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STABILITY INDICATING METHOD DEVELOPMENT & VALIDATION FOR SIMULTANEOUS ESTIMATION OF LORATADINE, AMBROXOL HYDROCHLORIDE AND GUAIPHENESIN IN BULK DRUG AND LIQUID DOSAGE FORM USING RP-HPLC METHOD

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Abstract

The Present study aim to develop and validate a simple, rapid, precise and economical Stability indicating RP-HPLC method for simultaneous estimation of Loratidine, Ambroxol Hydrochloride and Guaifenesin in bulk and pharmaceutical liquid dosage forms. The method was carried out on a HYPERSIL, ODS (C18 250x4.6 ID) 5µm column with a mobile phase of mixed phosphate buffer: Methanol: Acetonitrile (20:50:30 v/v) at a flow rate of 1.0 mL/min & PH of 5.5. Detection was carried out at 245 nm.The retention time of guaifenesin, Ambroxol Hydrochloride and Loratidine was 3.53, 5.667 and 7.660 min, respectively. Linearity was observed over concentration range of 60-140mcg/ml for Guaiphenesin, 36-84mcg/ml for Ambroxol Hydrochlordie & 6-14mcg/ml of loratadine. Co-efficient Regression for three drugs was found to be above 0.99% various other validation parameters were found to be satisfactory & lie in the range of acceptance. The drug was subjected to stress conditions as per ICH guidelines & was found to be stable. The method employed is indicating a promising approach for the estimation of Loratadine, Ambroxol Hydrochloride & Guaiphenesin drugs in Bulk & combined Pharmaceutical Liquid dosage forms.

Keywords: Isocratic elution; RP-HPLC Method development; stability indicating; Validation.

Introduction

Loratidine is chemically 4-[8-chloro-5, 6-dihydro-11H- benzo [5, 6] cyclohepta [1, 2-b] pyridin-11-ylidene]-1-piperidinecarboxylic acid ethyl ester1 with a potent antihistaminic activity. Loratadine competes with free histamine and

exhibits specific, selective peripheral H_1 antagonistic activity. This blocks the action of endogenous histamine, which subsequently leads to temporary relief of the negative symptoms (eg. nasal congestion, watery eyes) brought on by histamine.

Ambroxol hydrochloride is chemically, 1 ({[2 – Amino – 3, 5 dibromo phenyl] – methyl} amino) cyclohexanol monohydrochloride1 which is a semi synthetic derivative of vasicine from the Indian shrub "Adhatoda vasica". It is a mucolytic agent. Ambroxol hydrochloride is an N – desmethyl metabolite of bromohexine.

Guaifenesin (glyceryl guaiacolate) has the chemical name 3-(2-methoxyphenoxy)-1,2-propanediol.An expectorant that also has some muscle relaxing action. It is used in many cough preparations.

Many methods have been described in the literature for the determination of Loratidine and Ambroxol Hydrochloride and Guaifenesin individually and in combination with other drugs. However, there is no HPLC method reported for the simultaneous estimation of these drugs in combined dosage forms. Fixed dose combination containing Loratidine (5 mg) Ambroxol Hydrochloride (30 mg) and Guaifensin (50 mg) is available in the Syrup form in the market.

The aim of this work was to develop an RP-HPLC method with ultraviolet detection for the simultaneous determination of Loratidine ,Ambroxol Hydrochloride and Guaifensin in pharmaceutical dosage forms. The present RP-HPLC method was validated following the ICH guidelines.

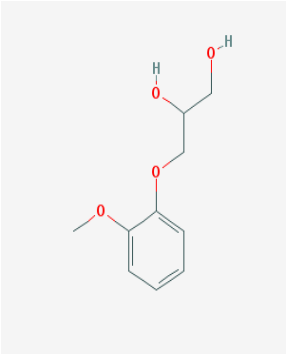

Fig-1 STR of GUAI

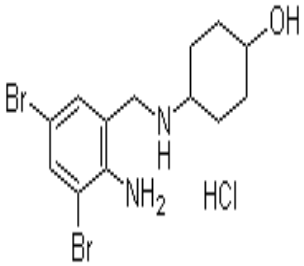

Fig-2 STR of AMB

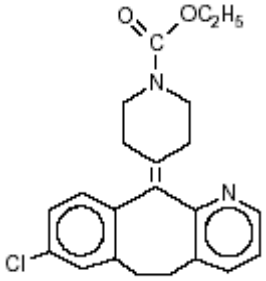

Fig-3 STR of LOR

Materials and Methods

Materials

Reagents & chemicals

LORATIDIN, AMBROXOL HCl, GUAIPHESNINE bulk drugs are obtained as Gift Samples obtained from PEGASUS FARMACO PVT LMT & syrup formulation was collected from local pharmacy with fixed dosage form of

