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Therapeutic drug monitoring for lisinopril in rats using dried blood spots

Bushra T AlQuadeib*#, Iman M Alfagih#, Basmah Aldosari and Alanood Almurshedi
Department of Pharmaceutics, College of Pharmacy, King Saud University, Saudi Arabia, Riyadh, Saudi Arabia

Abstract: A unique, easy, precise and exact high-performance liquid chromatographic-mass tandem (LCMS/MS) approach was created and validated for the measurement of the antihypertensive medicine Lisinopril (LIS) in dried blood spots (DBS). This was the first time according to our knowledge that LIS is being validated in DBS. Liquid chromatography mass tandem was utilized using the Water Acquity column as UPLC -HSS T3® column. Ten millimole ammonium formate, 0.2 percent formic acid, 0.2 percent trimethylamine, one percent acetonitrile (pH 3.0± 0.02) used as mobile phase (A), and a mobile phase (B) consisting of 0.2 percent formic acid in acetonitrile. The mobile phase lasts for 2.5 minutes at a flow rate of 0.2 ml/min. For the drug as well internal standard, the retention times (RT) obtained under optimal circumstances were 0.63±0.02 and 2.18±0.03 min, dried blood spot samples, offering consistent and quantitative drug recovery. The process was the shortest RT reported for the LIS, it is a linear relationship with concentrations from 10 - to 100ng/ml. A protein precipitation approach was used to measure the LIS. The method used to analyze DBS samples from rats receiving LIS.

Keywords: Lisinopril, validation, UPLC-MS/MS, dried blood spot.

INTRODUCTION

In the United States, hypertension disease (high blood pressure, BP) affects greater than 25% of adult population (Organization, 2002, Firke *et al*., 2020), and Saudi Arabia has similar fig. (El Bcheraoui *et al*., 2014). Keeping hypertension patients under control is essential to reducing their risk of coronary heart disease and stroke. Thiazide diuretics, ACE inhibitors, calcium channel blockers, -blockers and angiotensin II receptor antagonists are all used to treat hypertension. As a result, in the majority of hypertensive patients, the successful management of high blood pressure necessitates the administration of two or more medications at the same time (Jarari *et al*., 2015, Center, 2018). To receive effective therapy, patients must follow the recommendations for taking a medicine as well as the specified dose. Between 50 and 75 percent of hypertension patients do not respond to their medications (Organization, 2002, Kronish and Ye, 2013). Many physicians neglect to check blood pressure on a regular basis or to appropriately titrate and control pharmacological doses, resulting in ineffective treatment (Chernonosov, 2018, Center, 2018).

Lisinopril (LIS; $C_{21}H_{31}N_3O_5$), is an angiotensin-converting enzyme inhibitor commonly approved by FDA for hypertension treatment and also to reduce the chance of cardiac failure (Bicket, 2002, Yancy *et al*., 2013). Lisinopril is a hydrophilic molecule with carboxyl and amino groups (fig. 1). Lisinopril's bioavailability after oral dosage is roughly 25%, however, it varies greatly between people (6 to 60 percent) (Vlase *et al*., 2010).

Unfortunately, BP measurement is often sub optimally performed in clinical practice, which can lead to errors that inappropriately alter management decisions in 20% to 45% of cases. Measuring antihypertensive drug concentrations in blood is more reliable.

Dried blood spots (DBSs) can help to simplify drug concentration measurement techniques. Patients and caregivers don't need any special training to draw capillary blood from a finger prick using the minimally invasive DBS sampling procedure. This model method can aid in the recruitment of participants for preclinical and clinical investigations and is appropriate for routine clinical testing. DBS substantially lowers the risk of contracting HIV and other contagious infections. DBS also saves time and money by simplifying mail delivery and storage to the specified laboratory.

Various analytical techniques, including gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC) with ultraviolet or fluorescence detection (Khan *et al*., 2023), and liquid chromatography mass spectrometry (LC-MS/MS), were used to measure the level of LIS in body fluids (Hussein *et al*., 2019, Kousoulos *et al*., 2005, Qin *et al*., 2012, Shah *et al*., 2017, Vlase *et al*., 2010, Jacobs *et al*., 2022, Li *et al*., 2022, Jacobs *et al*., 2023). HPLC-based techniques often offer good selectivity but insufficient sensitivity to bioequivalence/pharmacokinetic research. Although gas chromatography-mass

**Corresponding author:* e-mail: bquadeib@ksu.edu.sa #Equally contributed.

spectrometry technologies are sufficiently sensitive, a separate derivatization step is still required for sample preparation, which is often complex and time-consuming (Vlase *et al*., 2010, Zhou *et al*., 2008, Qin *et al*., 2012). With one-step sample preparation, there may be a limit of quantification (LOQ) for LC-MS/MS assays as 1.29 or 2.0 ng/ml, making them the best method for drug quantification (Vlase *et al*., 2010).

