To extract it from the biological matrix an in-line pre-concentration unit was introduced using a column filled with Amberlite XAD-4 resin. This is an inexpensive resin that is a non-ionic crosslinked polymer, stable throughout the pH range of 1–13. These characteristics enable us to perform the retention of target analyte in the Amberlite XAD-4 sorbent under alkaline conditions (pH = 13), using 0.10 mol L^{-1} NaOH as the carrier. Standards and samples were also alkalinized to this pH. This was necessary to promote the retention of quinine which is predominantly in the non-ionic form at high pH values (Fig. 1, bottom). The pH shift towards acidic values which promoted the ionization of the retained quinine compound and thus its subsequent desorption from the non-ionic crosslinked polymer resin was carried out with sulfuric acid at a pH near the maximum emission of quinine and where the intrinsic fluorescence of some possible interferents such as chloroquine is almost null.[19]

The influence of the eluting agent concentration was then tested between 0.001 and 0.10 mol L^{-1}. For quinine concentrations between 12.5 and 50 ng mL^{-1}, calibration curves were established and an increase in sensitivity up to 0.05 mol L^{-1} H_2SO_4 was noticed (Fig. 2). The differences obtained with higher concentrations (only of about 0.5% for 0.10 mol L^{-1} H_2SO_4) did not justify its use. Therefore, for further experiments a 0.05 mol L^{-1} H_2SO_4 solution was chosen.

With this proper elution process, the adsorbed quinine was rapidly eluted, because of the high rate of diffusion of the eluting agent through the porous structure of the beads. In order to enhance the determination frequency, the influence of the eluent flow rate in the reaction was evaluated using the above-defined conditions. Flow rate values between 1.0 and 2.0 mL min^{-1} were tested and similar values of sensitivity were attained. Furthermore, an elution volume equal to 3.0 mL was sufficient to ensure the complete desorption of the quinine retained to the sorbent

Table 2 Protocol sequence for determination of quinine in urine samples

Step	MP_1	MP_2	MP_3	MP_4	Pulses	Time (s)	Flow rate (mL min^{-1})	Description
1	OFF	ON	OFF	OFF	200	0.30	2.0	Regeneration of the column with 2 mL of NaOH 0.1 M
2	OFF	OFF	ON	OFF	12	2.0	0.3	Insertion of the sample/quinine standard through the column
3	OFF	OFF	OFF	ON	200	0.30	2.0	Cleanup of the column and removal of matrix remains by propulsion of 2 mL of water
4	ON	OFF	OFF	OFF	300	0.30	2.0	Elution of retained quinine towards the detector with 3 mL of H_2SO_4 0.05 M

used. Flow rates higher than 2.0 mL min^{-1} were not applied to avoid the possible pressure build-up. Then, the value chosen was 2.0 mL min^{-1}. In a similar way, the volumes and flow rates of the regeneration and cleanup steps (Table 2, Steps 1 and 3, respectively) were evaluated with the aim of increasing the determination rate without compromising the performance of the method. The corresponding volumes used should be adequate to promote an effective regeneration of the resin (Table 2, Step 1) and the cleanup of the biological matrix that remained after the insertion of the sample (Table 2, Step 3) without the induction of a pre-elution of the analyte. Therefore, the corresponding volumes that passed through the column were equal to 2 mL. The flow rates of the regeneration and cleanup steps were evaluated and then fixed at 2.0 mL min^{-1}, as these flow rates did not practically affect the sensitivity of the method.

