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Simultaneous Determination of Amlodipine and Ramipril by High Performance Thin Layer Chromatography

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A simple, selective and validated high performance thin layer chromatographic method has been developed for the simultaneous estimation of amlodipine and ramipril in tablets. Aluminium plates precoated with silica gel G 60 F_{254} was used as stationary phase and toluene:ethanol:acetone: ammonia in the ratio of (7:2:1:0.3 v/v) was used as mobile phase. Quantification was carried out by the use of densitometric absorbance mode at 217 nm. The content of amlodipine and ramipril in tablet/capsule formulation was calculated and found to be 98.66/99.25, 100.17/99.36, 99.93/100.39 and 99.96/100.06 % by height and area for tablet and capsule, respectively. The proposed HPTLC method was quantitatively evaluated in terms of stability, precision, repeatability, accuracy and calibration correlation proving its utility in routine analysis of its tablet dosage form.

Key Words: HPTLC determination, Amlodipine, Ramipril.

INTRODUCTION

Amlodipine[1] (AM), chemically is 3-ethyl 5-methyl (4RS)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzenesulphonate. It is calcium channel blocker and used in treatment of hypertension and angina. Ramipril[2] (RAM), chemically is (2S,3aS26aS)-1-[(S)-2-[[(S)1-(ethoxycarbonyl)-3-phenylpropyl]-amino]propanoyl]octahydrocyclopenta [b]pyrrole-2-carboxylic acid. Literature survey reveals HPLC[3-5], spectrophotometric[6-8], colorimetric[9] methods for estimation of amlodipine alone or in combination with other drugs and similarly HPLC[10,11] and spectrophotometric[12-14] are reported for estimation of ramipril alone or in combination with other drugs. The present study described the development and validation of a simple, specific, accurate and precise HPTLC method for determination in pharmaceutical dosage forms.

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EXPERIMENTAL

Amlodipine working standard was a gift sample from Torrent Pharmaceuticals Ltd., Indrad and Ramipril from Astra-Zeneca Ltd., Bangalore. Silica Gel 60G F_{254} TLC plates (10 × 10 cm, Merck) were used as stationary phase. Tablets were purchased from local market. Ethanol, touene, acetone and ammonia of GR grade (E. Merck) purity were purchased from local supplier.

A CAMAG HPTLC system (Switzerland) comprising of CAMAG Linomat IV semiautomatic sample applicator, CAMAG TLC scanner 3, CAMAG twin trough chamber (10 × 10 cm), CAMAG CATS 4 software, Hamilton syringe (100 µL) were used during the study.

HPTLC method and chromatographic condition: The chromatographic estimation were performed using the following conditions, stationary phase, aluminium sheets precoated with silica gel 60 F_{254} (10 × 10 cm). Spotting parameters used were, 4 mm bandwidth, 4 mm space between two bands and a spryaing rate of 6 s/µL. Mobile phase used was toluene:ethanol:acetone:ammonia in the ratio of (7:2:1:0.3 v/v). The chamber saturation time employed was 10 min and the plates were developed using ascending technique to a distance of 7 cm. Scanning wavelength of 217 nm with a slit dimension of 3.0 × 0.45 mm.

Linearity of detector response: Aliquots of mix working standard of AT-RAM were spotted as sharp bands on the precoated TLC plate, using Camag linomat IV semiautomatic applicator under nitrogen stream. The plate was developed under chromatographic conditions described above. The plate was removed from the chamber and dried using in hot air dryer. Densitometric measurements were performed at 217 nm in absorbance mode. Data peak height and peak area of each band was recorded. The calibration curve was prepared by plotting peak height and peak *vs.* concentration corresponding to each spot.

Assay

Stock solution A: An accurately weighed quantity of amlodipine besylate (amlo-b *ca.* 25 mg) was transferred in to a 25 mL volumetric flask. It was dissolved and diluted up to the mark with methanol to give a standard stock solution of 1 mg/mL.