Liquid chromatography with tandem mass spectrometry (LC MS/MS) will be used in this investigation to measure LIS in DBS and to test a recently developed technique with one-step sample preparation and the smallest volume of DBS (30 µl). These qualities are critical in pharmacokinetic/bioequivalence studies and can be used to monitor patient adherence to medication on a regular basis. The technology was thoroughly validated and successfully applied to monitor patient adherence to LIS dosage and LIS stability in DBS using dried blood spots.

MATERIALS AND METHODS

Chemicals and reagents

Lisinopril hydrochloride was a gift from Al-Jazeera Pharmaceuticals Industries Company, Riyadh, Saudi Arabia. Donepezil HCl as internal standard (IS) was provided by Jai Radhe Sales (Gujarat, India). Whatman 903 protein saver card (Sigma Aldrich, Chemie Gmbh, Munich, Germany). Water was deionized and purified using a Milli-Q Reagent Grade water system (Millipore Corporation, Bedford, MX 01730, USA). All extra chemicals and reagents were analytical HPLC quality, and we used them exactly as they were provided.

Instrumentation

A Waters® Acquity HPLC system with a tandem mass spectrometer (triple quadrupole) and electrospray ionization (ESI) source, an Acquity binary solvent management pump and Mass lynx software, version 4.1, was used to carry out the experiment.

Condition for the mass spectrometry

The Water Acquity column as UPLC –HSS T3® (1.0 x 100 mm) with 1.7 mm particle size, water^{TM} with quarternary solvent pump, and triple quadrupole mass spectrometer was used for the chromatographic separations (both from Waters, Milford, MA). The program MassLynx version 4.1 was utilized to gather and process the data (Waters, Milford, MA, USA). During the chromatographic separation, the column temperature was maintained at 40 °C while the autosampler temperature was adjusted to 10 °C with a 10 µl injection volume. In positive ion detection mode, the mobile phase consists of solvent A, which is an aqueous solution of ten mM ammonium formate, 0.2 percent formic acid, 0.2 percent trimethylamine, and one percent acetonitrile (pH 3.0±0.02). Meanwhile, 0.2 percent formic acid in acetonitrile is considered solvent B. Each sample ran for 2.5 minutes with a 0.2 ml/min mobile phase flow rate. The gradient elution software was utilized in the following way: beginning with 20 % B; After 1.0 minutes, the proportion of B elevated to 80% for 2.3 minutes, at which point it declined again to 20%. The used technology utilized multiple reaction monitoring (MRM) chromatograms in the electrospray ionization (ESI) positive modes in conjunction with triple-quadruple LC/tandem mass spectrometric detection (Waters ^{TM}) to detect both LIS and IS, with a 0.5-second dwell time, the multiple reaction monitoring (MRM) transitions of m/z 406.90> 186.85 (CE 34 eV), 406.90> 360.75 (CE 8 eV) with cone voltage 10 for LIS and MRM transitions of m/z 380.00 > 91.02 (CE 35 eV) with cone voltage 30 for IS. Using Nitrogen as a desolvating gas, it flowed at a rate of 650 L/h. The source was 110 degrees Celsius, the nebulizer was 150 degrees Celsius, and the desolvation line was 500 degrees Celsius (7 psi), with a flow rate of the collision gas (argon) was 0.25 mL/min and a capillary voltage of 3.5 kV.

Calibration standards and DBS sample preparation

The standard solutions for the drug and internal standards (LIS and IS) were made as 1000 µg/ml in methanol (Stock solution) and kept in glass vials at -30°C until needed. A 30 µl aliquot of blood was transferred straight from the patient's finger to the Whatman 903 paper without disturbing the collecting area's surface for quality control and calibration curve DBS. DBS samples were taken from patients and kept in plastic bags with desiccants at room temperature after being dried at room temperature for 3 hours. An Eppendorf tube was used to transfer an 8mm disc that had been punched out of the middle of a 30 µl DBS sample. Having medication concentrations between 10 and 100 ng/ml), 300 µl of the methanolic solution was combined with 20 µl of IS. One minute of vortexing, followed by 30 minutes of temperature-controlled ultrasonication at 40 °C and ten minutes of centrifugation at 13200 rpm. Each supernatant was transferred in its whole, 270 µl, to a new microcentrifuge tube, where it was gently dried with an N_2 gas stream. Using 150 µl of methanol/water (40:60, v/v) containing 0.1 percent formic acid, the dried residue was rehydrated. The finished extracts were placed into auto-sampler vials for LC analysis. To complete the study, a 10 µl sample was injected into the LC-MS/MS apparatus. Quality control (QC) samples were also made by spiking drug-free dried blood spots with working solutions to achieve final concentrations that covered the quantification's lower limit. The LIS concentration solutions were 10, 50, and 100 ng/ml for QC concentrations used in this study. Until they were required, the calibration standards and QC samples were stored at a temperature of -30°C.

