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SIMULTANEOUS & STABILITY INDICATING METHOD FOR DETERMINATION OF CETRIZINE HYDROCHLORIDE AND AMBROXOL HYDROCHLORIDE IN SYRUP

M. Gnana Raja [1]*, G. Geetha [1], A. Sankaranarayanan [2], Karna Maga Ganiga Raju [2] and P. Saravanamuthu Kumar [3]

PSG College of Pharmacy [1], Coimbatore, Tamil Nadu, India
KMS Health Center [2], Padi, Chennai, Tamil Nadu, India
Apex Laboratories Pvt. Ltd., [3], Chennai, Tamil Nadu, India

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Correspondence to Author:

M. Gnana Raja

Manager, Analytical research and development, KMS Health Center, Padi, Chennai, Tamil Nadu, India

E mail: laconil2002@yahoo.com

ABSTRACT

A simple isocratic HPLC method has been developed and validated as per ICH Guidelines for simultaneous and stability indicating determination of Cetirizine Hydrochloride and Ambroxol hydrochloride in pharmaceutical liquid oral dosage form syrup. Separation was achieved in Hypersil BDS C18 column, 5 µ, and 250 mm x 4.60 mm id with flow rate of 1.5 ml/minute and the detection at 230nm. 0.2 molar Ammonium phosphate with the pH of 4.0 with dilute orthophosphoric acid of about 65 percentage in acetonitrile combination was used as a mobile phase. Developed method validated as per ICH Guidelines, prior all the parameter system suitability was carried with five repeated injection of standard solution at the concentration of 50 µg/mL and 30 µg/mL Cetirizine Hydrochloride and Ambroxol Hydrochloride respectively. Percent RSD of five replicate injections of Cetirizine Hydrochloride and Ambroxol Hydrochloride and Tailing factor was found below 2.0% and 2.0 overall analyses respectively. A linear response was observed over the concentration range of 25.06 to 75.18 and 15.13 to 45.38ppm for the assay of Cetirizine Hydrochloride and Ambroxol Hydrochloride respectively. The results of analysis were validated statically and by recovery studies. Hence, the proposed method was found to be accurate, precise, reproducible and specific and can be used for simultaneous analysis of these drugs in liquid formulation.

INTRODUCTION: Cetirizine Hydrochloride is chemically 2-[2-[4-[(4-chlorophenyl)-phenylmethyl] piperazin- 1-yl] ethoxy] acetic acid and the active metabolite of the piperazine H1- receptor antagonist Hydroxyzine, it is not metabolized. Cetirizine inhibits the release of histamine and of cytotoxic mediators from platelets, as well as eosinophil chemotaxis during the secondary phase of allergic response. Also it is used to treat chronic idiopathic, urticaria, perennial allergic rhinitis, seasonal allergic rhinitis, allergic asthma, physical urticaria and atopic dermatitis.

Cetirizine hydrochloride (**Figure 1** official in EP [1] and BP [2]) is a highly selective antagonist of the peripheral histamine H1-reseptor on effectors cell in the GIT, and blood vessels.

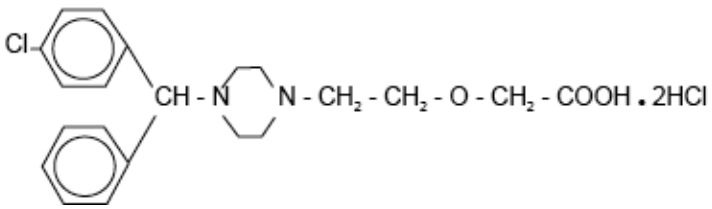

FIGURE 1: CHEMICAL STRUCTURE OF CETIRIZINE HYDROCHLORIDE

Ambroxol Hydrochloride (**Figure 2** official in EP [1] and BP [2]) is chemically Trans-4-(2-Amino-3, 5-dibrombenzylamino)-cyclohexanol Ambroxol is a clinically proven systemically active mucolytic agent. Administered orally onset of action occurs after about 30 minutes. The breakdown of acid mucopolysaccharide fibers makes the sputum thinner and less viscous and therefore more easily removed by coughing. Although sputum volume decreases, its viscosity remains low for as long as treatment is maintained. It is a metabolite of Bromhexine.