Stock solution B: An accurately weighed quantity of ramipril (RAM *ca.* 25 mg) was transferred in to a 25 mL volumetric flask. It was dissolved and diluted up to the mark with methanol to give a standard stock solution of 1 mg/mL.

Working solution A: From stock solution A 3.5 mL of amlo-b and 2.5 mL from stock solution B was pipetted out in 10 mL volumetric flask and diluted to 10 mL with methanol.

Working solution B: From stock solution **A** 7 mL of amlo-b and 2.5 mL from stock solution **B** was pipetted out in 10 mL volumetric flask and diluted to mark with methanol.

Preparation of sample solution

For market formulation (AR-1 and AR-2): 20 Tablets/capsules were accurately weighed and average weight was calculated. Accurately weighed quantity of tablet/capsule powder equivalent average weight of tablet 2.5 mg of ramipril was transferred in 10 mL volumetric flask. To it 6 mL of methanol was added and shaken for 10 min and volume was adjusted upto mark with methanol and then filtered.

On HPTLC plates, two spots of standard and six spots of the sample solution (AR-2) of equal volumes from each sample solution were applied. Similarly two spots of standard (working solution B) and six spots of the sample solution (AR-1) of equal volumes from each sample solution were applied. After development, the bands of the drugs were scanned at 217 nm. The peak height and area of standard and sample bands were compared to obtain the concentration. the amount of amlodipine and ramipril per tablet was calculated by applying suitable dilution factor and comparing peak height and peak area of the standard and sample solutions. Typical densitogram of ramipril and amlodipine from marketed formulation is shown in Fig. 1.

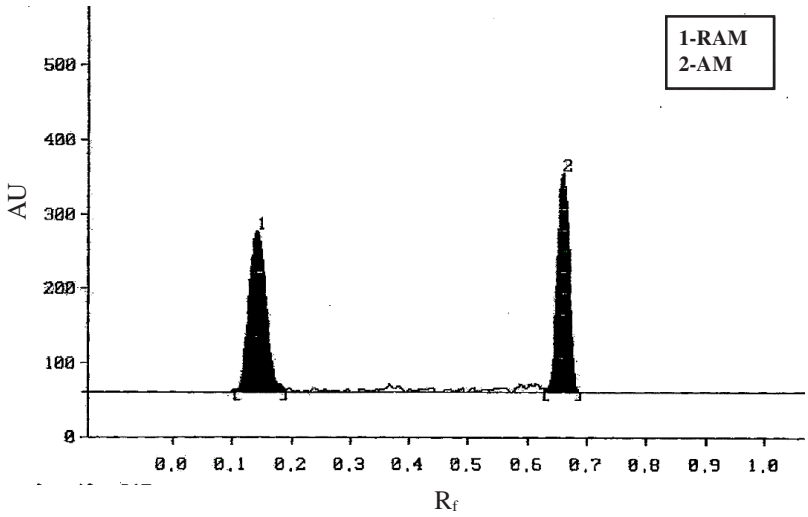

Fig. 1. Typical densitogram of ramipril (RAM) and amlodipine (AM) from marketed formulation

Method validtion

Accuracy: Accuracy of the mehtod was ascertained by performing recovery studies using standard addition method. To a fixed amount of preanalyzed pure drug were added at four different levels respectively. The total amount of drug was determined by above proposed method and the amount of pure drug recovered was calculated. The average pre cent recovery was found to be nearly 100 % (Table-1) following formula was used for calculating recovery of pure drugs:

$$\% \text{ Recovery} = \frac{T - A}{S} \times 100$$

where, T = total amount of drug estimated, A = Amount contributed by tablet powder (as per amount estimated by proposed method), S = amount of pure drug added.

Precision: Precision of analytical method is expressed as SD or RSD of series of measurement by replicate estimation of the drugs by proposed method (Table-1).