The study protocol was reviewed and approved by the Institutional Review Board of the King Saud University Institutional Review Board Committee (IRB) (Approved number, E-21-5855). It was carried and conducted in accordance with the major ethical principles of the Declaration of Helsinki and the Korean Good Clinical Practice Guidelines.

Ion Suppression Study

Six separate rats plasma samples were extracted, all of which were blank, without drugs and/or IS. The extracts were rehydrated using LIS at nominal amounts of 10, 50, and 100 ng/ml, with the same nominal amount of LIS in the mobile phase as unextracted reference standard solutions (n = 6). Then the peak areas of the test solutions were compared to those of the latter. Reconstituted extracts/reference solutions had a mean area ratio of 0.93, with a relative standard deviation of 3.9%. There was therefore no indication of ion suppression. This is done by utilizing Matuszewski *et al*. 1988 (Matuszewski *et al*., 1998) approach, which confirmed the lack of ion suppression.

Method validation

Validation of the analytical method was done at values between 10 and 100 ng/ml. Selectivity, accuracy, within-run precision, between-run precision, and stability of DBS samples were assessed. FDA guidelines and standards for validating bioanalytical methods (Meesters and Voswinkel, 2018, the European Pharmacopeia (Agency, 2011), and the applicable guidelines (International Conference on Harmonization (ICH)) were used to validate the method (Matuszewski *et al*., 1998).

Stability

Numerous factors, including storage conditions, the medication's chemical quality, and container design, might affect a drug's stability. Stability procedures must assess the analyte's stability during sample handling, short-term storage in an autosampler before analysis, and freeze-thaw cycles throughout the analytical process.

After preparation and storage at -30 °C for 0, 72 hours, 1 week, and 2 weeks, samples of 1 µg/ml were exposed to freeze (-30 ^{0}C) - thaw (room temperature at 25 ^{0}C) cycles to test their stability. By maintaining sample vials sealed with parafilm at 10°C for 72 hours, the stability of the processed samples in the autosampler was assessed. At 0, 24, 48, 72 hours, 1 week, and 2 weeks, the samples were examined.

Application of method (dried blood spots)

Samples were collected from two separate groups of rats (n=8). In the first group, and untreated (n=4) while in the second group, LIS was used for each as oral single dose as 15 mg/kg. Thirty µl samples were taken 4 to 8 hours after ingesting the medication. The three DBS samples (i.e., 3x30 µl) were typically obtained from each rat. Rats study was approved by Institutional Review Board Committee (IRB) (Approved number, E-21-5855).

Table 1: Summary of percentage accuracy and precision of lisinopril from DBS samples (n=18).

		Measured Drug Concentration (ng/ml)		
		Nominal Drug Concentration (ng/ml)		
Day	Intra-day statistics	10	50	100
Day 1		11	55	101
		8.9	51	98
		9.2	48	94
		10	51	105
		8.7	49	102
		10.3	48	95
	n	6	6	6
	Mean	9.68	50.33	99.17
	SD	0.90	2.66	4.26
	Precision (RSD%)	9.27	5.28	4.30
	Accuracy (RE%)	3.27	0.66	0.84
Day 2		8.1	53	113
		8.3	49	89
		10.6	47	101
		11	55	105
		10.5	51	107
		9.8	48	109
	n	6	6	6
	Mean	9.72	50.50	104.00
	SD	1.24	3.08	8.37
	Precision (RSD%)	12.75	6.10	8.04
	Accuracy (RE%)	2.92	0.99	3.85
Day 3		10.3	49	108
		11.3	54	103
		8.5	48	91
		9.7	48	105
		10.5	53	94
		8.2	52	98
	n	6	6	6
	Mean	9.75	50.67	99.83
	SD	1.20	2.66	6.62
	Precision (RSD%)	12.34	5.25	6.63
	Accuracy (RE%)	2.56	1.32	0.17
Inter-day statistics	N	18	18	18
	Mean	9.72	50.50	101.00
	SD	1.11	2.80	6.41
	Precision (RSD%)	11.7	9	9.8
	Accuracy (RE%)	-3.4	0.45	1.59
	P value	0.927	0.78	0.605

STATISTICAL ANALYSIS

All statistical calculations were performed with the Statistical Product and Service Solutions (SPSS) for Windows, version 10.0. Correlations were considered statistically significant if calculated *P* values were 0.05 or less.

RESULTS

Improvement of of Chromatographic Separation

Using positive ions and MRM modes, lisinopril was discovered at the following transitions of mass to charge (m/z):406.9 > 186.85 (CE 34 eV), 406.90> 360.75 (CE 8 eV) for LIS (fig. 2), while an internal standard (donepezil) detected at m/z 380.00 > 91.02 (CE 35 eV). The cone voltage was 10 V and 30 V for LIS and IS, respectively. With a flow rate of 643 L/h, the nitrogen desolvation temperature was 349 °C, the column temperature was 40 °C, and the degasser pressure was 0.69 psi. 3.50 kV is the capillary voltage that was used.

