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Simultaneous determination of five 1,4-dihydropyridines in pharmaceutical formulations by high-performance liquid chromatography–amperometric detection

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Abstract

A high-performance liquid chromatographic method with electrochemical detection was developed for the simultaneous determination of five 1,4-dihydropyridines: amlodipine, nitrendipine, felodipine, lacidipine and lercanidipine. These drugs are widely used in the treatment of hypertension, angina pectoris and the therapy of cerebrovascular spasms of various origins. The chromatographic separation was performed on a Supelcosil LC ABZ + Plus C_{18} column with a mobile phase consisting of acetonitrile–10 mM acetate buffer (72:28, v/v) at a flow rate of 1 ml/min. The temperature was set at 30 ± 0.2 °C. The amperometric detector, equipped with a glassy carbon electrode was operated at +1100 mV versus Ag/AgCl in the direct current mode. Under these chromatographic conditions, the drugs eluted in less than 12 min. The method showed to be linear over the range 4.5–15 μg/ml with a within-day and day-to-day repeatabilities in terms of R.S.D. lower than 15%, an accuracy greater than 98% and detection limits varying from 90 ng/ml (amlodipine) to 1.55 μg/ml (nitrendipine). The method was successfully applied to commercially available pharmaceuticals with relative errors lower than 5%. The validity of the method was examined comparing the results obtained with those of HPLC with photometric detection.

Keywords: Pharmaceutical analysis; Dihydropyridines; Pyridines; Amlodipine; Nitrendipine; Felodipine; Lacidipine; Lercanidipine

1. Introduction

Calcium antagonists block the influx of calcium ions through voltage-operated calcium channels located in the cell membrane. Among the different groups, dihydropyridines is the most numerous and includes the largest number of novel compounds.

They act upon the L-type channel, which has a specific dihydropyridine site in its extracellular surface and bind more selectively to vascular calcium channels than to those in the myocardium. Newer dihydropyridines exhibit greater selectivity, with evidence for specific vascular vessel bed binding. Each of these agents is effective in the treatment of hypertension and angina pectoris [1–3].

Since 1,4-dihydropyridines calcium antagonists (1,4-DHPs) have at least two electroactive groups, a reducible aromatic nitro group and an oxidizable dihydropyridine ring, they can be analysed electrochemically. Voltammetric studies on the redox mechanism, polarographic studies of photodegradation products and the development of different pulse voltamperometric methods for the quantification of some of these compounds in pharmaceutical preparations or in biological fluids have been published in literature over the last years [4–12].

Chromatographic techniques with different kinds of detection (photometric, fluorimetric, electrochemical, mass spectrometry, radio immunoassay) have been also used for the quantitative determination of nifedipine and some related 1,4-dihydropyridines in biological samples [13–22]. HPLC with photometric detection is the technique used in the Pharmacopoeia for the analysis of amlodipine [23], nitrendipine [24] and felodipine [25].

HPLC with electrochemical detection (ED) has been applied to the determination of several of the studied compounds and other 1,4-DHPs in biological fluids [19–22], but it has not been used for the analysis of these drugs in pharmaceutical formulations.

The compounds studied were: amlodipine {(*R*,*S*)-2[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine},

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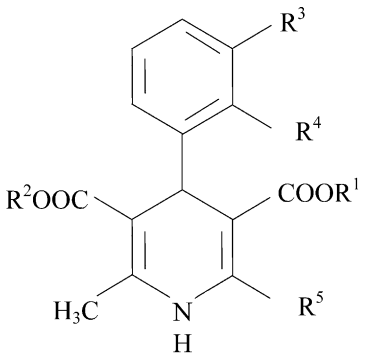

