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Simultaneous determination of atenolol and amlodipine in tablets by high-performance thin-layer chromatography

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Abstract

A new simple, precise, rapid and selective high-performance thin-layer chromatographic (HPTLC) method has been developed for the simultaneous determination of atenolol (ATL) and amlodipine (AMLO) in tablets, using methylene chloride:methanol:ammonia solution (25% NH_3) (8.8:1.3:0.1; v/v) as the mobile phase and Merck HPTLC plates (0.2 mm thickness) precoated with 60F254 silica gel on aluminium sheet as the stationary phase. Detection was carried out densitometrically using a UV detector at 230 nm. The retention factors of ATL and AMLO were 0.33 and 0.75, respectively. Calibration curves were linear in the range 10–500 μg ml^{-1} for both. Assays of ATL and AMLO were 49.87 mg per tablet (relative standard deviation (R.S.D.), 1.3%) and 4.90 mg per tablet (R.S.D., 1.38%) for brand I, and 49.27 mg per tablet (R.S.D., 1.12%) and 4.98 mg per tablet (R.S.D., 1.42%) for brand II, respectively. The percentage recoveries for ATL and AMLO for brands I and II were 99.06 and 99.30%, and 99.27 and 99.15%, respectively.

Keywords: High-performance thin-layer chromatography; Atenolol; Amlodipine; Simultaneous determination

1. Introduction

Atenolol (ATL), chemically (*R*,*S*)-4-(2-hydroxy-3-isopropyl-aminopropoxy) phenylacetamide, is a beta-adrenoceptor antagonist. It is official in the Indian Pharmacopoeia [1] and the British Pharmacopoeia [2]. There are various methods such as UV spectrophotometry [3–5], spectrofluorimetry [6], high-performance liquid chromatography (HPLC) [7,8] and gas–liquid chromatography [9] for the determination of ATL in single-dosage formulations.

Amlodipine (AMLO), chemically, 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylic acid 3-ethyl, 5-methyl ester, is an anti-hypertensive and an antianginal agent in the form of the besylate salt, amlodipine besylate. It is not official in any Pharmacopoeia. Some HPLC methods [10–14] are reported in the literature for its determination in pharmaceutical preparations (tablets) and biological fluids.

There are many combination dosages containing these two drugs in the market from Cipla Ltd (India) and Lyka Laboratories Ltd (India). How-

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ever, there is no method for the simultaneous determination of these two drugs by high-performance thin-layer chromatography (HPTLC), although we have recently reported an HPLC method [15]. HPTLC is a more effective technique for the simultaneous determination in single samples in routine analysis. The aim of the present investigation is to develop an HPTLC method for the simultaneous determination of ATL and AMLO. We have developed a method using methylene chloride:methanol:ammonia (25%NH_3) (8.8:1.3:0.1; v/v) as mobile phase on silicagel 60F254 HPTLC plates (0.2 mm; Merck). Quantitative estimation was accomplished by densitometric scanning with UV detector at 230 nm wavelength. The method was confirmed by application on authentic dosage forms.

2. Experimental

2.1. Instrumentation

A Camag Linomat IV sample applicator, a Camag TLC Scanner II controlled by Cats 3.15 version software and a Camag twin trough chamber were used. Merck HPTLC plates coated with silicagel 60 F 254 (0.2 mm thickness) on aluminium sheets were used as the stationary phase.

2.2. Solvents and chemicals

Reference standards of ATL and AMLO were procured from Qualirex Chemicals Pvt. Ltd (Aurangabad, India) and TATA Pharma Ltd (Patalganga, India), respectively. These standards were checked for their purities by non-aqueous titration using crystal violet indicator and found to be 99.81 and 99.31% pure, respectively. Two brands of tablets, Amlopres (Cipla Ltd) and Stamlobeta (Lyka Ltd), were procured from the market. The methylene chloride, methanol and ammonia solution (25% NH_3) used was of AR grade supplied by S.D. Fine Chemicals Ltd (Thane, India).

