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RESEARCH ARTICLE

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A simple and rapid external standard calibration HPLC method for determination of lumefantrine in dried blood spot samples from malaria patients in Botswana

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Abstract

A simple external calibration liquid chromatography-diode array detector method was developed, validated, and applied for the determination of lumefantrine (Lum) in dried blood spot (DBS) samples collected from malaria patients in Botswana. The samples were validated in accordance with the United States Food and Drug Administration guidelines for bioanalytical methods after sample preparation using solid–liquid extraction. Separation was achieved using an XTerra C18 column (50 × 4.6 mm, 5 μm), and a binary solvent system of acetonitrile and water adjusted to pH 2.3 was used as the mobile phase. The validated method was applied for the determination of Lum in DBS samples collected from malaria patients infected with *Plasmodium falciparum* in Botswana. The calibration curve was linear between 0.5 and 12 μg/mL with a coefficient of determination (R^2) of 0.9996. The limit of detection and the lower limit of quantification were 0.5 and 1.4 μg/mL, respectively. The efficiency of extraction measured as percentage recovery ranged between 84.2% and 107.8% at the three quality control (QC) levels, that is, low QC, mid QC, and high QC. In conclusion, data suggest that the method is suitable for the determination of trace Lum in biofluids and can also be used for therapeutic drug monitoring and pharmacokinetic profiling.

KEYWORDS

dried blood spot, HPLC, LLOQ, lumefantrine, malaria

1 | INTRODUCTION

Malaria is one of the most severe global public health problems. It is a leading cause of death and disease in many developing countries, especially in Africa (World Health Organization [WHO], 2023). It is transmitted between humans through *Anopheles* mosquito bites, thus making it one of the most dangerous parasitic infectious diseases globally. It is caused by a protozoan parasite of the genus *Plasmodium*, which includes *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, and *Plasmodium knowlesi* (Boudhar et al., 2016). The most dangerous among the malarial parasites is *P. falciparum* (WHO, 2023), the one responsible for the high cases of morbidity and mortality (mostly in Africa), followed by *P. vivax* which has a wide geographical spread (WHO, 2023).

Botswana adopted the use of artemisinin-based combination therapies (ACTs), particularly coartem, that is, a combination of artemether and lumefantrine (Lum), in 2007 (Bango et al., 2020; The Republic of Botswana Ministry of Health Department of Public Health, 2006) as a first-line treatment for malaria. Coartem (20 mg of artemether [Art] and 120 mg of lumefantrine) is an ACT used for the treatment of uncomplicated malaria. This therefore raised the need to develop methods for detecting Art and Lum in human fluids such as whole blood, plasma, and sera to facilitate therapeutic drug monitoring (TDM) as well as adduce pharmacokinetic (PK) profiles that are geographical disposition dependent.

Several methods have been reported for the determination of Lum in whole human blood and plasma (César et al., 2011; Huang et al., 2018; Khalil et al., 2011; Khuda et al., 2014; Lindegårdh et al., 2005; Ntale et al., 2008; Pingale & Mangaonkar, 2013; Silva et al., 2015). These methods employ either high-performance liquid chromatography–ultraviolet/visible (HPLC-UV/Vis.) detection or liquid chromatography–mass spectrometry (LC-MS). Suleman et al. (2015) reported the use of gas chromatography (GC) for the determination of Lum in pharmaceutical products after derivatization. However, derivatization has several disadvantages, the most important being the lack of universal and environmentally benign derivatization reagents. Thus, HPLC-UV and LC–MS/MS are the most widely used methods for the determination of Lum, and both are applied to the same extent, although in low- and middle-income countries (LMICs), low-cost and easy-to-maintain instruments such as HPLC are preferred. Precise and accurate analysis is usually preferred with other analytical considerations such as the use of internal standards, which is still controversial (Imre et al., 2019; Usher et al., 2015). The design of modern HPLC injectors is adequate to effect quantitative transfer of solutions from sample loops into columns for separation. This means that all quantitative analyses using HPLC can be accomplished without the use of internal standards, that is, using external standard calibration with minimal loss in precision and accuracy of the method. Poor precision and accuracy are usually characteristic of instruments in which injectates are prone to volume fluctuations resulting from variations in volumes injected due to solutions clinging to the walls of injection syringes in, for example, GC analyses. External standard calibrations simplify the analyses especially in LMICs, where additionally, procurement of deuterated materials that are mainly used as internal standards can be expensive and in events where an appropriate substance to use as internal standard is limited by availability. Therefore, a cheap, simple, and robust analytical method based on external calibrations in these environments can simplify the stereotype in bioanalysis. This is a figure of merit.

