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Simultaneous Estimation of Levocetirizine, Ambroxol, Phenylpropanolamine and Paracetamol in Combined Dosage Forms by RP-HPLC Method

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A simple, selective, rapid, precise and economical reverse phase-high performance liquid chromatography (RP-HPLC) method has been developed for the simultaneous estimation of levocetirizine, ambroxol, phenylpropanolamine and paracetamol from tablets. The method was carried out on a Hichrom C_{18} (25 cm × 4.6 mm i.d., 5 μ) column with a mobile phase consisting of acetonitrile : 0.5% triethylamine (adjusted to pH 3.5 using orthophosphoric acid) (30 : 70 v/v) at a flow rate of 1.2 mL/min. Detection was carried out at 238 nm. Tadalafil was used as an internal standard. The retention times of phenylpropanolamine, paracetamol, ambroxol, levocetirizine and tadalafil were 2.74, 3.34, 5.09, 6.86 and 8.73 min, respectively. The validation of the proposed method was also carried out. The proposed method can be used for the estimation of these drugs in combined dosage forms.

Key Words: RP-HPLC, Levocetirizine, Ambroxol, Phenylpropanolamine, Paracetamol.

INTRODUCTION

Levocetirizine is chemically (RS)-2-[2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]acetic acid, dihydrochloride[1]; it is used as antihistamine (H_1-receptor antagonist). Ambroxol is chemically *trans*-4-[[(2amino-3,5-dibromobenzyl)methyl]amino] cyclohexanol hydrochloride[2], it is used as expectorant. Phenylpropanolamine is chemically (1RS,2SR)-2-amino-1-phenyl-1-propanol hydrochloride[2], it is used as sympathomimetic. Paracetamol is chemically *N*-(4-hydroxyphenyl)acetamide[2] and it is used as analgesic and antipyretic. Many methods have been described in literature for the determination of levocetirizine (LC), ambroxol (AX), phenylpropanolamine (PP) and paracetamol (PT) individually and in combination with other drugs[3–14]. However, there is no HPLC method reported for the simultaneous estimation of these drugs in combined dosage forms. Fixed dose combination of 5 mg of LC, 60 mg of AX, 25 mg of PP and 500 mg of PT is available in tablet form in the market. The

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present work describes a simple, precise and accurate reversed phase HPLC method for the simultaneous estimation of LC, AX, PP and PT in combined dosage forms.

EXPERIMENTAL

Acetonitrile HPLC grade was procured from E. Merck (India) Ltd., Mumbai. triethylamine AR grade and orthophosphoric acid AR grade was procured from Qualigens Fine Chemicals, Mumbai. Water HPLC grade was obtained from a Milli-QRO water purification system. Reference standards of levocetirizine and phenylpropanolamine were procured from Intra Labs, Bangalore; Tadalafil was procured from Glenmark Pharmaceuticals Ltd., Ankleshwar and paracetamol was procured from Intermed Pharmaceuticals, Chennai. Ambroxol was procured from Cadilla Pharmaceuticals Ltd., Ahmedabad.

Chromatographic conditions

A Shimadzu® HPLC (LC-10AT *VP*) system was used for the analysis. The method was carried out on Hichrom C_{18} (25 cm × 4.6 mm i.d., 5 μ) column as a stationary phase and acetonitrile : 0.5% triethylamine (adjusted to pH 3.5 using orthophosphoric acid) (30 : 70 v/v) as the mobile phase at a flow rate of 1.2 mL/min. Rheodyne 7725i injector with 20 μL loop was used for the injection of samples. Detection was done at 238 nm. The mobile phase was filtered through 0.2 μ membrane filter and degassed.

Preparation of solutions

Standard stock solutions of 1 mg/mL of LC, AX, PP and PT were prepared separately using a solvent mixture of water and acetonitrile (1 : 1) (v/v). From this standard stock solution, the mixed standard solution was prepared to contain 0.5 μg/mL of LC, 6 μg/mL of AX, 2.5 μg/mL of PP, 50 μg/mL of PT and 60 μg/mL of TD as internal standard.

Twenty tablets, each tablet containing 5 mg of LC, 60 mg of AX, 25 mg of PP and 500 mg of PT were finely powdered and a quantity of powder equivalent to 0.5 mg of LC, 6 mg of AX, 2.5 mg of PP and 50 mg of PT were weighed and transferred to a sintered glass crucible. To this 6 mL of 1 mg/mL of TD was added and the drugs were extracted with three quantities, each of 20 mL of mixture of acetonitrile and water (1 : 1) (v/v). The combined extracts were made up to 100 mL with mobile phase and further dilutions were made to get a concentration of 0.5 μg/mL of LC, 6 μg/mL of AX, 2.5 μg/mL of PP and 50 μg/mL of PT (theoretical value) and 60 μg/mL of TD as internal standard and this solution was used for the estimation.