TABLE-1
SUMMARY OF RESULTS OF ESTIMATIONS

Marketed formulation	% of labeled claim*				Amount of pure drug added (mg)		Recovery* (%)			
	RAM		AM				RAM		AM	
	Peak height	Peak area	Peak height	Peak area	RAM	AM	Peak height	Peak area	Peak height	Peak area
Formulation	99.96	100.06	99.93	100.39	2.50	2.90	98.92	99.44	99.07	97.72
AR-1	±0.712	±0.888	±0.838	±0.911	5.10	5.20	100.10	99.57	100.44	98.46
Formulation	100.17	99.36	98.66	99.25	8.10	7.80	99.67	99.77	98.17	100.44
AR-2	±1.515	±1.442	±0.661	±0.600	10.10	10.80	98.14	101.81	100.23	99.58
					Mean per cent recovery		99.21 ±0.747	100.15 ±0.967	99.48 ±0.918	99.05 ±1.040

*Standard deviation of five observation.

Ruggedness: It was ascertained by analyst to analysts variation (Table-2).

Stability: The stability indicating ability of the proposed method was investigated by deliberately degrading the sample preparation. The stress conditions applied were acidic (1 M HCl), alkaline (1 M NaOH) and oxidizing condition (3 % H_2O_2) for 24 h at 50ºC. Also, heat (60ºC) and UV-exposure for 24 h was studied. The assay values for amlodipine and ramipril when calculated considering peak height and area in all five conditions was found to be different (Table-3).

TABLE-2
METHOD VALIDATION PARAMETERS

Parameters		Results (by height & by area)
Linearity range:	Amlodipine	1.4-7.6 µg
	Ramipril	1.0-5.4 µg
Correlation coefficient:	Amlodipine	0.9919/0.9941
	Ramipril	0.9936/0.9979
Inter-day RSD % (n = 3):	Ramipril	0.536/0.768
	Amlodipine	1.353/1.102
Intra-day RSD % (n = 3):	Ramipril	0.526/0.459
	Amlodipine	0.4323/0.279
Analyst to analyst RSD % (n = 3):	Ramipril	0.412/0.147
	Amlodipine	0.049/1.185
Specific		Specific

TABLE-3
RESULTS OF SPECIFICITY STUDIES

Sample (treated)	% of labeled claim*			
	Ramipril		Amlodipine	
	Height	Area	Height	Area
Acid	93.42	87.11	104.75	102.23
Alkali	Nearly 100	Nearly 100	28.24	24.00
Oxide	97.54	92.18	97.22	90.39
UV	114.07	120.12	92.72	100.76
Heat	111.73	116.22	95.12	102.69

*Each results of mean of six observations.

RESULTS AND DISCUSSION

Amlodipine and ramipril were completely extracted from tablet matrix with methanol. Combination of toluene, chloroform, acetone and ammonia offered optimum migration and resolution of amlodipine and ramipril from other components of formulation matrix (Fig. 1).

The amount of amlodipine and ramipril in tablet/capsule formulation was calculated on applying suitable dilution factor and comparing peak height and peak area of the standard and sample solutions. The content of amlodipine and ramipril in tablet/capsule formulation was calculated and found to be 98.66/99.25, 100.17/99.36, 99.93/100.39 and 99.96/100.06 % by height and area for tablet and capsule, respectively (Table-1).

The linearity of response for amlodipine and ramipril was found in the range of 1.4-7.6 and 1.0-5.4 µg. The per cent recovery was calculated

using suitable diluting factor. The average recovery value was found to be 99.48/99.05 and 99.21/100.15 % for amlodipine and ramipril, respectively (Table-1).

The intra-day and inter-day RSD was found to be in the range, respectively. Lower values of intra-day and inter-day variation in the analysis indicate that the method is precise. Different validation parameters for the proposed HPTLC method for determination of amlodipine and ramipril have been summarized in Table-2.

The results of the stability studies in different stress condition as per peak height and peak area, respectively were different indicating degradation has taken in all the stress condition which is seen from the different peak of the degraded drug/distortion in the peak height and area. The proposed HPTLC method was found to be simple, specific, precise and accurate. Thus it can be employed for routine analysis of amlodipine and ramipril from pharmaceutical dosage forms.

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