The use of dried blood spot (DBS) sampling for whole blood for the determination of Lum has been reported only by Ntale et al. (2008) and applied in malarial samples obtained from Uganda. The use of DBS is attractive especially in LMICs due to its simplicity, storage, and transportation over conventional whole blood, plasma, and sera (Li & Tse, 2010; McDade, 2014; McDade et al., 2007; Wilhelm et al., 2014). Its collection is said to be minimally invasive and relatively painless with low risk, thus simplifying the recruitment of subjects (Parker & Cubitt, 1999). In this study, the simplicity of DBS filters and the advantages described here motivated their use in combination with HPLC-diode array detection (DAD). The aim of this study was to develop and validate a bioanalytical method using HPLC-DAD without the use of an internal standard, that is, external standard calibration, and apply it for the determination of Lum (whose structure is shown in Figure 1), in selected DBS samples from malaria patients in Botswana. It was anticipated that the method would later be adapted for TDM and PK profiling of Lum.

2 | MATERIALS AND METHODS

2.1 | Reference standards and chemicals

Lum standard was purchased from the United States Pharmacopeia Convention (Rockville, USA). All solvents used were of analytical grade. HPLC-grade acetonitrile and methanol (99.9% purity) were obtained from Fisher Scientific, (Loughborough, UK) and Sigma-Aldrich (St. Louis, USA), respectively. Formic acid (FA, purity: 85%) was purchased from Sigma-Aldrich. *n*-Hexane was supplied by Chemcity (Gaborone, Botswana), and ethyl acetate was obtained from Minema (Johannesburg, South Africa).

2.2 | Apparatus and instrumentation

High-purity water used in this study was processed using a Heal Force Super series NW ultra-pure water system (Shanghai, China). A Thermo Scientific SL 16R centrifuge (Rochester, NY, USA) was used for centrifugation experiments. A Lab Companion shaking incubator SI-300 (San Diego, CA, USA) was used for vortexing. The neat calibration standards were weighed using a Sartorius microbalance. The mobile phase pH was measured using a pH meter from HANNA Instruments (Smithfield, USA). The concentrations of the analytes were determined using an Agilent 1100 HPLC system coupled to a diode array detector (Agilent Technologies, Palo Alto, CA, USA). This system consists of a quaternary pump (G1311A), a vacuum degasser (G1379A), an autosampler (G1313A), and a thermostated column

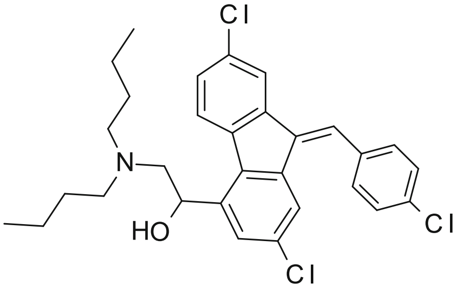

FIGURE 1 Chemical structure of lumefantrine.

compartment (G1316A) and a diode array detector. The quaternary pump comprises four solvent reservoirs connected to a vacuum degasser.

2.3 | Ethical approval

Ethical approvals for the project were obtained from the University of Botswana (ref.: UBR/RES/IRB/BIO/GRAD/007) and the Ministry of Health (ref.: DPH 20/4 XIII (99)).

