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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF *LORATADINE, AMBROXOL HYDROCHLORIDE AND GUAIPHENESIN* USING REVERSE PHASE HPLC METHOD IN BULK AND LIQUID DOSAGE FORM

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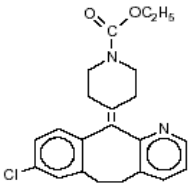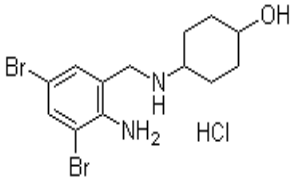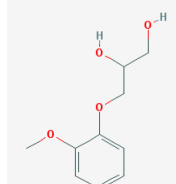*[3]Center for Pharmaceutical Sciences, JNTU-H Hyderabad.*

ABSTRACT

A new simple, accurate, precise and reproducible RP-HPLC method has been developed for the simultaneous estimation of loratadine, ambroxol hydrochloride and guaiphenesin in bulk drug & liquid dosage form using C18 column (Waters, 250 x 4.6 mm, 5 μm) in isocratic mode. The mobile phase consisted of 0.1 M di potassium Phosphate buffer (pH 5.5) and methanol in the ratio of 50:50 v/v. The detection was carried out at 245 nm. The method was linear over the concentration range for Loratadine 6-14μg/ml, ambroxol hydrochloride36-84μg/ml and guaiphenesin 60-140μg/ml. the recoveries of loratadine, ambroxol hydrochloride and guaiphenesin were found to be 100.68-98.8%, 100.34-98.52%, 100.71-98.54% respectively. The validation of method was carried out utilizing ICH-guidelines. The described HPLC method was successfully employed for the analysis of pharmaceutical formulations containing combined dosage form.

Keywords: Loratadine, Ambroxol Hydrochloride and Guaiphenesin, Reverse Phase HPLC. Validation.

INTRODUCTION:

Table 1: Drug profile

Description	Loratadine	Ambroxol Hcl	Guaiphensine
Structure			
IUPAC name	ethyl 4-(8-chloro-5,6-dihydro-11H-benzo[5,6] cyclohepta[1,2-b]pyridin-11-ylidene) -1-piperidinecarboxylate	2-Amino-3,5-dibromo-N-[trans-4-hydroxycyclohexyl]benzylamine	3-(2-methoxyphenoxy)-1,2-propanediol
Mol. formula	$C_{22}H_{23}ClN_2O_2$	$C_{13}H_{19}Br_2ClN_2O$	C10H14O4
Pka	5.0		13.62
Mol.wt	382.89	414.56376	198.21
Category	Anti-Allergic Agents, Antipruritics, Histamine H1 Antagonists, Non-Sedating	Expectorants	Expectorants

Loratadine is a derivative of azatadine and a second generation histamine H1 receptor antagonist used in the treatment of allergic rhinitis and urticaria. Unlike

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most classical Antihistamines (histamine H1 antagonists) it lacks central nervous system depressing effects such as drowsiness. Ambroxol is a secretolytic agent used in the treatment of respiratory diseases associated with viscid or excessive mucus. It is the active ingredient of Mucosolvan, Lasolvan or Mucoangin. The substance is a mucoactive drug with several properties including secretolytic and secretomotoric actions that restore the physiological clearance mechanisms of the respiratory tract which play an important role in the body's natural

defence mechanisms. It stimulates synthesis and release of surfactant by type II pneumocytes. Surfactants acts as an antiglue factor by reducing the adhesion of mucus to the bronchial wall, in improving its transport and in providing protection against infection and irritating agents. Guaifenesin An expectorant that also has some muscle relaxing action. It is used in many cough preparations. Guaifenesin (glyceryl guaiacolate) has the chemical name 3-(2-methoxyphenoxy)-1,2-propanediol. Its molecular formula is $C_{10}H_{14}O_4$ with a molecular weight of 198.21. It is a white or slightly gray crystalline substance with a slightly bitter aromatic taste. One gram dissolves in 20 mL water at 25°C; it is freely soluble in ethanol. It is Guaifenesin is readily absorbed from the GI tract and is rapidly metabolized and excreted.

According to literature survey few spectrophotometric methods have been reported for the determination of LOR, AMB, GUA in single and in combination with other drugs. Simultaneous determination of LOR and AMB, GUA in bulk and liquid dosage form were reported by using spectrophotometric,. However very few HPLC methods were reported for the simultaneous estimation of LOR and AMB,GUA in liquid dosage form. The aim of present work was to develop and validate as per ICH guidelines [20], a sensitive HPLC method that can be applied for simultaneous estimation of LOR and AMB, GUA.

