Importance of Pre-Analytical Factors Contributing to Measurement Uncertainty, When Determining Sulfadoxine and Sulfamethoxazole from Capillary Blood Dried on Sampling Paper

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Abstract

A bioanalytical method is developed and validated for determination of sulfadoxine (SD) and sulfamethoxazole (SM) in 100 µL capillary blood dried on sampling paper (Whatman 31ET Chr). SD and SM are extracted with 2000 µL perchloric acid and the liquid phase is loaded onto ENV+ solid-phase extraction columns. SD, SM, and the internal standard are separated on a Purospher STAR RP-18 liquid chromatography column (150 × 4.6 mm) with a mobile phase consisting of acetonitrile–sodium acetate buffer pH 5.2, I = 0.1 (33:67, v/v). Analytes are detected with UV at 256 nm. Lower limit of quantitation is 5 µmol/L, where precisions are 4.2% and 3.9% for SD and SM, respectively. Three brands of sampling papers have been compared with respect to absorption properties, extraction recoveries, and variations. Punching out dried blood spots (DBS) instead of cutting spots into strips prior to extraction has been evaluated by examining precision and accuracy of SD and SM determinations. Importance of uniformity of types of sampling paper, sampling volume and biological matrix, benefit of punching out discs from DBS, and impact on absorption properties of different brands of sampling papers are discussed. Avoiding pre-analytical errors whenever possible results in concentrations determined being more accurate and precise.

Introduction

Malaria and pneumonia are the leading causes of child death in malarious countries (1). Children with overlapping symptoms of malaria (fever) and pneumonia (cough and increased respiratory rate) receive dual treatment with both sulfa-based antibiotics [i.e., sulfamethoxazole (SM)] and antimalarials [i.e., sulfadoxine (SD)] (2). Although most African countries have now changed their malaria treatment policies from SD (i.e., Fansidar, SD, and pyrimethamin) or chloroquine to artemisinin-based combination therapy, it is likely that SD will be used for routine treatment of malaria for some time further (3). SM (i.e., Co-trimoxazole; SM, and trimethoprim) is being administered as prophylaxis to people with HIV in Africa, and concerns are that this might speed up the spread of SD-resistant malaria (3). Drug determination is crucial in order to measure frequency of dual antimalarial-antibiotic medication and whether dangerously high sulfa drug levels are reached through dual treatment. Both antimalarial and antibiotic drug resistance is driven by frequent use of non-therapeutic dosages (4), thus it is important to be able to measure blood levels of drugs administered in order to determine if treatment failure is due to drug resistance being developed (5).

For biological samples, when using matrixes such as venous blood and plasma, there must be facilities for storing samples at the sampling location. However, for clinical studies concerning drugs against tropical diseases, samples can be collected at sites remotely located from the laboratory and storage as well as transportation might be problematic. Capillary blood on sampling paper, dried blood spots (DBS), can be dried at room temperature and sent by ordinary mail. In a patient perspective, there are important advantages, especially when sampling children, because only 100 µL capillary blood is needed and no venipuncture is necessary. Risk of HIV virus transmission is almost eliminated, as active virus concentration (infectivity titer) is greatly reduced when blood is dried on sampling paper (6–8). Hepatitis virus may survive for an extended period of time in dried blood (6), but exposure to viruses during transportation could only be achieved if a suitable liquid penetrates the sealed envelope, mixes with dried blood, escapes in sufficient quantity, and comes in contact with the bloodstream of the person handling the package. Therefore, transmission of HIV virus or hepatitis virus when handling DBS is highly unlikely (7). However,

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when analyzing samples, precautions must be taken [i.e., heat-deactivation, thereby completely inactivating HIV viruses and significantly reducing hepatitis C viral activity (8,9)].

Methods to measure either SD in DBS (10,11), SD in human plasma, red blood cells, and whole blood (12), or SM in plasma (13) have been described. In our department we have previously developed a method for simultaneous determination of SD and SM in DBS (14).

In one study, SM concentrations in plasma were determined in healthy volunteers, after a single dose of two tablets of co-trimoxazole containing 400 mg SM per tablet. Maximum concentration in plasma was approximately 156 µmol/L and minimum concentration 20 µmol/L 30 h after dosage; with half-life of 9.6 h (13).