DRUG	R^1	R^2	R^3	R^4	R^5
Amlodipine	$-CH_2CH_3$	$-CH_3$	-H	-Cl	$-CH_2O(CH_2)_2NH$
Nitrendipine	$-CH(CH_3)_2$	$-CH_2CH_2OCH_3$	$-NO_2$	-H	$-CH_3$
Felodipine	$-CH_3$	$-CH_2CH_3$	-Cl	-Cl	$-CH_3$
Lacidipine	$-CH_2CH_3$	$-CH_2CH_3$	-H	$CH=CHCOOC(CH_3)_3$	$-CH_3$
Lercanidipine	$-CH_3$	$-CH_2(CH_3)_2CH_2N(CH_3)CH_2CH_2CH(C_5H_5)_2$	$-NO_2$	-H	$-CH_3$

Fig. 1. Structures of the 1,4-dihydropyridines studied.

nitrendipine [1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinecarboxylic acid ethyl methyl ester], felodipine [ethyl methyl 4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridine dicarboxylate], lacidipine ((*E*)-4-{2-[3-(1,1-dimethyletoxy)-3-oxo-1-propenyl]phenyl}-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylic acid diethyl ester) and lercanidipine {methyl 1,1-dimethyl-2-[*N*-(3,3-diphenylpropyl)-*N*-methylamino] ethyl-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate hydrochloride}. The formulas of these drugs are shown in Fig. 1.

The aim of this work is the development of a robust, fast and simple chromatographic method with amperometric detection for the simultaneous determination of these drugs in tablets. The results obtained will be compared with those calculated using a previous chromatographic method with UV detection developed in our laboratory [26].

2. Experimental

2.1. Apparatus and column

The HPLC system consisted of a Waters 510 (Milford, MA, USA) HPLC pump and Rheodyne (Cotati, USA) 7125 (Pharmacia, Barcelona, Spain) injector fitted with a 20 μl injection loop. The electrochemical detector was a PAR (EG&G) model 400 and operated in the dc mode at +1100 mV versus a Ag/AgCl/NaCl 3 M reference electrode. A glassy carbon electrode was used as working electrode and a platinum rod as auxiliary electrode, with a 5 s low pass filter time constant and a current range of 200 nA.

Chromatograms were recorded with the aid of a computer and data were treated with the Millennium 3.2 Chromatography Manager software from Waters (Barcelona, Spain).

A Supelcosil ABZ + Plus, 25 cm × 4.6 mm i.d., 5 μm (Supelco, Barcelona, Spain) HPLC column with a μBondapak C_{18} precolumn module (Waters) was used. The column was kept at constant temperature using a Waters TMC temperature control system.

2.2. Reagents and solutions

The 1,4-dihydropyridines were kindly supplied by the pharmaceutical companies: amlodipine and nitrendipine, by Bayer (Barcelona, Spain), felodipine by Astra (Barcelona, Spain), lacidipine by Glaxo (Madrid, Spain) and lercanidipine by Recordatti (Madrid, Spain).

Solvents were purchased from Lab-Scan (Dublin, Ireland) and were HPLC grade. The other reagents were analytical-reagent quality and supplied by Merck (Darmstadt, Germany). Water used in all experiments was obtained from Milli-Ro and Milli-Q systems (Millipore, Bedford, MA, USA).

Stock solutions of each drug studied (1000 μg/ml) were prepared in methanol, stored at 4 °C and protected from light. Working solutions were prepared by appropriate dilution in mobile phase just before use, utilizing amber glass volumetric flasks in order to avoid degradation.

2.3. Chromatographic conditions

Acetonitrile—10 mM CH_3COOH/CH_3COONa at pH 5 (72:28, v/v) was used as mobile phase. The buffer served as supporting electrolyte. The mixture was filtered through a 0.45 μm pore membrane, and the air was removed from the phase by bubbling helium through.

The μBondapak C_{18} column head-pressure was 110 bar at a flow rate of 1.0 ml/min. The injection volume was 20 μl. The column temperature was kept constant at 30.0 ± 0.2 °C. A potential of +1100 mV was used as working potential.

2.4. Electrode maintenance

The electrode was cleaned electrochemically at the end of each working day by keeping it at −600 mV for 5 min

and after that at +1600 mV for 10 min. This operation was carried out using the mobile phase at a flow rate of 1 ml/min.

