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Technical note

Sensitive high-performance liquid chromatographic analysis of amlodipine in human plasma with amperometric detection and a single-step solid-phase sample preparation

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Abstract

A narrow-bore HPLC assay with electrochemical detection for the determination of the calcium antagonist amlodipine in human plasma samples is presented. By using a single-step solid-phase extraction procedure on Bond Elut C_2 columns, the sample preparation step has been considerably simplified and less time-consuming compared to earlier presented works. With a linear and reproducible calibration curve over the range 0.5–20 ng ml^{-1} plasma, the assay has successfully been used in the analysis of more than 500 plasma samples from a multicenter trial.

1. Introduction

Amlodipine belongs to the dihydropyridine-type calcium antagonists. Only a few chromatographic methods for the determination of amlodipine in subnanogram ml^{-1} plasma levels have been described in the literature. The method most frequently used in clinical studies is a sensitive gas chromatographic assay with electron capture detection after derivatization with trimethylacetyl chloride [1]. This method, however, requires special arrangement to reduce the risk of thermal decomposition of amlodipine to the pyridine analogue that also is present as metabolite in human plasma samples. An HPLC assay with electrochemical detection described by Shimooka et al. [2] showing about the same limit of quantitation as the GC assay has also been used for determination of amlodipine in human plasma. However, these two chromatographic methods described above are both using time-consuming liquid–liquid sample preparation procedures in two steps or more. Our aim was to develop a robust HPLC assay for amlodipine with high sensitivity and a simple reproducible sample preparation procedure.

2. Experimental

2.1. Chemicals and materials

Acetonitrile (far UV) and methanol, both HPLC grade, were purchased from LabScan Analytical Sciences (Dublin, Ireland). Acetic acid (100%), ammonia (25%), disodium hydrogenphosphate-2-hydrate, sodium acetate and ethylenediaminetetraacetic acid disodium

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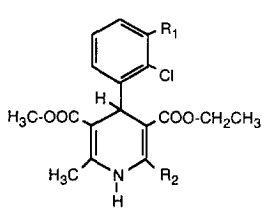
salt dihydrate (EDTA) all pro analysi, were purchased from Merck (Darmstadt, Germany). Potassium dihydrogenphosphate was from Fluka (Buchs, Switzerland) and sodium dodecylsulfate (SDS) was from Kebo (Stockholm, Sweden). Racemic amlodipine (maleate) and UK52.829 (fumarate) were obtained from Central Research, Pfizer (Sandwich, UK) (Fig. 1).

Bond Elut C_2 extraction columns, with a sorbent mass of 100 mg in 3 ml cartridges, were purchased from Analytichem (Harbor City, CA, USA).

2.2. Apparatus

The HPLC system consisted of a Gynkotek 480 pump (München, Germany), an Antec Decade amperometric detector (Leiden, Netherlands), a Waters 717 autosampler (Milford, MA, USA) and an EZChrom Scientific Software integrator system (San Ramon, CA, USA). The built-in detector oven housed an Antec VT-03 analytical cell with a 50-μm spacer, an ESA 5020 guard cell (Bedford, MA, USA), an Antec LO-Pulse pulse damper, a Scantec PEEK inlet filter (Partille, Sweden) and the analytical column. Chromatography was carried out on a Zorbax SB-Phenyl column 150 × 2.1 mm I.D. (Rockland Technologies, USA).

Extraction was carried out on an IST VacMaster preparation system (Hengoed, UK) and organic solvents were evaporated with a Heto Lab Equipment vacuum concentrator, Hetovac VR-1, CT 110 (Birkerød, Denmark).



	R_1	R_2
Amlodipine	H	CH_2-O-CH_2-CH_2-NH_2
UK52.829	Cl	CH_2-O-CH_2-CH_2-NH_2

Fig. 1. Molecular structures.

2.3. Chromatographic conditions

The mobile phase consisting of methanol and 0.1 *M* acetate buffer, pH 4.0, in a ratio of 65:35 (v/v) with 2 m*M* SDS and 1 mg EDTA l^{-1} was passed through a 0.22-μm filter and recycled in the chromatographic system. A flow-rate of 0.3 ml/min, at a column and detector oven temperature of 30°C, was used. The analytical cell had an applied voltage of 0.95 V and the nanoampere sensitivity range was used. The applied voltage of the guard cell was 0.5 V.