When comparing SD concentration ratios in whole blood to plasma between healthy cases and *P. falciparum* cases, ratios were lower for those with falciparum malaria, due to differences in protein binding and hematocrit (12). Whole blood concentration of SD decreased as percentage hematocrit increased (10). Percentage hematocrit also varied during course of treatment (15). Subjects received two tablets of Fansidar (consisting of 500 mg SD). On day 7, SD concentrations were approximately 107 (91–123) µmol/L in healthy volunteers, while being 143 (112–175) µmol/L in patients with malaria (12). In a more recent study, SD concentrations in DBS were 124 (93–180) µmol/L on day 7, and half-life was 6.7 days, after administering a single dose of Fansidar (25 mg/kg body weight) to children between one and five years old (16). Day 7 measurements of SD accurately predict response to treatment (16), and it is recommended to measure drug concentrations at least at this time point for drugs with half-lives greater than 12 h. For drugs with shorter elimination half-lives, more frequent earlier sampling is required (17).

The method described in this paper aims to improve an earlier developed method for determination of SD and SM simultaneously in DBS (14) by adding solid-phase extraction (SPE). In the previous method, extracts of SD and SM from DBS are injected directly into the liquid chromatograph (LC). In order to obtain chromatograms as free from interfering substances as possible, a clear elute without blood components is preferred. Among extraction fluids evaluated, perchloric acid resulted in superior extraction recovery, with extract being clear and colorless. A Purospher STAR-RP 18 LC column is used in order to minimize risk of damaging the LC column. In order to prevent the corrosive perchloric acid from harming the injector, it is recommended that the injector port is extensively flushed. In the method described in this paper, SPE is added in order to remove perchloric acid, thereby reducing risk on equipment. The resulting method has been validated according to the FDA guideline (18). Apart from method development and validation, impact on concentration determinations if accidentally mixing types of papers in a study is being discussed, by evaluating differences in extraction recoveries of SD and SM from three brands of papers. Punching out blood spots (19) in comparison to cutting the full blood spot into strips during sample preparation, are also described, as correct sampling volume for accurate and precise quantification of analytes is of utmost importance and if personnel collecting samples are not properly informed, DBS can vary in size, resulting in measurements being incorrect. Importance of uniformity of types of sampling paper, sampling volume and biological matrix, benefit of punching out discs from DBS, and impact of different absorption properties of sampling papers are being discussed. Avoiding pre-analytical errors whenever possible results in concentrations determined being more accurate and precise.

Experimental

Chemicals and materials

SD, SM, and sulfadimethoxine (IS) were of analytical grade from Riedel-de Haën (Seelze, Germany) and structures are shown in Figure 1. Perchloric acid 70% p.a. was from Riedel-de Haën; methanol and acetonitrile Chromasolv were from Sigma-Aldrich (St Louis, MO). Acetic acid 100% anhydrous p.a. and sodium acetate anhydrous p.a. were purchased from Merck (Darmstadt, Germany). Sampling papers used were Schleicher & Schuell 2992 (Dassel, Germany), Whatman 31ET Chr, and Whatman 903 from Whatman International (Maidstone, UK). Drug-free venous heparinised human blood was obtained from the Department of Blood Transfusion, Falun Central Hospital (Falun, Sweden).

SPE

Extraction was carried out on an automated Aspec XL4 from Gilson (Middleton, WI) using ENV+ extraction columns (25 mg, 1 mL from Biotage, Uppsala, Sweden). ENV+ is a hydroxylated polystyrene-divinylbenzene polymeric sorbent. The ASPEC system uses a positive air pressure in order to push fluids through the column.