2.4 | Sample collection

Blood spots were collected on Munktell TFN filter papers (Ahistrom GmbH, Bärenstein, Germany) from patients on oral Art/Lum combination therapy. All the patients were diagnosed using the rapid diagnostic test for uncomplicated malaria. All the DBS filter paper samples were donated by the University of Botswana–University of Pennsylvania (UB–UPenn) Partnership from an archive sample repository under the Botswana malaria program. They were collected in accordance with the World Health Organization guidelines under the malaria program. The guidelines (not shown here) included recording the baseline characteristics of the patients from whom the coded samples were collected longitudinally on days 0, 3, 7, 14, 21, and 28 after dosing using a single blind technique. DBS samples with ~30 μL of blood spotted on filters were collected by laboratory personnel in two Ministry of Health districts, that is, Ngamiland and Central districts. Nine samples collected from Ngamiland district and three from Central district were used in this study. The DBS filters were punched into the centrifuge tubes for extraction during analysis.

2.5 | Stock solutions

Stock solutions of Lum (1000 ppm) were prepared in the mobile phase; 25 mg was dissolved in 25 mL of acetonitrile containing 0.15% FA. The stock solution was stored in an amber-colored bottle in a refrigerator. The working standards were prepared by serially diluting the stock solution in acetonitrile before analysis. All working standards were stored at 4°C.

2.6 | DAD wavelength column, mobile phase selection, and optimization of the flow rate

The maximum absorption wavelength (λ_{max}) was selected by scanning a solution containing Lum using a double-beam scanning UV/Vis. spectrophotometer. Selection of the analytical column was limited by availability. However, several column types, including the Waters C18 XTerra column, were tested. Acetonitrile and methanol were compared for suitability as mobile phases, whereas the flow rate was optimized by increasing the values from 0.2 to 0.8 mL/min in increments of 0.2 mL/min. The data were converted to linear flow rate (in cm/s); the linear flow rate was plotted as the abscissa, and the height equivalent to a theoretical plate (HETP) obtained using the Agilent ChemStation acquisition software was plotted as the ordinate. The resultant plot provided the optimum flow rate that was used throughout these experiments.

2.7 | High-performance liquid chromatography

Chromatographic separations were achieved using optimized conditions on an XTerra column (50 × 4.6 mm, 5 μm particle size). The mobile phase consisting of acetonitrile and water was adjusted to pH 2.3 using FA, filtered through a 0.45-μm filter paper, and degassed ultrasonically for 15 min. Gradient elution was used (as shown in Table 1) using the optimized gradient parameters. The mobile phase flow rate was 0.6 mL/min, and the injection volume was 10 μL. Separation was performed at 25°C because Lum is thermally labile. Lum was detected at 242 nm using the diode array detector. Data processing and control were performed using Productivity ChemStation software, version 10.02.

2.8 | Sample preparation using solid–liquid extraction

The extraction procedure for DBS was adopted from Khalil et al. (2011) and slightly modified. The modification was that no buffer was used to adjust the pH of the samples. Instead of plasma samples, DBS samples collected on filter papers were used. Control DBS samples that were screened for Lum and found to contain none were used for validation. DBS filters devoid of Lum were first spiked by spotting 30 μL of spiked bovine whole blood at the required concentration and drying them before extraction. This procedure enabled the calculation of percentage recoveries. After spiking, 3 mL of *n*-hexane:ethyl acetate (70:30, %v/v) was added to each of the centrifuge tubes containing the punched DBS filters. The mixture was vortexed for 15 min vigorously and further centrifuged for 10 min at 4000 rpm. The resulting organic phase was transferred and evaporated to dryness under a stream of nitrogen gas at room temperature. The residue was reconstituted in 0.15 mL of the mobile phase, that is, acetonitrile:water (pH 2.3) (80:20, %v/v), and vortexed for ~1 min. Ten microliters of the extract was then injected into the HPLC system for analysis. This procedure was also applied to real DBS samples.

TABLE 1 Optimum gradient elution program.