Guaiphenesin 50mg, Ambroxol Hydrochloride 30mg & Loratadine 5mg. Other chemicals such as Sodium dihydrogen ortho phosphate & Potassium Dihydrogen ortho Phosphate are of AR Grade and water, Acetonitrile & Methanol is of HPLC Grade

Instrumentation

HPLC from Agilent 1220 with HPLC Column as HYPERSIL, ODS (C18 250x4.6 ID) 5µm and Hamilton Syringe of 20µl system was controlled using spin chrome version 3.00 data station was applied for data collecting software, other instruments used were UV-Visible Spectrophotometer of PG Instruments Lmt, Model T60 ,Electronic balance of Wensar weighing scales Model PGB-600 , pH meter of Systronics Digital & Ultra sonicator from PCI analyte ,

Method

Preparation Mobile Phase

A mixture of 20 volumes of Mixed Phosphate buffer pH 5.5, 30 volumes of Acetonitrile & 50 volume of methanol. The mobile phase was sonicated for 10min to remove gases.

Preparation of Mixed Phosphate buffer:

1.625 gm of Potassium di Hydrogen ortho phosphate and 0.3 gms of Di Potassium Hydrogen ortho phosphate was weighed and dissolved in 100ml of water and volume was made up to 550ml with water. Adjust the pH to 5.5 using ortho phosphoric acid. The buffer was filtered through 0.45µ filters to remove all fine particles and gases.

Determination Of Working Wavelength (λmax)

Standard solutions of 10 µg /ml of AMB,LOR & GUAI were prepared individualy & each drug was scaned individually & in combined form in uv range of 200-400nm, the absorption curve shows characteristic absorption maxima at 308 nm for Ambroxol HCl, 271 nm for Guaiphensine and 245 nm for loratidine & 245 nm for the combination

Experimental Trials

Preparation of mixed standard stock solution

Weigh accurately 5 mg of loratidine to these add 30mg of ambroxol HCl & guaiphensine 50mg & makeup the volume to 50ml. from the above pipette out 5ml solution make up to 50ml using mobile phase. it gives the concentration as 100mcg of Guaifensine, 60mcg Ambroxol Hcl & 10mcg of Loratadine This solution is used for recording chromatogram using following chromatographic conditions

Preparation of Sample stock 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 50 ml.

Chromatographic conditions

Tab-1: Optimized chromatographic conditions.

Mobile phase	Phosphate buffer: methanol: ACN (20:50:30)
pH	5.5
Column	ODS C18 (250×4.6 ×5µ)
Flow rate	1.0ml/mint
Column temperature	ROOM TEMPERATURE
Sample temperature	ROOM TEMPERATURE
Wavelength	245nm
Injection volume	0.02ml
Run time	10mint
Retention time	Last drug-7.660

