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NOTE

Estimation of Acipimox in Bulk Drug and Capsules by RP-HPLC Method

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A reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the estimation of acipimox in bulk drug and pharmaceutical dosage forms. The quantification was carried out on Luna C_{18} column in isocratic mode, with mobile phase consisting of 0.1 % v/v phosphoric acid in water and acetonitrile in the ratio of 95:5 [v/v]. The mobile phase was pumped at a rate of 1.0 mL/min and the detection was carried out at 229 nm and the linearity was found to be in the range of 20-300 µg/mL. The regression equation was found to be Y = 33672x + 6367.2 with correlation coefficient [r^2] of 0.9998. The percentage recovery values were found to be in the range of 99.96-100.07 %. Validation of the proposed method has also been done.

Key Words: Acipimox, RP-HPLC.

Acipimox, chemically 5-methylpyrazine carboxylic acid 4-oxide, is a nicotinic acid analogue which is an antilipolytic drug used in the management of different forms of hyperlipidemia[1,2]. Literature survey reveals that the drug can be estimated by HPLC[3], UV estimation in formulation[4]. The aim of this study is to develop a rapid, economical, precise and accurate RP-HPLC method for the determination of acipimox in capsules.

HPLC experiments were performed on a Shimadzu HPLC system equipped with Phenomenex Luna C_{18}, 5 µm (4.6 mm × 250 mm) column, two LC-20AD pumps, SCL-10AVP system controller, SIL-20A auto injector, SPD-20A UV-visible detector and LC solution software was used. The mobile phase consisted of 0.1 % v/v phosphoric acid in water and acetonitrile in the ratio of 95:5 (v/v) that was set at a flow rate of 1 mL/min.

Commercially available 250 mg capsules of acipimox were used. HPLC grade acetonitrile and phosphoric acid were obtained from Merck Germany. HPLC grade deionized water (Nanopure Diamond, Barnstead Thermolyne, USA) was used. Fresh working solutions were prepared daily. All solutions were filtered through 0.45 µm filter and degassed using a sonicator.

Chromatographic conditions: The mobile phase used was of 0.1 % v/v phosphoric acid in water and acetonitrile in the ratio of 95:5 (v/v). The analysis was carried out under isocratic conditions using a flow rate 1.0 mL/min at room temperature. Chromatograms were recorded at 229 nm using a SPD-20A UV-visible detector. The samples were introduced by injector with a 10 µL sample loop. A typical chromatogram is shown in Fig. 1.

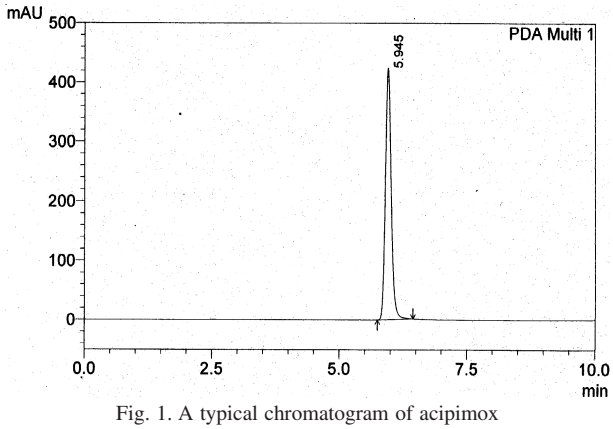

Fig. 1. A typical chromatogram of acipimox

Working standard of drug solution: About 100 mg of acipimox was weighed accurately and dissolved in 100 mL of mobile phase in a 100 mL volumetric flask and diluted up to the mark with the same to get the concentration of 1 mg/mL.

Linearity: Aliquots of standard acipimox stock solution were taken in different 100 mL volumetric flasks and diluted up to the mark with the mobile phase such that the final concentrations of acipimox are in the range of 20-300 µg/mL. Each of these drug solutions (10 µL) was injected into the column and the peak areas and retention times were recorded. A

TABLE-2
ASSAY RESULTS AND PRECISION STUDIES

Formulation	Labeled amount (mg/capsules)	Amount found (mg/ capsules)	Label claim* ± SD (%)	Precision**		
				Repeatability	Inter-day	Intra-day
Acipimox capsules	250	249.99	99.99 ± 0.0004	0.0002	0.0004	0.0002

*Average of six determinations. **RSD % of five determinations.

