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A Simple and Sensitive HPTLC Method for the Determination of Content Uniformity of Atorvastatin Calcium Tablets

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A simple, sensitive HPTLC method was developed for the analysis of atorvastatin calcium in its commercial single component tablet formulations (10 mg/tablet). The stationary phase was precoated silica gel 60 F_{254}. The mobile phase used was a mixture of benzene: methanol, (7:3 v/v). Combination of benzene: methanol offered optimum migration (R_F=0.46±0.02). Detection of the spots was carried out at 281 nm. The method was validated in terms of linearity (200-600 ng/spots), precision (intra-day variation: 0.25 to 1.01%, inter-day variation: 0.21 to 0.88%), accuracy (99.2±0.48) and specificity. The limit of detection and limit of quantification for atorvastatin calcium were found to be 40 ng/spot and 200 ng/spot, respectively. The proposed method was successfully applied to determine atorvastatin calcium content of 10 individual tablet units of two market formulations, after extracting atorvastatin calcium with methanol. Both the formulation complied with the USP specifications (RSD less than or equal to 6 %) of the content uniformity test. The proposed HPTLC method can analyse ten or formulation units simultaneously on a single plate and provides a faster and cost-effective quality control tool for routine analysis of atorvastatin calcium tablet formulation.

Atorvastatin calcium chemically [R-(R*-R*)]-2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethy1)-3-pheny1-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoicacid, calcium salt (2:1) trihydrate, is a synthetic lipid-lowering agent. Atorvastatin is an inhibitor of 3-hydroxy-3-methylglutaryl Co-enzyme A (HMG-Co A) reductase. This enzyme catalyses the conversion of HMG-Co A to mevalonate, an early and rate limiting step in cholesterol biosynthesis[1-2]. Various methods like HPLC[3-5], GC-MS[6], LC-MS[7], and HPLC-Electrospray tendem mass spectrometry[8] have been reported for the estimation of atorvastatin calcium from its formulations.

Recently various formulations (10, 20, 40 mg tablets) of atorvastatin calcium have been introduced in the market. As it is evident that content uniformity requirements may be applied where the product to be tested contains 50 mg or less of active ingredients which comprises 50 % or less by the weight of dosage form unit[9], it is essential to determine content uniformity for atorvastatin calcium formulations. The present study describes development and validation of a simple, specific, sensitive, accurate and precise HPTLC method for the determination of atorvastatin calcium in tablets and its application estimating the content uniformity atorvastatin calcium tablets.

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MATERIALS AND METHODS

Atorvastatin calcium working standard was a gift sample from E. Merck (India) Ltd, Mumbai. Silica gel 60 F_{254} TLC plates (20×20 cm, layer thickness 0.2 mm, E. Merck, Germany) were used as stationary phase. Two single com-

ponent film-coated tablet formulations atorvastatin calcium (10 mg, formulation 1-Tonact 10 mg of atorvastatin tablets procured from Lupin Pharmaceuticals, Mumbai and formulation 2-Lipicor 10 mg of atorvastatin tablets procured from Intas Pharmaceuticals, Ahmedabad). All chemicals and reagents used were of analytical grade. A Camag HPTLC system (Switzerland) comprising of Camag Linomat IV semiautomatic sample applicator, Hamilton syringe (100 µl), Camag TLC Scanner 3, Camag CATS 4 software covering single level content uniformity option, Camag Twin-trough chamber (20×10 cm) and Remi centrifuge (Model C30) were used during the study.

Preparation of standard atorvastatin calcium solution and samples:

Atorvastatin calcium (10 mg) was weighed accurately and transferred to 10 ml volumetric flask. It was dissolved in 10 ml of methanol and adjusted to the mark with methanol. One millilitre of the solution was further diluted with methanol to obtain the final concentration 100 µg/ml of atorvastatin calcium. Ten film-coated tablets (each containing 10 mg atorvastatin calcium) were taken and tablet was finely powdered, powder of each tablet was dissolved in 5 ml of methanol. The solution was centrifuged for about 15 min at 600 rpm. The solution was then filtered through Whatman filter paper No. 41 and the residue was washed with methanol and the volume was adjusted to 10 ml with the same solvent. One millilitre of the solution was further diluted to 10 ml to have concentration of atorvastatin calcium equivalent to 100 µg/ml.

HPTLC method and chromatographic conditions:

TLC plates were pre-washed with methanol. Activation was done in oven at 50° for 5 min. The plate was allowed to cool at room temperature. The chromatographic estimations were performed using following conditions, Stationary phase, pre-coated silica gel 60 F_{254} aluminium sheets (20×10 cm); mobile phase, benzene: methanol (7:3 v/v); chamber saturation 30 min, wavelength of scanning, 281 nm; slit dimensions, 5×0.45 mm. Following spotting parameters were used- band width 6 mm, space between two bands 7 mm and spraying rate 10 s/µl.