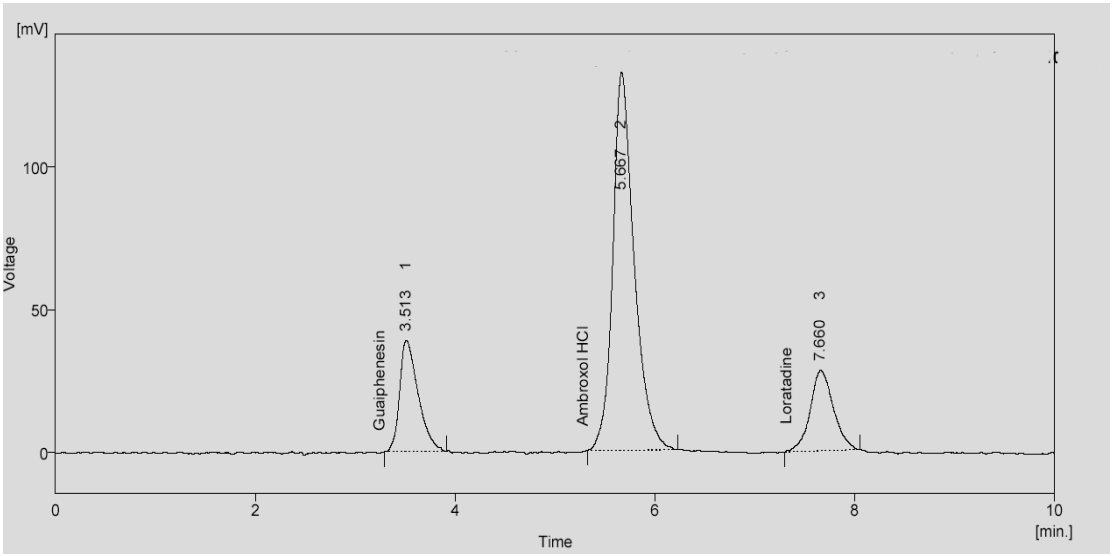

Fig-4: Chromatogram of Loratidin, Ambroxol HCl, Guaiphesnine by using mobile phase.

Tab-2: Result of Loratidin, Ambroxol HCl, Guaiphesnine by using mobile phase.

S.No.	Name	Rt (min)	Peak Area	Efficiency	Asymmetry	Resolution
1	**Guaiphensine**	3.513	548.118	3768	1.975	-
2	**ambroxolHCL**	5.667	2164.007	3676	1.564	6.082
3	**Loratidine**	7.660	522.492	5490	1.254	5.063

Discussion: The peak Asymmetry factor was less than 2 for Loratidin, Ambroxol HCl, Guaiphesnine and the efficiency also good, and the retention time was also satisfactory for Loratidin, Ambroxol HCl, Guaiphesnine

Assay:

Assay of the method was carried out by 5 replicate analysis of 100% target concentration of Guaiphensine,Ambroxol Hydrochloride & Loratidine. Percentage assay was calucated for all the three drugs. Results depicted in Table No: 3 & **Fig: 5, 6**

$$\% \text{ Assay} = \frac{\text{AT}}{\text{AS}} \times \frac{\text{WS}}{\text{DS}} \times \frac{\text{DT}}{\text{WT}} \times \frac{\text{P}}{100} \times \frac{\text{AW}}{\text{LC}} \times 100$$

Where:

$\mathbf{A_T}$ = Average area counts of injections for test peak in the chromatogram of sample solution.

$\mathbf{A_S}$ = Average area count of five replicate injections for std peak in the chromatogram of standard solution.

$\mathbf{D_S}$ = Dilution factor of standard solution (weight÷dilution).

$\mathbf{D_T}$ = Dilution factor of sample solution.

P = Percentage purity of working standard used.

Method validation

Method validation was performed by considering the following parameters such as System suitability,specificity,assay,linearity,range,accuracy,precision,LOD,LOQ,robustness, ruggedness and forced degradation studies.

System suitability

Method: To ensure that the analytical system is working properly & can give accurate & precise results Standard solutions of GUAI, AMB & LOR were diluted into five replicates 100% Standard stock solution injected into the chromatographic system. The system suitability parameters like Tailing & % RSD for all the three drugs were evaluated & Depicted in Table 4.

Linearity

Method

Linearity of method was determined by taking calibration curve of 5 standard solutions at 60%, 80%, 100%, 120% and 140% levels.

Method was validated for Linearity by taking samples in..

Level – I 0.6ml of standard solution is diluted to 10ml gives 6mcg of loratadine, 36 mcg of Ambroxol Hcl & 60mcg of guaifensine.