In previously reported lisinopril literatures, it stated that the [M+H]+ ion (m/z 406) following the collision that caused the dissociation of produced some abundant one ion (m/z 349.2 (Zhou *et al*., 2008) or m/z 246 (Hussein *et al*., 2019, Tanna *et al*., 2018) or m/z 84 (Kousoulos *et al*., 2005, André, 2017) or m/z 114 (Shah *et al*., 2017). Another author's reported two degradation daughters such as m/z 246 and 84 (Qin *et al*., 2012, El-Leithy *et al*., 2017). Others reported three and more degradation daughters such as m/z 245, 246, 291, 309 (Vlase *et al*., 2010) or as m/z 360, 309, 291, 263, 246, 84 (Bernieh, 2017) were obtained.

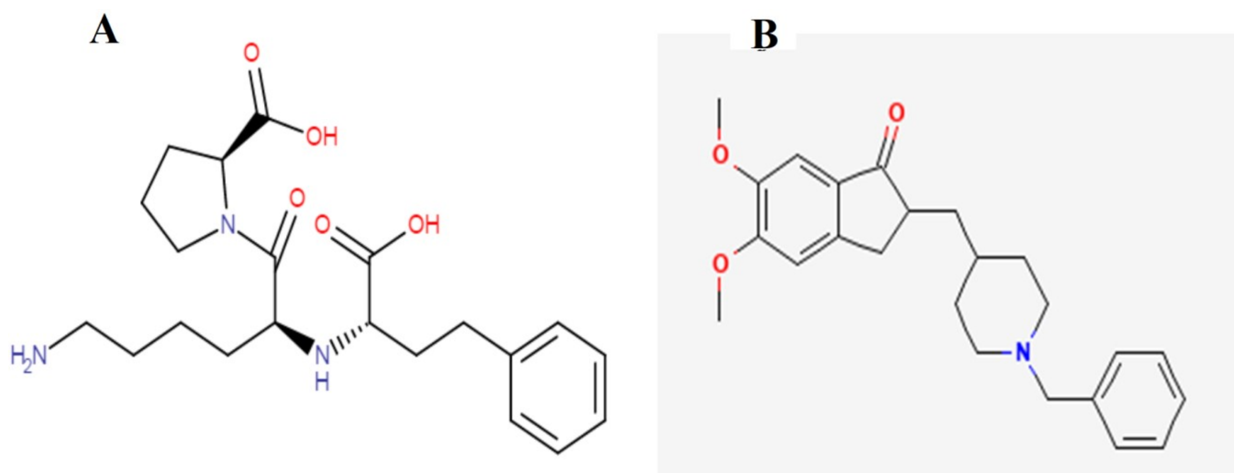

Fig. 1: Structure of Lisinopril (A) and Donepezil (B)