Time (min)	Acetonitrile	Water (pH 2.3)
0.0	82	18
0.91	50	50
1.0	82	18

2.9 | Method validation

The United States Food and Drug Administration (USFDA) guidelines (USFDA, 2018) were used for validating the method. A partial validation strategy was chosen for this purpose. The following analytical performance parameters were investigated: linearity, limit of detection (LOD), lower limit of quantification (LLOQ), precision, accuracy, percentage recovery, and percentage matrix effect (%ME). After the method was confirmed fit for the determination of Lum, it was applied to selected DBS samples from patients infected with malaria in Botswana.

2.10 | LOD and LLOQ

Nine solutions of Lum were prepared in acetonitrile containing 0.15% FA for constructing the calibration curve. The concentrations of the calibrators were 1.4, 2, 3, 4, 5, 6, 8, 10, and 12 μg/mL, a zero calibrator; that is, a blank was added as recommended by the USFDA guidelines. At each concentration level including the zero calibrator, a punch of blank control DBS filter was introduced. Vials were left to stand for 30 min and then shaken vigorously for 2 min; then 10 μL was injected into the HPLC system. Using optimized HPLC conditions, three determinations were made at each concentration level, and the peak areas of standards were used to construct the calibration curve. Using the constructed curve, the LOD thrice the signal-to-noise (S/N) ratio and the LLOQ 5 times the S/N ratio were estimated using Equation (1) (González & Alonso, 2020):

$$\mathrm{LLOQ} = \left(\frac{y_{\mathrm{blank}} + 5_{\sigma}}{b}\right), \tag{1}$$

where y_{blank} is the background signal or the signal of the blank equivalent to $s_{y/x}$ which is the standard deviation of the line of regression of y on x, that is, the calibration curve. The upper limit of quantification (ULOQ) was set at 12 μg/mL considering the therapeutic range for Lum is 1–10 mg/L (Silva et al., 2015). The calibration range was selected so that the therapeutic range would be covered and any sample concentration that exceeded the ULOQ would be analyzed by dilution (González & Alonso, 2020).

2.11 | Percentage matrix effect

%ME is defined in the USFDA guidelines (USFDA, 2018) as an alteration or interference in response by any unintended analytes present in the sample/matrix. This was calculated using Equation (2) (Matuszewski et al., 2003):

$$\%\mathrm{ME} = \frac{B - A}{A} \times 100, \tag{2}$$

where B is the average peak area of the matrix standard and A is the average peak area of the solvent standard.

2.12 | Selectivity, linearity, and extraction efficiency

These parameters were determined by analyzing different blank samples at least five times for each. These blanks were of similar matrix (surrogate) as the analyte of interest. A method is said to be selective if no interference is observed at the retention times of the analyte. Linearity was measured as the coefficient of determination, that is, R^2 value of the calibration curve.

The extraction efficiency expressed as percentage recovery was measured by spiking blanks of the DBS filters with known concentrations of the analyte at three quality control (QC) concentrations, that is, low, mid, and high set for the estimation of precision and accuracy. The solid–liquid extraction (SLE) sample preparation technique was used to extract the analytes for five replicates at the low-, mid-, and high QC levels, and five determinations were made per replicate. Recoveries were calculated as the ratio of the recovered concentration to the spiked concentration expressed as a percentage.

2.13 | Accuracy and precision

Four QC samples were prepared by spiking blank DBS filters at four concentration levels, as recommended by González and Alonso (2020), and extracting using the SLE procedure. The QC nominal concentrations were spiked at the LLOQ, at thrice the LLOQ (low QC) sample, at mid-calibration curve (mid-QC), and at 75% of the ULOQ (high QC) level. For intra-assay precision and accuracy determination, five replicates and five measurements of each QC sample were made, whereas for inter-day assay precision and accuracy, the same procedure was repeated on five different days. According to the USFDA guidelines (USFDA, 2018), the within- and between-run accuracy should not exceed 15% of nominal concentration except at the LLOQ where it should not exceed 20%. The precision expressed as the relative standard deviation (%RSD) was calculated as the ratio of the standard deviation to the average concentration expressed as a percentage. The USFDA guidelines (USFDA, 2018) recommend that the within- and between-run precision, that is, %RSD, should not exceed 15% of the nominal concentration except at the LLOQ, which should not exceed 20% coefficient of variance.