Level – II 0.8ml of standard stock solution is diluted to 10ml gives 8mcg of loratadine, 48 mcg of Ambroxol Hcl & 80mcg of guaifensine

Level – III 1ml of the stock solution was taken & diluted to 10ml which gives 10mcg of loratadine, 60 mcg of Ambroxol Hcl & 100mcg of guaifensine

Level – IV 1.2ml of standard stock solution was pipeete out & dilute to 10ml which gives 12mcg of loratadine, 72 mcg of Ambroxol Hcl & 120mcg of guaifensine

Level – V 1.4ml of standard stock solution was taken & diluted to 10ml gives 14mcg of loratadine, 84 mcg of Ambroxol Hcl & 140mcg of guaifensine

Linearity results for Guaifensine,Ambroxol Hcl & Loratadine with their respective chromatogram as in Linearity plot was also plotted among peak area and concentration and the response peak was found to be linear over the range of 6 to 14mcg of loratadine , 36 to 84 mcg of ambroxol Hcl & 60 to 140 mcg of guaifensine of targeted concentration.

Ref: 5 table & 7 fig

Accuracy

The accuracy of method was demonstrated by preparing recovery samples(spiking) with known quantities of Guaifensine,Ambroxol Hcl and Loratadine , at the level of 80, 100, 120 % of targeted concentration. Recovery samples are prepared on triplicate at each level, the samples at different levels are chromatographed and the percentage mean recovery for the amount added was calculated

Preparation of 80 % solution: 0.2ml of sample solution was pipette out & spiked with 0.8 ml of standard stock solution which gives the concentration of 100mcg of guaiphenesin, 60mcg of ambroxol hydrochloride & 10mcg of loratadine

Preparation of 100 % solution: 0.2ml of sample solution was pipette out & spiked with 1ml of standard stock solution which gives the concentration of 120mcg of guaiphenesin , 70mcg of ambroxol hydrochloride & 12mcg of loratadine

Preparation of 120 % solution: 0.2ml of sample solution was pipette out & spiked with 1.2 ml of standard stock solution which gives the concentration of 140mcg of guaiphenesin , 84 mcg of ambroxol hydrochloride & 14mcg of loratadine

Ref: Table 6.

Precision:

System precision: System suitability was demonstrated by injecting six replicate injections of standard solution as per test method and are chromatographed tabulated in table 7

Method Precision:

Method precision was demonstrated by preparing Six samples as per test method representing single batch and were chromatographed. The precision of the method was evaluated by computing the %RSD of the Retention time & peak area and were found as in table 8,

Limit of Detection: It Is Defined As Lowest Amount Of Analytic Which Can Be Detected In A Sample The Detection Limit Is Determined By Analysis Of A Sample With Known Concentration Of Analyte & By Establishing The Minimum Level At Which The Analyte Can Be Detected

These limits are normally applied to related substances in the drug substance or drug product. Specifications on these limits are submitted with the regulatory impurities method relating to release and stability of both drug substance and drug product.

$$\text{LOD} = \frac{3.3\sigma}{S}$$

Where, σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Limit of Quantification:

$$\text{LOQ} = \frac{10\sigma}{S}$$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Ref: Table-9

Robustness: robustness of a method is the measure of its capacity to remain unaffected by small but deliberate variation in method parameters & provide an indication of its relaibaility during its normal storage. Robustness was performed by using working standard concentration of Standard stock solution & varying the method condtions such as wavelength from 245nm to 243nm & 247. And flow rate is adjusted from 1ml/mint to 1.2ml/mint & 0.8l/mint.

Ref: tab 10

Ruggedness

This is to prove the lack of influence of operational & environmental variables on test method ruggedness is the measure of reproducibility of test results under the variation in conditions normally expected from analyst to analyst The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts

Ruggedness was performed by injecting the standard & sample solution of drug by two different analysts & calculate the percentage assay & % RSD

Ref: tab-11

Stability indicating forced degradation studies

Standard solution of GUAI, AMB and LOR is prepared & is subjected to stress conditions in presence of alkali, acid, high temperature & photolytic effects

Hydrolytic degradation under acidic condition:

subjecting the drug content in 3ml of 0.1 N HCl at normal condition for 3 hr and then the mixture was neutralized with 0.1 N NaOH

Hydrolytic degradation under alkaline condition:

subjecting the drug content in 3ml of 0.1N NaOH at normal condition for 3 hr. and then the mixture was neutralized with 0.1N Hcl

Thermal induced degradation: it was performed by refluxing the drug content for 3 hr at 60^0c temperature

Photolytic degradation: it was performed by subjecting the drug content to uv light for about 3hr.

