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Quantitative determination of atenolol in dried blood spot samples by LC–HRMS: A potential method for assessing medication adherence

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ABSTRACT

The use of blood spot collection cards was investigated as a means of obtaining small volume samples for the quantification of therapeutic drugs for assessing medication adherence. A liquid chromatography–high resolution TOF mass spectrometry (LC–HRMS) method, based on the measurement at the accurate mass to charge ratio of the target analyte, was used to ensure specificity for atenolol in the dried blood spot (DBS) samples. A working method was developed and validated. For the preparation of DBS samples whole blood spiked with analyte was used to produce 30 μl blood spots on specimen collection cards. A 5 mm disc was cut from the dried blood spot and extracted using methanol:water (60:40, v/v) containing the internal standard, atenolol-d_7. Extracts were vortexed, sonicated and then centrifuged. Gradient chromatographic elution was achieved using an Ascentis Express C18 100 mm × 2.1 mm column and a mobile phase flow rate of 0.2 ml/min and the column oven temperature at 30 °C. MS detection was carried out in electrospray positive ion mode for target ions at accurate mass m/z 267.1703 for atenolol and 274.2143 for the IS. Drug extraction efficiency from spiked blood spots was demonstrated to be 96 ± 5% and the drug was stable in DBS for at least 10 weeks. The developed LC–HRMS method was linear within the tested calibration range of 25–1500 ng/ml and validation showed the accuracy (relative error) and precision (coefficient of variation) values were within the pre-defined limits of ≤15% at all concentrations with a limit of quantification of 25 ng/ml. Factors with potential to affect drug quantification measurements such as the matrix effects, volume of blood applied onto the collection card and effect of different sampling cards were investigated. The developed LC–HRMS method was applied to blood spots on sampling card taken from adult healthy volunteers previously administered a 50 mg atenolol tablet and a DBS concentration–time profile was obtained for atenolol. Requiring only a micro volume (30 μl) blood sample for analysis, the developed DBS based assay has the potential to assess patient adherence to atenolol.

1. Introduction

Cardiovascular medication non-adherence is a growing concern to clinicians, other healthcare professionals and health service providers because of mounting evidence that it is prevalent and associated with considerable morbidity, mortality and higher costs of care [1–3]. There is evidence that ~50–60% of patients prescribed cardiovascular drugs do not adhere to their prescribed regimen [4,5].

Atenolol (Fig. 1), [4-(2′-hydroxy-3′-isopropylaminopropoxy) phenyl acetamide], is a selective β_1 receptor antagonist belonging to a class of drugs known as β-blockers widely used for the treatment of cardiovascular disease including hypertension, angina pectoris, myocardial infarction, and arrhythmia. Determining the plasma concentration of atenolol, once important for investigating the pharmacokinetics of the drug, can now be used for the assessment of patient adherence to a prescribed regimen. For a single oral drug dose the expected concentration in the blood would rise to a maximum (C(max)) over a period of approximately 1–3 h followed by the concentration then falling exponentially, with a characteristic half-life. The requirements of an analytical method to monitor adherence to therapy are to detect the residual levels of the drug up to 24 h after the initial dose. At this time a repeat dose should be taken. Information from the literature derived from plasma for atenolol concentrations suggests a C(max) concentration of 330 ng/ml at a t(max) of 2.74 h and residual levels of 25 ng/ml atenolol 24 h after dosing [6].

Dried blood spots are an established small blood volume (typically ≤50 μl) collection technique used routinely for newborn

Abbreviations: PK, pharmacokinetics; DBS, dried blood spot; LC–HRMS, liquid chromatography–high resolution mass spectrometry.

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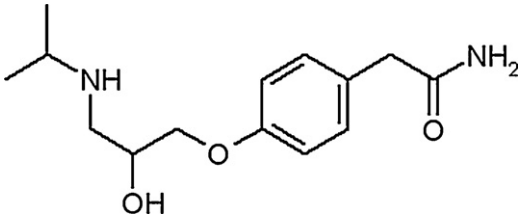

Fig. 1. Chemical structure of atenolol.

screening purposes where the sample is obtained via a simple heelstick prick procedure [7,8]. For adults DBS capillary blood is obtained from a finger prick with a lancet by the patient themselves. The DBS sampling technique is minimally invasive and therefore ideal for routine clinical testing. Sample storage and transmission between sites is easy since the samples are dried blood spots on sample collection cards and therefore pose less of a biological hazard. After drying, the paper with the blood spot sample is sent by mail to the analytical laboratory. The laboratory punches out a disk from the blood spot and then the disk is extracted for target drug(s). The size of the paper disk provides a volumetric measurement that is similar to liquid measurement devices. The advantages of DBS based methods coupled with improved analytical instrumental capability [9] has led to a surge in the use of this methodology for various applications including therapeutic drug monitoring [10–13], toxicokinetic (TK) [14,15] and paediatric pharmacokinetic (PK) [16–19] studies. Numerous bioanalytical methods using DBS sampling to quantify small molecules including dexamethasone [17], paracetamol [10], metformin [11], tacrolimus [13], metronidazole [18], canrenone [19] and rifampicin [12] are documented.