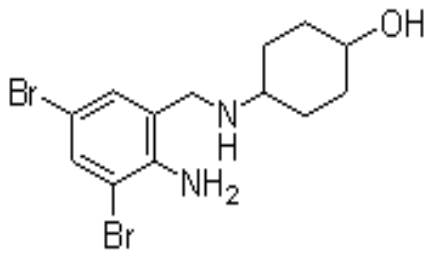

FIGURE 2: CHEMICAL STRUCTURE OF AMBROXOL HYDROCHLORIDE

Objective of study: As far as literature concerned, numbers of methods are reported for RP-HPLC and spectrophotometric estimation [3-4] of ambroxol hydrochloride and Cetirizine Hydrochloride in combined dosage form, Spectrophotometric [5-6] estimation of ambroxol hydrochloride and cetirizine hydrochloride in tablet formulation. Simultaneous analysis ambroxol hydrochloride and cetirizine hydrochloride in tablet dosage form by RP-HPLC [7-9] method.

A rapid, stability indicating RP-HPLC [10] method for simultaneous determination of ambroxol hydrochloride, cetirizine hydrochloride and antimicrobial preservatives in liquid pharmaceutical formulation, high performance liquid chromatographic method for cetirizine and ambroxol in human plasma and urine [11]. But this study was worked on the simultaneous and stability indicating method for determination of cetirizine Hydrochloride and ambroxol hydrochloride and validation performed as per ICH [12], Q2B- Validation of Analytical Procedure.

MATERIAL AND METHODS: HPLC grade acetonitrile, reagent grade Ortho -phosphoric acid, ammonium phosphate, sodium hydroxide, Hydrochloric acid, 6% hydrogen peroxide were purchased from Merck (Darmstadt, Germany).

Distilled water, purified using a Millipore Milli-Q plus water system was used to prepare the mobile phase and standard solutions.

The development and validation of the assay was performed on a HPLC Quaternary system with VWD Model Agilent 1200 series, consisting of a Quaternary pump-G1311A, a Degasser- G1322A, a ALS- G1329A, a TCC-G1316A and Waters-2996 Photodiode Array Detector, a Waters HPLC with PDA Detectors, Waters 2695- Separation Module with EMPOWER software.

The analytical column used to achieve chromatographic separation was Hypersil BDS column 250 x 4.6 mm, 5µm particle size. The peak purity was determined on a 2996 Photodiode Array Detector (PDA).

Procedure

Mobile phase preparation: 0.2 m Ammonium phosphate buffer pH adjusted to 4.0 with orthophosphoric acid and 65 percentage of the buffer in acetonitrile used as a mobile phase. 1.5 mL/minutes flow rate of the mobile phase with the column temperature of about 25°C was giving the consistent of retention time of about 18.6 and 5.8 of Cetirizine Hydrochloride and Ambroxol Hydrochloride respectively.

Standard and Test solution preparation: Standard and sample solution was prepared at the concentration of about 50µg/mL and 30µg/mL of Cetirizine Hydrochloride and Ambroxol Hydrochloride respectively with the mobile phase as a diluent by sonication of about 15 minutes and the final solution filtered through 0.45 micron filter paper.

Method Validation: Precision was performed with six repeated sample preparation of nominal concentration, accuracy was performed 50% to 150% of five concentration level Cetirizine Hydrochloride and Ambroxol Hydrochloride by the preparation sample was weighed as equivalent to 50%, 75%, 100%, 125% and 150% of the nominal concentration respectively.

At low level concentration and highest level concentration accuracy proved with six replicate preparation and medium level triplicate preparations was done.