2.4. Extraction procedure

The Bond Elut C_2 extraction columns were conditioned before use with 2 ml acetonitrile, 1 ml water and 1 ml phosphate buffer (0.025 *M*, pH 7.0). Into the column cartridge were added 0.5 ml phosphate buffer (0.025 *M*, pH 7.0), 50 μl internal standard solution (0.2 μg/ml UK52.829 in methanol–water, 1:1, v/v), 1 ml plasma sample and another portion of 0.5 ml buffer. The solution was applied to the columns with a gentle underpressure of 2–5 mmHg (ca. 270–670 Pa). The columns were then washed with 2 ml 20% acetonitrile in water and dried by vacuum suction for 5 min, and finally washed with 1 ml acetonitrile. The samples were eluted with 1 ml of a solution of 2.5% ammonia in acetonitrile and the solvent was evaporated with the Hetovac vacuum concentrator. Samples were resolved in 50 μl of a solution of methanol and 0.1 *M* acetate buffer, pH 4.0, (1:1, v/v) and then transferred into microvials for the autosampler.

3. Results and discussion

3.1. The HPLC system

Chromatography was carried out on a narrow-bore column with Zorbax stable bond phenyl materials which are developed for the analysis of basic compounds. When a narrow-bore column with NovaPak phenyl materials first was tested, neither amlodipine nor UK52.829 were eluted within 90 min, probably due to extensive interac-

tions with uncapped silanol residues. To reduce secondary interactions and achieve migration an amine (i.e. *n*-octylamine) had to be added to the mobile phase. However, this system could not be used together with electrochemical detection since the amino additive was readily oxidized causing a very high background. With the stable bond phenyl materials no amine additive was necessary and the background was low. The use of a narrow-bore column (I.D. 2 mm) and consequently a reduced flow-rate further improved the signal-to-noise ratio. Amlodipine as well as the internal standard UK52.829 was eluted within 15 min (Fig. 2).

According to findings by Shimooka et al. [2], an applied voltage of 0.95 V on the analytical cell was found to give optimal and stable detection conditions. A linear detector response for the peak-height ratio of amlodipine and UK52.829 was observed in a broad concentration range between 0.5 and 20 ng ml^{-1} plasma with a correlation coefficient above 0.999. Calculated coefficients of variation at four levels (0.2, 0.5, 5 and 20 ng/ml plasma) and between-day variation at two levels (control samples) are listed in Table 1. The limit of quantitation with a coefficient of variation below 15% was determined as 0.2 ng amlodipine ml^{-1} plasma. Both the precision of the standard curve and the dynamic range over two decades indicate that the presented HPLC assay is robust.

3.2. Sample preparation

By the use of solid-phase extraction twenty samples were processed in a single step within 35