2.14 | Determination of Lum in real DBS samples

DBS samples from malaria patients on Art/Lum were collected as previously described. The samples and blank controls were extracted using the modified SLE procedure. Lum was eluted from the column and detected from the extracts. Concentrations were calculated, and the logarithmic concentration–time curves were plotted to determine the time domain trend of the samples. Baseline data such as age and gender were not considered because the focus was to develop and validate a bioanalytical method that could effectively separate, identify, and quantify Lum from DBS samples.

3 | RESULTS

3.1 | DAD wavelength column, mobile phase selection, and flow rate optimization

Lum with the maximum wavelength of 242 nm was dissolved in acetonitrile:water (pH 2.3). The Waters XTerra (50 × 4.6 mm, 5 μm) column exhibited good peak symmetry for Lum with minimal interfering peaks. The mobile phase composition of acetonitrile:water adjusted to pH 2.3 using FA yielded the best peak shape compared to methanol (result not shown). Figure 2 shows the van Deemter plot of HETP versus the linear flow rate of the mobile phase.

Using the SLE procedure and the optimum flow rate, chromatograms obtained after elution of Lum in the surrogate matrix and an extract of a spiked DBS sample at the LLOQ are shown in Figure 3.

Chromatograms obtained after elution of sample extracts of sample 376 from Ngamiland district and sample 171 from Central district 14 days after Lum dose are shown in Figure 4.

Real DBS peaks for samples 171 and 376 were lower in peak areas compared to those of 1-μg/mL Lum in the matrix and in the extract of a matrix spike at 1.3 μg/mL because after sample extraction using SLE, all samples were diluted by a factor of 5, which was used to correct the concentrations.

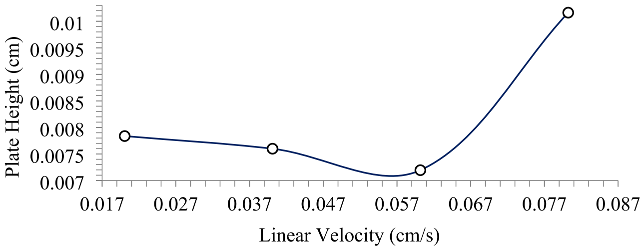

FIGURE 2 The van Deemter plot for optimization of flow rate.

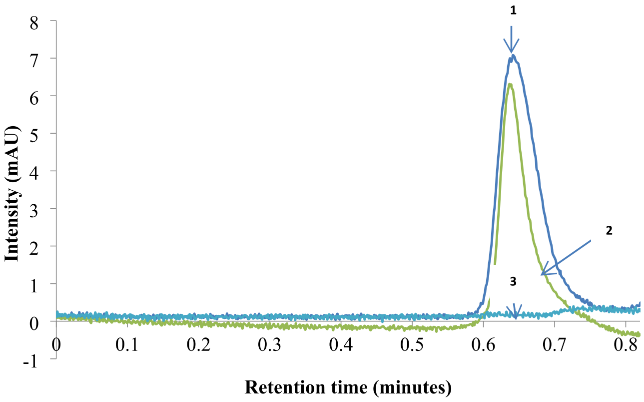

FIGURE 3 Chromatograms of (1) 1 μg/mL Lum (lumefantrine) in the matrix; (2) extract of a matrix spike at the LLOQ (lower limit of quantification), that is, 1.3 μg/mL; and (3) matrix blank.

3.2 | Method validation

3.2.1 | Calibration curves

A calibration curve with nine calibrators was constructed. The curve enabled the estimation of the LOD, which was used as a reference for spiking blank DBS filters to enable the calculation of the recovery, accuracy, and precision.