2.3. Standard stock solutions

Standard stock solutions of 10 mg ml^{-1} ATL and 1.0 mg ml^{-1} AMLO were prepared by dissolving 250 mg standard ATL and 25 mg standard AMLO in 25 ml methanol.

2.4. Working standard solution

Four millilitres of each of the standard stock solutions was diluted to 100 ml with methanol to give a concentration of 0.4 mg ml^{-1} ATL and 0.040 mg ml^{-1} AMLO. This solution was used as the working standard for analysis of all samples.

2.5. Sample preparation

Twenty currently marketed pharmaceutical forms (Amlopres and Stamlo beta, both containing 50 mg ATL and 5 mg AMLO per tablet) were assayed. They were crushed to a fine powder and appropriate amounts of each one, corresponding to about 100 mg ATL and 10 mg AMLO, were weighed in a 50 ml volumetric flask. After addition of 40 ml methanol and sonication (30–45 min), the samples made up to volume with methanol and filtered through a Whatman paper (No.1). An aliquot (2 ml) of the filterate solution was taken in a 10 ml volumetric flask and diluted to the mark with methanol, and used for the analysis.

2.6. Mobile phase

Methylene chloride:methanol:ammonia solution (25%NH_3) (8.8:1.3:0.1; v/v) was mixed and centrifuged. Centrifugate was used as mobile phase.

2.7. Calibration procedure

Aliqotes of standard stock solution of ATL and AMLO were taken in eight different 10 ml standard volumetric flasks and diluted to the mark with the methanol, such that the final concentrations of ATL and AMLO were in the range 10–500 μg ml^{-1}. Ten microlitres of each of these solutions were spotted on HPTLC plates as 8 mm bands and saturated in a twin trough chamber. The plates were developed with the mobile phase up to 80 mm height. The plates were removed and then dried. Each band was scanned densitometrically at 230 nm, and the peak areas were recorded

to plot the peak areas versus concentrations in μg ml^{-1}.

2.8. Assay procedure

Ten microlitres of each of the working standard and the sample solution were spotted in duplicate on HPTLC plates as 8 mm bands and developed as per the calibration procedure, and the peak areas were recorded.

The amounts of ATL and AMLO were then computed using the formula:

$$A = \frac{R_{spl} \times C \times D \times W}{R_{std} \times w} \times \text{factor}$$

where A is the amount of ATL/AMLO per tablet (mg), C is the concentration of standard ATL/AMLO (mg ml^{-1}), D is the dilution factor, factor is the conversion factor for amlodipine besylate to AMLO base = 0.78 or the conversion factor for ATL = 1, R_{spl} is the area of ATL/AMLO in sample solution, R_{std} is the area of ATL /AMLO in standard solution, W is the average weight of tablets, and w is the weight of a tablet powder taken for analysis (mg).

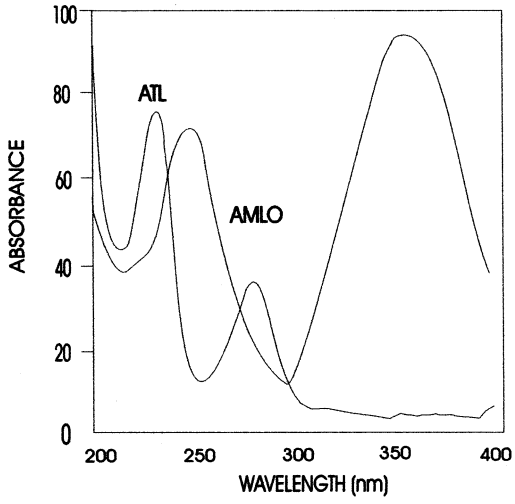

Fig. 1. Absorption spectra recorded for atenolol (continuous line) and amlodipine (dotted line).