The aim of this work was to develop a simple, sensitive and selective liquid-chromatography high-resolution/accurate mass TOF mass spectrometry (LC–HRMS) method for the quantification of atenolol in dried blood spots (DBS), to be applied specifically in adherence studies. Most of the documented assays for atenolol determination in plasma, for example high-performance liquid chromatography (HPLC) [6,20–25], liquid chromatography–mass chromatography (LC–MS), liquid chromatography–tandem mass spectrometry (LC–MS/MS) [26–28], or gas chromatography–mass spectrometry (GC–MS) [29,30] are based on large blood samples (typically >0.5 ml) which require visits to a specialist clinic. Dried blood spot (DBS) sampling, which needs no specialised collection clinic, combined with high-resolution, accurate mass spectrometry detection coupled to liquid chromatography separation has the potential to offer improved selectivity and resolution [31–33]. This analytical technique is at least comparable, in performance, to tandem mass spectrometry. The TOF mass spectrometer would allow high mass resolution and accurate mass determination (~1 ppm with internal calibration). TOF instruments also offer the advantage of fast data acquisition due to the fact that all ions are acquired in each spectrum with this non-scanning technology and furthermore it is possible to perform a retrospective analysis of the stored data.

2. Experimental

2.1. Chemicals and materials

Acetonitrile, methanol and water, LC–MS grade, were purchased from Fisher Scientific (Loughborough, UK). Formic acid (≥98%), specimen collection filter paper type 903, autosampler vials with 0.3 ml inserts with caps, microcentrifuge tubes (1.5 ml), volumetric pipettes, pipette tips and polyethylene bags were also obtained from Fisher Scientific (Loughborough, UK). Atenolol (R-(+), 99%) and atenolol-d_7 analytical standard were purchased from Sigma–Aldrich (Poole, UK). Ahlstrom 226 sampling paper was obtained from ID Biological Systems (Greenville, SC, USA) and Agilent DMS sampling card was obtained from Agilent Technologies (Santa Clara, CA, USA). A 5 mm diameter punch was obtained from Maun Industries Ltd. (Nottingham, UK). Lithium heparin coated blood collection tubes were purchased from International Scientific Supplies Ltd. (Bradford, UK). Fresh blood was obtained from informed volunteers in line with De Montfort University Ethics Protocols.

2.2. Preparation of atenolol standard stock and working solutions

A standard stock solution of atenolol was prepared in methanol at a concentration of 1 mg/ml. Atenolol working solutions for spiked blood spots were prepared as follows: The stock solution was diluted with methanol:water (60:40, v/v) to produce different working solutions of 15,000; 10,000; 5000; 2500; 1000; 500 and 250 ng/ml. All working solutions were prepared freshly. Spiked blood standards were prepared by spiking different samples of fresh blood (900 μl) with 100 μl of one of the above mentioned working solutions to yield final blood atenolol concentrations of 1500; 1000; 500; 250; 100; 50; 25 ng/ml. A zero (blank) atenolol blood sample was prepared by spiking with 100 μl of methanol:water (60:40, v/v).

A stock solution of the internal standard, atenolol-d_7 was prepared by diluting the analytical standard (1 mg/ml) in methanol:water (60:40, v/v) to produce a 10 μg/ml concentration. This was further diluted with methanol:water (60:40, v/v) to produce an extraction solvent containing 250 ng/ml of IS.

2.3. Preparation of calibration standards and validation samples

Thirty microlitres of calibration standards in blood across the concentration range 1500, 1000, 500, 250, 100, 50, 25 and 0 ng/ml were spotted directly onto the sampling paper type 903 using a volumetric pipette. The spotted samples were allowed to air dry overnight at room temperature prior to processing. A 30 μl volume applied onto sampling paper gave a spot of size of ~9.5 mm in diameter.

2.4. Dried blood spot analyte solvent extraction

A 5 mm disc (approximately 15 μl of blood) was punched from the centre of the DBS sample and transferred to a 1.5 ml microcentrifuge tube. A 200 μl volume of extraction solvent consisting of methanol/water (60:40, v/v) plus IS (250 ng/ml) was added to this. Sample tubes were then vortexed for 1 min and sonicated for 30 min. Thereafter they were centrifuged for 10 min at 13.2 × *g* and each extract was transferred to an autosampler vial for analysis by LC–HRMS.

2.5. LC–high resolution MS analyses

The chromatographic system consisted of an Agilent 1290 LC which was coupled to an Agilent 6530 QTOF mass spectrometer, used in the TOF mode, i.e. the quadrupole was not operating as a mass spectrometer. The target drug was analysed on an Ascentis Express C18 column (Sigma–Aldrich, Poole, UK, 100 mm × 2.1 mm i.d., 2.7 μm particle pore size) which was preceded by a 0.3 μm inline filter (Agilent Technologies, Chesire, UK). The column oven temperature was set to 30 °C. Sample injection volume was 5 μl. The mobile phase consisted of water containing 0.1% (v/v) formic acid (eluent A) and acetonitrile containing 0.1% (v/v) formic acid (eluent B) and was delivered at 0.2 ml/min with gradient elution. The mobile phase was initiated at 5% B and maintained for 1.0 min before increasing to 70% B by 6.0 min and held until 7.0 min before

returning to 5% B. The gradient elution programme was then held for 2.5 min to re-equilibrate the column prior to the next injection.

Operation of the mass spectrometer was in electrospray positive ion mode. The MS source and chamber conditions were optimised to give maximum analyte signal intensity as follows: Fragmentor voltage: 200 V; Skimmer: 65 V; Gas Temperature: 300 °C; Dry Gas: 5 l/min; Nebuliser: 50.0 psig; Sheath Gas Temperature: 350 °C; Sheath Gas Flow: 12 l/min. Scan Range: 100–1000 m/z; Scan Rate: 1 Hz. HRMS lock reference masses: 121.0508 m/z and 922.00987 m/z.