Chromatographic separation:

Five microlitres of standard solution of atorvastatin calcium (100 µg/ml) was applied on the pre-washed and activated plate under nitrogen stream using semiautomatic spotter. It was developed at constant temperature in a Camag twin-trough chamber previously saturated for 30 min with 10 ml mixture of benzene: methanol (7:3 v/v) as the mobile phase. The plate was removed from the chamber and dried in air. Densitometric measurements were performed at 281 nm in reflectance mode with Camag TLC Scanner 3 using CATS 4 software incorporating the track optimisation option. For the preparation of a calibration curve, aliquots of 2, 3, 4, 5, 6 µl of standard solution of atorvastatin calcium (100 µg/ml) were applied on the TLC plates using semiautomatic spotter under nitrogen stream. The TLC plate was dried, developed and densitometrically analysed as described earlier.

Validation of method:

The method was validated in terms of linearity, accuracy, and specificity, intra-day and inter-day precision, repeatability of measurement of peak area as well as repeatability of sample application. The limit of detection and limit of quantification for atorvastatin calcium were also determined.

Determination of atorvastatin calcium in formulation:

Five microliters of sample solution for formulation 1 and 2 (100 µg/ml) were applied on TLC plates, developed and scanned as described earlier. Amount of atorvastatin calcium present in the sample solution was determined by using single level content uniformity test option in Camag CATS 4 software.

RESULTS AND DISCUSSION

Literature survey indicated that various methods have been reported for analysis of atorvastatin calcium. Most of them are HPLC, GC-MS and LC-MS which are sophisticated, costly and time consuming. Therefore it was thought to utilize HPTLC, a versatile, speedy and cost effective technique. Keeping physiochemical properties of atorvastatin calcium in mind, HPTLC methods was developed and determine atorvastatin calcium of the tablets.

Since atorvastatin calcium is freely soluble in methanol, tablet powder was extracted with methanol. Centrifuge for 15 min at 600 rpm helped to extract completely atorvastatin calcium from tablet matrix. Various solvent systems like mixtures of chloroform:methanol, chloroform:methanol : acetic acid, chloroform:methanol : formic acid were tried to separate and resolve spot of atorvastatin calcium from the spots of impurities and other excipients of formulations. The mixture of benzene:methanol (7:3 v/v) could resolve atorvastatin calcium spot with better peak shape. Combination of benzene and methanol offered

optimum migration (R_F=0.46±0.02) and resolution of atorvastatin calcium from other components of formulation matrix (fig.1). Even pre-saturation of TLC chamber with mobile phase for 30 min assured better reproducibility in migration of atorvastatin calcium and better resolution.

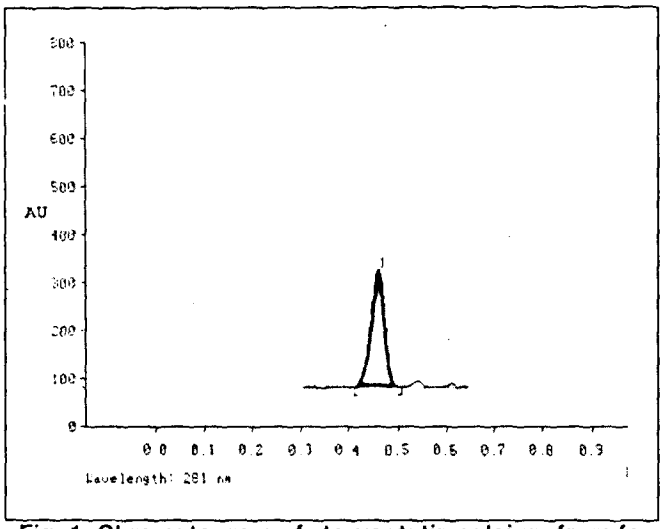

Fig. 1: Chromatogram of atorvastatin calcium from formulation.

Chromatogram showing resolution of atorvastatin calcium (peak 1) from components of formulation matrix.

The linearity of response for atorvastatin calcium was determined by analysing corresponding standards for each concentration in the range of 200 to 600 ng/spot in 6 replicates. It was observed that the responses for the various concentrations of standard atorvastatin calcium were linear in the range of 200 to 600 ng/spot, with a correlation coefficient of 0.999. The limit of detection and limit of quantification for atorvastatin calcium were found to be 40 ng/spot and 200 ng/spot, respectively.

Accuracy of analysis, in terms of systemic error involved, was determined by calculating recovery of atorvastatin calcium standard addition method at 3 levels of the calibration curve (n=3). The results indicated that the recovery of added atorvastatin calcium 99.2±0.48 ensuring that the method is accurate.