3.2.2 | Linearity, LOD, and LLOQ

The calibration curve constructed using the matrix of interest was linear with a regression equation of $y = 58.25x - 16.713$ and a coefficient of determination (R^2) of 0.9996, indicating good linearity, from which the LOD was estimated to be 0.4 μg/mL. This detection limit was considered to be the method detection limit because the calibration curve was constructed in the matrix of interest. The LLOQ calculated using Equation (1) at five times the S/N ratio was 0.5 μg/mL. The calibration was linear throughout the selected calibration range, that is, 0.5–12 μg/mL. Table 2 presents the nominal concentrations that were used to construct the calibration curve recalculated using the regression equation with associated error analysis.

3.2.3 | Accuracy and precision

Table 3 presents the intra- and inter-day assay precisions and accuracies for the four QC samples.

3.2.4 | Percentage recoveries

Percentage recoveries were estimated at the three QC concentrations used for validating precision and accuracy. At each nominal concentration, three replicate sample aliquots were made with five

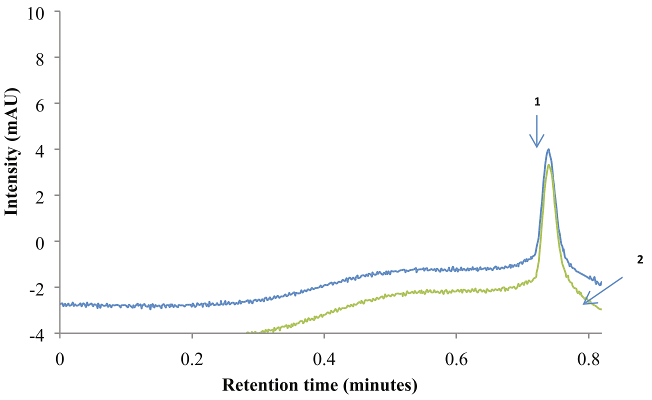

FIGURE 4 Chromatograms of (1) sample 171 from a Central district sample 14 days after Lum (lumefantrine) dose and (2) sample 376 from a Ngamiland sample 14 days after Lum dose.

TABLE 2 Error analysis for recalculation of concentrations using nominal calibration concentrations and the regression equation.

Nominal concentration (μg/mL)	Mean back-calculated concentration (μg/mL) ($n = 3$)	% Bias ($n = 3$)	%RSD ($n = 3$)
0.5	0.52	−4.00	7.09
2.0	1.92	4.00	0.28
3.0	3.08	−2.67	1.79
4.0	3.86	3.50	0.56
5.0	5.06	−1.20	0.49
6.0	6.06	−1.00	0.57
8.0	8.01	−0.12	1.07
10.0	10.08	−0.80	0.08
12.0	11.97	0.25	0.36

Note: $R^2 = 0.9991$.
Abbreviation: RSD, relative standard deviation.

TABLE 3 Intra- and inter-day accuracy and precision of four QC samples at the LLOQ, low QC, mid-QC, and high QC.

	Intra-assay ($n = 5$)			Inter-assay ($n = 5$)		
Nominal QC concentration (μg/mL)	Measured concentration (μg/mL)	% Bias	%RSD	Measured concentration (μg/mL)	% Bias	%RSD
0.5 (LLOQ)	0.49	2.00	3.41	0.51	−2.00	4.45
1.5 (low QC)	1.46	2.67	12.93	1.56	−4.00	13.11
6.0 (mid-QC)	5.61	6.50	4.52	5.72	4.67	4.86
9.0 (high QC)	8.15	9.44	3.79	7.78	13.56	6.39

Abbreviations: LLOQ, lower limit of quantification; QC, quality control; RSD, relative standard deviation.

determinations per replicate per day. Recovery studies were performed within an interval of 3 days parallel with precision and accuracy estimations. The recoveries expressed as % ± *s*, where *s* is the standard deviation, were 107.8 ± 11.1% at the low QC, 96.7 ± 11.1% at the mid-QC, and 84.2 ± 5.5% at the high QC nominal concentrations.

3.2.5 | Matrix effects

The USFDA guidelines (USFDA, 2018) recommend that the selectivity, that is, the response in the blank matrix and calibrators, should not deviate by more than 5%. The results determined using Equation (2) are presented in Table 4.