Assay method

With the optimized chromatographic conditions, a steady baseline was recorded, the mixed standard solution was injected and the chromatogram was

recorded. The retention times of PP, PT, AX, LC and TD were 2.74, 3.34, 5.09, 6.86 and 8.73 min, respectively. This procedure was repeated for the sample solution obtained from the formulation. The response factor (peak area ratio of standard peak area and internal standard peak area) of the standard solution and sample solution were calculated (Table-2). The concentrations of the drugs were calculated (Table-1) using the following formula

$$\text{Concentration of drugs} = \frac{\text{Response factor of the sample}}{\text{Response factor of the standard}} \times \text{Concentration of standard}$$

Method validation

Accuracy of the method was studied by recovery experiments. To the powdered tablet formulation (0.5 mg of LC, 6 mg of AX, 2.5 mg of PP and 50 mg of PT), 6 mL of 1 mg/mL of TD solution and reference standard drugs were added at the levels of 25, 50 and 100% of the label claim. The extraction of drugs were followed using sample preparation procedure and these were analyzed. The percentage recovery was calculated and presented in Table-1. Precision of the method was demonstrated by repeatability studies. This was done by injecting consecutively the standard solution for 10 times and passing them through the assay procedure.

TABLE-1
RESULTS OF ANALYSIS OF FORMULATION AND RECOVERY STUDIES

Drug	Amount (mg/tab)		Label claim* (%)	Recovery* (%)
	Label claim	Found ± SD*		
Levocetirizine	5	4.97 ± 0.016	99.46	99.58
Ambroxol	60	60.04 ± 0.087	100.07	99.99
Phenylpropanolamine	25	24.98 ± 0.010	99.94	99.87
Paracetamol	500	500.01 ± 0.044	100.00	100.00

Average of 6 determinations.
Formulation I, LCZ Plus (Rapross Pharmaceuticals) each tablet containing 5 mg of LC, 60 mg of AX, 25 mg of PP and 500 mg of PT.

Linearity and range of the method was determined by analyzing mixed standard containing 0.15–1.0 μg/mL of LC, 1.5–12.0 μg/mL of AX, 0.75–5.0 μg/mL of PP and 15–120 μg/mL of PT (50–150% of targeted level of the assay concentration) containing 60 μg/mL of TD as internal standard, respectively. The calibration curve was plotted using response factor *vs.* concentration of standard solution; the values are presented in Table-2. The limit of detection (LOD) and limit of quantification (LOQ) of the method was determined by injecting

progressively low concentration of the standard solutions with the optimized chromatographic conditions.

TABLE-2
LINEARITY AND RANGE

Internal standard peak area (60 µg/mL Tadalafil)	Levocetirizine			Ambroxol			Phenylpropanol-amine			Paracetamol		
	Concentration (µg/mL)	Peak area	Response factor	Concentration (µg/mL)	Peak area	Response factor	Concentration (µg/mL)	Peak area	Response factor	Concentration (µg/mL)	Peak area	Response factor
	0.15	12364	0.0064	1.5	99378	0.051	0.75	57398	0.029	15	489471	0.253
	0.25	23547	0.0120	3.0	198752	0.103	1.5	114792	0.059	30	965919	0.500
1931837	0.50	47093	0.0240	6.0	397512	0.206	2.0	153058	0.079	60	1955019	1.012
	0.75	70640	0.0370	9.0	596258	0.309	3.0	267895	0.139	80	2932529	1.518
	1.00	94660	0.0490	12.0	795012	0.412	5.0	420904	0.218	120	3910038	2.024