MassHunter Workstation Software (Agilent Technologies) was used to operate the system and acquire all data and the data processed using Qualitative Analysis B.02.00 and Quantitative Analysis B.01.04 software (Agilent Technologies). External calibration of the TOF mass spectrometer was performed daily before starting the analysis.

2.6. Validation

2.6.1. Selectivity

To demonstrate the selectivity of the LC–HRMS method, blank blood spots and atenolol spiked blood spots were analysed and subsequently processed. Representative extracted ion chromatograms for protonated atenolol using mass tolerance filters at (a) m/z 267.1 ± 0.5 (i.e. ±500 ppm) (b) m/z 267.1 ± 0.1 (i.e. ±100 ppm) and (c) m/z 267.170 ± 0.001 (i.e. ±1 ppm) were generated to show that other components that could be present in the sample matrix or from the sampling paper were resolved from the target analyte.

2.6.2. Linearity and sensitivity

Calibration standards were prepared in replicate (n = 6) and analysed on three separate days. A calibration plot of analyte/IS peak area ratio against nominal atenolol concentration was produced and an equally weighted linear regression was applied.

The quantification limits of atenolol in the DBS extracts were determined. The limit of quantification (LOQ) was based a signal-to-noise ratio of 10 in a 0.5 min window around the elution time of the analyte in an analytical blank whilst exhibiting an inaccuracy and imprecison of ≤15%.

2.6.3. Accuracy and precision

Inter and intra-day accuracy and precision for the assay were determined from the analysis of replicate (n = 6) calibration standards at seven atenolol concentrations within the range of 25–1500 ng/ml on three separate days. The accuracy was expressed as the relative error (RE%) and precision as the coefficient of variation (CV%). A RE and CV of ≤15% at all concentrations was considered acceptable, in line with international recommendations [34].

2.6.4. Matrix-effects

Replicate (n = 6) samples of analyte spiked in extracted blank whole blood to 50, 500 and 1500 ng/ml were produced to evaluate suppression or enhancement of the detector response due to constituents within dried blood spots. The samples prepared were compared to standards of the same concentration spiked into methanol:water (60:40, v/v). The matrix effect was calculated by $(B/A - 1) \times 100$. Where A represents atenolol spiked into pure solvent and B represents atenolol spiked into extracted blank whole blood.

2.6.5. Recovery of atenolol from dried blood spot

The extraction efficiency or the recovery of atenolol from the spiked dried blood spots was determined by comparing peak area ratios from DBS with those obtained by direct injection of atenolol in the extraction solvent (methanol:water (60:40, v/v)) at the same concentration as the spiked blood spots. Recovery was calculated using the following equation: % recovery = (peak area of dried blood spot extract/peak area of standard atenolol solution) × 100. The extraction efficiency of atenolol was determined at three concentrations (50, 500 and 1500 ng/ml). All determinations were repeated six times.

2.6.6. Blood spot size

The need for quantitative reproducibility raises the question of the physical size of the sample taken from the DBS for subsequent extraction. The approach adopted was to punch a disk from within the DBS with the logical compromise of the largest diameter disks consistent with a typical DBS from normal clinical practice. The purpose of this series of experiments was to show that the results obtained were independent of the size of the blood spot collected. The analysis of a fixed sample size disc should produce extract data which is directly related to the concentration of atenolol in the original blood sample, assuming the spot has spread uniformly throughout the paper.

To assess the effect of blood volume collected on atenolol quantification 20, 30 and 40 μl blood spots at 50, 500 and 1500 ng/ml were prepared in replicate (n = 6). Five millimetre discs (approximately 15 μl of blood) were sampled from the centre of the 20, 30 and 40 μl volume DBS of 50, 500 and 1500 ng/ml atenolol. These spots were of different diameters corresponding to the volume of sample deposited. The atenolol was extracted as described in Section 2.4 using methanol/water (60:40, v/v) containing IS as the extraction medium and analysed as specified in Section 2.5. The concentrations of extracts were determined using the linear regression equation generated from a calibration produced from 30 μl dried blood spots.

2.6.7. Stability of dried blood spots

The stability of dried blood spot samples during storage for 10 weeks at room temperature was determined by analysing blood spots spiked with atenolol at the level of 50 ng/ml, 500 ng/ml and 1500 ng/ml (n = 6). The aim of this was to investigate if batch wise preparation and subsequent storage would be possible. Five millimetre discs (approximately 15 μl of blood) were punched from the centre of the 30 μl volume DBS of 50, 500 and 1500 ng/ml atenolol. The atenolol was extracted as described in Section 2.4 using methanol/water (60:40, v/v) containing IS as the extraction medium and analysed as stated in Section 2.5.

2.6.8. Effect of different sampling substrates

Three different sampling papers were evaluated and the extraction efficiency of atenolol assessed. These were Whatman 903, Ahlstrom 226 and Agilent DMS card. The 903 and 226 are cellulose based in comparison with the Agilent DMS card which is non-cellulose based.

2.7. Application of method

The developed LC–HRMS DBS methodology was applied to a series of dried blood spots collected from two healthy volunteers receiving a course of atenolol. A 100 mg atenolol tablet had been orally administered to the healthy volunteers. The study has received ethical approvals from the De Montfort University Research Ethics Committee.