The sample volume was one of the most important parameters of the developed methodology in terms of the sensitivity of the determination but also in terms of sampling strategy. Sample volumes between 120 and 1000 μL were tested and the calibration curves (fluorescent intensity (FI) = 0.1437 (±0.0081) $C_{quinine}$ (ng mL^{-1}) − 0.2378 (±0.6509), R^2 = 0.9968; FI = 0.5742 (±0.0457) $C_{quinine}$ (ng mL^{-1}) + 0.1547 (±1.994), R^2 = 0.9952; FI = 0.8346 (±0.0415) $C_{quinine}$ (ng mL^{-1}) + 0.1231 (±0.8467), R^2 = 0.9975) obtained showed linear responses in concentration ranges of 6.25–150 ng mL^{-1}, 3.12–100 ng mL^{-1} and 0.78–50 ng mL^{-1} for sample volumes of 120, 500 and 1000 μL, respectively. Thus, the developed method provided a wide dynamic concentration range achieved by just changing the sample volume (Step 2, Table 2). A volume of 120 μL was selected for further experiments as it led to a higher sampling frequency and the sensitivity achieved enabled quinine determination within the normal quinine ranges present in human urine.

Table 3 Range of values used in the study of system parameters and selected values for its operation

Parameter	Range	Selected value
Eluent concentration (mol L^{-1})	0.001–0.10	0.05
Elution solution volume (μL)	3000–5000	3000
Eluent flow rate (mL min^{-1})	1.0–2.0	2.0
Loading flow rate (mL min^{-1})	0.25–2.0	0.3
Column dimensions	i.d. 2–4 mm; length 3–4 cm	i.d. 3 mm; length 4 cm
Sample volume (μL)	120–1000	120

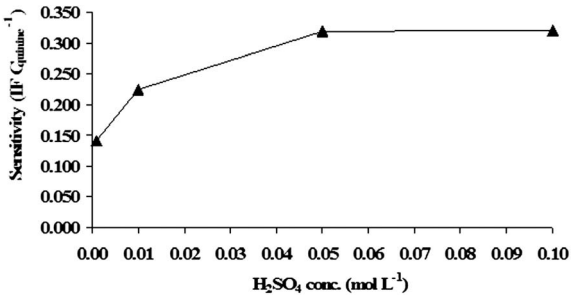

Fig. 2 Influence of the sulfuric acid concentration on the elution rate and subsequent sensitivity of the presented method. *Experimental conditions*: sample volume of 500 μL.

The influence of other factors affecting the quinine pre-concentration efficiency was examined, namely the column dimensions, the loading flow rate and the nature of the resin, besides the key elution operation.

Using the conditions described before, studies were performed with three different columns: two columns with internal diameters of 3.0 and 4.0 mm and lengths of 4.0 cm and one column with an internal diameter of 2.0 mm and a length of 3.0 cm. The slope value obtained for the 3.0 cm column was about 61% of that obtained for the 4.0 cm column with 3.0 mm inner diameter. This difference could be attributed to the lower quantity of the macroreticular resin used in the 3.0 cm column resulting in a lower quinine retention rate. The internal diameter of the column was also varied, keeping the column length at 4.0 cm. Moreover, the sensitivity obtained for the 4.0 mm internal diameter column was about 86% of that obtained when using the 3.0 mm inner diameter column. Perhaps, in spite of achieving multiple bead pores available at the time of the arrival of the analyte, as a consequence of using a higher quantity of resin, there was a higher dead volume which carried out a considerable sample dispersion increase and consequently an analytical signal decrease. Therefore, the column with 3.0 mm diameter and 4.0 cm length was chosen for further studies.

To avoid the formation of a liquid gap at the top of the column and the consequent pressure build-up, the operations of loading and elution were performed in opposite directions. Therefore, the connections between the MP_1, responsible for propelling the eluent, and the extraction column and between the MP_3, responsible for the loading the sample, and the extraction

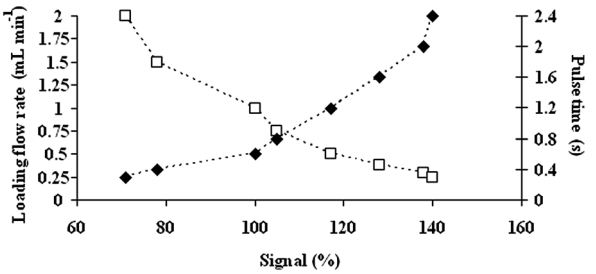

Fig. 3 Variation of the analytical signal magnitude (%) by changing the loading flow rate (□) and the MP_3 pulse time (◆). *Conditions*: aspiration of 120 μL of sample volume.