The linearity was performed for the standard solution of Cetirizine Hydrochloride and Ambroxol Hydrochloride from the level of 50% to 150% of about five different concentrations from nominal concentration of standard and sample solution. Range of the method has been captured from precision, accuracy and linearity section. Deliberate change of the chromatographic condition will affect the method reproducibility so the method accuracy was proved by robustness and ruggedness experiments of 20% of flow rate, 10 % of the organic modification, 5% of the column temperature and pH of the mobile phase, and the system suitability was proved.

Degradation Study: Proving the method as stability indicating method, sample and placebo were forcefully degraded, Acid degradation by 5 m hydrochloric acid, Base hydrolysis by 5m sodium hydroxide and 6% hydrogen peroxide oxidation, UV visible light as per ICH guidelines in the chamber, 105°C of temperature was maintained for 24 hours for thermal degradation, saturated KNO3 was used for degrading the sample by moisture and aqueous degradation was done by water.

RESULTS & DISCUSSION: Developed analytical method was validated and system suitability was performed prior to each parameter and the result was found satisfactory for all the parameter. Percent RSD of overall analysis was about 1.6% to 1.0% and overall tailing factor was found 1.2 and 1.3 Cetirizine Hydrochloride and Ambroxol Hydrochloride respectively. Refer chromatogram of blank, standard and sample **Figure 1, Figure 2 and Figure 3**.