3. Results and discussions

3.1. Selectivity

Since extracted ion chromatograms (EICs), based on the accurate mass measurement, were to be used for quantification of atenolol

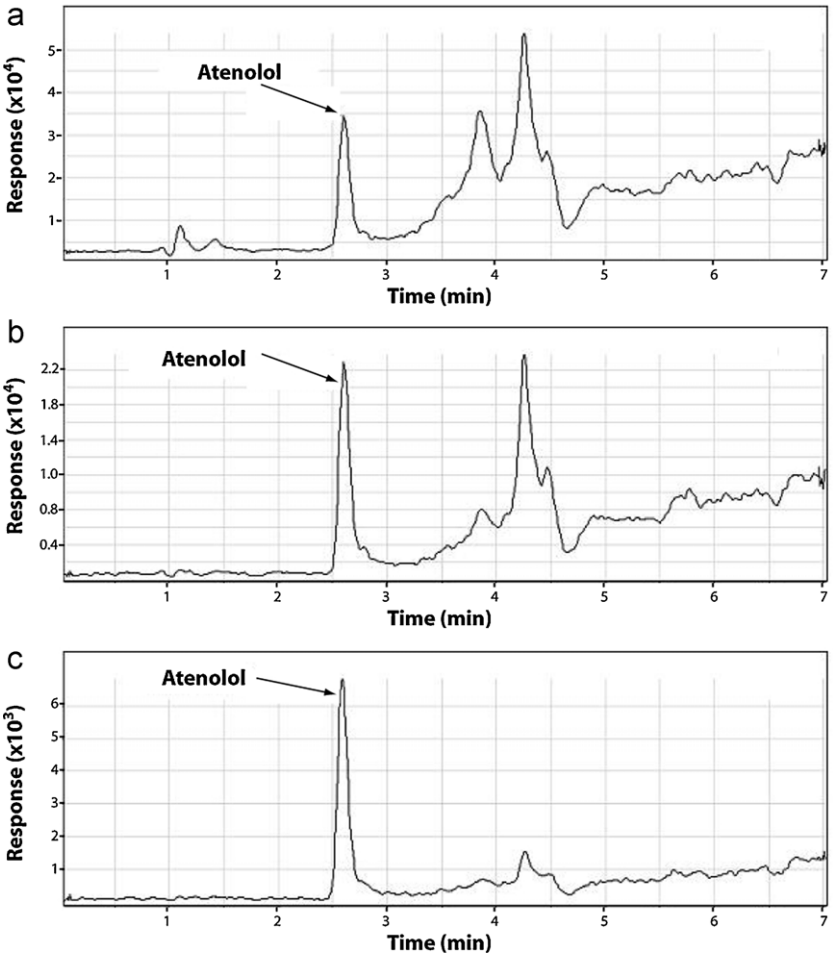

Fig. 2. Demonstration of enhanced selectivity resulting from the use of narrow mass tolerance windows for 50 ng/ml atenolol DBS. EIC of the protonated atenolol of *m/z* 267.1703 using mass tolerance filters of (a) ±500 ppm, (b) ±100 ppm and (c) ±1 ppm.

it was necessary to determine the accurate masses of the analyte and internal standard. Standard mass spectra in the full scan range 100–1000 *m/z* were obtained by injection of standard solutions of atenolol (500 ng/ml) and IS (250 ng/ml) in a mixture of methanol and water (60:40, v/v). The molecular ions $[M+H]^+$ for atenolol and IS showed a high intensity signal at *m/z* 267.1703 and *m/z* 274.2143 respectively. The developed LC–HRMS method for atenolol in DBS demonstrated good selectivity as the extracted ion chromatograms showed there were no interferences at the same retention time of atenolol (2.7 min). Selectivity enhancement obtained by narrowing the *m/z* extraction window is demonstrated in Fig. 2. Atenolol is detected with several other compounds when a mass tolerance of greater then 100 ppm is used whereas at 1 ppm the target compound gives the most intense signal and therefore better selectivity is achieved.

3.2. Linearity and sensitivity

The calibration curves were produced in replicate ($n = 6$) by plotting atenolol/IS peak area ratio against nominal atenolol concentration and an equally weighted linear regression applied. The response was linear over the calibration range 25–1500 ng/ml. The slope (0.036 ± 0.002), the intercept (0.007 ± 0.001) and the mean correlation coefficient $R^2 = (0.991 \pm 0.001)$ were determined.

The limit of quantification (LOQ) with a signal-to-noise ratio of ≥10 and acceptable assay accuracy and precision was 25 ng/ml in whole dried blood.

3.3. Accuracy and precision

The intra and inter-day performance of the assay was measured by analysing five spiked samples of atenolol at each concentration on three separate days (Table 1). Accuracy was expressed as the mean relative error (RE%) and precision was expressed as the coefficient of variation (CV%) and values for both were within the pre-defined 15% limit for all concentrations in each run. The overall variation in assay performance between runs was also ≤15%.

3.4. Matrix effect

No significant (<5%) ion suppression or enhancement of the analyte signal due to endogenous components of blood or the sampling paper was observed at the three tested atenolol concentrations (Table 2). The three concentrations were chosen from the low, medium and high range of the calibration curve (50, 500 and 1500 ng/ml). These results provide assurance on the selectivity of the extraction procedure and the ionisation method.