When high noisy or a baseline drift was observed, the glassy carbon electrode was cleaned with a tissue wetted with methanol in order to remove any possibly adsorbed compounds, and rinsed with deionised water.

2.5. Procedure for tablets

Five pharmaceutical formulations have been analysed using the HPLC method developed in this work: Astudal (amlodipine 5 mg) from Almirall Prodesfarma, Baypresol (nitrendipine 20 mg) from Bayer, Plendil (felodipine 5 mg) from Laboratorio Astra España, Lacipil (lacidipine 4 mg) from Glaxo Wellcome and Lercadip (lercanidipine 9.4 mg) from Biohorm.

The same procedure was applied to the sample preparation of all formulations. Five tablets were weighed and then mixed and crushed in a mortar. A suitable amount of powder was accurately weighed and dissolved in methanol. The mixture was sonicated for 10 min and centrifuged at 800 × *g* for 5 min. The clear solution was transferred into a volumetric flask, and the solid washed twice with methanol in order to dissolve completely the active component. The clear solutions were also added into the volumetric flask and finally made up to a fixed volume with methanol. Aliquots of these solutions were diluted in mobile phase and injected three times in the chromatographic system.

2.6. Calibration curves

A multi-drug stock solution of 200 μg/ml of each 1,4-DHP studied was prepared from 1000 μg/ml stock solutions, in methanol.

Calibration standards covering the range 4.5–15 μg/ml were prepared from the multi-drug stock solution in mobile phase. The calibration curves were obtained by plotting chromatographic peak area versus the nominal concentration of each compound. The slopes and intercepts of the calibration curves were calculated by unweighed least-squares regression analysis.

2.7. Repeatability, accuracy and detection limit

The intra-day and inter-day repeatabilities were calculated by injecting replicate samples ($n = 10$) at three levels of concentration of each 1,4-DHP, and are expressed as relative standard deviation (R.S.D.).

The accuracy of the method is defined by the analysis of five samples of each 1,4-DHP at three levels of concentration. Accuracy is defined as mean (found concentration/actual concentration) × 100%. The detection limit of the method was defined as the quantity of compound which produced a chromatographic peak with a signal-to-noise ratio of 3.

3. Results

Under static conditions, these compounds are oxidised at the glassy carbon electrode, giving rise to voltammetric peaks whose potential and intensity are pH dependent [27]. The anodic process can be attributed to the oxidation of the dihydropyridine ring to the pyridine derivative compound [4–10].

The simultaneous determination of a mixture of these compounds by voltammetric methods is not possible, due to the overlapping of the peaks, thus it led us to the development of a chromatographic system with amperometric detection.

3.1. Optimisation of the chromatographic system

The composition of the mobile phase that allowed the complete resolution of the chromatographic peaks was obtained by means of experimental design and HPLC–UV [26]. In this previous work we studied the chromatographic behaviour of the compounds depending on the composition, concentration of the buffer and pH value of the mobile

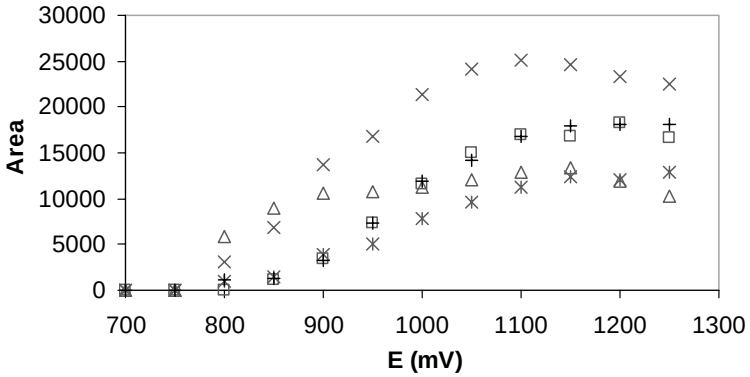

Fig. 2. Hydrodynamic voltammogram of: (△) amlodipine; (□) nitrendipine; (×) felodipine; (*) lacidipine; and (+) lercanidipine. Amount of drug injected: 10 μg/ml. For chromatographic conditions see Section 2.3.

phase, and temperature of the analytical column. This study was done by means of experimental design using for this purpose a fractionated factorial design and a composite design.