Percentage recovery & percentage degradation was calculated for the stressed sample solution & is compared with that of standard unstressed solution.

Ref:tab-12

Results & Discussion

Assay:

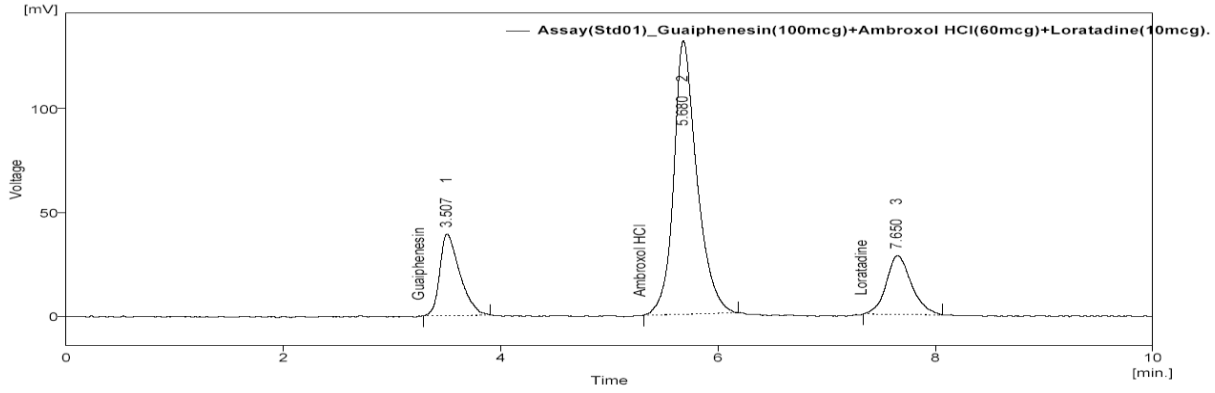

Fig-6: Assay standard injection.

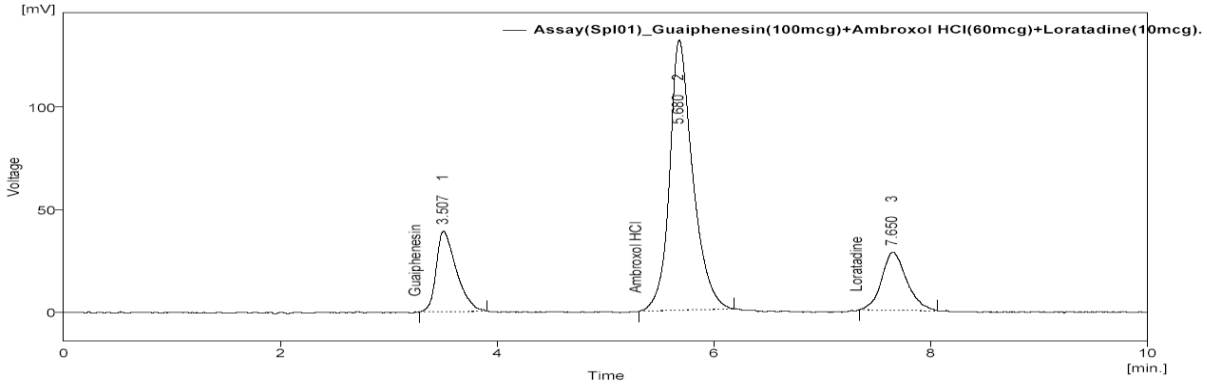

Fig-5: Assay sample injection.

Table 3: **Assay Results.**

Parameters	**Guaiphenesin**	**Ambroxol HCl**	**Loratadine**
Standard Area avg	502.850	1968.245	458.4458
Sample area avg	503.811	1967.583	465.1908
Tablet avg weight	5	5	5
Standard weight	50	30	5
Sample weight	5	5	5
Label amount	50	30	5
std.purity	99.2	99.3	99.3
Cal.:	**49.69**	**29.78**	**5.04**
%Assay	**99.39**	**99.27**	**100.76**

Acceptance value: 90-110 %

Discussion: The amount of AMBROXOL HCl, GUAIFENSINE AND LORATADINE present in the taken dosage form was found to be 99.27 % and 99.39% and 100.76 % respectively.