TABLE 4 Matrix effects expressed as percentage.

Concentration (μg/mL)	% Matrix effect
1.4	−4.5
2.0	−4.7
3.0	−3.6
4.0	−3.8
5.0	−6.7
6.0	−5.6
8.0	−6.9
10.0	−4.2
12.0	−5.2

3.2.6 | Application of DBS sample analysis

Tables 5 and 6 present average concentrations of Lum in 12 malaria patients from Ngamiland and Central districts over a period of 28 days collected on different days. The tables present the results for samples collected after 3, 14, and 28 days for simplicity.

Figure 5 shows log concentration versus time plots for two subjects, that is, sample 591 from Ngamiland district and sample 085 from Central district, that were plotted to mimic those that are used to adduce PK profiles. The log concentration versus time plot for the samples collected after the administration of Lum at 0, 3, 7, 14, and 28 days was used for clarity. Assuming that the variances of the data were not equal and to demonstrate the usability of the concentration–time profiles, the log concentration versus time plots were used because their slopes in PK profiling yield the elimination constants (K_e). The log concentration–time plot can also be used to predict the distribution of the drug and can distinguish between an orally and intravenously administered drug.

In contrast with other studies that have been reported in the literature, the nonspecificity of LC-UV can be observed in the differences in the LOD and LOQ values estimated in this study compared to the LC–MS methods, as seen in Table 7.

TABLE 5 Average concentrations of Lum in DBS filters collected from Ngamiland.

Subject code	Day 3 Average concentration (μg/mL) (*n* = 3)	Day 14 Average concentration (μg/mL) (*n* = 3)	Day 28 Average concentration (μg/mL) (*n* = 3)
588	3.2	4.9	4.4
576	2.7	3.6	5.9
375	3.6	4.2	4.0
376	3.5	4.6	5.8
400	3.3	3.9	5.1
129	3.2	2.8	4.0
591	4.6	3.1	3.0
587	3.0	2.9	4.2
590	3.2	3.2	3.7

Abbreviations: DBS, dried blood spot; Lum, lumefantrine.

TABLE 6 Average concentration of Lum in DBS filters collected from Central district.

Subject code	Day 3 Average concentration (μg/mL) (*n* = 3)	Day 14 Average concentration (μg/mL) (*n* = 3)	Day 28 Average concentration (μg/mL) (*n* = 3)
085	3.8	4.5	3.4
167	3.5	3.9	3.7
171	4.2	3.4	5.4

Abbreviations: DBS, dried blood spot; Lum, lumefantrine.

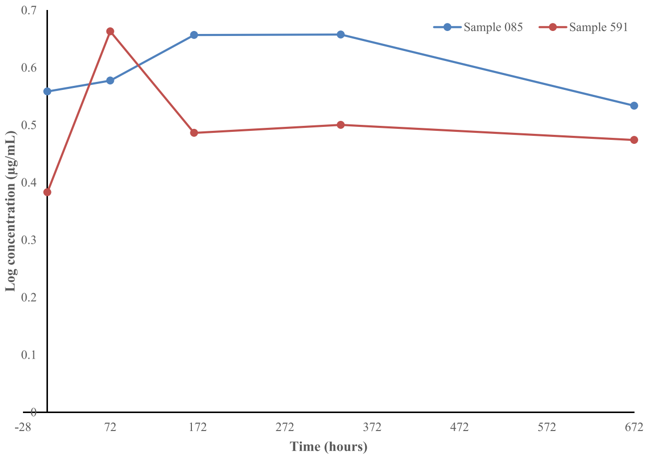

FIGURE 5 Logarithmic concentration–time graph over a 28-day period for a patient from sample 591 from Ngamiland district and sample 085 from Central district receiving fixed artemether–lumefantrine dose containing 120 mg of lumefantrine.