column were placed in different commutation valves (sv_1 and sv_2, respectively). The influence of the loading flow rate in the analytical signal was tested between 0.25 and 2.0 mL min^{-1} as shown in Fig. 3. Considering the starting conditions of 0.6 seconds for the pulse time, which corresponds to a loading flow rate of 1 mL min^{-1}, it was verified that for low pulse times (*e.g.* 0.3 s) (and hence higher flow rates), the analytical signal declined (Fig. 3). However as the pulse time increased and consequently the loading flow rate decreased the analytical signal incremented up to a pulse time of 2 s. This could be explained by a longer residence time in the column with the resin and consequently favourable conditions created for the analyte that reached the bead pores and become entrapped within the sorbent, without increasing the sample dispersion. The difference obtained with higher pulse times (only of about 2% for a pulse time of 2.4 s) did not substantiate its use. As a result, a loading flow rate of 0.3 mL min^{-1} (corresponding to a 2 s pulse time) was adopted.

Finally, a comparative study was carried out using the Amberlite XAD-2 resin, which has been widely used to adsorb organic compounds even from biological matrices.[20] For quinine concentrations up to 50 ng mL^{-1}, calibration curves were then established (FI = 0.0780 (±0.0120) $C_{quinine}$ (ng mL^{-1}) − 0.0337 (±0.3426), $R^2 = 0.9975$; FI = 0.1443 (±0.0351) $C_{quinine}$ (ng mL^{-1}) + 0.2506 (±1.006), $R^2 = 0.9936$) using the Amberlite XAD-2 and XAD-4 resins, respectively, both packed in acrylic columns (4 cm length; 3 mm inner diameter). It was verified that the slope value obtained for the column filled with Amberlite XAD-4 was about 2 times higher than that obtained when using the Amberlite XAD-2.

3.2. Evaluation of the method and its application to urine samples

Calibration curves for urine were constructed by transferring aliquots of the standard solutions of quinine into a blank pooled drug-free urine sample to give final concentrations within the range of concentrations studied. A blank quinine-free urine sample was also considered.

Under the above-defined optimal conditions, the calibration curve (FI = 0.1331 (±0.0096) $C_{quinine}$ (ng mL^{-1}) + 0.3909 (±0.5936), $R^2 = 0.9948$) obtained showed linear responses in concentration ranges of 12.5–150 ng mL^{-1}. A decrease in sensitivity of about 7% was noticed when compared with the calibration of quinine standards in aqueous medium.

The detection limit was estimated[21] as three times the standard deviation of 10 blank measurements and a value of 9.7 ng mL^{-1} was obtained. Furthermore, the developed methodology can be particularly interesting for the analysis of samples that demand lower detection limits by just increasing the sample volume.

The repeatability of the flow procedure was estimated by calculating the relative standard deviation (R.S.D.) from 10 consecutive injections of urine samples. Values of 1.7% for 12.5 ng mL^{-1} quinine and 1.4% for 100 ng mL^{-1} quinine spiked urine samples were obtained, showing small differences over a significant range of concentrations. The time required for a complete analytical cycle is the sum of the time required for each step and for proper micropumps activation and deactivation. Almost 260 s were required to complete an analytical cycle and hence the determination rate equated to about 14 determinations per hour. Therefore, the present flow-solid-phase extraction technique represents a noteworthy improvement in the efficiency and quickness when compared to the available procedures, where the sample pre-treatment besides being time-consuming is almost completely separated from the analysis procedure.

In order to validate the proposed method for the determination of quinine in biological fluids, analyses of spiked urine samples were performed. Results of recovery studies of quinine for spiked urine samples within the normal quinine ranges present in these biological fluids are given in Table 4. Each value represents the mean of three measurements carried out. It was verified from these studies that quinine recovery varied in the range 85–115% for all samples studied over the entire range of concentration and the majority of RSD values were lower than 5%.