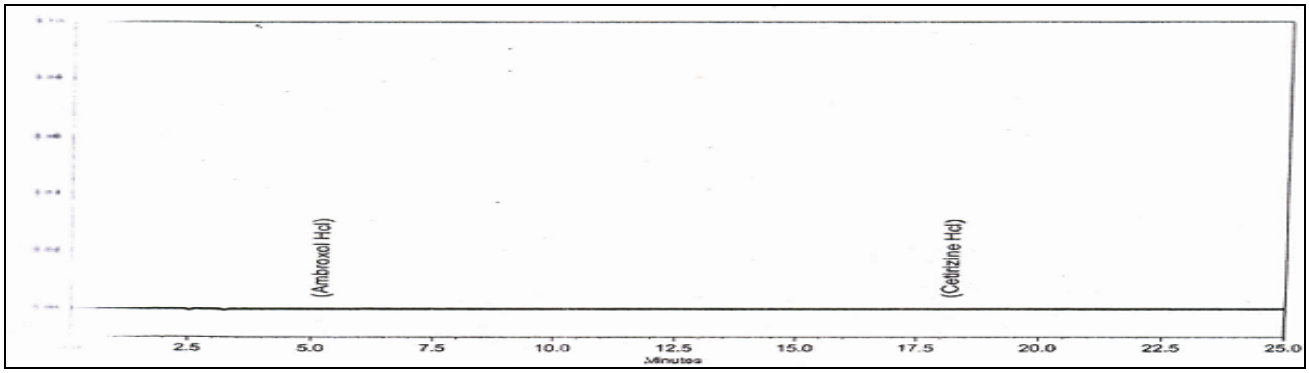

FIGURE 1: CHROMATOGRAM OF BLANK

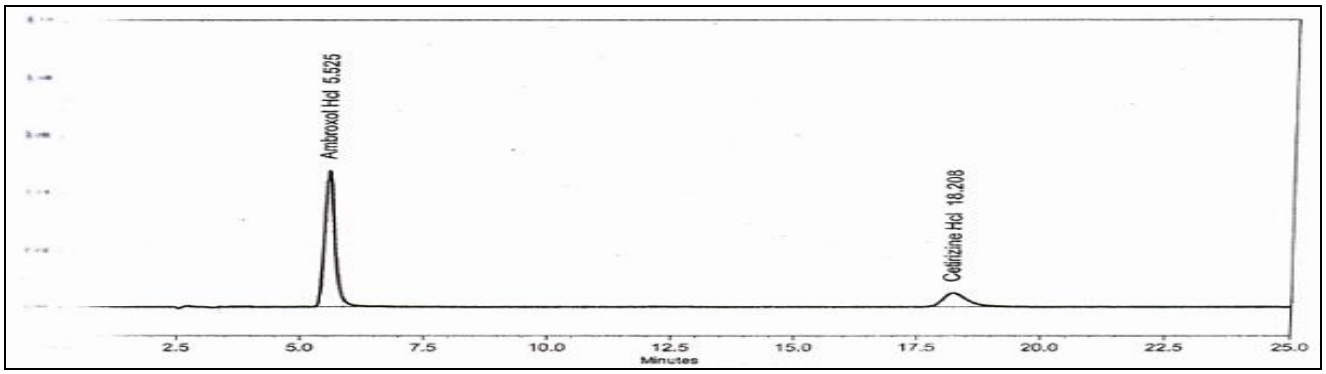

FIGURE 2: CHROMATOGRAM OF STANDARD

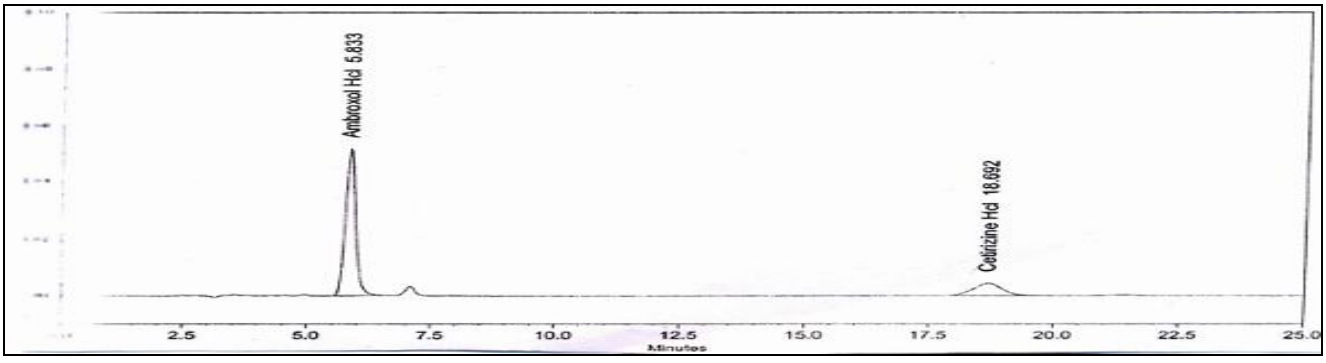

FIGURE 3: CHROMATOGRAM OF SAMPLE

Six replicate preparations was done for precision and the average assay of six replicate preparations of about 99.6%, 99.7% and the percent RSD of assay was found 1.6%, 1.0% for Cetirizine Hydrochloride and Ambroxol Hydrochloride respectively (**Table 1**).

Linearity of Cetirizine Hydrochloride and Ambroxol Hydrochloride of the concentration of 25.1 to 75.02 and 15.1 to 45.4µg/mL respectively, the correlation of coefficient was found to be 0.999 and 0.999 respectively (**Figure 4 and Figure 5**).

TABLE 1: PRECISION

S. No.	Precision	
	CTH	AXH
	Nominal	
1	99.3	99.5
2	97.2	98.2
3	98.5	101.1
4	100.2	100.5
5	101.2	99.8
6	101.1	99.2
Average	99.6	99.7
% RSD	1.6	1.0