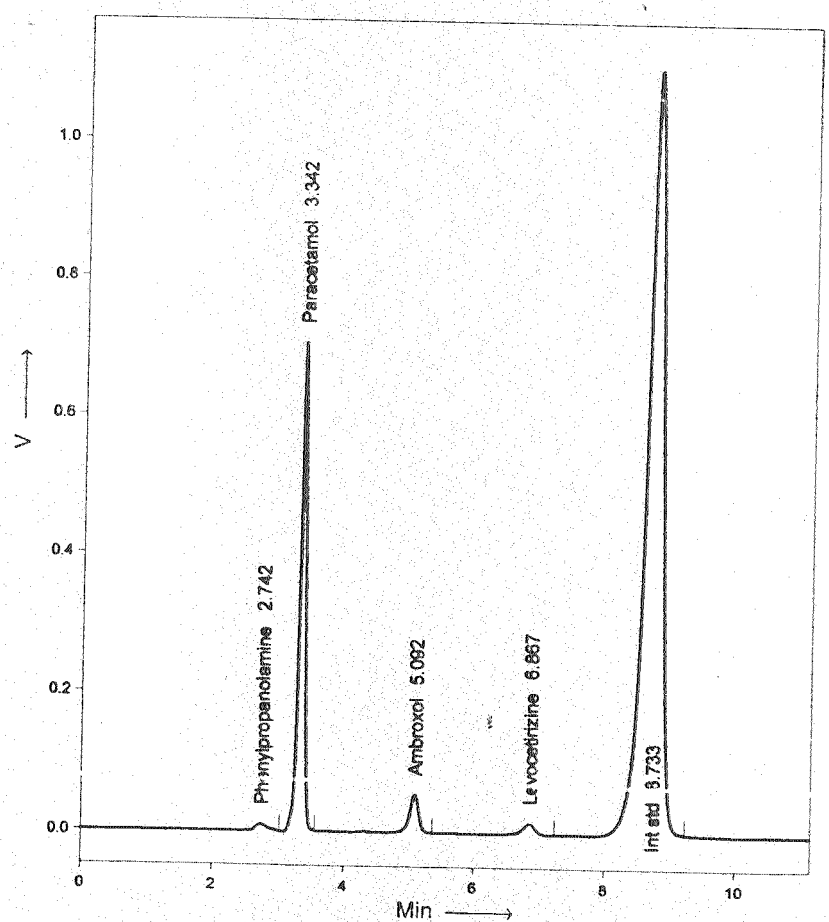

Fig. 1. Typical chromatogram of sample solution

RESULTS AND DISCUSSION

The chromatograms of mixed sample solutions are presented in Fig. 1. The accuracy of the method was determined by recovery studies and the percentage of recovery was calculated. From the data obtained, recoveries for the standard drugs were considered accurate. The precision procedure was satisfactory. The concentration range from 0.15–1.0 μg/mL of LC, 1.5–12.0 μg/mL of AX, 0.75–5.0 μg/mL of PP and 15–120 μg/mL of PT were examined by the assay procedure and the calibration curves were plotted (Fig. 2.). The calibration curve shows linear response over the range of concentration used in the assay procedure. The calibration curve passes through the origin, which justifies the use of single point

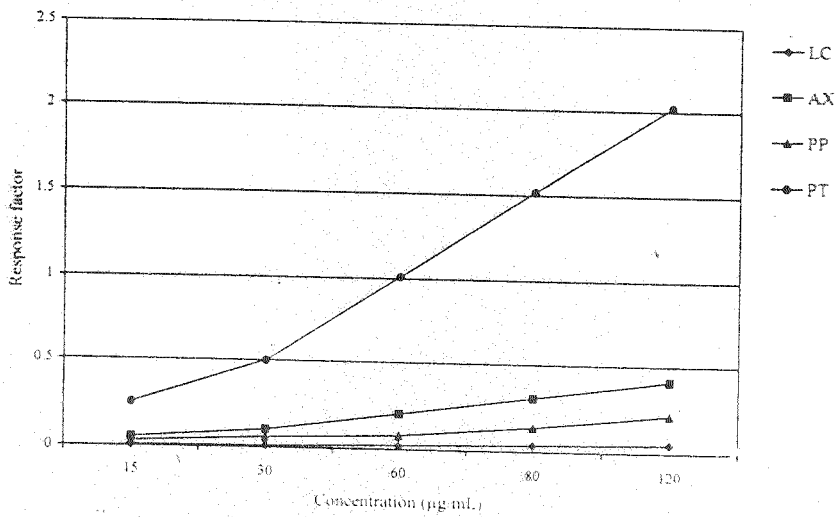

Fig. 2. Calibration curve for LC, AX, PP and PT

calibration and the proximity of maximum points to the calibration line demonstrated that the method has adequate linearity to the concentration of the analyte.

The limits of detection (LOD) for LC, AX, PP and PT were found to be 5 ng/mL, 25 ng/mL, 50 ng/mL and 5 ng/mL, respectively and the limits of quantification (LOQ) were 15 ng/mL, 100 ng/mL, 200 ng/mL and 15 ng/mL for LC, AX, PP and PT (Table-3). The ruggedness of the method was determined by carrying out the experiment with different instruments like Shimadzu HPLC (LC-10AT), Agilent HPLC and Water's Breeze HPLC by different operators using different columns of similar type like Hypersil, Phenomenex LUNA and Hichrom. Robustness of the method was determined by making slight changes in the chromatographic conditions. Further, there is no interference due to excipients. The system suitability studies were also carried out to determine column efficiency, resolution and peak asymmetry (Table-3). The proposed HPLC method is simple, selective, precise, rugged, robust, linear and rapid. Hence, this method

can be applied for the quality control of raw materials, formulations and dissolution studies.

TABLE-3
SYSTEM SUITABILITY STUDIES

S. No.	Parameters	Levocetirizine	Ambroxol	Phenylpropanolamine	Paracetamol
1.	Theoretical plates/metre	25784	26897	29874	28479
2.	Resolution factor	2.6	4.2	1.6	
3.	Asymmetry factor	1.0	1.01	1.07	0.99
4.	LOD (ng/mL)	5	25	50	5
5.	LOQ (ng/mL)	15	100	200	15

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