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Isocratic, simultaneous reversed-phase high-performance liquid chromatographic estimation of six drugs for combined hypertension therapy

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Abstract

We report an isocratic, HPLC procedure for assay of the orally administered hypertension drugs [atenolol, amlodipine, nifedipine, nitrendipine, nimodipine and felodipine given in retention order] of which atenolol, an aryloxypropanolamine β-blocker is administered with anyone of the other five dihydropyridine calcium channel blockers in combined hypertension therapy. The drugs were dissolved in methanol and 20 μl of a mix of the drugs was injected onto a reverse phase JASCO-metaphase ODS (250×4.0 mm) 5 μ column. Any one of the six drugs could be used as the internal standard. The drugs were resolved by elution with a pH 4.5 equivolume mobile phase of acetonitrile–0.01 *M* KH_2PO_4, with pH adjustments done with H_3PO_4 (flow-rate 1.5 ml min^{-1}). The column effluent was monitored at 250 nm. The detector response (peak height ratio) was linear in the dynamic range of 25–3200 ng ml^{-1} of these drugs, with the detection limits at ~15 ng ml^{-1}. Full statistical evaluation of the data including linear regression (least-square fit) analysis was performed. The suggested procedure has the advantage that all the five dihydropyridine derivatives can be quantified alone or in formulation with atenolol.

Keywords: Atenolol; Amlodipine; Nifedipine; Nitrendipine; Nimodipine; Felodipine

1. Introduction

The calcium channel blockers, or calcium antagonists, are used in the management of angina pectoris and hypertension and work through the inhibition of the cellular influx of calcium which is responsible for the maintenance of the plateau phase of the action potential. Most of these drugs are chemically related to a dihydropyridine structure. Of these, amlodipine, felodipine, nifedipine, nimodipine, and nitrendipine are the most commonly employed in therapy. Beta-blockers are competitive antagonists at β-adrenergic receptor sites and are used against cardio vascular disorders such as hypertension, angina pectoris, myocardial infarction and cardiac arrhythmias. Atenolol, an aryloxypropanolamine blocker, is one of the most used, showing longer half-life and minor of side effects than other members of its class of drugs. Combination therapy with a β-blocker and a calcium channel blocker becomes often necessary and such dosage forms have been formulated [1]. Literature survey reports several methods [2] for the individual assay of atenolol and the five calcium channel blockers. However no method has been reported for the simultaneous assay of this drug when associated with other calcium-blockers. The present paper, thus

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reports an isocratic, simultaneous, RP-HPLC method for the assay of atenolol, Amlodipine, Nifedipine, Nitrendipine, Nimodipine and Felodipine, thus condensing assay information of five reports into one single report.

2. Experimental

2.1. Materials and reagents

Atenolol (ATN), felodipine (FEL), nifedipine (NIF), nimodipine (NIM) and nitrendipine (NIT), all free bases, and amlodipine (AML) besylate were supplied by local pharmaceutical firms as standard certified samples. Methanol and acetonitrile of HPLC grade, potassium dihydrogen phosphate and phosphoric acid of analytical grade, were from E. Merck.

2.2. Solutions

Standard solutions were prepared by dissolving 500 mg of individual drugs in 500 ml of methanol and then diluting them in ten-fold stages to the appropriate concentration with methanol. The buffer was prepared by dissolving 1.361 g of potassium dihydrogen phosphate in distilled water and diluting to 1000 ml in a volumetric flask. After mixing equal volumes of acetonitrile and buffer solution, final adjustment to pH 4.5 was made with 1 *M* phosphoric acid.

2.3. Apparatus and chromatographic conditions

The apparatus used was a JASCO–HPLC-900 Series chromatograph equipped with a PU-980 intelligent pump, a Mod. 975 UV–Visible detector and a Mod. 7125 Rheodyne injector with a 20 μl external loop. The column was a JASCO-Metaphase ODS (250×4.0 mm, 5 μm) operating at room temperature. The elution was carried out under isocratic condition at a flow-rate of 1.5 ml min^{-1}. Mobile phase consisted of a solution of 0.01 *M* phosphate buffer–acetonitrile 1: 1 by volume, adjusting to pH 4.5. Detector was set at the wavelength of 250 nm. Responses were recorded and integrated using Borwin chromatographic software.

2.4. Sample preparation

Dosage forms formulated with atenolol (50 mg) as a common additive and NIM (20 mg) and AML (5 mg) were assayed. Twenty tablets of the dosage forms were crushed to powder and homogenized. Amounts of these were extracted in 50 ml of methanol and the solution filtered (on a 0.45 μm pore size filter). An appropriate amount of FEL was added as internal standard and the solution diluted to obtain final concentrations within the values of the calibration curves.

2.5. Calibration and calculation

For linearity studies eight different concentrations (25, 50, 100, 200, 400, 800, 1600 and 3200 ng ml^{-1}) of a mixture of each drug, were prepared and assayed ($n=5$/concentration). Felodipine was used as internal standard at a concentration of 800 ng ml^{-1}. Responses were measured as peak heights and peak height ratios of the drug/I.S. were plotted against concentration.