4 | DISCUSSION

Figure 2 shows that the linear flow velocity corresponding to the minimum plate height was taken as the optimum flow rate. This point was located at 0.057 cm/s with a corresponding plate height of 0.00706 cm. The linear flow rate was converted to volumetric flow rate, which was 0.570, that is, ~0.6 mL/min. Figure 3 shows that the chromatogram of the blank matrix, that is, trace (3), exhibited no interference at the retention time of the analyte. This was an indication of excellent selectivity and specificity. The analyte response in the matrix spike at the LLOQ was seven times the blank matrix response; that is, it exceeded the minimum five times response recommended by the USFDA guidelines (USFDA, 2018); the sensitivity can be inferred to be acceptable.

Table 2 presents the error analysis for recalculating the concentrations using the nominal calibration concentrations and the regression equation. All the calibration standards satisfied the USFDA requirement; that is, 75% of the calibration standards should be within 15% of the nominal value (20% for the LLOQ). The three QC values, that is, low, mid, and high in duplicate, 12 values (100%), were within 15% of the nominal value (20% for LLOQ), and this qualified the calibration curve as valid. The precision and accuracy of the method were extremely good, as observed in Table 3, with all values conforming to the USFDA guidelines. The extraction efficiency of the SLE method yielded percentage recoveries above 86.8%. This is considered excellent in this laboratory. MEs evaluated based on USFDA guidelines, as described by González and Alonso (2020) and presented in Table 4, showed that all the deviations were negative. The negative values indicated signal suppression/reduction probably because of the use of HPLC employing UV/Vis. detection, which can be nonspecific.

Figure 5 shows that the method satisfied the requirements for adducing PK profiling data if a complete set of baseline data were available. Patients 591 and 085 showed concentration peaks of Lum

TABLE 7 Comparison of data from this study with literature values.

Sample type	Method	Extraction method	LOD (ng/mL)	LOQ (ng/mL)	% Recovery	Reference
Human plasma	LC–MS/MS	PPT	5.3	21	<30	Silva et al. (2015)
DBS	LC-UV	LLE	–	158.7	45–51	Ntale et al. (2008)
Plasma	LC-UV	Automated SPE	–	24	63–75	Lindegårdh et al. (2005)
Human plasma	LC–MS/MS	PPT	–	200	93.16	Pingale and Mangaonkar (2013)
DBS	LC-UV	SLE	500	1400 (LLOQ)	84.2–107.8	This study

Abbreviations: DBS, dried blood spot; LOD, limit of detection; LLE, liquid liquid extraction; LLOQ, lower limit of quantification; SLE, solid–liquid extraction.

on or after day 3 within the error margin defined in the validation section. The concentrations increased and reached a peak before they decreased. This is an expected phenomenon in PK and TDM studies. This confirmed that this method could produce data to study the PK profiles of Lum in patients affected with malaria. This method can also be used to adduce TDM data, especially in infants with uncomplicated *P. falciparum*.

In contrast with literature values for similar studies as observed in Table 7, the percentage recoveries obtained in this study were orders of magnitude better due to the simplicity of the extraction method on relatively clean sample collection media. The applicability was also justified due to the high therapeutic range of Lum in both malaria-free and malaria-infected subjects, which averages ~1–10 μg/mL (González & Alonso, 2020).

5 | CONCLUSIONS

An LC-DAD method for the quantification of Lum in human samples collected on DBS filters for application in TDM and PK profiling was successfully developed and validated using the USFDA guidelines. This fit-for-purpose method satisfied the guidelines and was applied for the quantification of Lum in selected DBS samples from patients treated for malaria from Botswana. This method was deemed suitable for application in TDM and PK studies.

AUTHOR CONTRIBUTIONS

Blondie O. Brooks conducted the investigations, applied software, wrote the original draft, and performed formal analysis. Giacomo Maria Paganotti contributed to conceptualization, provision of resources, project administration, and supervision. Amos Massele was involved in funding acquisition and provided resources. Enoch Sepako contributed to project administration and funding acquisition. Paul Adiukwu contributed to writing—review and editing. Kwenga Festus Sichilongo contributed to method development, conceptualization, supervision, software, project administration, and writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

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