

DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING HPTLC METHOD FOR ANALYSIS OF AMLODIPINE BESILATE, LOSARTAN POTASSIUM AND HYDROCHLOROTHIAZIDE IN PHARMACEUTICAL DOSAGE FORM

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Received on : 12.09.2009

Revised : 19.03.10

Accepted : 20.03.10

ABSTRACT

The present work describes a stability-indicating HPTLC method for simultaneous analysis of Amlodipine besilate (AMLO), Losartan Potassium (LOS) and Hydrochlorothiazide (HCTZ) in bulk and pharmaceutical dosage form. Aluminium plate precoated with silica gel 60 F₂₅₄ was used as stationary phase. The separation was carried out using chloroform: ethyl acetate: methanol: ammonia (4: 4: 2:0.2 v/v/v/v) as mobile phase. The densitometric scanning was carried out at 232 nm. The linearity was obtained in the range 100–800 ng per band for amlodipine besilate (Correlation coefficient: 0.9954), 400-2400 ng per band for hydrochlorothiazide (Correlation coefficient: 0.9952) and 1000-8000 ng per band for losartan potassium (Correlation coefficient: 0.9947). The method was validated as per ICH guidelines. The combined dose tablet formulation was subjected to forced degradation by acid, alkali, oxidation, dry heat and exposure to ultraviolet radiations at 254 nm. The degradation products were well resolved from the pure drug with significantly different R_f values.

Keywords: *Amlodipine besilate (AMLO); Losartan Potassium (LOS); Hydrochlorothiazide (HCTZ); HPTLC; Validation; Stability Studies.*

INTRODUCTION

Amlodipine Besilate (AMLO), chemically it is 3, 5-Pyridinedicarboxylic acid, 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-, 3-ethyl 5-methyl ester, (±)-, monobenzenesulfonate¹, it is a long acting calcium channel blocker used as an antihypertensive agent. Losartan Potassium (LOS), chemically it is 2-Butyl-4-chloro-1-[p-(o-1H-tetrazol-5-ylphenyl)benzyl] imidazole-5-methanol, mono - potassium salt¹, it is angiotensin II receptor blockers used as antihypertensive agent. Hydrochlorothiazide (HCTZ), chemically it is 6-Chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide¹, used as diuretic. Literature survey revealed that a number of UV-Spectrophotometric, colorimetric, RP-HPLC, and HPTLC methods have been reported for estimation of AMLO, LOS and HCTZ individually or in combination with other drugs²⁻¹¹. Since no analytical method is reported for simultaneous estimation of these drugs in combined dose formulation, in the present work a successful attempt has been made to estimate these drugs simultaneously by high performance thin layer chromatography (HPTLC).

EXPERIMENTAL

Materials

Standard gift sample of Amlodipine Besilate was provided by Emcure Pharmaceuticals Ltd., India,

Losartan Potassium by Cipla Ltd, India, and Hydrochlorothiazide by Unichem Laboratories, India. Combined dose tablet formulation (TRILOPACE, containing 05 mg amlodipine besilate, 12.5 mg hydrochlorothiazide and 50 mg losartan potassium, manufactured by SUN Pharmaceutical Industries, Dadra, India), were procured from local market. All chemicals and reagents used were of HPLC grade.

Instrumentation and chromatographic conditions

The standard solutions containing AMLO, HCTZ and LOS were applied as band (Band size: 5 mm) under a continuous flow of nitrogen on aluminium plates precoated with silica gel 60 F₂₅₄ plates (E. Merck, Darmstadt, Germany; supplied by Merck India, Mumbai, India) with a 100 μ l syringe (Hamilton, Bonaduz, Switzerland) using automatic sample applicator Camag Linomat V (Muttentz, Switzerland). It was developed in a Camag twin trough glass chamber which was already saturated for 20 min. with the mobile phase. The mobile phase consisted of chloroform: ethyl acetate: methanol: ammonia (4: 4: 2:0.2 v/v/v/v). After chromatographic development, plate was air dried and observed under UV chamber. The resolved bands of drugs were scanned at 232 nm with Camag TLC scanner III densitometer controlled by WinCAT's software version 1.4.3.6336.

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Standard solutions and calibration graphs

Stock solutions were prepared individually by dissolving 20 mg of AMLO, 50 mg of HCTZ and 200 mg of LOS in 50 ml methanol. Stock solutions of each drug was appropriately diluted and were applied on a separate HPTLC plate to reach a final concentration range of 100–800 ng per band for AMLO, 400-2400 ng per band for HCTZ and 1000-8000 ng per band for LOS, respectively. The plates were developed in the above mentioned mobile phase and the resolved bands of drugs were scanned at 232 nm. The peak areas were plotted against the corresponding concentrations to obtain the calibration curves.