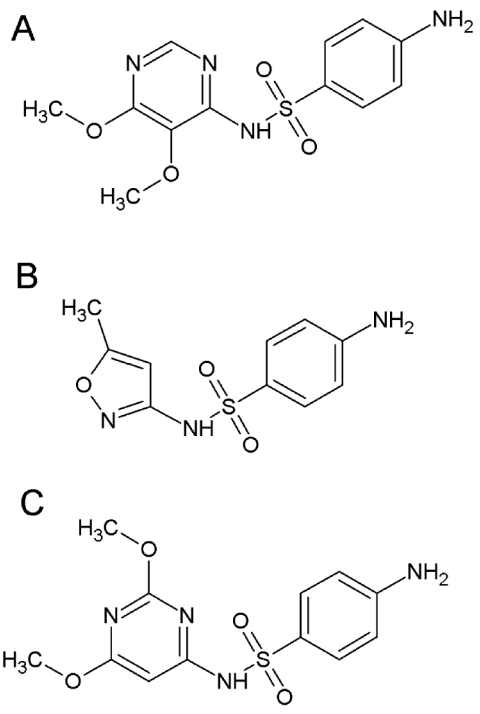

Figure 1. Structures of SD (A), SM (B), and IS (C).

Instrumentation and chromatographic conditions

The LC system consisted of a Waters 515 isocratic pump from Waters (Milford, MA), a Gilson 234 injector from Gilson, a spectroflow 757 absorbance detector at 256 nm from ABI Analytical (Kratos Division, Weiterstadt, Germany), and a VICI Jour Research solvent saver from VICI AG International (Schenkon, Switzerland). Data acquisition was performed with CSW 32 chromatography station from DataApex Ltd. (Prague, the Czech Republic). The mobile phase was acetonitrile–sodium acetate buffer pH 5.2, I = 0.1 (33:67, v/v) with flow rate 1 mL/min. The LC column was Purospher STAR RP-18 endcapped, 150 × 4.6 mm from Merck with a guard column SecurityGuard C18 from Phenomenex (Torrance, CA). Statistical calculations were performed with Statgraphics Plus for Windows 4.0 from Statgraphics centurion (Herndon, VA).

Preparation of calibration standards and quality control samples

Stock solutions of SD and SM were prepared in deionized water with 0.075M sodium hydroxide and IS was prepared in methanol. SD and SM stock solutions were used to prepare working standard solutions in deionized water (Milli-Q). To obtain seven calibration standards, ranging between 5 and 800 µmol/L, and quality control (QC) samples at four concentration levels (15, 75, 250, and 600 µmol/L), working standard solutions were added to drug free venous heparinised blood. Calibration standards and QC samples were then applied in 100 µL aliquots onto sampling paper and left to dry at ambient temperature over night. Samples were stored at –80°C until analysis.

Sample preparation

Samples were cut into four strips or punched out in 16 mm discs and placed in polypropylene tubes. Two-thousand microliters 0.5 mol/L perchloric acid with 25 µmol/L IS was added. Thereafter, samples were shaken on a mechanical shaker for 30 min followed by centrifugation at 2230 × g for 10 min. The liquid phase was decanted into new polypropylene tubes and loaded onto the SPE columns (Table I). Eluates were evaporated at 70ºC under a gentle stream of air until dryness, and reconstituted in 200 µL acetonitrile–deionized water 30:70 (v/v). Twenty microliters of each sample was injected into the LC system.

Comparison of sampling papers

Three brands of sampling papers, S&S 2992, Whatman 31ET Chr, and Whatman 903, were investigated with respect to extraction recovery and precision of SD and SM. Comparison was made with QC samples at two concentrations (15 and 600 µmol/L) in triplicates. Absorption of blood (being linked to drying time, as poor absorption, results in prolonged time until dryness) and formation of uniform, circular blood spots on papers were visually evaluated.

Table I. ASPEC SPE Procedure for Extraction of SD and SM in DBS

SPE-step	Liquid dispensed	Dispensing volume (µL)	Dispensing flow rate (µL/min)	Pressuring air volume (µL)
Conditioning	Deionized water	1000	1000	0
Sample loading	Sample	2000	500	0
Washing	Methanol–deionized water (40:60, v/v)	1000	1000	700
Elution	Methanol–ammonia (95:5, v/v)	500 × 2	500	700

