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Quantification of amlodipine in dried blood spot samples by high performance liquid chromatography tandem mass spectrometry

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ABSTRACT

A sensitive and specific method, utilising high performance liquid chromatography tandem mass spectrometry (HPLC–MS/MS) was developed for the quantitative determination of amlodipine in dried blood spot (DBS) samples. Chromatographic separation was achieved using a Waters XBridge C18 column with gradient elution of a mixture of water and acetonitrile containing 0.1% formic acid (v/v). Amlodipine was quantified using a Waters Quattro Premier mass spectrometer coupled with an electro-spray ionization (ESI) source in positive ion mode. The MRM transitions of 408.9 *m/z* → 238.1 *m/z* and 408.9 → 294.0 *m/z* were used to quantify and qualify amlodipine, respectively. The method was validated across the concentration range of 0.5–30 ng/mL by assessing specificity, sensitivity, linearity, precision, accuracy, recovery and matrix effect according to the Food and Drug Administration (FDA) guidelines. This method was also validated clinically within a large pharmacoepidemiological study in which amlodipine blood concentration was determined in patients who had been prescribed this medication.

1. Introduction

Amlodipine (Fig. 1) is a long-acting calcium channel blocker medication [1]. It is effective in hypertensive patients via direct anti-inflammatory effects as well as via inhibition of TNF-α and IL-1β on vascular smooth muscle cells [2]. Amlodipine is commonly used in the treatment of hypertension, angina and coronary artery disease (CAD) at the dosage of 5 or 10 mg per day in the morning [3]. As part of a large ongoing pharmacoepidemiology study, we are currently evaluating the adherence of patients to amlodipine treatment. Previous observational data indicated that fewer than 10% of patients strictly followed recommended dosage of amlodipine when taken in a fixed dose combination with atorvastatin [4]. The present work is part of a research project which combines blood level determination (in dried blood spot samples) with population pharmacokinetic modelling [5].

Conventionally, the determination of drug plasma concentrations has been widely used for pharmacokinetics studies [6]. The drug plasma concentrations are typically measured using LC–MS/MS [7–9], with the use of an internal standard and solid phase extraction in order to achieve recommended repeatability, precision and accuracy (< 15%). Recently, DBS samples have been suggested as an alternative sampling matrix for pharmacokinetics studies [10,11]. Our research group has published analytical methods using DBS samples of various medications [5,12,13]. DBS sampling is particularly recommended for use with neonates and infants for clinical and ethical reasons since very small volume samples are required [14].

In this study, we developed an HPLC–MS/MS method for the determination of amlodipine in DBS samples (15 μL) with a limit of quantification (LOQ) of 0.5 ng/mL. This method has been fully validated and was shown not to be influenced by other drugs (n = 7) likely to be taken concomitantly by patents. This is the first published method using HPLC–MS/MS for quantifying amlodipine in DBS samples on its own, however, another paper has described determination of amlodipine using a method which was initially validated for other compounds [15]. The method is currently being employed in the assessment of medication exposure/adherence in a large cohort study of patients (≥50 years old) in the Northern Ireland.

2. Material and methods

2.1. Chemicals materials

Amlodipine besylate (purity ≥98%), formic acid (for mass spectrometry, ≥98%), methanol and acetonitrile (CHROMASOLV®, for HPLC, ≥99.9%) were all purchased from Sigma-Aldrich (Poole, UK). HPLC grade water was obtained using a Millipore Direct-Q™ water

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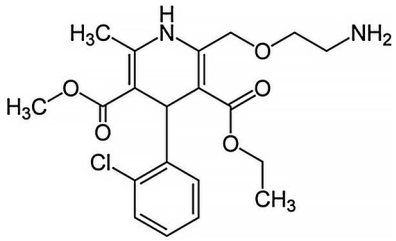

Fig. 1. Chemical Structure of Amlodipine.

system (Watford, UK). The School of Pharmacy Ethical Committee (013PMY2009) approved the collection of blank blood from healthy human volunteers for assay development.

All materials for DBS sample preparation have been described in our previous publications [14,16,17]. Briefly, Ahlstorm 226 (Guthrie cards) together with storage pouches were purchased from 3 M Security Systems Division (Oldham, England). The 8 mm, single hole punch (2700–62) used to cut out the DBS disks from the cards was purchased from Darice, Strongsville, Ohio, USA. Zip-loc bags, silica gel pouches and polypropylene freezer storage boxes were procured from Amazon UK. A Zymark Turbo Vap® LV Evaporator workstation (Zymark, Runcorn, UK) was used to remove solvent from sample extracts under controlled temperature. A Stuart rotator SB2 and vortex mixer SA8 (Bibby Scientific, Staffordshire, UK) were used to mix the spiked blood samples and vortex samples, respectively.