Materials

LOR and AMB, GUA were received gratis from Hetero drugs, Hyderabad and were used as received. HPLC grade acetonitrile was purchased from SD Fine Chem Pvt. Ltd. (Mumbai, Maharashtra). Ultra-pure water was obtained from ELGA (Bucks, UK) water purification unit. .All other chemicals were of analytical reagent grade.

EXPERIMENTAL WORK:

Chromatographic conditions

The HPLC system (Agilent 1220 series) consisted of quaternary gradient system (600 Controller), in-line degasser (Agilent), UV detector and Manual sampler. Data was processed using EZchrome software (Agilent). Isocratic elution of the mobile phase 0.1 M Dipotassium Phosphate buffer (pH 7) and acetonitrile in the ratio of 70:30 v/v with the flow rate of 1 ml/min. Separation was performed on a C_{18} (250 x 4.6 mm i.d, 5 μ particle size) analytical column and a pre-column to protect the analytical column from strongly bonded material. Integration of the detector output was performed using the EZ chrome software to determine the peak area. The contents of the mobile phase were filtered through a 0.45 μm membrane filter and degassed by sonication before use. Mobile phase was used as diluents. The flow rate of the mobile phase was optimized to 1 ml/min which yields a column back pressure of 110–112 kg/cm. The run time was set at 10 min and a column temperature was maintained at 35°C.

The volume of injection was 10 μl, prior to injection of the analyte, the column was equilibrated for 30–40 min with the mobile phase. The eluents were detected at 245 nm. The developed method was validated in terms of specificity, linearity, accuracy, limit of detection (LOD), limit of quantification(LOQ), intra-day and inter-day precision and robustness for the assay of LOR and AMB,GUA as per ICH guidelines.

Preparation of standard solutions:

Standard stock solutions of Guaifensine, Ambroxol Hcl & Loratadine (microgram/ml) were prepared by dissolving 50mg Guaifensine, 30mg Ambroxol Hcl & 5mg Loratadine dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5min and dilute to 100 ml with mobile phase. Pipette out 5ml & further dilute to 50ml.

Preparation of sample solution:

Pipette out 5ml of the syrup solution and dissolved in 50 ml of mobile phase sonicate the solution for about 30mints and filter through 0.45 micron filter from this pipette out 5ml and make up to 50ml

RESULTS AND DISCUSSION:

Method Development:

Number of mobile phase and their different proportions were tried and finally was selected as 0.1 M Dipotassium Phosphate buffer (pH 7) and acetonitrile in the ratio of 70:30 v/vappropriate mobile phase which gave good resolution and acceptable system suitability parameters. The results of system suitability parameters were shown in table 3. The chromatogram of working standard solution is shown in Fig 1. The summaries of Chromatographic conditions were given in table 2.

Table 2: Summary of Chromatographic conditions

S. No	Parameter	Description/Value
1.	Stationary Phase	Water's C18 (250X4.6X5)
2	Mobile Phase	0.1 M Dipotassium Phosphate buffer (pH 7) and acetonitrile in the ratio of 70:30 v/v
3	Flow rate	1 ml/min
4	Detection Wavelength	245nm
5	Detector	UV detector
7	Rt's	LOR – 7.0503 Min AMB- 5.280Min GUA – 3.107Min
8	Injection volume	10 μl
9	Column Temperature	35 °C
10	Run time	10 mins
11	Diluent	Mobile Phase

Fig. 1: Typical Chromatogram of *Loratadine, Ambroxol hydrochloride and Guaiphenesin*
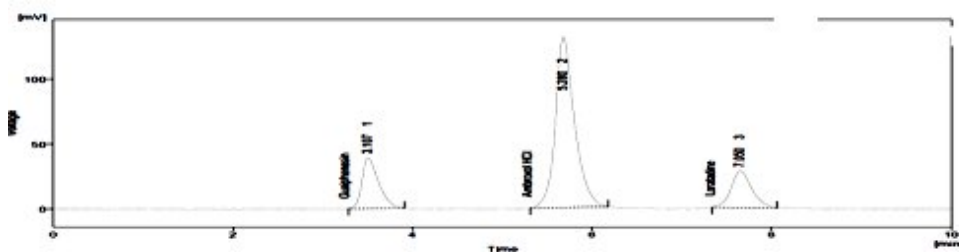
Table 3: System suitability parameters