3. Results and discussion

Octyl and octadecyl columns were both tried for separation and resolution. It was found that the octadecylsilane offered more advantages over the decyl column. Individual drug solutions were injected into the column at the concentration of 100 μg ml^{-1} and both elution pattern and resolution parameters studied as a function of pH. In addition, the UV spectra of individual drugs were recorded in the wavelength range from 210 to 300 nm and compared. The pH effect showed that optimized conditions are reached when the pH value is 4.5, producing good and well shaped peaks for all drugs assayed. The choice to use a common wavelength set at 250 nm was considered satisfactory, permitting the detection of all drugs with adequate sensitivity.

In the case of ATN the LOD was only 600 ng ml^{-1} due to high noise produced by the mobile phase. In the case of AML, NIF and NIT distinct deviations were observed in the absorbance linearity

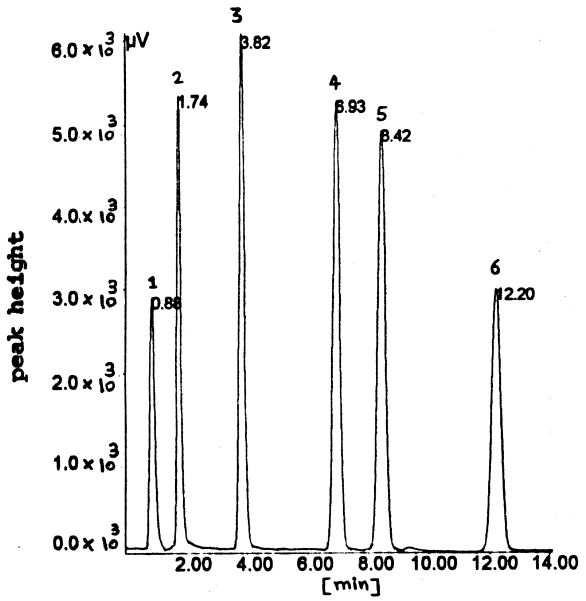

Fig. 1. A typical chromatogram of the six anti-hypertensive drugs analyzed. [1] Atenolol, [2] Amlodipine, [3] Nifedipine, [4] Nitrendipine, [5] Nimodipine, [6] Felodipine (I.S.).

Table 1
Chromatographic resolution parameters for the peaks recorded in analysis of the assayed drugs

Drug	t_R	k'	R	N	T
Atenolol	0.88	0.76	–	1780	1.02
Amlodipine	1.74	2.48	1.80	3780	1.13
Nifedipine	3.82	6.64	4.00	4935	1.01
Nitrendipine	6.93	12.86	4.80	6878	1.03
Nimodipine	8.42	15.84	3.60	7952	1.00
Felodipine	12.20	23.40	6.00	9967	1.02

t_R=Retention time; k'=capacity factor; R=resolution; N=number of theoretical plates; T=peak symmetry factor.

and hence the concentration ranges were divided into two. The data was analysed by the linear least squares fit method and the results are presented in Table 2. The calculations were done as per Gordus [3]. A typical chromatogram of the five drugs assayed and the internal standard, FEL, is depicted in Fig. 1. The relative chromatographic resolution parameters are reported Table 1. The good chromatographic separation indicated that anyone of the drugs can be used as internal standard for the assay of the other drugs. The results obtained in analysis of reference calibration samples are reported in Table 2.

Peak to noise ratio [N_{p-p}] was measured at 250 nm and the minimum detectable quantity (absolute amount injected), defined as $3\times N_{p-p}$, was found to be 500 pg for AML, FEL, NIF, NIM and NIT while for the least sensitive drug, ATN, it was of the order of 1600 pg. If higher injection volumes are used and with a better choice of wavelength this could be still brought down to two or three times, if only one or two of the drugs are to be assayed.

Accuracy of the method was evaluated as the percentage error of the mean assayed concentration relative to the spiked concentration at three different levels of each analyte studied. Inter-day and intra-day performance studies at each of these three concentrations showed that the C.V. never exceeded 6%.

Replicate analysis [$n=7$] of dosage forms yielded, on average, the values of 49.8±0.3 mg for ATN, 19.6±0.5 mg for NIM and 4.9±0.1 mg for AML. None of the excepients, like starch, lactose, hydroxy propyl methyl cellulose and others, was found to interfere in the analysis of the drugs.

Table 2
Linear regression (least squares fit) calibration data ($n=5$)

Drug	Conc. range (ng ml^{-1})	Slope $m\pm S_m$	Intercept $b\pm S_b$	r	S_{yx}
Atenolol	800–3200	0.659±0.021	−0.003±0.002	0.999	0.007
Amlodipine	25–200	1.475±0.239	0.004±0.027	0.998	0.010
	200–3200	0.930±0.07	0.112±0.064	0.999	0.024
Nifedipine	25–200	1.089±0.230	0.009±0.003	0.999	0.010
	200–3200	1.298±0.028	−0.049±0.020	0.999	0.010
Nitrendipine	25–200	1.009±0.400	0.011±0.053	0.999	0.010
	200–3200	1.124±0.073	−0.021±0.067	0.999	0.025
Nimodipine	25–3200	0.996±0.041	0.002±0.038	0.999	0.014

S_{yx}=point error.

4. Conclusions

The present method has demonstrated that at least five Ca-channel blockers can be simultaneously assayed with a β-blocker, ATN in bulk and dosage forms using RP-HPLC.

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