The intra-day precision was determined by analysing standard atorvastatin calcium solutions in the concentration range of 200-600 ng/spot for three times on the same day while inter-day precision was determined by analysing corresponding standards daily for 3 days over a period of one week. The intra-day and inter-day coefficients of variation were found to be in the range of 0.25 to 1.01% and 0.21 to 0.88%, respectively. The smaller values of intra-day and inter-day variation in the analysis indicate that the method is precise.

Repeatability of measurement of peak area was determined by spotting 6 µl of atorvastatin calcium standard solution on TLC plate and developing the plate. The separated spot of atorvastatin calcium was scanned seven times without changing position of the plate and relative standard deviation (RSD or CV) for measurement of peak area was calculated and was found to be 0.044%.

Repeatability of sample application was assessed spotting 6 µl of atorvastatin calcium standard solution seven times on a TLC plate by semiautomatic spotter, followed by development of plate and recording the peak areas for five spots. The RSD for the peak area values was calculated and was found to be 0.31%. Both the RSD values, for measurement of peak area and sample application, were below the instrumental specifications (1% and 3%, respectively), ensuring proper functioning of HPTLC system.

To confirm the specificity of the proposed method atorvastatin calcium tablet solution was spotted on the TLC plate, developed and scanned as described earlier. It was observed that excipients present in formulation did not interfere with peak of atorvastatin calcium (R_f= 0.46±0.02). The spectrum of atorvastatin calcium extracted from tablet was also compared with spectrum of standard atorvastatin calcium, which showed good c orrelation. Different validation parameters for the proposed HPTLC method for determining atorvastatin calcium content are summarized in (Table 1). The proposed HPTLC method was found to be rapid, simple, specific, sensitive, precise and accurate.

This method was applied to determine the content of atorvastatin calcium in two different market samples of single component atorvastatin calcium. The atorvastatin calcium content of individual tablet unit (10 replicates) for both the formulations was calculated by comparing the area of atorvastatin calcium from formulation unit with that of standard atorvastatin calcium. The corresponding data along with RSD in the content for each formulation are given in (Table 2). The diagrammatic representations generated using Camag CATS 4 software single level content uniformity option for 10 units of the tablet formulations.

According to USP if the average of the limits specified in the potency definitions in the individual monograph is

TABLE 1: SUMMARY OF VALIDATION PARAMETERS

PARAMETERS	RESULTS
Linearity range	200-600 ng/spot
Correlation coefficient	0.999
Limit of detection	40 ng/spot
Limit of quantification	200 ng/spot
Accuracy	99.2±0.48
Precision (% CV)	
Repeatability of sample application (n=7)	0.31%
Repeatability of measurement of peak area (n=7)	0.044%
Inter-day (n=3)	0.21-0.88 %
Intra-day (n=3)	0.25-1.01 %
Specificity	Specific

Different validation parameters of the proposed HPTLC method for determination of atorvastatin calcium in formulation.

according to USP, both formulation 1 and 2 comply with the content uniformity test of USP.

Since, to perform content uniformity test employing other methods, like HPLC or UV/Visible spectrometry, one has to analyse the specified number of dosage form units serially, which proves to be time consuming. On the other hand, using proposed HPTLC method one can analyse 10 or more dosage form units on a single plate simultaneously. Thus, proposed method proves to be very fast and cost-effective and can be employed in pharmaceutical industry for determination of content uniformity of single component film-coated atorvastatin calcium tablet dosage forms on routine basis.

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TABLE 2: ANALYSIS OF ATORVASTATIN CALCIUM TABLET DOSAGE FORMS FOR CONTENT UNIFORMITY

Label claim (mg)	Formulation 1		Formulation 2	
	Amount found (mg)	% Recovery	Amount found (mg)	% recovery
10	09.95	99.6	08.59	85.9
10	08.80	88.0	08.70	87.1
10	09.68	96.8	09.77	97.7
10	10.50	105.0	10.47	104.7
10	09.51	95.2	09.36	93.7
10	09.96	99.6	09.97	99.7
10	10.54	105.4	10.54	105.4
10	10.20	102.1	10.22	102.2
10	10.28	102.8	10.37	103.8
10	10.50	105.0	10.57	105.7
% RSD		5.50%		5.92

Amount of atorvastatin calcium according to label claim=10 mg and USP specifications for content uniformity: Range for drug content 85 to 115%; % CV for drug content = 6%. Formulation 1-Tonact (10 mg), Lupin Pharmaceuticals, Mumbai and formulation 2-Lipicor (10 mg) Intas Pharmaceuticals, Ahmedabad.

100% or les unless otherwise specified in the individual monograph, the requirement for dose uniformity are met if the amount of an active ingredient in each of 10 dosage units as determined from weight variation or content uniformity method is within the range of 85 to 115% of the label claim and RSD is less than or equal to 6%. Thus, since the content of individual tablet unit and the RSD between the content of 10 tablet units fall within the permissible limits

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