System suitability

Tab-4: System suitability.

S.NO	**Drugs**	**Mean of RT**	**Mean of peak area**	**Std dvt of RT**	**Std dvt of peak area**	**%RSD of Rt**	**%RSD of Peak area**	**Theoretical plates (TP)**	**Tailing factor (TF)**
1	**GUAI**	3.5112	504.151	0.0082	6.040	0.23	1.20	37613	1.926
2	**AMB**	5.686	1969.290	0.013	14.723	0.23	0.75	38073	1.518
3	**LOR**	7.649	460.689	0.017	4.390	0.22	0.95	56287	1.313

Acceptance criteria

1. The % RSD for the retention times of all three drugs Peaks from 6 replicate injections of each Standard solution should be not more than 2.0 %
2. The % RSD for the peak area responses of all three drugs peaks from 6 replicate injections of each standard solution should be not more than 2.0%.
3. The number of theoretical plates (N) for the three drugs peaks peaks is not less than 2000.
4. The Tailing factor (T) for the three drugs peaks peaks is not more than 2.0.

Discussion

The % RSD for the retention times and peak area of all three drugs were found to be less than 2%. The plate count and tailing factor results were found to be satisfactory and are found to be within the limit.

Hence it can be concluded that system suitability parameters meets the requirements of method validation

Linearity

Table-5: linearity of Guaiphensine, Ambroxol Hcl and Loratadine.

linearity of Guaiphensine			linearity of Ambroxol Hcl		linearity of Loratadine	
S.No.	**Conc.(µg/ml)**	**Area**	**Conc.(µg/ml)**	**Area**	**Conc.(µg/ml)**	**Area**
1	60	304.033	36	1181.540	6	314.128
2	80	399.371	48	1585.082	8	392.201
3	100	548.118	60	2164.007	10	522.492
4	120	682.836	72	2593.381	12	610.296
5	140	789.865	84	3095.008	14	717.025

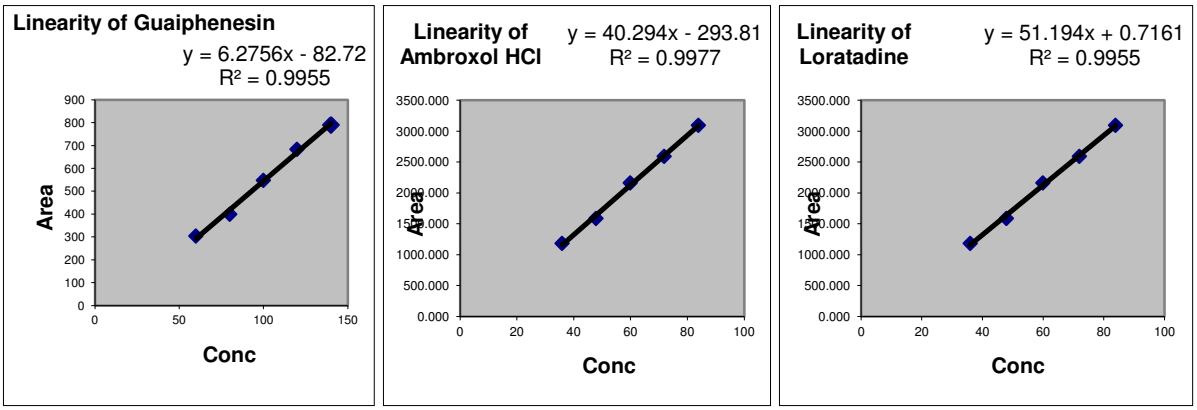

Fig-7: Linearity graphs.

Acceptance criteria

N.L.T=0.99%

Discussion: The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of Guaiphensine, ambroxol Hcl and Loratadine is 0.995, 0.997 and 0.9955. The relationship between the concentration of Guaiphensine, ambroxol Hcl and Loratadine and area of Guaiphensine, ambroxol Hcl and Loratadine is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits

Accuracy

Table-6: Recovery study of GUAI, AMB and LOR.