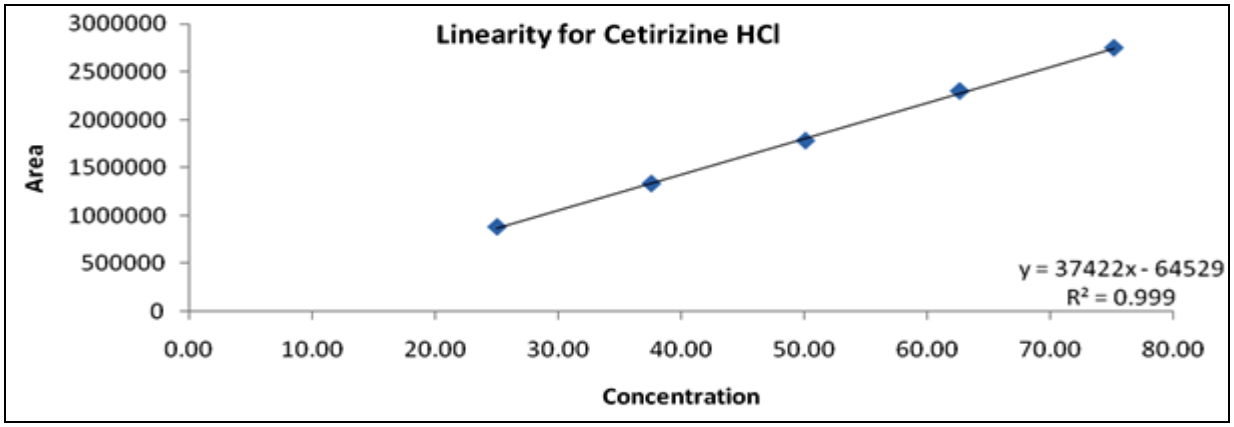

FIGURE 4 LINEARITY OF CETIRIZINE HYDROCHLORIDE

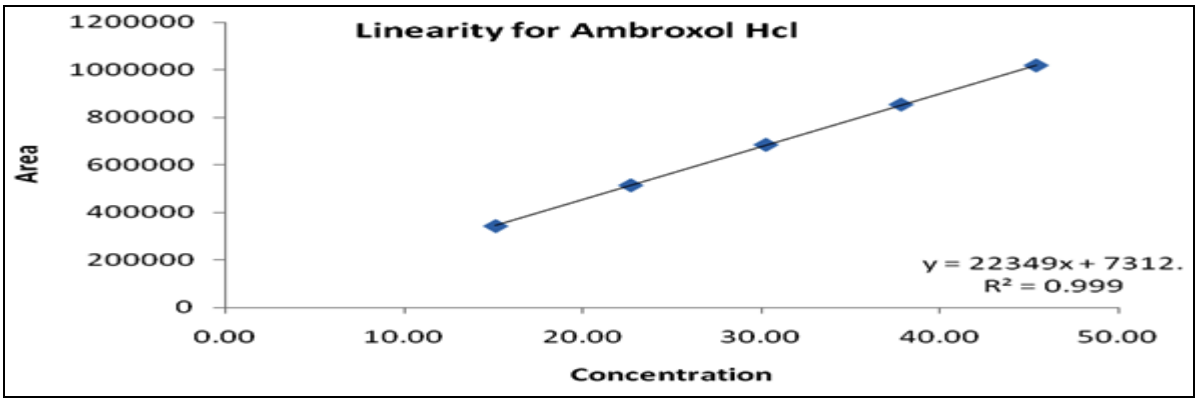

FIGURE 5 LINEARITY OF AMBROXOL HYDROCHLORIDE

Accuracy of the both the drugs was proved 50.0% to 150.0% form the nominal concentration and the percent RSD of each level and average of overall was found 0.3% and 1.8% . Average percent recovery of each level was found 99.2% to 101.0% (**Table 2**).

Ruggedness or intermediate precision was proved with the sample preparation of six replicates with intra day, another analyst, different column and different system, the percent RSD was found 0.7 and 0.9 for Cetirizine Hydrochloride and Ambroxol Hydrochloride respectively (**Table 3**).

Robustness was proved with the chromatographic condition is deliberately changed of about 20% of flow rate, 10 % of the organic modification, 5% of the column temperature and pH of the mobile phase, in this condition system suitability was proved (**Table 4**).