The hydrodynamic voltammograms of each compound were performed in order to select the optimum potential value for their detection. For this purpose, a potential scan in the range +700 up to +1300 mV was applied to a solution containing 10 μg/ml of each of the compounds. Three replicates of each injection were made and the mean value of peak areas obtained was plotted versus potential, Fig. 2. Potential values higher than +1300 mV produced a lack of response. A potential of +1100 mV was chosen as the working potential, since it was the lowest potential that gave rise to reproducible areas with the maximum sensitivity for the studied compounds without producing a high background current.

The effect of the electrolyte and its concentration on the signal-to-noise ratio was studied. For this purpose, acetate and phosphate buffers were tested at three different concentrations (5, 10 and 15 mM). In general the background noise was higher in the case of using phosphate buffer. The sensivity for all the drugs improved as the concentration of the acetate buffer was increased, but an increase of the background noise was observed at the highest concentration. The optimal electrolyte concentration was chosen as 10 mM acetate buffer.

The mixture acetonitrile–10 mM CH_3COOH/CH_3COONa (pH 5) (72:28, v/v) showed a good resolution in terms of k' (amlodipine 0.53, nitrendipine 0.73, felodipine 1.33, lacidipine 2.05, lercanidipine 2.61) and an analysis time lower than 12 min were achieved for the five 1,4-dihydropyridines studied when a potential of +1100 mV was used, Fig. 3.

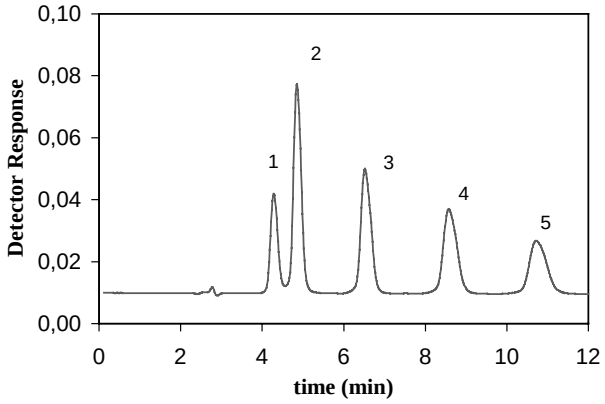

Fig. 3. Chromatogram obtained for 10 μg/ml standard solution of: (1) amlodipine; (2) nitrendipine; (3) felodipine; (4) lacidipine; and (5) lercanidipine. For chromatographic conditions see Section 2.3.

3.2. Analytical applications

3.2.1. Validation of the HPLC–ED method

Once optimal chromatographic conditions have been established, a quantitative method for the simultaneous determination of these compounds in their pharmaceutical formulations was carried out. The results of the validation of the method are shown in Table 1.

Table 1
Quantitative determination of amlodipine, nitrendipine, felodipine, lacidipine and lercanidipine at μg/ml levels[a] with HPLC–ED

1,4-DHP	Retention time (min ± S.D.)	Linear range (μg/ml)	Slope[b]	Intercept	r^2	Repeatability (R.S.D., %)		Detection limit (μg/ml)(S/N = 3)
						Intra-day	Inter-day	
Amlodipine	4.29 ± 0.01	4.5–15	7879 ± 41	442.6 ± 432	0.9999	1.5[c] 0.7[d] 1.4[e]	12.2[c] 12.6[d] 14.5[e]	0.09
Nitrendipine	4.87 ± 0.01	4.5–15	7607 ± 433	8401 ± 4522	0.9990	1.8[c] 2.4[d] 3.6[e]	13.7[c] 12.0[d] 13.1[e]	1.55
Felodipine	6.54 ± 0.01	4.5–15	1163 ± 503	5524 ± 5252	0.9994	1.6[c] 1.4[d] 3.1[e]	9.5[c] 6.9[d] 8.4[e]	0.82
Lacidipine	8.56 ± 0.01	4.5–15	6395 ± 341	1276 ± 3559	0.9992	2.0[c] 2.9[d] 3.9[e]	11.2[c] 13.0[d] 14.7[e]	0.62
Lercanidipine	10.14 ± 0.03	4.5–15	10544 ± 467	8727 ± 4872	0.9994	4.8[c] 4.3[d] 4.1[e]	12.4[c] 12.7[d] 14.2[e]	1.17