Regression model selection

Although the UV-detector is a linear method of detection, deviation from linearity can be observed due to differences in extraction recoveries. Also, variances in responses are often dependent on concentration (i.e., heteroscedasticity). If heteroscedasticity is confirmed, weighted models as well as models using transformation should be evaluated (20). Models evaluated were ordinary linear regression, weighted linear regression (weights $1/x^{0.5}$, $1/x$, $1/x^2$, $1/y^{0.5}$, $1/y$, and $1/y^2$), and double logarithmic transformation with linear regression. Two calibration curves each day for three days were used to create accuracy profiles. In accuracy profiles, the 90% confidence limits of each mean concentration are included and must be within the fixed limits (21). Deviations of mean values from added concentrations as well as the spread of replicate measurements are taken into account at each concentration level. If more than one model still performed acceptably, precision and accuracy of QC samples, at four concentration levels in five replicates over three days, were used to select optimal regression model.

Method validation

Selectivity

Selectivity was recently evaluated for SD and SM. One hundred microliters blank heparinised blood from six different donors were dried onto sampling paper and extracted with 2000 µL 0.5 mol/L perchloric acid. Twenty microliters of each sample was injected in the LC and evaluated for interferences. Also, a number of antimalarials (pyrimethamine, trimethoprim, pyronaridine, amodiaquine, chloroquine, proguanil, quinine, lumefantrine, artemether, dihydroartemisinin, piperaquine, atovaquone), some of their most common metabolites (desethyl-amodiaquine, desethyl-chloroquine, and cykloguanil), and other commonly used drugs (paracetamol and amoxicillin) were injected at concentrations corresponding to therapeutic concentrations determined in blood or plasma (14).

Stability

Stabilities of long term, short term, and freeze-thaw for SD and SM in DBS, as well as stock solution stability of SD, SM, and IS have also been evaluated and were observed to be stable (14). Long-term stability was evaluated in blood on sampling paper at two concentration levels (25 and 250 µmol/L) at day 1, 5, 10, and 40 at three temperatures (4, 22, and 37°C). Short-term stability was evaluated at room temperature (22°C) for 24 h, in DBS as well as in the extraction fluid. For determination of freeze-thaw stability, DBS, in triplicate at the two concentration levels (25 and 250 µmol/L), were submitted to three cycles of freezing (–86°C) and thawing. Stability of stock solutions

of SD, SM, and IS were assured at room temperature for six hours. ANOVA or *t*-test was used for determination of stability; limits of ± 15% were used as complement (18). Stability of SD and SM with respect to heat treatment for HIV viral deactivation and hepatitis C virus reduction (9) were evaluated at low and high QC levels in DBS. Three DBS samples at 15 and 600 µmol/L were stored at 50ºC for 3 h and compared to non-treated samples. *t*-Test was used for determination of stability, in combination with limits of ± 15% deviation of treated samples compared to non-treated.

Accuracy, precision, and lower limit of quantitation

Accuracy and precision were determined using QC samples at four concentrations (15, 75, 250, 600 µmol/L), five replicates for five days (n = 25 at each concentration level). Within- and between-day precisions were calculated with concentrations determined from calibration curves prepared at the respective day. Lower limit of quantitation (LLOQ) was determined at the concentration where accuracy was within ± 20% and precision 20%, with signal-to-noise ratio > 5 for SD and SM (18). LLOQ was determined during regression model selection, from calibration graphs in duplicate generated over three days. The selected LLOQ was verified during method validation.

Extraction recovery

Extraction recoveries were calculated by comparing peak heights by direct injection of standard solutions of SD and SM with the same nominal concentrations as QC samples. Samples used for determination of accuracy and precision were evaluated for extraction recovery.

Calibration

Calibration graphs in the interval 5–800 µmol/L were constructed with the ratio of SD or SM peak area and IS peak area, using the regression model optimal for the data as determined during pre-validation. One calibration curve was analyzed each day for five days and correlation, intercept, and slope were calculated (mean ± s, n = 5).

Comparison of punched and cut blood spots

Precision and accuracy of SD and SM determined in punched blood spots were compared to determinations of the analytes in blood spots being cut out in full and divided into four strips. Fifteen percent limits of precision and ± 15% accuracy were used, as suggested by the FDA guidelines (18). Calibration curves were prepared in the same manner as samples (i.e., calibration standard blood spots were either punched out or cut into strips). Determinations were made from triplicates of QC samples at four concentration levels (15, 75, 250, and 600 µmol/L) for two days.