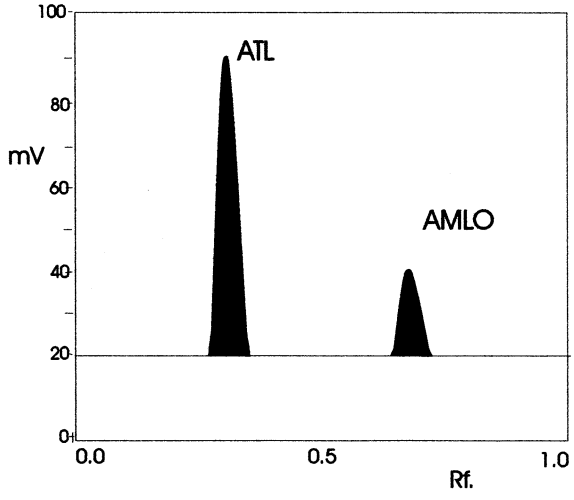

Fig. 2. Typical chromatograms obtained in analysis for atenolol (1) and amlodipine (2) by HPTLC.

3. Results and discussion

3.1. Chromatography

The UV spectrum of ATL and AMLO (concentration, 10 μg/ml each in methanol) is as shown in Fig. 1. A wavelength of 230 nm was chosen as a common wavelength to match the concentration ratio of the drugs present in the formulation. The mobile phase of methylene chloride:methanol: ammonia solution (25%NH_3) in the proportion 8.8:1.3:0.1 (v/v) was selected because it gave highest resolution, minimum tailing and R_f values of 0.33 and 0.75 for ATL and AMLO, respectively (Fig. 2).

3.2. System suitability

To ascertain the resolution and reproducibility of the chromatographic method, system suitability tests were carried out using working standard solutions of ATL and AMLO. This solution was spotted five times, parameters such as tailing factor, resolution factor, limit of detection (LOD) and limit of quantification (LOQ) were studied. Their average values, along with relative standard deviation (R.S.D.) values, are presented in Table 1. The R.S.D. of LOQ for ATL and AMLO was

Table 1
System suitability and detection/quantification limits in atenolol and amlodipine determination ($n = 5$)

Parameters	ATL	AMLO
Tailing factor	1.3	1.2
Resolution factor		2.18
R.S.D. (%)	1.05	1.51
LOD	1.0 μg ml^{-1}	2.0 μg ml^{-1}
R.S.D. (%)	18.0	10.9
LOQ	3.0 μg ml^{-1}	6.0 μg ml^{-1}
R.S.D. (%)	8.5	4.62

8.5 and 4.62%, respectively. These values are considered to be good enough for a reasonable accuracy in most of the laboratories worldwide.

3.3. Linearity

The plot of peak areas versus the respective concentration of ATL and AMLO were found to be linear in the concentration range 10–500 μg ml^{-1} (Table 3). They were represented by the linear regression equation

$$Y_{\mathrm{ATL}} = 4.54x + 63.46 \quad \Gamma = 0.9991$$

$$Y_{\mathrm{AMLO}} = 5.71x + 178.85 \quad \Gamma = 0.9994$$

The Γ value for ATL and AMLO were found to be close to 1. Apart from the Γ value, the intercept value was not more than 2% of the response obtained for 100% concentration in both the cases; hence, single point calibration was used.

3.4. Pharmaceutical preparation assay, and accuracy and precision evaluation

The amounts of ATL and AMLO were found by the number of replicates of both pharmaceutical preparations ($n = 5$) performed by the inter-day assays. The statistical parameter and results are reported in Table 2. These results were in close agreement to the label claim of both the

Table 2
Evaluation of atenolol and amlodipine amonts in pharmaceutical formulations ($n = 5$ per tablet)

Brand	Sr. number	Amount of ATL (mg per tablet) (label claim, 50 mg per tablet)	Amount of AMLO (mg per tablet) (label claim, 5 mg per tablet)
I (Amlopres-AT; Cipla Ltd)	1	49.97	4.94
	2	49.73	4.90
	3	49.82	4.82
	4	49.89	4.93
	5	49.92	4.89
	Mean assay	49.87	4.90
	Mean assay (%)	99.70	98.60
	R.S.D. (%)	0.19	0.90
II (Stamlo Beta; Lyka Labs)	1	48.98	5.02
	2	49.12	4.95
	3	49.15	4.89
	4	49.80	4.92
	5	49.30	5.10
	Mean assay	49.27	4.98
	Mean assay (%)	98.54	99.60
	R.S.D.(%)	0.65	1.71

