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

Graham Lawson, Elizabeth Cocks, Sangeeta Tanna*

Leicester School of Pharmacy, Faculty of Health and Life Sciences, De Montfort University, Leicester LE1 9BH, UK

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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.

* Corresponding author.
E-mail addresses: glawson@dmu.ac.uk (G. Lawson), ecocks@dmu.ac.uk (E. Cocks), stanna@dmu.ac.uk (S. Tanna).

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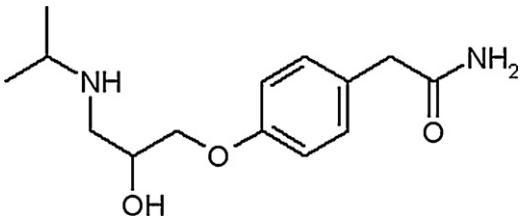

Fig. 1. Chemical structure of atenolol.

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