HPTLC Method for the Simultaneous Determination of Amlodipine and Benazepril in Their Formulations

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Abstract

A new, simple, precise, rapid, and selective high-performance thin-layer chromatographic (HPTLC) method is developed for the simultaneous analysis of amlodipine and benazepril in pharmaceutical formulations. The method uses zolpidem as an internal standard (IS). The stationary phase used is silica gel 60 F_{254} prewashed with methanol. The mobile phase consists of an ethyl acetate–methanol–ammonia solution (8.5:2.0:1.0, v/v/v). Detection and quantitation are performed densitometrically at λ = 254 nm. The R_f values of amlodipine, benazepril, and zolpidem (IS) are 0.58, 0.50, and 0.78, respectively. The limits of detection of amlodipine and benazepril are 0.02 and 0.2 µg; linearity ranges are 0.1–0.8 and 0.2–2.0 µg; and the percentage recoveries are 99.79% and 100.25%, respectively.

Introduction

Amlodipine, 2-[(2-amino ethoxy) methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridine dicarboxylic acid-3-ethyl-5-methyl ester, and benazepril, (3s)-1-(carboxymethyl)-[(cis)-1- (ethoxy carbonyl)-3-phenyl propyl amino]-2,3,4,5-tetrahydro-1H-[1]benzazepin-2-one, are used as antihypertensive agents. They are, respectively, listed in the Martindale Pharmacopoeia (1,2). A combination of 10 mg of amlodipine and 10 mg of benazepril (3–5) is commercially available in tablet form. A literature survey reveals that only one high-performance liquid chromatography method (6) is available for its estimation in their dosage forms. The present work describes the application of high-performance thin-layer chromatographic (HPTLC) estimation of amlodipine and benazepril from its formulations. The method is simple, precise, rapid, and selective.

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Experimental

Solvents and chemicals

Reference standards of amlodipine (98.66%), benazepril (99.90%), and zolpidem [internal standard (IS)] were procured from Cadila Healthcare Ltd. (Ahmedabad, India), Novartis (Mumbai, India), and Unichem Ltd. (Mumbai, India), respectively. Tablet formulation was procured commercially. Chromatographic-grade ethyl acetate, methanol, and ammonia (AR) were obtained from Merck (Mumbai, India).

Standard solution

Amlodipine and benazepril (100 mg) were accurately weighed into a 100-mL volumetric flask, dissolved in methanol (50 mL), and the solution was diluted to volume with the same solvent to furnish a working standard.

Sample solution

Twenty tablets were weighed and finely powdered. The accurately weighed powder equivalent to 100 mg of amlodipine and benazepril was transferred to a 100-mL volumetric flask, dissolved in methanol (50 mL), and shaken on a mechanical shaker for 15 min. The solution was then diluted to volume with the same solvent, mixed, and finally filtered through Whatmann No. 42 filter paper. A sample (1 mL) of the filtrate was diluted to 10 mL with methanol in a volumetric flask; this solution was used for analysis.

Chromatography

Chromatography was performed on aluminium-backed silica gel 60 F_{254} HPTLC plates prewashed with methanol; the plates were developed with ethyl acetate–methanol–ammonia solution (10%) (8.5:2.0:1.0, v/v/v) in a Camag twin-trough chamber (Muttenz, Switzerland).

Standard solutions of amlodipine and benazepril were transferred to different 10-mL volumetric flasks and diluted to volume with the methanol so that the final concentrations of amlodipine

and benazepril were 0.1–0.8 and 0.2–2.0 µg/µL, respectively, containing 0.8 µg zolpidem (IS) each. Both standards and samples (5.0 µL amlodipine and benazepril containing 0.8 µg zolpidem) were applied to the plates as 6-mm bands by means of a Camag Linomat IV sample applicator (Camag).

After development and drying of the plates, evaluation of both drugs was performed by scanning densitometry at l = 254 nm by means of a Camag TLC Scanner III controlled by CATS.V.4.06 software (Camag). Peak areas were recorded for all the peaks. The amount of amlodipine and benazepril was computed from the peak area by use of the formula:

$$\text{Amount of Amlodipine and Benazepril} = (\text{Rspl} \times \text{C} \times \text{D} \times \text{Average wt})/(\text{Rstd} \times \text{W}) \quad \text{Eq. 1}$$

where, Rspl is the area of the amlodipine or benazepril sample peak, Rstd is the area of the amlodipine or benazepril standard peak, C is the concentration of standard solution (mg/mL), D is the dilution factor, and W is the weight of the tablet (mg).

Results and Discussion

Chromatography

The mobile phase resolved the two drugs very efficiently, as is shown in Figure 1. The R_f values of amlodipine, benazepril, and zolpidem (IS) were 0.58, 0.50, and 0.78, respectively. The wavelength maxima of amlodipine, benazepril, and zolpidem (IS) were 362, 240, and 298 nm, and a wavelength of 254 nm was selected for detection because this resulted in similar detection sensitivities for both drugs. The molar absorptivity of amlodipine and benazepril were 1.9954×10^4 and 3.8396×10^4, respectively.