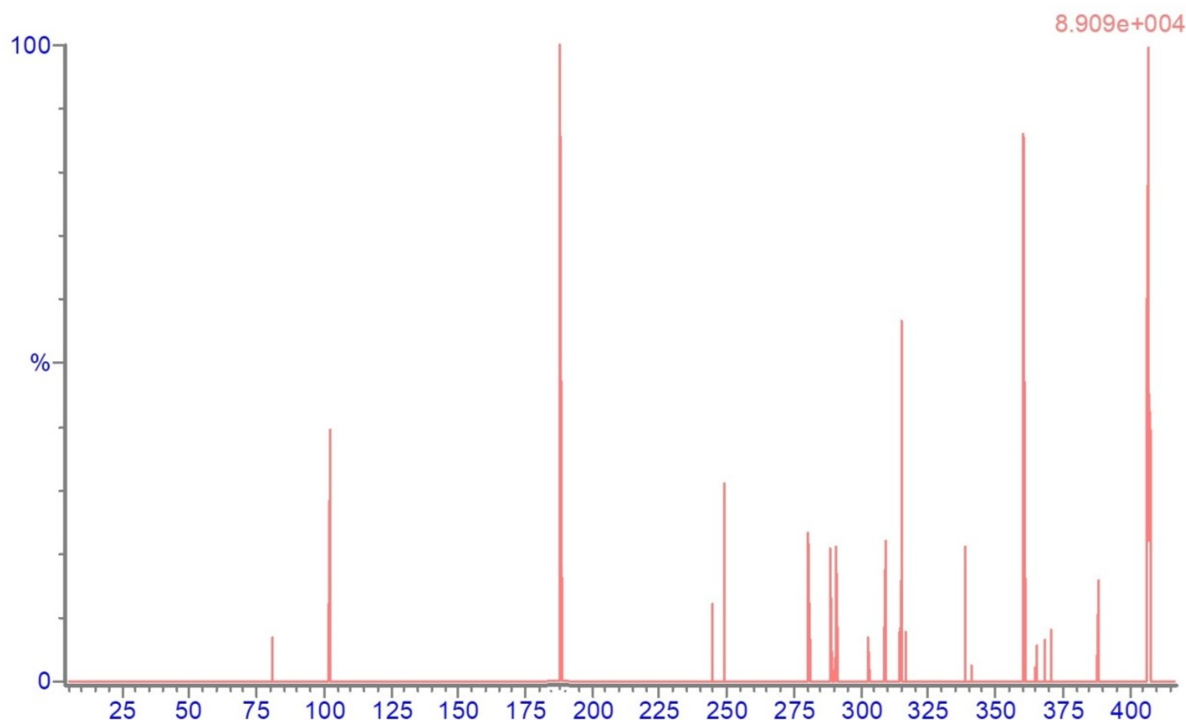

Fig. 2: Tandem mass spectra and its fragmentation of Lisinopril in positive ion mode

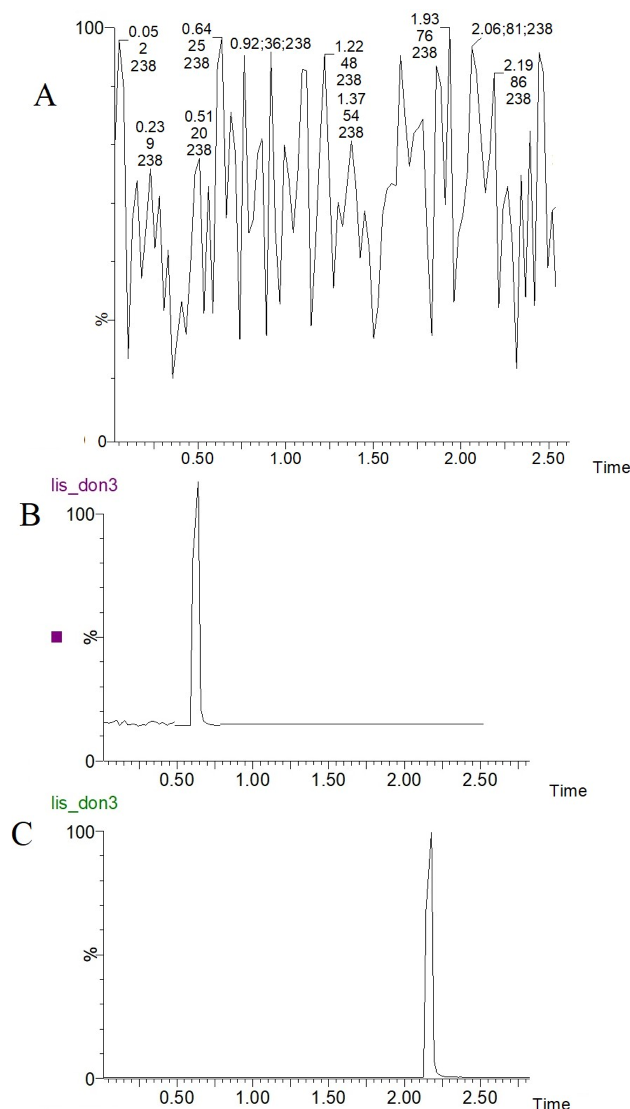

Fig. 3: Mass chromatograms obtained with DBS analyses. A; Blank DBS. B; DBS spiked with Lisinopril. C; DBS spiked with donapezil (IS).

We optimized the chromatographic conditions using a Waters Acquity UPLC –HSS T3® column that was kept at 40 ^{0}C with a 10 µl injection volume. A simple aqueous ; solvent A; ten mM ammonium formate with 0.2% formic acid, 0.2% trimethylamine and 1% acetonitrile (pH 3.0 ± 0.02) as well as; solvent B; 0.2% formic acid in acetonitrile was pumped under an gradient condition with 0.2 ml/min as a flow rate. We used the following gradient elution program: initially started with 20% B; the percentage of B ramped to 80% after 1 minute; till 2.3 minutes; the percentage of B ramped to the initial condition (20% B). For lisinopril and the IS to have a good chromatographic peak shape and resolution, the mobile phase's composition and pH are crucial, as reported by (Zhou *et al*., 2008). Reduce intensity and sharpen peaks to lessen the mobile phase's acidity. The entire analysis time under these circumstances is 2.5 minutes. Based on its chromatographic and extraction characteristics, donepezil was chosen as the IS.