S. No	Parameter	Results		
		Loratidine	Ambroxol HCL	Guaiphenesin
1	Retention Time (min)	7.0503	5.280	3.107
2	Tailing	1.313	1.518	1.926
3	Theoretical Plates (n)	56287	38073	37613
4	Resolution factor (R)	6.188		
5	Similarity Factor	1.0124 (Limit: 0.98 – 1.2)		

Method Validation:

Accuracy

Recovery assessment was obtained by using standard addition technique which was by adding known quantities of pure standards at three different levels in 50%, 100%and 150% to the pre analyzed sample formulation. From the amount of drug found, amount of drug recovered and percentage recovery were calculated which sense to conformation that the proposed method was accurate. The results were tabulated in Table 4.

Table 4: Results of Accuracy

S. No	% conc at specific level	GUA			AMB			LOR		
		Amount added(μg\ml)	Amount found (μg\ml)	Mean % recovery	Amount added (μg\ml)	Amount found (μg\ml)	Mean % recovery	Amount added (μg\ml)	Amount found (μg\ml)	Mean % recovery
1	80*	100	100.71	100.71	60	59.60	99.34	10	9.89	98.9
2	100**	120	119.33	99.44	72	72.25	100.34	12	12.08	100.68
3	120*	140	137.96	98.54	84	82.76	98.52	14	13.93	99.48

*Mean % Recovery of 6 replicates; **Mean % Recovery of 3 replicates

Precision

The intraday and inter day precision of the proposed method was determined by analyzing mixed standard solution of GUA and AMB,LOR at concentration 50μg/mL and 30μg/mL, 5μg/mL 3 times on the same day and on 3 different days. The results shown in table 4 were reported in terms of relative standard deviation.

Table 5: Results of Precision (%Assay)

Sample No.	LORATIDINE		AMBROXOL		GUAIPHENESIN	
	Sample Area - 1	Rt	Sample Area - 2	Rt	Sample Area - 3	Rt
1	494.509	3.107	1917.821	5.270	461.950	7.060
2	498.118	3.113	1964.007	5.267	452.492	7.060
3	509.122	3.107	1987.675	5.280	464.347	7.080
4	495.529	3.151	1970.719	5.290	464.243	7.080
5	498.800	3.103	1953.816	5.283	459.677	7.067
6	499.820	3.113	1974.276	5.287	461.425	7.060
Avg	499.316	3.1157	1961.386	5.280	460.689	7.065
STD		0.0177	STD	0.009	STD	0.008
% RSD		0.57	% RSD	0.17	% RSD	0.11

Linearity:

Calibration graphs were constructed by plotting peak area vs concentration of GUA and AMB and LOR the regression equations were calculated. The calibration graphs were plotted over 5different linear concentrations in the range of 60-140μg/ml for GUA and 36-84μg/ml for AMB,6-14μg/ml. Aliquots (10 ml) of each solution were injected under the operating chromatographic condition described above [Number of replicates (n =5)]. The linearity graphs were shown in fig 2& 3, 4.
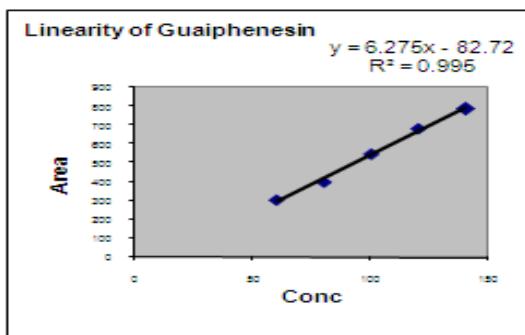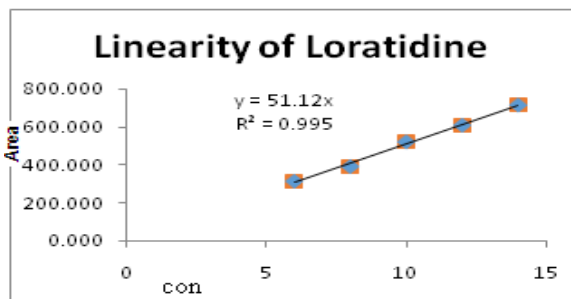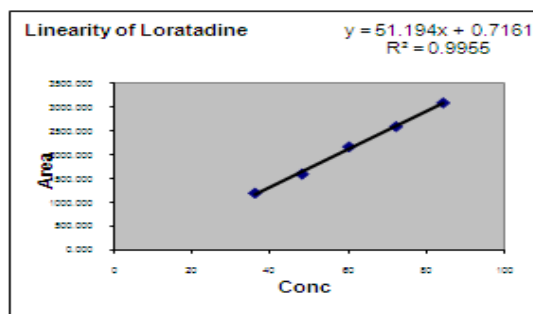