Results and Discussion

Comparison of sampling papers

When evaluating absorption of blood and formation of uniform, circular blood spots differences between brands of papers were noticeable. Whatman 31ET Chr as well as 903 absorbed blood well and formed uniform, circular blood spots; both types of papers differing from 2992 where blood dried slowly, forming irregular spots. Both 2992 and 903 had significantly higher extraction recoveries as compared to 31ET Chr (Figure 2). Disadvantage of 903 sampling paper was greater variance, at least at high concentration level SD and SM, as compared to 31ET Chr (Figure 2). As 2992 had the drawback of blood forming irregular spots and drying slowly, this paper was not suitable for the method being developed. The 903 paper is commonly used for neonatal screening, while 31ET Chr often is used in electrophoresis. At least from our experience, 903 is much more difficult to purchase. This might be due to the fact that 903 is registered as an in vitro medical device. After Whatman incorporated Schleicher and Schuell, 2992 is no longer available, and to the best of our knowledge, there is no interchangeable Whatman grade. Due to accessibility, absorption properties, and less variation between samples, 31ET Chr was selected for the method described in this paper.

Regression model selection

The optimal regression model for SD and SM was double logarithmic transformation with linear regression. Model selection was made from calibration curves prepared from blood spots cut into strips, and the same regression model was applied when evaluating samples prepared from punched blood spots.

Method validation

Selectivity

No chromatographic interferences were found in blood from six different donors. None of the injected pharmaceuticals interfered with determination of SD, SM, and the IS (14).

Stability

Both SD and SM were stable during all circumstances evaluated, within the ± 15% limit. Also, the IS was stable for at least 6 h at room temperature (14). Stability was verified for SD as well as SM in DBS when heat-treated at 50°C for 3 h.

Accuracy, precision, and LLOQ

LLOQ was 5 µmol/L for both SD and SM, where precision and accuracy were 4.2% and –1.3% for SD, and 3.9% and –0.4% for

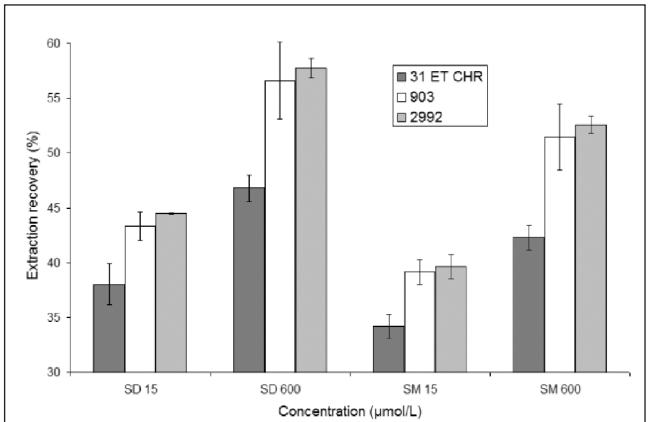

Figure 2. Extraction recovery of SD and SM on Whatman 31ET Chr, 903, and 2992 sampling paper (mean ± s, n = 3).

SM. The signal-to-noise ratio was 7 for SM and 14 for SD at LLOQ. SD could have been determined at somewhat lower concentrations. As assay sensitivity was satisfactory, the LLOQ was set at 5 µmol/L for SD and SM alike, allowing the same calibration range to be used. Accuracy and precision of quality control samples are shown in Table II. Overlaid chromatograms of a blank sample, a sample at LLOQ, and a QC sample (15 µmol/L) are shown in Figure 3.

Extraction recovery

Extraction recoveries for SD and SM are shown in Table II. In earlier work with SD and SM, extraction recoveries were 66–77% for SD and 55–75% for SM when extracting SD and SM from DBS (14). Extraction recoveries of IS at each concentration level of SD and SM are also shown in Table II.

Calibration

Linear calibration graphs in the interval 5–800 µmol/L were obtained with correlation coefficients (r) 0.9998 ± 0.0001 for SD and 0.9997 ± 0.0003 for SM. Slopes were 1.062 ± 0.022 and 1.093 ± 0.008 for SD and SM, respectively, and intercepts were –3.054 ± 0.059 and –3.399 ± 0.039 (mean ± s, n = 5).