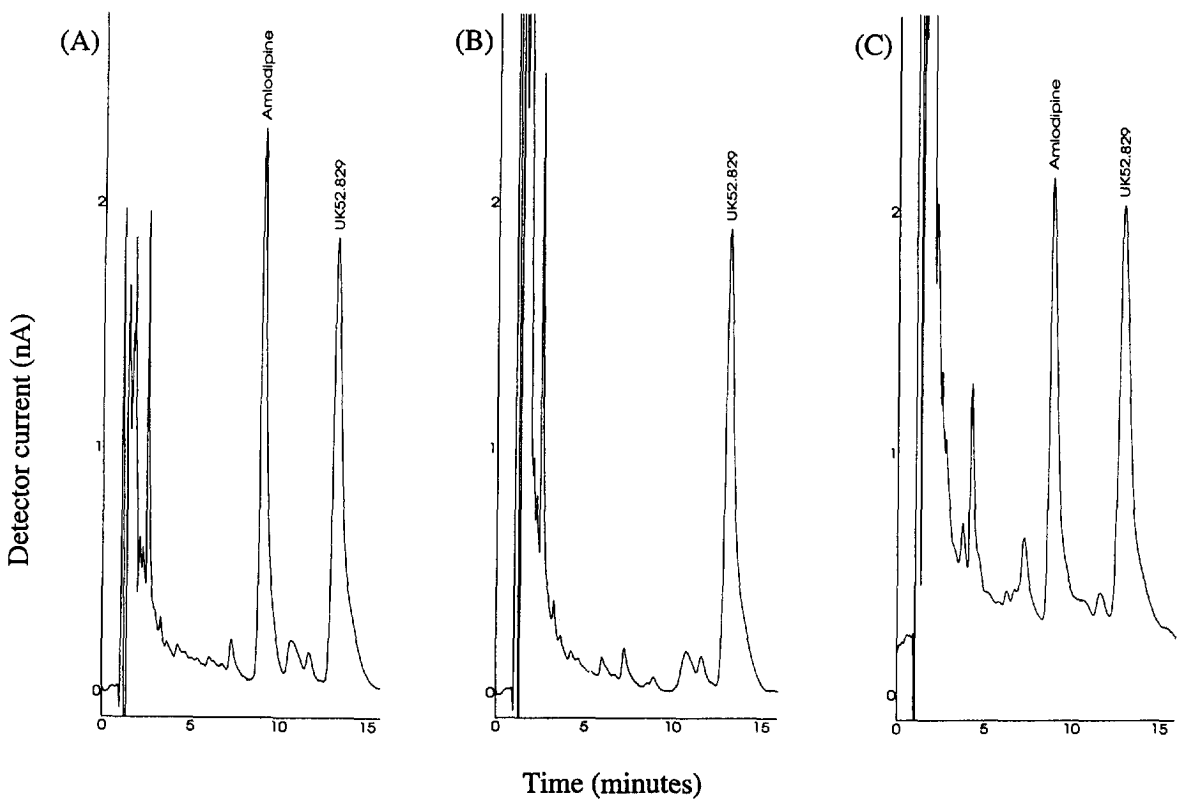


Fig. 2. Chromatograms showing the separation of amlodipine and the internal standard UK52.829. (A) Human plasma spiked with 6 ng ml^{-1} amlodipine and 10 ng ml^{-1} internal standard, (B) human plasma just spiked with 10 ng ml^{-1} of the internal standard and (C) 1 ml of a human plasma sample at steady state after once daily administration of 5 mg amlodipine, spiked with 10 ng ml^{-1} of the internal standard.

Table 1
The variation in peak-height ratio of spiked human plasma samples with amlodipine added at four different levels (a) and between-day variation in peak-height ratio of control samples at two levels (b), with the internal standard UK52.829 added in an amount of 10 ng ml^{-1}

Added amount (ng ml^{-1})	Number of samples	Mean ± S.D. (ng ml^{-1})	Coefficient of variation (%)
0.2 (a)	4[a]	–	8.9
0.5 (a)	5	–	5.6
5.0 (a)	5	–	5.4
20 (a)	5	–	2.5
1.3 (b)	5	1.27 ± 0.11	9.0
6.0 (b)	5	6.10 ± 0.34	8.3

[a] One sample, clearly out of range, excluded.

min and with a total use of less than 5 ml organic solvents (i.e. acetonitrile) per sample. In the liquid–liquid sample preparation procedure described by Shimooka et al. [2], there were three extraction and washing steps with time-consuming centrifugations and decantations in between, and the recovery was low (i.e., <60%). Furthermore, the extraction solvent (i.e. diethyl ether) used had to be washed in three steps and distilled before use. Finally, an extra component, the 2,3-dichlorophenyl analogue to amlodipine, had to be added to the plasma samples to reduce glass absorption. This analogue was eluted as the last peak in the chromatogram.

In our solid-phase extraction system amlodipine and UK52.829 were both extracted on Bond Elut C_2 columns with a recovery of 98% ($n = 5$) and 95% ($n = 5$), respectively, and a coefficient of variation below 3%. The strong interaction of amlodipine and UK52.829 with the C_2 material at the selected pH 7.0, was probably partially due to secondary interactions of the basic primary amino group, on the side chain of the dihydropyridines, with silanol residues. It has been shown that even in rigorously end-capped materials 30% or more uncapped silanol groups are still present [3]. This strong silanol interaction allowed a strong washing step with 100% acetonitrile. Methanol, however, was not suitable in the washing step since it was able to elute amlodipine and UK52.829, probably due to its proton donor capacity. With 2.5% ammonia in acetonitrile the hydrophobic interaction with the C_2 chains as well as the ionic secondary interactions with the silanol residues were broken and the dihydropyridines were eluted. The same columns were reused for several times without cross-contamination or loss of capacity.

4. Conclusion

By using a solid-phase extraction technique, the sample preparation step has been considerably simplified and is less time-consuming compared to earlier described amlodipine assays. Furthermore, since the same extraction columns can be used several times without reduction in recovery or any risk of cross-contamination, the costs are kept low. Partly due to the Antec Decade detector system with the detector cell as well as the HPLC column housed in an oven, a robust assay has been achieved. Finally, the use of narrow-bore chromatography and consequently low flow-rates results in a low background current in the amperometric detector, high sensitivity and low solvent consumption.

References

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