[a] For chromatographic conditions, see the Section 2.
[b] Area ($\mu g^{-1}\,ml^{-1}$).
[c] Ten determinations at the 5 μg/ml level.
[d] Ten determinations at the 10 μg/ml level.
[e] Ten determinations at the 15 μg/ml level.

Table 2
Determination of the studied compounds in their pharmaceutical formulations

Pharmaceutical formulation	Compound	Certified value (mg/tablet)	HPLC–ED		HPLC–UV	
			Experimental value[a] (mg/tablet)	Relative error (%)	Experimental value[a] (mg/tablet)	Relative error (%)
Astudal	Amlodipine	5.0	4.9 ± 0.2	−2.0	5.1 ± 0.2	+2.0
Baypresol	Nitrendipine	20.0	19.6 ± 0.5	−2.0	19.7 ± 0.8	−1.5
Plendil	Felodipine	5.0	4.9 ± 0.2	−2.0	5.1 ± 0.2	+2.0
Lacipil	Lacidipine	4.0	3.9 ± 0.2	−2.5	3.9 ± 0.2	−2.5
Lercadip	Lercanidipine	9.4	9.2 ± 0.2	−2.1	9.2 ± 0.2	−2.1

[a] Amount ± *ts*, where *t* is student parameter, *s* is the standard deviation and *n* is the number of replicates; $n = 5$ different samples and 3 replicates of each sample.

As can be seen the relative standard deviation of the retention time was <0.05%, thus indicating high stability of the system. Linearity range 4.5–15 μg/ml was chosen for calibration purposes as in the case of pharmaceutical formulations there is no need to work at lower values of concentration.

The within-day and day-to-day repeatabilities were <5 and <15%, respectively. For this kind of detection, detection limits ranged from 0.09 to 1.55 μg/ml, depending on the compound. Acceptable accuracy was obtained in the assay for each compound: 102 ± 1% amlodipine, 100 ± 1% nitrendipine, 99 ± 1% felodipine, 102 ± 2% lacidipine and 102 ± 1% lercanidipine.

3.2.2. Analysis of pharmaceutical formulations

The method developed was applied to the determination of the studied compounds in pharmaceutical formulations, obtaining values in accordance with those certified by the pharmaceutical companies, with relative errors lower than 5%. The results obtained by HPLC–ED are shown in Table 2.

4. Discussion

The method described for the determination of five 1,4-DHPs is simple, sensitive and rapid enough for its application to routine analysis of the active components in their pharmaceutical formulations. The validation parameters were adequate in terms of repeatability, linearity and accuracy (Table 1).

The chromatographic method with amperometric detection presents as advantage over other static voltammetric methods, the possibility of simultaneous determination of several 1,4-DHP drugs with similar oxidation potentials which would give rise to overlapping of the signals.

If we compare the data obtained in this work with those reported in an earlier HPLC–photometric detection work [26], it can be concluded that both kinds of detections allow the quantification of the unchanged 1,4-DHP drugs without interference from the excipients of the tablets. Good values for intra-day repeatability and accuracy are achieved using both methods. However, inter-day repeatability values were better using photometric detection, with lower values of R.S.D. This could be explained due to the great variability of signal produced in the electrochemical detector, probably because of adsorption effects of these compounds at the concentration levels assayed.

The low concentration levels of this drugs (ng/ml) found in plasma and serum only would allow the use of the HPLC with amperometric detection method developed for the determination of amlodipine (90 ng/ml).

Moreover, the known selectivity of the electrochemical detection would make possible the application of this method to the monitorization of this compound in plasma obtained from patients under cardiovascular treatment.

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