3.5. Recovery

The overall recovery of atenolol from dried blood samples for concentrations 50, 500 and 1500 ng/ml was between 96% and 100%. Consistent recovery values at low, medium and high concentrations indicate the extraction process is acceptable across this range. Table 2 gives the overall extraction recoveries obtained for each

Table 1
Intra- and inter-day accuracy and precision data for atenolol in whole dried blood samples ($n = 6$ at all concentration levels).

	Nominal conc. (ng/ml)						
	25	50	100	250	500	1000	1500
Intra-day							
Run 1							
Mean conc. (ng/ml)	23.2	57.8	110.3	263.6	486.5	977.2	1504.7
SD	3.1	1.0	12.2	13.5	43.3	101.8	74.4
Accuracy (RE%)	−1.6	14.1	10.3	5.4	2.7	2.3	0.3
Precision (CV%)	13.3	1.7	11.1	5.1	8.9	10.4	4.9
Run 2							
Mean conc. (ng/ml)	27.5	54.4	107.0	240.1	454.7	918.3	1579.4
SD	1.6	5.8	6.3	23.0	32.2	70.0	87.6
Accuracy (RE%)	8.4	10.6	7.0	3.9	9.0	8.1	5.2
Precision (CV%)	5.9	8.9	5.9	8.6	7.0	7.6	5.5
Run 3							
Mean conc. (ng/ml)	24.5	53.8	110.4	248.3	457.6	979.7	1601.1
SD	2.0	3.7	9.8	14.1	38.7	48.7	62.9
Accuracy (RE%)	1.9	7.6	10.4	13.8	7.5	12.0	6.7
Precision (CV%)	8.1	5.8	8.9	6.6	8.4	9.5	3.9
Inter-day							
Mean conc. (ng/ml)	26.5	54.3	100.5	253.8	496.6	995.7	1537.9
SD	1.3	4.9	5.2	17.7	63.4	45.2	76.9
Av. accuracy (RE%)	6.1	8.7	0.5	1.9	0.7	0.4	1.1
Overall precision (CV%)	7.7	9.7	5.1	6.9	12.7	4.5	5.0

Table 2
Matrix effect data at three concentrations of atenolol and recovery data from dried blood spots at three atenolol concentrations ($n = 6$).

	Nominal conc. (ng/ml)		
	50	500	1500
Matrix effect data			
Matrix effect % (mean)	3.4	0.2	4.9
Precision (CV%)	4.1	1.9	2.6
Recovery data			
Recovery (%)	99.7	96.3	97.5
SD	9.6	6.6	15.2
Precision (CV%)	9.6	7.0	15.6

concentration level. The high recoveries observed indicate analyte stability under the extraction conditions applied and good extraction.

3.6. Blood spot size

Method precision and accuracy were evaluated using the atenolol extraction data from the 5 mm discs sampled from the centre of the 20, 30 and 40 μl volume DBS of 50, 500 and 1500 ng/ml atenolol. Intra-day precision and accuracy of the method was determined using 6 determinations for each concentration level and are shown in Table 3. All values for accuracy and precision were less than 15% and therefore considered acceptable.

The purpose of this series of experiments was to show that the results obtained were independent of the size of the blood spot collected. The analysis of a fixed sample size disc should produce extract data which is directly related to the concentration of atenolol in the original blood sample. The hypothesis here is that each blood spot will spread evenly and uniformly across the sampling card. This hypothesis has been questioned especially for neonate blood samples where the haematocrit levels are known to vary widely with age [35–37]. There is no evidence to suggest this wide variation occurs in the population of concern with the current research. The results in Table 3 show that within experimental error for the therapeutically relevant concentration range the data output is the same for each sample volume chosen. In this respect these findings are analogous to other recent published data for the quantitative analysis from dried blood spots of dexamethasone [17], acetaminophen [15] and tacrolimus [13].

3.7. Stability

The mean and standard deviation for the extraction recoveries carried out after a 10-week interval for atenolol from a DBS sample ($n = 6$) was 99.6 ± 9.4%. Table 4 shows the atenolol concentrations from the dried blood spots after this time period. These results indicate that atenolol is stable in the dried blood spots for at least 2 months when stored at room temperature. Further tests are underway to determine long-term stability of analyte on the sampling paper.

Table 3
Effect of varying blood spot size on accuracy and precision of assay of atenolol at three concentrations ($n = 6$).

Atenolol concentration in whole blood (ng/ml)	DBS volume (μl)	Mean concentration found ±SD (ng/ml) ($n = 6$)	Accuracy (RE%)	Precision (CV%)
50	40	47.6	7.2	4.8
	30	49.9	8.9	0.2
	20	50.4	0.1	11.7
500	40	548.0	3.6	9.6
	30	454.7	9.0	7.1
	20	565.6	13.1	5.1
1500	40	1568.8	11.3	12.9
	30	1500.4	0.1	3.9
	20	1645	9.7	14.5

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Table 4
Accuracy, precision and quantification of atenolol in DBS samples after 10 weeks of storage.