Fig 2

Fig 3

Fig 4

Limit of detection (LOD) and limit of quantitation (LOQ):

The limit of detection (LOD) and limit of quantitation (LOQ) of GUA and AMB,LOR were determined by calculating the signal-to-noise(S/N) ratio of 3:1 and 10:1, respectively according to International Conference on Harmonization guidelines. LOD values for GUA and AMB, LOR were found to be 16.63μg/mL & 1.55μg/mL, 0.20μg/mL respectively. LOQ values for GUA and AMB, LOR were found to be50.44μg/mL & 4.71μg/mL, 0.62 μg/mL respectively.

Assay of the liquid dosage form:

The proposed validated method was successfully applied to determine GUA and AMB,LOR in liquid dosage form. The results obtained for GUA and AMB, LOR were comparable with corresponding labeled amounts. The results were tabulated in table 4.

Table 6: Assay of the dosage form

Sample No.	GUA			AMB			LOR		
	Labeled amount (mg/ tablet)	Standard Area	Sample Area	Labeled amount (mcg/ tablet)	Standard Area	Sample Area	Labeled amount (mg/ tablet)	Standard Area	Sample Area
1		504.162	504.162		1956.535	1956.535		455.941	455.941
2		510.658	500.513		1974.511	1974.424		453.077	474.012
3	50	497.429	506.293	30	1953.899	1965.211	5	459.598	476.167
4		505.043	510.658		1962.761	1974.511		450.249	453.077
5		496.956	497.429		1993.521	1967.236		473.364	453.077
6		510.658	506.293		1974.511	1965.211		450.249	466.757
	Average	502.850	503.811	Average	1968.245	1967.583	Average	458.4458	465.1908
STD PURITY			99.2	STD PURITY		99.3	STD PURITY		99.3
CAL			49.69 mg	CAL		29.78mg	CAL		5.04mg
% Assay:			99.39	% Assay:		99.27	% Assay:		100.76

FORCED DEGRADATION:

a) Hydrolytic degradation under acidic condition: to sample 0.1N HCl is added and refluxed for 30 minutes at 60°C.
b) Hydrolytic degradation under alkaline condition: 0.1N NaOH refluxed for 30 minutes at 60°c.

c) Oxidative degradation: to sample 1% of H_2O_2 is added and refluxed for 30minutes at 60°
d) Thermal degradation: Sample is exposed to 105°C for about 1 hours.
e) Photolytic degradation: Sample was exposed to sunlight for 24 hours

Table 7: Forced degradation study

Parameter	Area of GUA	Area of AMB	Area of LOR	% Degradation of GUA	% Degradation of AMB	% Degradation of LOR
Acid	493.836	1949.45	456.360	9.9	9.9	12.6
Base	502.845	1993.305	479.344	8.2	7.8	8.2
Sun light	510.34	1990.47	471.478	3.6	5.1	4.2
Heat	527.851	2052.678	500.124	6.8	8.0	9.7

Formula to calculate assay

$$Assay = \frac{sample\ area}{stadard\ area} \times \frac{conc\ of\ std}{conc\ of\ sample} \times \frac{purity\ of\ working\ std}{100} \times \frac{Avg\ weight}{label\ claim} \times 100$$

Net degradation = Assay of sample – Assay of degradation sample.

Acceptance criteria for forced degradation: the net degradation should be between 1%-50%

Result: Forced degradation is done to establish that the method is suitable even at a high temperature. In table 7 it is seen that Even after the degradation there is no shift in retention time. This shows that even after degradation there is no impact on method. By this it can be said that this method indicates "Stability".

CONCLUSIONS:

The proposed method has advantage of simplicity and convenience for the separation and quantization of GUA and AMB, LOR in the combination which can be used for the assay of their dosage form. Also, the low solvent consumption and short analytical run time lead to environmentally friendly chromatographic procedure. The method is accurate, precise, rapid and selective for simultaneous estimation of loratadine, ambroxol hydrochloride and guaiphenesin in its dosage form. Hence it can be conveniently adopted for routine analysis.

ACKNOWLEDGMENTS:

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