For comparison purposes, a linear relationship C_{MPFS} (ng mL^{-1}) = C_0 + $SC_{SPIKING}$ (ng mL^{-1}) was established. The equation found was C_{MPFS} = −0.0006 (±0.0047) + 0.9917 (±0.087) $C_{SPIKING}$, where the values in parentheses are the limits

Table 4 Results of recovery assays of quinine from urine samples

Samples	Concentration of spiked urine (ng mL^{-1})	Corresponding mean concentration found (ng mL^{-1})	Recovery percentage	RSD (%)
Urine 1	15	13 (±1)	86.9	3.9
	30	27 (±1)	91.4	3.9
	60	69 (±1)	114.9	1.9
Urine 2	30	34 (±2)	113.7	5.5
	36	33 (±1)	91.4	3.7
	42	38 (±1)	90.9	3.7
Urine 3	30	33 (±1)	109.1	3.1
	42	38 (±1)	89.9	1.6
	75	75 (±1)	99.5	0.5
Urine 4	36	33 (±1)	91.1	2.8
	48	42 (±1)	87.1	1.4
	62.5	61.5 (±3.0)	98.3	4.4
Urine 5	36	34 (±2)	94.9	6.5
	46	49 (±2)	106.6	5.1
	100	98 (±2)	97.5	2.2
Urine 6	48	48 (±1)	100.5	2.0
	50	49 (±1)	98.8	2.4
	87.5	79.3 (±1.1)	90.6	1.0
Urine 7	15	16 (±1)	104.0	6.5
	60	52 (±3)	87.3	6.3
	90	95 (±1)	105.6	1.5

of the 95% confidence intervals. From these figures it is clear that the estimated intercept and slope do not differ significantly from the values 0 and 1, respectively. Thus, there is no evidence of systematic differences between the two sets of results obtained by the proposed methodology and the corresponding concentration levels of spiked urine. Furthermore, when a paired *t*-test[21] was performed on the data obtained for quinine content, a *t* value of 1.14 was obtained. The comparison between that value and the critical value tabulated $t = 2.08$ illustrates the absence of statistical differences at the 95% confidence level for the results of the concentration of spiked urine and the corresponding concentration found.

4. Conclusions

The proposed miniaturized methodology was successfully applied to the determination of quinine in human urine, with results comparable to those obtained by the available procedures. It combines the automatic flow methodology with an in-line preconcentration unit in order to extract the analyte from the biological sample, allowing its determination in a fully aqueous environment. Therefore this strategy offered an environmentally friendly alternative to the organic solvent extraction usually performed.[10,11,19,22,23]

Compared to other previously described methods for quinine determination in biological samples,[22] the developed method did not explore the quinine metabolism. In fact, quinine is eliminated in urine mainly as unchanged drug and its major metabolite, 3-hydroxyquinine, represents only 14%.[24] Additionally, some high-performance liquid chromatography methodologies also enabled the determination of the synthetic anti-malarial drug chloroquine.[11,12,23] However, in recent years new outbreaks of chloroquine-resistant strains of malaria are guiding the driving forces behind the renewed interest in the use of the naturally occurring alkaloid quinine in the treatment of this parasitical disease.[19] Recently, two flow injection analysis methods with chemiluminometric detection for the determination of quinine were developed[13,14] with detection limits identical to those achieved here. However the former[13] requires an off-line organic solvent liquid–liquid extraction before the introduction to the flow system, besides the use of the organic solvent extractor as a carrier, and the latter one[14] comprises a protein precipitation step and subsequent centrifugation within the sample pre-treatment.

The proposed method provided recovery rates from urine samples in the range from 85 to 115%, which means that their extraction efficiency is very satisfactory. These values are comparable and even higher than those reported in the literature.

The fluorometric determination of quinine, implemented in a fully automatic controlled multipumping flow system, resulted in a simple, low cost, sensitive, and precise methodology that could be a choice for implementation in clinical laboratories in routine assessment as a reliable analytical alternative to the available procedures.

Finally, the proposed automatic methodology could be a valuable strategy for the screening of other organic compounds from biological matrices.

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