Atenolol concentration in whole blood (ng/ml)	Mean concentration (ng/ml) ($n = 6$)	Accuracy (RE%)	Precision (CV%)	Extraction efficiency ±SD (%)
100	91.0	−8.9	6.3	91.1 ± 11.4
500	571.0	14.2	6.8	114.1 ± 8.4
1000	932.1	6.7	9.3	93.7 ± 8.5

3.8. Effect of different dried blood spot sampling papers

The DBS sampling paper type has been reported to affect the recovery and quantification of analytes [9,38]. Three DBS sampling types were evaluated for the accuracy and precision of for three atenolol DBS concentrations. These were the Whatman 903, Ahlstrom 226 and the Agilent DMS sampling papers. The Whatman 903 and the Ahlstrom 226 are cellulose based papers whilst the Agilent DMS is a non cellulose based paper. The thicknesses of each of the different sampling paper types were measured and found to be 466 ± 27 μm for the 903 paper; 526 ± 9 μm for the 226 paper and 729 ± 74 μm for the DMS cards. It was noted that all spots on the Agilent DMS cards were of a smaller diameter compared to the same volume spotted on the two cellulose based papers. The same volume of blood sample was therefore concentrated into a smaller volume of paper by virtue of the increased paper thickness. As a result of this a 5 mm diameter disc from the papers would contain a higher volume of blood which would give a higher level of atenolol in the subsequent determination. This will then be interpreted as a higher concentration. It was further noted that a 30 μl injection volume on an Agilent DMS card did not saturate both sides of the sampling paper. This suggests that the paper pore size and thickness affect the loading capacity and spreadability of blood onto the DBS paper. From a 5 mm fixed punch size, the atenolol concentrations determined were lower with the 903 paper for the three atenolol concentrations investigated suggesting that the thinnest sampling paper contained the least amount of target drug. For the three atenolol DBS concentrations investigated the accuracy expressed as the mean relative error was below the acceptable 15% for all concentrations and papers

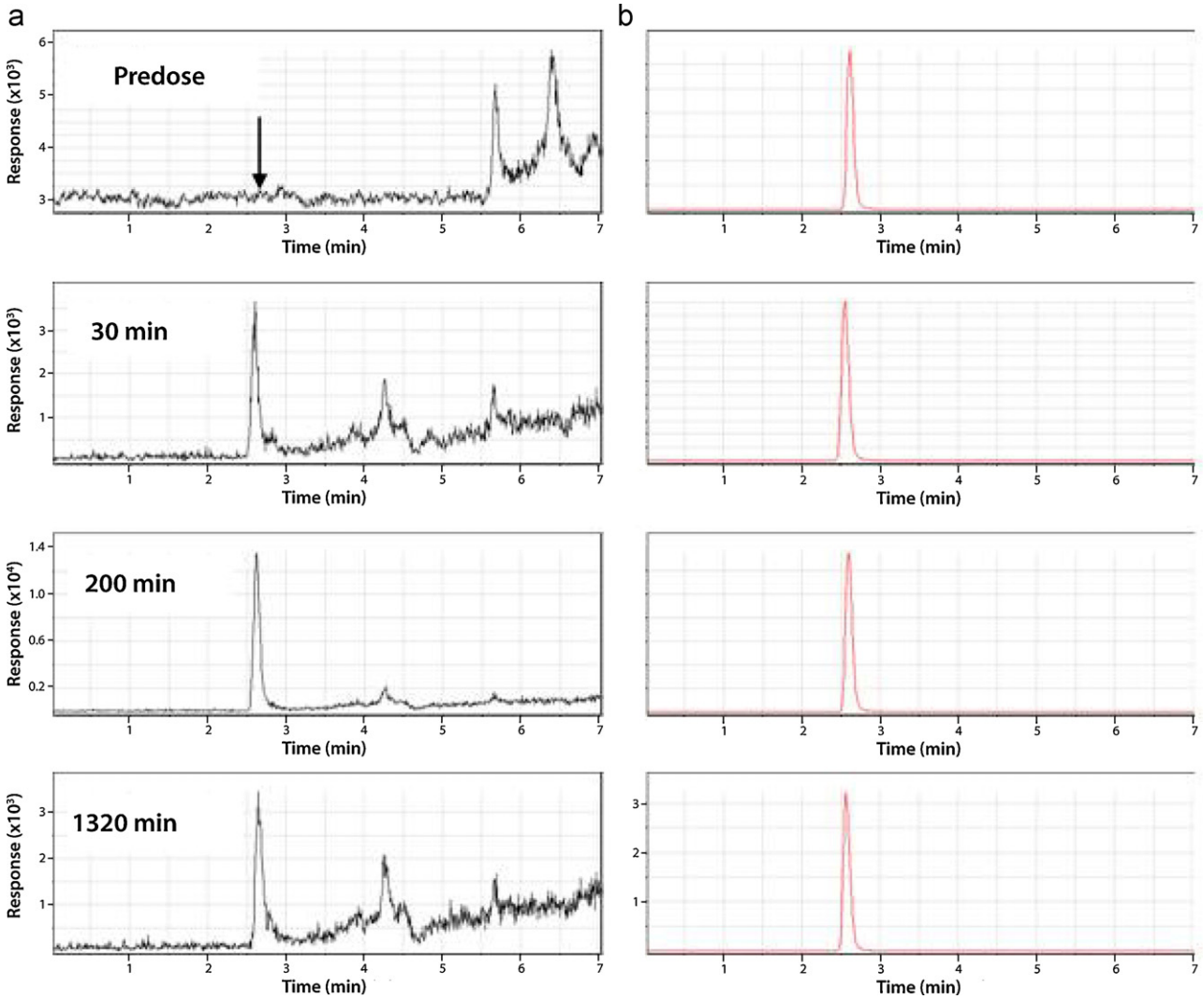

Fig. 3. Representative extracted ion chromatograms at m/z 267.1703 for atenolol (a) and at m/z 274.2143 for IS (b) for DBS samples obtained from a volunteer participated in a clinical study (pre-dose, 30 min, 200 min and 1320 min after single dose administration).