Degradation study was performed for placebo and drug product, and the placebo chromatogram shows no peak at the interested peak retention time and peak purity of Cetirizine Hydrochloride and Ambroxol hydrochloride passes and the percentage degradation is 4.21, 1.21 and 3.23, 1.02% in acid, 6% hydrogen

peroxide degradation and acid, Humidity degradation of maximum and minimum percentage degradation of Cetirizine Hydrochloride and Ambroxol hydrochloride respectively (**Table 5**).

The finalized chromatographic condition and validation summary refer **Table 6 and Table 7** respectively.

TABLE 2: ACCURACY

S. No.	Accuracy									
	CTH	AXH	CTH	AXH	CTH	AXH	CTH	AXH	CTH	AXH
	50%		75%		100%		125%		150%	
1	97.5	100.2	99.2	99.5	100.2	100.1	98.5	99.3	99.5	102.3
2	98.6	97.9							102.3	99.5
3	101.2	98.5	99.8	98.6	100.3	99.5	99.6	99.5	102.4	99.5
4	102.1	99.5							99.1	101.2
5	100.2	101.5	100.2	102.1	99.8	99.9	99.5	102.2	98.1	102.2
6	100.8	102.3							99.7	101.2
Average	100.1	100.0	99.7	100.1	100.1	99.8	99.2	100.3	100.2	101.0
% RSD	1.7	1.7	0.5	1.8	0.3	0.3	0.6	1.6	1.8	1.2

TABLE 3: INTERMEDIATE PRECISION

S. No.	Analyst I		Analyst II	
	CTH	AXH	CTH	AXH
1	99.3	99.5	99.5	99.9
2	97.2	98.2	100.2	100.2
3	98.5	101.1	100.3	100.3
4	100.2	100.5	101.2	101.2
5	101.2	99.8	99.8	102.2
6	101.1	99.2	99.3	101.2
Average	99.6	99.7	100.1	100.8
% RSD	1.6	1.0	0.7	0.9

TABLE 4: ROBUSTNESS

S. No.	Type of modification	System Suitability	
		Cetirizine Hydrochloride	Ambroxol Hydrochloride
		% RSD	Tailing factor
1	Organic variation +10%	1.1	1.3
2	Organic variation -10%	1.3	1.2
3	pH +5%	1.1	1.3
4	pH -5%	1.2	1.2
5	Column temperature +5%	1.0	1.2
6	Column temperature -5%	1.1	1.4
7	Flow +20%	1.4	1.2
8	Flow -20%	1.3	1.1

TABLE 5: DEGRADATION STUDY

S. No.	Type of Degradation	Degradant	% Degradation	
			Cetirizine	Ambroxol
1	Acid hydrolysis	5 N Hydrochloric acid	4.21	3.23
2	Base hydrolysis	5 N Sodium hydroxide	2.84	2.10
3	Oxidation	6% hydrogen Peroxide	1.21	1.25
4	Humidity	95% moisture by using saturated KNO3	2.31	1.02
5	Aqueous	Water	4.20	3.20
6	UV visible	Chamber as per ICH	2.10	1.85
7	Thermal	105°C for 1 Hour	1.78	1.68

TABLE 6: CHROMATOGRAPHIC CONDITION

S. No.	Variable	Condition
1	Column	Hypersil BDS, 250*4.6, 5µ
2	Phase	BDS
3	Mobile phase & Diluent	65% of 50mM of Ammonium phosphate buffer in Acetonitrile.
4	Flow rate	1.5 mL / Minutes
5	Temperature	25°C
6	Injection volume	20 µL
7	Wave length	230 nm

TABLE 7: VALIDATION SUMMARY

S. No.	Parameter	Cetirizine Hydrochloride	Ambroxol Hydrochloride
1	Precision (% RSD)	1.6	1.0
2	Intermediate Precision (%RSD)	0.7	0.9
3	Accuracy (Overall recovery)	99.9	100.2
4	Linearity	0.999	0.999

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