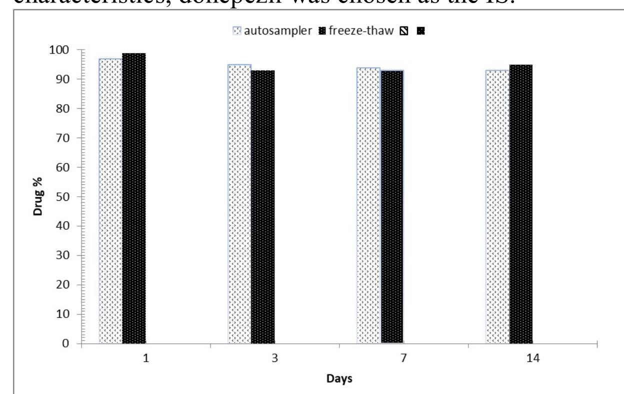

Fig. 4: Stability of Lisinopril in autosampler and after freezing–thaw cycles for 2 weeks (n = 6).

LIS and IS had retention times of 0.63± 0.02 and 2.18 ± 0.03 min, respectively, according to the chromatographic conditions specified in this paper (fig. 3), without any conflicting peaks in the DBS samples examined. The retention time for LIS was significantly less than previously reported estimates of 0.85 minute (Kousoulos *et al*., 2005, Hussein *et al*., 2019, Tanna *et al*., 2018),1.2 min (Qin *et al*., 2012), 1.44 min (Shah *et al*., 2017), 2.1 min (Zhou *et al*., 2008), 2.8-5.9 min (André, 2017), 4.5 min (Vlase *et al*., 2010), 6.63 min (El-Leithy *et al*., 2017).

Only one previous study of DBS LIS determination looked at the simultaneous determination of eleven possible cardiovascular medicines. This article, however, did not conduct any specific DBS validation experiments (Bernieh, 2017).

DISCUSSION

The sample preparation used in this investigation was straightforward and based on a single-step methanol extraction, which allowed accurate quantification of LIS from the limited amount of sample present in DBS (approximately 30 µl). There are presently no established validation guidelines for dried blood spot (DBS) assays (Enderle *et al*., 2016). Hence, the validation of the developed DBS assay was conducted based upon international guidelines such as FDA and EU that bioanalytical techniques were used for analyzing rats sample analyte concentrations (Guideline, 1994, Araujo, 2009, Agency, 2011).

UPLC MS/MS validation

None of the spiked DBS samples obtained from various rats eluted any endogenous DBS components during LIS or IS retention times (fig. 3). Due to low background baseline noise, the excellent separation between LIS and IS was achieved (fig. 3). In an illustrated chromatogram, rats DBS samples to spike or quality control samples displayed the same chromatographic behavior, indicating

Table 2: Lisinopril concentrations from eight rats

N	Sex	Administered Drug	Time after Oral intake (h)	Concentration (µg/ml) ± (SD)
1	M	10 mg once daily	4	88.1 ± 11.23
2	F	15 mg once daily	8	49.23 ± 6.4
3	F	15 mg once daily	5	55.93 ± 5.9
4	M	10 mg once daily	6	76.28 ± 9.7
5	F	20 mg once daily	7	56.83 ± 4.9
6	M	10 mg once daily	4	72.45 ± 8.1
7	F	10 mg once daily	5	85.35 ± 7.3
8	F	20 mg once daily	8	69.45 ± 7.5

high test selectivity. The LOD in rats DBS was 1ng/ml with an RSD of 17% at a signal-to-noise ratio of >4.7, whereas the LLOQ for this test was 4ng/ml at a signal-to-noise ratio of >9 with an RSD of 14% at an injection volume of 10 µl.

Calibration DBS standard solutions (n=6) were freshly produced and tested in triplicate over a range of 1-100 ng/ml on three different days. Excellent linear relationships were obtained, with an average linear slope of 0.0488 (SD 0.017) and an average intercept of 0.193 (SD 0.021), and a correlation coefficient (r) value greater than 0.993. The percentage differences achieved in the calibration curve experiment met the requirements, according to the results. In contrast to inter-assay precision, which varied from 6.59 to 9.33%, intra-assay precision ranged from 5.89 to 8.41%. The accuracy varied between 98.3% and 104.9% (table 1). The recommendations considered the intra-day and inter-day precisions to be acceptable when they were less than 20%. At greater concentrations, the percentage RSD was lower and for the majority of analytes, the intra-day accuracy was lower than the inter-day precision. The lowest limit of quantification for LIS in DBS using LC ms/ms was 1 ng/ml, which is less than the anticipated therapeutic levels of LIS. The detection thresholds for LIS in DBS were also 1 ng/ml (Vlase *et al*., 2010) and 0.1 ng/ml (Swaisland, 1991).

However, the detection limit for LIS in plasma was obtained at 0.5 ng/ml (Qin *et al*., 2012), or 1 ng/ml (Shah *et al*., 2017), and was previously reported to be 1.29 ng/ml in rats plasma (Kousoulos *et al*., 2005).