TABLE-3
RECOVERY STUDY

Drug	Label claim (mg/ capsules)	Spike level (%)	Amount of drug added (µg/mL)	Amount of drug recovered (µg/mL)	Percentage recovery ± SD*
Acipimox capsules	250	75	75	74.97	99.96 ± 0.0613
		100	100	100.07	100.07 ± 0.0374
		125	125	125.02	100.01 ± 0.0226

*Mean of six determinations.

calibration graph was obtained by plotting peak area *versus* concentration of acipimox.

Application of the method to capsules: Commercially available capsules of acipimox were taken for the estimation of total drug content per capsule by proposed method. Twenty capsules containing acipimox were weighed accurately and emptied. An accurately weighed quantity of powder equivalent to 50 mg of acipimox was transferred into 50 mL volumetric flask and dissolved in 25 mL of mobile phase and sonicated for 5 min for complete extraction of the drug and the solution was diluted to volume with mobile phase. The solution was centrifuged at 4000 rpm for 10 min and the clear supernatant was collected. From this solution 5 mL was taken and diluted to 50 mL with mobile phase, to furnish a 100 µg/mL solution, of which 10 µL was injected for HPLC analysis.

Selection and optimization of analytical method: In order to obtain the best chromatographic conditions, the wavelength for detection, the column and the mobile phase composition must be adequately selected. Column chemistry, solvent type, solvent strength, detection wavelength and flow rate were varied to determine the chromatographic conditions giving the best separation. The mobile phase conditions were optimized so there was no interference with the acipimox peak from solvent or excipient peaks. An absorbance maximum for drug was 229 nm. Solutions of the drug in the mobile phase were injected directly for HPLC analysis and the responses (peak area) were recorded at 229 nm.

Validation of the method

System suitability: The system suitability was assessed by six replicate analyses of the drug at a concentration of 100 µg/mL. The acceptance criterion was ± 2 % for the per cent coefficient of variation (CV %) for the peak area and retention time. The percentage coefficient of variation of peak area and retention time for drug is within 2 % indicating the suitability of the system (Table-1).

TABLE-1
SYSTEM SUITABILITY PARAMETERS

Parameter	Result
Theoretical plates (N)	78279
Tailing factor	1.117
Limit of detection (µg/mL)	6.12
Limit of quantification (µg/mL)	18.57

Linearity: The plot of peak areas of each sample against respective concentration of acipimox was found to be linear in the range of 20-300 µg/mL with correlation coefficient of 0.9998. The regression of acipimox concentration over its peak area was found to be Y = 33672x + 6367.2, where Y is the mean peak area and X is the concentration of acipimox.

Precision: The precision of the method was demonstrated by interday and intraday variation studies. In the intraday studies, solutions of sample were repeated five times in a day and percentage relative standard deviation (RSD %) for response factor was calculated. In the interday variation studies, injections of sample solutions were made on 5 consecutive days and RSD % was calculated. The results of precision studies are expressed in Tables-2. From the data obtained, the developed RP-HPLC method was found to be precise.

Accuracy: Drug assay was performed in triplicate after spiking raw material in volumetric flasks with amounts of acipimox equivalent to 75,100 and 125 % of the standard concentration of acipimox (100 µg/mL) as in the analytical method. Recovery was with in the range of 99.96-100.07 % which indicates that the method was accurate (Table-3).

Limit of detection (LOD) and limit of quantitation (LOQ): Limit of detection and limit of quantification were calculated using standard deviation of the response and slope of calibration curve. The LOD for acipimox was found to be 6.12 µg/mL. The LOQ was 18.57 µg/mL.

Application of the method to dosage forms: The method was used for determination of acipimox in a capsules formulation. The results obtained (Table-2) showed the amount found was that expected and RSD % values were low, which confirms the method is suitable for routine analysis of the compound in pharmaceutical preparations.

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