Table 5
Effect of different DBS sampling papers on quantification of atenolol.

Atenolol concentration in whole blood (ng/ml)	Sampling card	Mean concentration (ng/ml) ($n = 6$)	Accuracy (RE%)	Precision (CV%)
50	903	49.9	8.9	0.2
	226	52.4	4.8	14.1
	DMS	52.3	4.6	10.1
500	903	454.7	9.0	7.1
	226	580.7	11.7	4.1
	DMS	510.4	2.1	11.0
1500	903	1568.8	11.3	12.9
	226	1634.9	8.9	8.1
	DMS	1663.4	10.9	10.9

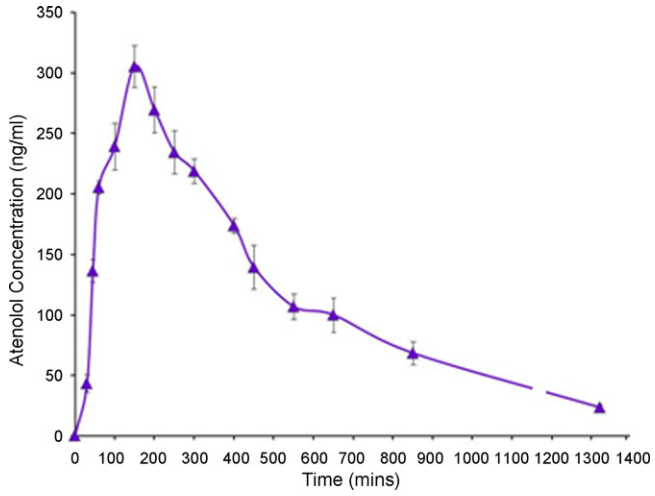

Fig. 4. Blood spot concentration–time plot of atenolol in a healthy volunteer after a single 50 mg oral dose of atenolol. Data were represented as mean ± SD.

(Table 5). The mean precision value of the measurements expressed as the coefficient of variation was the lowest for the Whatman 903 sampling paper. The poor precision from the Agilent DMS cards could be attributed to the thickness of these sampling cards and the visible evidence of the blood not being able to saturate the paper.

3.9. Application of assay: concentration–time profile

The validated method was successfully applied to the assay of atenolol in dried blood spots obtained from a healthy volunteer who received a single oral dose of 50 mg of atenolol. The representative extracted ion chromatograms showed a peak at 2.7 min for atenolol and the IS and are depicted in Fig. 3a and b. The peaks for atenolol were well within the limit of quantification of the DBS assay. The pharmacokinetic plot (Fig. 4) demonstrated the ability of the DBS method to reproduce published data for blood levels of atenolol [6] and indicates the detection levels required as a function of time after dosing, to ensure that the output from the test regime is meaningful. In order to monitor adherence to therapy the analytical method must be able to detect the residual levels of the drug up to 24 h (1440 min) after the initial dose. The results of the pharmacokinetic study (Fig. 4) demonstrate this capability and indicate that such a micro-sampling assay could therefore be usefully applied in a routine clinical setting to assess atenolol adherence and the DBS-based assay could be adapted to assess adherence of other medications.

There is close agreement between these results and the work of de Abreu et al. [6] who determined the levels of atenolol following oral administration of 50 mg of atenolol. For adult volunteers they found the maximum atenolol concentration (C(max)) to be 240–350 ng/ml with the time to C(max) of 2.08–3.25 h. The data obtained in this investigation (Fig. 4) is in very close agreement therefore confirming the validity of the DBS method.

4. Conclusion

The LC–HRMS method developed was rapid in terms of instrument time and provided high sensitivity determination of atenolol in DBS samples. The validated method has been shown to be accurate and precise with a RE and CV ≤15% at all tested concentrations. Stability of atenolol within DBS samples has been shown following storage at room temperature for up to ten weeks. There is no doubt that the triple quadrupole tandem mass spectrometer will remain the primary instrument used in quantitative therapeutic drug determinations where the target analytes are readily predetermined and new electronic developments will allow many more compounds to be monitored by the MRM process during a single LC–MS/MS run. One of the advantages of high-resolution full scan mass spectrometry is that all the mass spectral information, available in positive ESI mode, in the sample is collected and offers the potential for the data to be re-interrogated subsequently if deemed clinically relevant. Another consideration is that, since data is obtained in full scan mode, it is also possible to directly monitor interfering ions in the sample matrix that could produce ion suppression [31].

In this preliminary investigation a LC–HRMS-dried blood spot method for the quantification of atenolol in human whole blood has been described. The method seems promising for assessing adherence to atenolol and could be adapted to assess adherence to other cardiovascular medications. The degree of flexibility around blood volume collection is particularly advantageous to non-clinical test sites, i.e. at home or where accurate pipetting may be difficult to achieve, for instance in the therapeutic drug monitoring of elderly patients. The concept of monitoring atenolol concentrations in blood using DBS as a sampling tool arises from the need to assess adherence to medications in patients administered cardiovascular medications. Monitoring adherence is important to ensure that clinicians decide that treatment was ineffective only when it was taken as prescribed. Unnecessary dose adjustment, or the addition of other medication is avoided and a better healthcare regimen is created. The method is currently being applied to the analysis of blood spots taken from cardiovascular patients to assess adherence to cardiovascular medications including other β-blockers, ace inhibitors and statins.