Assay

The method was used to determine the amlodipine and benazepril content of two commercial brands of tablets; the results are shown in Table I. The low relative standard deviation (RSD) values are indicative of the high accuracy and precision of the method.

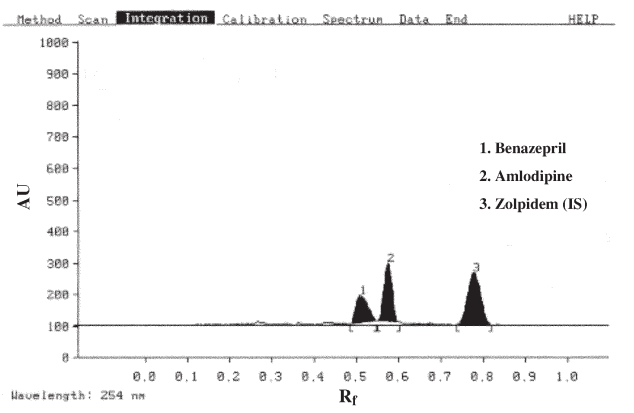

Figure 1. Typical chromatogram of amlodipine, benazepril, and zolpidem (IS).

System suitability

Linearity and limits of quantitation and detection

Calibration plots of peak area against concentration were linear in the range 0.1–0.8 µg for amlodipine and 0.2–2.0 µg for benazepril, respectively, and the intercept values were not significantly different from zero. The calibration lines were represented by the linear equations:

$$Y_{\text{AMLODIPINE}} = 2.46 + 123.45X \quad \text{Eq. 2}$$

$$Y_{\text{BENAZEPRIL}} = 1.22 + 356.19X \quad \text{Eq. 3}$$

For each equation the correlation coefficient (r) was 0.99.

The limits of quantitation (LOQ) and detection (LOD) were calculated on the basis of the equations:

$$\text{LOD} = 3 \times \text{N/B} \quad \text{Eq. 4}$$

$$\text{LOQ} = 10 \times \text{N/B} \quad \text{Eq. 5}$$

where N is the standard deviation of the peak areas of the drugs (n = 5), taken as a measure of the noise, and B is the slope of the

Table I. Results from HPTLC assay of Amlodipine and Benazepril

Brand	Component	Label claim (mg)	Amount found by proposed method (mg)	RSD (%, n = 5)
Brand I	Amlodipine	10	10.51	0.55
	Benazepril	10	10.12	0.24

Table II. Results from Recovery Analysis

Brand	Component	Amount added (mg)	Amount recovered (mg)
Brand I	Amlodipine	0	10.1
		1	10.9
		2	11.8
		3	13.1
Mean recovery = 99.79 (n = 5)			
Brand I	Benazepril	0	10.3
		1	11.1
		2	12.2
		3	13.5
Mean recovery = 100.25 (n = 5)			

Table III. Results from Ruggedness Studies

	Brand I amlodipine*	Brand I benazepril*
Analyst I	99.79	100.2
Analyst II	100.25	101.4

* All values are percentage recoveries.

Table IV. Results from Robustness Studies

Development distance (cm)	Amlodipine assay (%) Brand I	Benazepril assay (%) Brand II
7.0	101.1	99.5
7.5	103.3	100.2
8.0	105.2	101.3

corresponding calibration curve. The LOQs were 0.08 and 0.6 µg for amlodipine and benazepril, respectively; the respective LODs were 0.02 and 0.2 µg.

Accuracy and precision

The accuracy and precision of the method were studied by performing experiments by the standard addition technique. Three different levels of the standards were added to a previously analyzed sample, with each level being repeated three times. The amount (mg) of drug found by the method (*y* axis) was plotted against the amount of the standard drug added (*x* axis). The intercept on the *y* axis indicates the amount of the drug (mg) present per tablet. The percentage recovery was calculated from the amount of the drug found by use of the formula:

$$\%\text{Recovery} = [n\,(\Sigma XY) - (\Sigma X)\,(\Sigma Y)]/[n\,(\Sigma X^2) - (\Sigma X)^2] \times 100 \quad \text{Eq. 6}$$

where X is the amount of standard drug added, Y the amount of drug found by the proposed method, and n is the number of observations.

The recoveries of amlodipine and benazepril obtained were 99.79% and 100.25%, respectively, as is shown in Table II. This shows that there is no interference from the excipients in the tablets.

Ruggedness and robustness

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating conditions from instrument to instrument and from analyst to analyst. The results of ruggedness testing are reported in Table III. Robustness is a measure of the capacity of a method to remain unaffected by small but deliberate variations in method conditions, and is an indication of the reliability of the method. Typical results from robustness studies are shown in Table IV.

Conclusion

The HPTLC method proposed for the simultaneous determination of amlodipine and benazepril in solid dosage forms is accurate, precise, rapid, and selective. It can, therefore, be easily and conveniently adopted for routine quality control analysis.

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