Comparison of punched and cut blood spots

The use of punched blood spots instead of cutting spots into strips during sample preparation gave satisfactory results (Table III), well within the ± 15% limit (18). This approach can be useful in field situations where exact sample volumes cannot be collected (11). It is important to keep in mind that when punching DBS, samples need to be uniform as well as circular and this is highly dependent on absorption properties of sampling paper used. Another issue to be concerned with is the fact that method sensitivity and size of punched spots are strongly linked. Diameter of the punch used in this paper is only slightly smaller than the DBS and therefore sampling volume needs to be 100 µL or more, although not needing to be exact. Using a smaller punch would be possible as no difference has been noticed between punching blood spots in the centre and in the periphery of the DBS when comparing concentrations determined; however, one needs to keep in mind that this will reduce sensitivity of the method described. Changing punch size may therefore require a partial validation (18).

Discussion

A method for simultaneous determination of SD and SM has been developed. Blood spots are cut into strips, SD and SM are extracted with perchloric acid, extracts are loaded onto ENV+ SPE columns, and eluates are injected onto the LC after evaporation and reconstitution. This method provides an alternative to the method previously developed in our department, where extracts (SD and SM in 0.5 mol/L perchloric acid) were directly injected into the LC (14), by adding a SPE step, and thereby removing the perchloric acid prior to injection. This reduces maintenance required of equipment, but increases sample preparation time.

During SPE method development, a number of stationary phases were evaluated based on different separation mechanisms [i.e., reversed-phase (CN, C2, C4, C6, C8, and C18), ion exchange (SAX and SCX-2), hydrophilic interaction (Zic-Hilic), and polymeric stationary phases (ENV+ and HLB)]. ENV+ is hydrolyxated while HLB contains *N*-vinylpyrrolidone groups. Retention was only achieved on C8, ENV+, and HLB for SD, SM, and the IS. Due to variations noticed with respect to extraction recoveries, and concern for the robustness of the resulting method, C8 and HLB were excluded. ENV+ had the drawback of poor extraction recoveries although still high enough for the desired assay sensitivity. When attempting to improve extraction recovery, basic elution was evaluated; assuming retention would be less as SD and SM are more polar at high pH. Initially, the 100 mg bed was used. When evaluating bed masses between 25 and 100 mg, smaller masses corresponded to higher extraction recoveries. Therefore, in the resulting method the ENV+ column with bed mass of 25 mg was used.

In order to enhance simplicity of the method developed, precision and accuracy of SD and SM extracted from punched DBS were evaluated. Punched DBS did perform well and could be used to eliminate problems with differences in size of spots; especially if this is combined with pre-printed circles on the sampling paper aiming to secure that blood volume is large enough. The method described in this paper uses Whatman 31ET Chr sam-

Table II. Precision, Accuracy, and Extraction Recovery for the Determination of SD and SM in Blood Applied onto Whatman 31ET Chr Sampling Paper (n = 25)

	Added (µmol/L)	Found (µmol/L)	Within-day (%, n = 25)	Between-day (%, n = 5)	Accuracy (%)	Recovery (%, mean ± s)	Recovery IS (%, mean ± s)
SD	15	16	5.7	4.9	4.0	40 ± 4	61 ± 6
	75	77	3.1	2.4	3.0	41 ± 5	59 ± 5
	250	253	3.4	0.1	1.1	46 ± 4	63 ± 4
	600	605	2.6	3.0	0.8	52 ± 3	67 ± 4
SM	15	16	5.5	2.6	7.0	30 ± 3	61 ± 6
	75	79	3.2	1.1	5.0	33 ± 4	59 ± 5
	250	250	4.2	1.1	0.1	37 ± 3	63 ± 4
	600	614	3.1	4.0	2.5	44 ± 2	67 ± 4

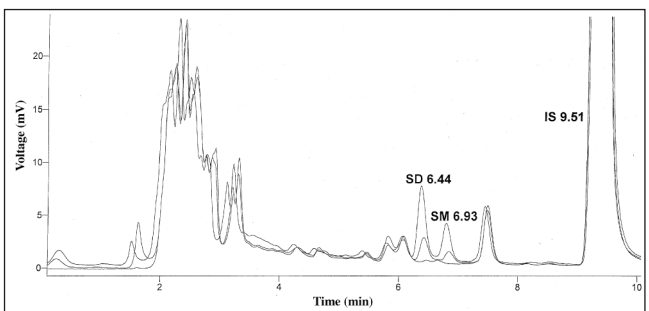

Figure 3. Chromatogram with blank sample, LLOQ sample, and QC (15 µmol/L) sample.