Conflict of interest

None declared.

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References

[1] P.M. Ho, C.L. Bryson, J.S. Rumsfeld, Circulation 119 (2009) 3028.
[2] J.B. Garner, Am. J. Cardiol. 105 (2010) 1495.
[3] H.B. Bosworth, B.B. Granger, P. Mendys, R. Brindis, R. Burkholder, S.M. Czajkowski, J.G. Daniel, I. Ekman, M. Ho, M. Johnson, S.E. Kimmel, L.Z. Liu, J. Musaus, W.H. Shrank, E.W. Buono, K. Weiss, C.B. Granger, Am. Heart J. 162 (2011) 412.
[4] A. Shroufi, J.W. Powles, J. Epidemiol. Community Health 64 (2010) 109.
[5] S. Baroletti, H. Dell'orfano, Circulation 121 (2010) 1455.
[6] L.R.P. De Abreu, S.A.C. De Castro, J. Pedrazzoli, AAPS Pharmsci. 5 (2003).
[7] L.L. Mccabe, E.R.B. Mccabe, Mol. Genet. Metab. 75 (2002) 299.
[8] A.F. Olshan, Environ. Health Perspect. 115 (2007) 1767.
[9] S. Tanna, G. Lawson, Anal. Methods 3 (2011) 1709.
[10] E.J. Oliveira, D.G. Watson, N.S. Morton, J. Pharm. Biomed. Anal. 29 (2002) 803.
[11] S. Aburuz, J. Millership, J. Mcelnay, J. Chromatogr. B 832 (2006) 202.
[12] A.L. Allanson, M.M. Cotton, J.N.A. Tettey, A.C. Boyter, J. Pharm. Biomed. Anal. 44 (2007) 963.
[13] K. Hoogtanders, J. Van Der Heijden, M. Christiaans, P. Edelbroek, J.P. Van Hooff, L.M.L. Stolk, J. Pharm. Biomed. Anal. 44 (2007) 658.
[14] M. Barfield, N. Spooner, R. Lad, S. Parry, S. Fowles, J. Chromatogr. B 870 (2008) 32.
[15] N. Spooner, R. Lad, M. Barfield, Anal. Chem. 81 (2009) 1557.
[16] G. Lawson, S. Tanna, H. Mulla, H. Pandya, J. Pharm. Pharmacol. 61 (2009) 42.
[17] P. Patel, S. Tanna, H. Mulla, V. Kairamkonda, H. Pandya, G. Lawson, J. Chromatogr. B 878 (2010) 3277.
[18] M.F. Suyagh, G. Iheagwaram, P.L. Kole, J. Millership, P. Collier, H. Halliday, J.C. Mcelnay, Anal. Bioanal. Chem. 397 (2010) 687.
[19] M.F. Suyagh, K.P. Laxman, J. Millership, P. Collier, H. Halliday, J.C. Mcelnay, J. Chromatogr. B 878 (2010) 769.
[20] R.B. Miller, J. Pharm. Biomed. Anal. 9 (1991) 849.
[21] R.B. Miller, Y. Guertin, J. Liq. Chromatogr. 15 (1992) 1289.
[22] G. Egginger, W. Lindner, S. Kahr, K. Stoschitzky, Chirality 5 (1993) 505.
[23] F.C.K. Chiu, J.N. Zhang, R.C. Li, K. Raymond, J. Chromatogr. B 691 (1997) 473.
[24] L. Vlase, S. Leucuta, S. Imre, Rev. Chim. 58 (2007) 3.
[25] R. Bhushan, S. Tanwar, Biomed. Chromatogr. 23 (2009) 787.
[26] W.K. Li, Y.H. Li, D.T. Francisco, N.D. Weng, Biomed. Chromatogr. 19 (2005) 385.
[27] J.S. Ni, O.Y. Hui, M. Aiello, C. Seto, L. Borbridge, T. Sakuma, R. Ellis, D. Welty, A. Acheampong, Pharm. Res. 25 (2008) 1572.
[28] A. Khuroo, S. Mishra, O. Singh, S. Saxena, T. Monif, Chromatographia 68 (2008) 721.
[29] B. Yilmaz, S. Arslan, Chromatographia 70 (2009) 1399.
[30] B. Yilmaz, V. Akba, Asian J. Chem. 23 (2010) 54.
[31] T. Zhang, D.G. Watson, C. Azike, J.N.A. Tettey, A.T. Stearns, A.R. Binning, C.J. Payne, J. Chromatogr. B 857 (2007) 352.
[32] G. Lawson, H. Mulla, H. Pandya, G. Ross, A. Sage, S. Tanna, 1st International Pediatric Biomarker Symposium, Innsbruck, Austria, 2010.
[33] J.L.H. Jiwan, P. Wallemacq, M.F. Herent, Clin. Biochem. 44 (2011) 136.
[34] Guidance for Industry Bioanalytical Method Validation, Food and Drug Administration, Rockville, 2001.
[35] J.V. Mei, J.R. Alexander, B.W. Adam, W.H. Hannon, J. Nutr. 131 (2001) 1631S.
[36] X.A. Ren, T. Paehler, M. Zimmer, Z.Y. Guo, P. Zane, G.T. Emmons, Bioanalysis 2 (2010) 1469.
[37] S. O'broin, Clin. Chem. 39 (1993) 1354.
[38] W.K. Li, F.L.S. Tse, Biomed. Chromatogr. 24 (2010) 49.