Stability Studies

The processed samples were stored in the autosampler for 24 hours at 10°C, where LIS was stable and the mean calculated results were within 1.4% of the nominal concentration (fig. 4). It is suggested to keep LIS in the autosampler for more than a day. Even after the third cycle, 2.6 percent of the LIS was lost with an RSD of 5.1 percent, with mean calculated values being within 2.5 percent of the nominal concentration. After 72 hours, the freeze-thaw temperature cycles had no influence on LIS stability (p>0.05).

These findings matched those of a recent study in which LIS remained stable for more than 45 days at hot (45 °C) and low (4°C) temperatures, with a maximum fluctuation of 5.02 percent of nominal concentrations. It has also proven LIS stability at room temperature for up to 20 days. As a result, after keeping the DBS at room temperature, no significant instability was seen (Vlase *et al*., 2010).

Method application

The quantitative analysis of LIS for DBS samples acquired from healthy or hypertensive subjects undergoing LIS treatment to manage hypertension symptoms was successfully applied using the created and verified LC MS/MS method. The assay was used to determine the LIS concentration in eight patients who had been treated with LIS and three healthy volunteers using DBS. LIS was given to patients as a single antihypertensive medication or in combination with other antihypertensive medications. Patients were given a 5-40 mg dose orally once a day and DBS samples were taken between 4 and 8 hours after the treatment was given (table 2). DBS samples from subjects who did not receive any medication revealed no misleading signals. table 2 shows that the measured DBS drug concentrations, which were in the range of 49.23 to 88.1ng/ml, were within the therapeutic range. When taken alone, the highest serum levels of lisinopril were 44ng/ml (Bernieh *et al*., 2017) and reported to be 50-88ng/ml (Hussein *et al*., 2019). The drug concentrations reported in this investigation were similar to, but not identical to, the Cmax concentrations reported in other studies. These findings clearly show that the volunteers who were chosen were following the prescribed LIS with a fast test with a small amount of blood in the DBS sample would suggest that the dose has been taken.

CONCLUSION

The newly created and tested LC-MS/MS method for determining Lisinopril in DBS samples provides a quick analysis of the time and sensitivity needed for LIS determination in DBS samples. The approach yielded less than 15 percent accuracy (RE) and precision (CV). The stability of LIS in DBS after storage at 10 and 30 degrees

Celsius was up to two weeks. This DBS approach allows self-patients to sample by simply dropping from a finger pick. This allows for the handling and transportation of samples from rare areas to the laboratory for analysis. This method offers a lot of promise in terms of assisting clinicians in indicating adherence to prescribed medication so that patients' treatment can be optimized.

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REFERENCES

Agency EM (2011). Guideline on bioanalytical method validation. EMEA/CHMP/EWP/192217/2009.

André MB (2017a). Development and optimization of an UPLC-MS/MS method for the simultaneous detection and quantification of 14 antihypertensive drugs in human urine samples. PhD, Faculdede De Farmacia - Universidade Do Porto, pp.1-238.

Araujo P (2009). Key aspects of analytical method validation and linearity evaluation. *J. Chromatogr. B,* **877**(23): 2224-2234.

Bernieh D (2017). Application of microsampling methods to cardiovascular medication adherence assessment. Ph.D. Thesis, Faculty of Health and Life Sciences, Leicester School of Pharmacy, De Montfort University, UK, pp.1-303.

Bernieh D, Lawson G and Tanna S (2017). Quantitative LC–HRMS determination of selected cardiovascular drugs, in dried blood spots, as an indicator of adherence to medication. *J. Pharm. Biomed. Anal.,* **142**(8): 232-243.

Bicket DP (2002). Using ACE inhibitors appropriately. *Am. Fam. Physician,* **66**(3): 461-469.

Chernonosov A (2018). The Use of dried blood spots for the quantification of antihypertensive drugs. *Int. J. Anal. Chem.,* 1-**12**

El-Leithy E, El-Fiky G and Nabil M (2017). Characterization of degradation products resulted from acidic hydrolysis of lisinopril under drastic conditions. *J. Adv. Pharm. Res.,* **1**(4): 201-208.

El Bcheraoui C, Memish ZA, Tuffaha M, Daoud F, Robinson M, Jaber S, Mikhitarian S, Al Saeedi M, Almazroa MA and Mokdad AH (2014). Hypertension and its associated risk factors in the Kingdom of Saudi Arabia, 2013: A national survey. *Int. J. Hypertens,* **8**: 1-9.

Enderle Y, Foerster K and Burhenne J (2016). Clinical feasibility of dried blood spots: Analytics, validation, and applications. *J. Pharm. Biomed. Anal.,* **130**(10): 231-243.

Firke SD, Bhoi KR, Patil KR, Jayswal SD, Tekade AR and Chalikwar SS (2020). A state-of-the-art review on applications of different analytical techniques for some ace inhibitors. *Anal. Chem. Lett.,* **10**(2): 60-79.