Table III. Precision and Accuracy for the Determination of SD and SM in DBS, Blood Spots Cut Into Strips in Comparison to Punched Blood Spots (*n* = 6)

	Added (µmol/L)	Found (µmol/L, mean ± s)	Within-day (%, *n* = 6)	Between-day (%, k = 2)	Accuracy (%)
DBS cut into strips					
SD	15	15.6 ± 0.6	4.2	4.5	4.0
	75	78 ± 3	3.8	1.5	3.7
	250	254 ± 8	3.3	4.2	1.5
	600	601 ± 15	2.4	1.2	0.2
SM	15	15.8 ± 0.7	4.5	4.6	5.0
	75	77 ± 3	4.2	1.1	2.5
	250	250 ± 8	3.0	3.5	0.0
	600	619 ± 17	2.8	1.2	3.2
Punched DBS samples					
SD	15	15.7 ± 0.3	2.0	8.6	4.5
	75	76 ± 1	1.8	4.7	1.3
	250	251 ± 5	1.9	6.4	0.5
	600	601 ± 41	6.8	3.1	0.1
SM	15	16.1 ± 0.5	2.8	11.8	7.6
	75	77 ± 2	2.3	5.6	2.7
	250	247 ± 5	2.0	6.5	–1.3
	600	615 ± 44	7.1	3.1	2.6

pling paper, as blood is absorbed rapidly, resulting in uniform, circular spots, and the sampling paper is easy to purchase. Drawback of 31ET Chr in comparison to 903 is increased loss in extraction recovery with the former. However, as both SD and SM are at fairly large concentrations in biological fluids, sensitivity is adequate with the 31ET Chr sampling paper. Differences in extraction recoveries of SD and SM between sampling papers also highlights the importance of using the same type of paper in patient samples as in calibration standards and QC samples. It is also of great significance that patient samples as well as calibration standards and quality control samples are of the same blood volume (or being punched out to discs with the same diameter). Changing sample volume to a smaller one than the method is being developed for during a clinical study, results in loss in assay sensitivity. If sampling volume and (or) sampling paper differ from those stated in the method developed and validated, additional error in drug concentration determination is introduced. When determining SD, concentrations can diverge between healthy volunteers and people with falciparum malaria, due to differences in hematocrit between groups (12). Also, hematocrit varies during course of treatment (15). As single day 7 measurements of SD accurately predicts response to treatment (16), correction of SD concentration with respect to percentage hematocrit appears not to be needed.

To conclude, there are some reasonably controllable factors contributing to errors in drug determination of SD and SM. It is important that sampling volume and sampling paper type are correct. If DBS will be punched out, spots need to be circular and uniform. Avoiding pre-analytical errors whenever possible results in concentration determinations being more accurate and precise, and accurate blood concentration is essential to curing malaria (17). As mentioned previously in this paper, drug determination is also crucial for evaluating frequency of dual antimalarial-antibiotic medication and whether dangerously high sulfa drug levels are reached through dual treatment with both SD and SM (in combination with pyrimethamin and trimethoprim, respectively). Also, it is important to be able to measure blood levels of drugs so that reasons for treatment failure can be known (5).

Conclusion

A method for measuring SD and SM simultaneously, with a simple sampling method such as DBS, is highly needed, especially in countries undergoing Home Based Management of Fever strategies where compliance is important to monitor. The method described in this paper provides a simple field sampling method in combination with a sample preparation method being less demanding on the LC system, which can be used to increase successful treatment of malaria and pneumonia in low-income settings. Importance of reducing pre-analytical errors when determining concentrations for SD and SM has been highlighted.

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