Table 3
Calibration curve data of atenolol and amlodipine

Concentration	ATL (area)	AMLO (area)	Concentration	ATL (area)	AMLO (area)
10	108.7	239.9	10	109.4	238.5
50	2.81	468.2	50	295.2	465.8
100	506.4	750.5	100	524.6	748.8
200	975.1	1295.8	200	991.5	1314.1
400	1927.2	2474.1	400	1875.5	2468.5
500	2283.5	2942.2	500	2308.5	2950.1
Slope	5.526404	5.580364	Slope	4.490288	5.590045
Intercept	63.10515	189.907	Intercept	74.48955	190.3571
Coefficient of regression	0.999396	0.999652	Coefficient of regression	0.999922	0.999747
10	110.5	240.5	10	105.6	241.3
50	292.6	461.21	50	293.5	467.3
100	524.5	749.5	100	518.6	752.3
200	990.5	1314.6	200	995.5	1312.5
400	1888.5	2469.3	400	1896.2	2473.5
500	2308.6	2953.6	500	2310.5	2951.6
Slope	4.504005	5.597111	Slope	4.521424	5.591025
Intercept	73.35894	189.39	Intercept	70.48424	192.3014
Coefficient of regression	0.999897	0.999759	Coefficient of regression	0.999842	0.999726
10	106.8	240.8			
50	294.2	463.5			
100	518.9	751.6			
200	993.4	1315.5			
400	1888.6	2470.6			
500	2312.4	2948.5			
Slope	4.515076	5.587515			
Intercept	70.88409	191.7052			
Coefficient of regression	0.999891	0.999722			

pharmaceutical preparations, and the relative standard deviation observed for both the drugs were very low. To confirm the accuracy of the proposed method, recovery experiments were carried out by standard addition technique by adding a known amount of standard at four different levels to the pre-analysed sample. Each level was repeated three times ($n = 3$) and the amount of drug found by the assay method. Results and statistical parameters are reported in Table 4. From the amount of drug found, the percentage recovery was calculated by the following equation:

$$\%\text{Recovery} = \frac{Y}{X + X_1}$$

where Y is the amount of drug found by the proposed method, X the amount of pre-analysed sample, and X_1 the amount of standard drug added.

These results show that the method is precise and accurate.

4. Conclusion

This method was developed for the first time on HPTLC to estimate the two drugs in formulation, in order to analyse more samples at a time. The method is easy to perform, precise, and accurate. The whole procedure may be extended to pharmaceutical preparations and other applications on the same drugs for routine screening.

Table 4
Accuracy and precision evaluation in atenolol and amlodipine analysis of pharmaceutical formulations ($n = 3$)

Brand	Drug	Amount of drug added (mg)	Amount found (mg)	Recovery (%)
I (Amlopres-AT; Cipla Ltd)	ATL	0	49.85	99.70
		10	58.97	98.28
		20	69.82	99.74
		30	78.80	98.50
			Mean	99.06
			R.S.D. (%)	0.78
	AMLO	0	4.95	99.00
		10	14.90	99.33
		20	24.78	99.12
		30	34.90	99.71
			Mean	99.30
			R.S.D. (%)	0.31
II (Stamlo Beta; Lyka Labs)	ATL	0	49.89	99.78
		10	58.98	98.30
		20	69.90	99.86
		30	79.32	99.15
			Mean	99.27
			R.S.D. (%)	0.73
	AMLO	0	4.90	98.00
		10	14.89	99.27
		20	24.92	99.68
		30	34.87	99.63
			Mean	99.15
			R.S.D. (%)	0.79

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