Guideline IHT (1994). Text on validation of analytical procedures. International Conference on Harmonization, Geneva, Switzerland, pp.1-5.

Hussein RF, Alvi SN and Hammami MM (2019). Rapid determination of lisinopril level in human plasma by LC-MS/MS. *Acta* Sci. *Med*. Sci., **3**(9): 3-8.

Jacobs C, Kunz M, Wagmann L, Mahfoud F and Meyer M (2022). Adherence monitoring of antihypertensive drugs in finger prick blood microsamples-Development and validation of an analytical procedure. *Toxicol. Anal. Clin.,* **34**(3): S41-S48.

Jacobs CM, Kunz M, Mahfoud F, Wagmann L and Meyer MR (2023). Closing the gap development of an analytical methodology using volumetric absorptive microsampling of finger prick blood followed by LC-HRMS/MS for adherence monitoring of anti-hypertensive drugs. *Anal. Bioanal. Chem*, **415**(1): 167-177.

Jarari N, Rao N, Peela JR, Ellafi KA, Shakila S, Said AR, Nelapalli NK, Min Y, Tun KD and Jamallulail SI (2015). A review on prescribing patterns of antihypertensive drugs. *Clin. Hypertens,* **22**(1): 1-8.

Khan MFS, Tahir L, Zhou X, Bary G, Sajid M, Shahzad AK, Khan I, Mohamed A and Ahmad R (2023). Method development for simultaneous estimation of Amlodipine Besylate and Perindopril Tertbutyl amine in fixed-dose. *Heliyon,* **9** (In Press).

Kousoulos C, Tsatsou G, Dotsikas Y and Loukas YL (2005). Development of a rapid liquid chromatography tandem mass spectrometry method for the determination of lisinopril, applicable for a bioequivalence study, employing a 96-well format solid phase extraction protocol. *Anal. Chim. Acta,* **551**(1-2): 177-183.

Kronish IM and Ye S (2013). Adherence to cardiovascular medications: lessons learned and future directions. *Prog. Cardiovasc. Dis.,* **55**(6): 590-600.

Li T, Liu YP, Liu SQ, Shi P, Jiang X, Tao Y, Gao XM, Ma YP and Cao Y (2022). Bioequivalence evaluation and food effect assessment of Lisinopril/amlodipine tablets in healthy Chinese subjects under fasting and fed conditions. *BMC Pharmacol. Toxicol.*, **23**(45): 1-11.

Matuszewski B, Constanzer M and Chavez-Eng C (1998). Matrix effect in quantitative LC/MS/MS analyses of biological fluids: a method for determination of finasteride in human plasma at picogram per milliliter concentrations. *Anal. Chem.,* **70**(5): 882-889.

Meeters R. and Voswinkel, S. 2018. Bioanalytical method development and validation: from the USFDA 2001 to the USFDA 2018 guidance for industry. *J. Appl. Bioanal*, **4**: 67-73.

Organization WH (2002). The world health report 2002: reducing risks, promoting healthy life. World Health Organization, pp.1-250.

Qin F, Wang D, Yang S, Jing L, Xiong Z and Li F (2012). Quantitative determination of lisinopril in human plasma by high performance liquid chromatography tandem mass spectrometry and its application in a pharmacokinetic study. *Biomed. Chromatogr.,* **26**(6): 691-696.

Shah JV, Shah PA, Shah PV, Sanyal M and Shrivastav PS (2017). Fast and sensitive LC-MS/MS method for the simultaneous determination of lisinopril and hydrochlorothiazide in human plasma. *J. Pharm. Anal.,* 7(3): 163-169.

Swaisland A (1991). The pharmacokinetics of co-administered lisinopril and hydrochlorothiazide. *J. Hum. Hypertens,* **5**(2S): 69-71.

Tanna S, Alalaqi A, Bernieh D and Lawson G 2018. Volumetric absorptive microsampling (VAMS) coupled with high-resolution, accurate-mass (HRAM) mass spectrometry as a simplified alternative to dried blood spot (DBS) analysis for therapeutic drug monitoring of cardiovascular drugs. *Clin. Mass Spectrom,* **10**(12): 1-8.

Vlase L, Popa DS, Muntean D and Achim M (2010). A new LC/MS/MS method for determination of lisinopril in human plasma. *Stud. U Babes-Bol. Che.,* **55**(1): 91-101.

Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE, Drazner MH, Fonarow GC, Geraci SA, Horwich T and Januzzi JL (2013). 2013 ACCF/AHA guideline for the management of heart failure: A report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *J. Am. Coll. Cardiol.,* **62**(3): e147-e239.

Zhou N, Liang YZ, Chen BM, Wang P, Chen X and Liu FP (2008). Development and validation of LC-MS method for the determination of lisinopril in human plasma and its application in a bioequivalence study. *J. Chromatogr. Sci.,* **46**(1): 848-853.