2.2. HPLC–MS/MS conditions

Liquid chromatography was performed on an Alliance E2695 HPLC separation system (Waters Corp., Milford, MA) with an XBridge C18 column (4.6 mm × 100 mm, 3.5 µm particle size) coupled with a C18 guard cartridge (130 Å, 4.6 mm × 20 mm, 3.5 µm particle size; Waters, Dublin, Ireland). The column was operated at 40 °C. The mobile phase consisted of water and acetonitrile containing 0.1% formic acid (v/v). A gradient elution with initial mobile phase composition of 20% acetonitrile and 80% water containing 0.1% formic acid was maintained for 2 min. Between 2 and 3 min, the percentage of acetonitrile was changed to 50% and maintained for 4 min. Between 7 and 7.5 min, the percentage of acetonitrile was further increased to 60% and then it was maintained for 3.5 min. The acetonitrile was increased to 90% at 11 min and the column was flushed with this mobile phase composition for 5 min. The percentage of acetonitrile was returned to 20% at 16.5 min and the column was equilibrated until 22 min. The mobile phase was delivered at a flow rate of 0.3 mL/min throughout. The sample injection volume was 20 µL using an auto-sampler system maintained at 10 °C.

A Waters Micromass Quattro Premier™ tandem quadrupole (Waters, Manchester, UK) was used for mass spectrometry detection. The conditions for the positive electrospray ion mode (ESI +) were optimized as follows: Cone gas (nitrogen) 80 L/h, desolvation gas (nitrogen) 800 L/h, desolvation temperature 300 °C, source temperature 120 °C and capillary voltage 3.30 V. Argon gas was used as the collision gas. The MRM transitions for quantification and qualification of amlodipine ion were $408.9\,m/z \rightarrow 238.1\,m/z$ and $408.9 \rightarrow 294.0\,m/z$, respectively (Fig. 2). Other parameters, i.e. cone voltage, collision energy and dwell time were set at 15 V, 10 V and 0.3 s, respectively. Data acquisition and processing were performed using Waters Quanlynx 4.0 software and Microsoft Excel 2016.

2.3. Standard solutions, matrix-matched calibrators and quality controls (QCs)

The amlodipine concentration was calculated and prepared according to its corresponding amlodipine besylate solution. A stock solution of amlodipine was firstly calculated and prepared for 0.1 mg/mL in absolute ethanol. This stock solution was further diluted using methanol to make the standard solutions at 25, 50, 100, 250, 500, 1000 and 1500 ng/mL for preparation of matrix-matched calibrators. Similarly, working solutions at concentrations of 50, 375 and 1250 ng/mL were prepared for quality control samples (QCs). All the above solutions were stored at 4 °C and were brought to room temperature (20 °C) before use.

To every 980 µL of whole blood, 20 µL aliquots of the appropriate amlodipine standard solutions (25, 50, 100, 250, 500, 1000 and 1500 ng/mL) were added to give final concentrations of 0.5, 1, 2, 5, 10, 20 and 30 ng/mL, respectively. The QCs at concentrations of 1 (low QC), 7.5 (middle QC) and 25 ng/mL (high QC) were prepared in the same way of matrix-matched calibrators.

2.4. DBS sample preparation

To allow the equilibration of amlodipine slowly in the blood, spiked blood solutions were mixed for 30 min at 37 °C before spotting onto Guthrie cards. The blood spots were prepared by accurately pipetting 15 µL of the spiked blood (calibrators and QCs) onto the middle of marked sample circle on the Guthrie cards. The samples were dried at room temperature in the dark (cupboard) overnight. Thereafter, they were individually placed in cellophane envelopes and placed in zip lock bags together with silica gel pouches (dehydration agent). The cards were then stored in polypropylene freezer storage sealed containers at −20 °C until analysis.

2.5. Amlodipine extraction from DBS samples

The DBS samples were brought to room temperature before analysis. An 8 mm disk was punched from the card; this disk size incorporated with complete blood sample. Each disk was then transferred to a 2 mL micro tube and was extracted by addition of 1 mL of methanol containing 0.1% formic acid followed by vortex mixing for 30 min. The extracts were then transferred to a fresh 2 mL micro tube. The extraction solvent was evaporated under a nitrogen stream using the Zymark Turbovap at 37 °C and reconstituted with 75 µL of mobile phase (water: acetonitrile, 50:50, containing 0.1% formic acid). The reconstituted solution was then centrifuged (9000*g*; 4 °C) for 15 min followed by injection of 20 µL of the supernatant onto the HPLC–MS/MS.

2.6. Method validation

The FDA guidelines were applied for all the validation work in this study [18].

2.6.1. Linearity

Linearity of the method was assessed using 7-point calibration curves analysed on 3 consecutive days. Peak areas against their corresponding concentrations were used to assess the relationship. The calibration curves were linear and R^2 values were calculated for each calibration curve developed.

2.6.2. Sensitivity

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated based on the peak-to-noise ratio at 3:1 and 10:1, respectively.

2.6.3. Selectivity

The selectivity of the method was determined by analysing blank DBS samples from five different sources.

2.6.4. Precision and accuracy

Five replicated sets of QCs (1, 7.5 and 25 ng/mL) were used to determine the accuracy and precision of the assay. The accuracy was calculated by comparing the measured concentration with the true