Analysis of marketed formulation

Twenty tablets were weighed and average weight was calculated. Tablets were crushed to obtain fine powder. Tablet powder equivalent to about 05 mg AMLO, 12.5 mg HCTZ and 50 mg LOS was transferred to 25 ml volumetric flask, 15 ml of methanol was added, sonicated for 10 min and the volume was made up to the mark with methanol. The content of the flask was mixed and filtered through Whatman filter paper No. 41. The filtrate was appropriately diluted to obtain final concentration of 40 ig/ml, 100 ig/ml and 400 ig/ml of amlodipine besilate, hydrochlorothiazide and losartan potassium, respectively. Eight bands of diluted sample solution and two bands of mixed standard solution, 10 µl each, were applied on HPTLC plate. The plate was chromatographed and scanned under the above mentioned chromatographic conditions. The peak area of the bands was measured at 232 nm and concentration of each drug in the tablet sample was calculated by comparing peak area of sample with peak area of respective standard.

Method validation¹²

The method was validated in compliance with ICH guidelines. Results of Validation studies are summarized in Table. 1

Table 1: Result of validation parameters

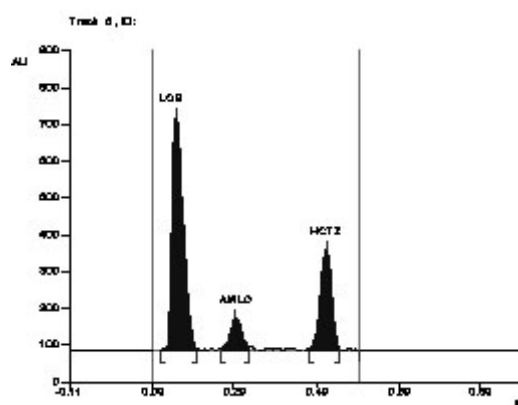
Parameters	LOS	HCTZ	AMLO
Linearity range (ng/spot)	1000-8000	400-2400	100-800
Accuracy*: % Recovery	100.46 %	100.00 %	99.07 %
(% R.S.D)	(0.8143)	(1.0710)	(0.5653)
Precision (% R.S.D)			
Intra-day (n = 3)	0.3471	0.3895	0.8544
Inter-day (n = 3)	0.3298	0.3429	0.4462
LOD (ng/spot)	40.26	5.2029	6.4222
LOQ (ng/spot)	121.99	15.766	19.461
Robustness	Robust	Robust	Robust

*mean of six determinations

RESULT AND DISCUSSION

Different solvents like chloroform, ethyl acetate, methanol, toluene acetone, tri-ethyl amine, ammonia in varying proportions were tried for mobile phase selection. Finally the mobile phase containing chloroform: ethyl acetate: methanol: ammonia (4: 4:

2:0.2 v/v/v/v) was found to effectively resolve the peaks for AMLO, HCTZ and LOS. The bands obtained were dense, compact and symmetrical in nature with the Rf value of 0.14 for LOS, 0.32 for AMLO and 0.52 for HCTZ (Fig 1). The analytical concentration range over which the drugs obey linearity range was found to be 100–800 ng per band for amlodipine besilate, 400-2400 ng per band for hydrochlorothiazide, 1000-8000 ng per band for losartan potassium with correlation coefficients (r^2) of 0.9954, 0.9952 and 0.9947, respectively. Percent label claim estimated by the proposed method for AMLO, HCTZ and LOS was found in the range of 98.53 % - 100.5 % and % RSD values less than 2 indicated that there was no interference from the excipients present in tablet formulation.

Fig. 1 HPTLC chromatogram of LOS, AMLO and HCTZ

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 80%, 100% and 120%. Known amounts of standard drugs were added to pre-analyzed samples and were subjected to the proposed HPTLC method. Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. Percentage relative standard deviation (% RSD) was found to be less than 2 % for within a day and day to day variations, which proves that method is precise. The standard deviation of peak areas was calculated for each set of conditions and % RSD was found to be less than 2 %. The low values of RSD obtained after introducing small deliberate changes in the chromatographic conditions indicate the robustness of the method. The LOD and LOQ values for AMLO, HCTZ and LOS were determined by the proposed method using calibration standards. LOD and LOQ values were calculated as $3.3 \sigma/S$ and $10 \sigma/S$, respectively. Where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation ($n = 5$).

The analytes were not stable in solution, and a decrease in drug concentration and an increase in the amount of degradation product was observed after 12 h. It was therefore inferred that analysis of AMLO, HCTZ and LOS should be performed within 12 h after

preparation of standard and sample. Forced degradation studies were performed by deliberate degradation of tablet sample under different stress conditions viz. 0.1 M HCl, 0.1 M NaOH, 3 % H₂O₂ and exposure of tablet sample to heat and UV radiations, for 24 hours. The selected mobile phase was able to resolve the peak of active components for the degraded product peaks indicating the selectivity of the proposed HPTLC method.

CONCLUSION

Based on the results obtained it can be concluded that the developed HPTLC method is accurate, precise, selective and less time consuming and can be employed for the simultaneous estimation of Losartan potassium, Amlodipine besilate and Hydrochlorothiazide in tablet formulation.

ACKNOWLEDGEMENT

The authors are very thankful to Dr. Avinash D. Deshpande, Director of Pharmacy, Pad. Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune for providing necessary facilities. The authors are also thankful to Emcure Pharmaceuticals Ltd., Unichem laboratories and Cipla Ltd for providing gift samples of drugs.

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