Drug	concentration	Std Area	Recovery level	Amt taken(mcg/ml)	Avg area	Amount recovered (mcg/ml)	%Recovery	Average %Recovery
GUAI	80	399.371	80	100	502.752	100.71	100.71	**99.68%**
	100	548.118	100	120	654.091	119.33	99.44	
	120	682.836	120	140	785.012	137.96	98.54	
AMB HCl	48	1585.082	80	60	1968.254	59.60	99.34	**99.4%**
	60	2164.007	100	72	2605.660	72.25	100.34	
	72	2593.381	120	84	2980.861	82.76	98.52	
LOR	8	392.20	80	10	484.821	9.89	98.89	**99.56%**
	10	522.492	100	12	631.261	12.08	100.68	
	12	610.296	120	14	708.306	13.93	99.48	

Acceptance criteria: The % recovery should lie between 98% and 102%.

Discussion: The percentage mean recovery is 99.56%, 99.4% and 99.68% for GUAI, AMB and LOR respectively.

Precision: System Precision:

Table-7: system precision of GUAI, AMB & LOR.

System Precision						
Drugs	**Avg of RT**	**Avg of peak area**	**St dev of RT**	**St dev of Peak area**	**% RSD of RT**	**% RSD of peak area**
GUAI	3.5088	499.316	0.0039	5.207	0.11	1.04
AMB	5.663	1961.386	0.046	24.110	0.82	1.23
LOR	7.6678	460.689	0.0098	4.390	0.13	0.95
%RSD- NMT2.00%						

Table-8: Method precision of GUAI, AMB & LOR.

Method precision						
Drugs	**Avg of RT**	**Avg of peak area**	**St dev of RT**	**St dev of Peak area**	**% RSD of RT**	**% RSD of peak area**
GUAI	3.5078	503.261	0.0028	4.771	**0.08**	**0.95**
AMB	5.678	1968.724	0.003	7.233	**0.05**	**0.37**
LOR	7.636	460.689	0.008	4.390	**0.10**	**0.95**
%RSD- NMT2.00%						

Discussion: Test results are showing that the %RSD of Assay results are within limits. The results were shown in table

LOD & LOQ

Tab-9: Limit of detection & limit of quantification.

	Guaiphenesin		**Ambroxol HCl**		**Loratadine**	
	mcg	**Area**	**mcg**	**Area**	**mcg**	**Area**
	60	304.033	36	1181.540	6	314.128
	80	399.371	48	1585.082	8	392.201
	100	548.118	60	2164.007	10	522.492
	120	682.836	72	2593.381	12	610.296
	140	789.865	84	3095.008	14	717.025
SD	31.6	199	18.974	765	3.2	162
Slope	6.27		40.294		51.19	
	mcg	Area	mcg	Area	mcg	Area
LOD	16.63	104.68	1.55	62.69	0.20	10.46
LOQ	50.44	317.22	4.71	189.96	0.62	31.70

Discussion:

LOD for this method was found to be 16.63 µg/ml & area 104.68 for **Guaiphenesin** and 1.55 µg/ml & area 62.69 for Ambroxol Hcl and 0.20 µg/ml & area 10.46

The LOQ for this method was found to be 50.44 µg/ml & area 317.22 for **Guaiphenesin** and 4.71 µg/ml & area 189.96 for Ambroxol Hcl and 0.62 µg/ml & area 31.70

Robustness

Table-10: Result of Robustness study.

Parameter	Guaifensine		Ambroxol Hcl		Loratadine	
	Retention time(min)	Tailing factor	Retention time(min)	Tailing factor	Retention time(min)	Tailing factor
Flow Rate						
0.8 ml/min	4.353	1.961	7.070	1.672	9.513	1.358
1.2 ml/min	2.947	1.941	4.770	1.500	6.417	1.259
1ml/min	3.513	1.975	5.667	1.564	7.660	1.254
Wavelength						
243nm	3.510	1.900	5.703	1.491	7.677	1.308
247 nm	3.507	1.974	5.690	1.500	7.647	1.279
245nm	3.513	1.975	5.667	1.564	7.660	1.254

Discussion: From the observation it was found that the system suitability parameters were within limit at all variable conditions.

Ruggedness

Acceptance criteria:

The % Relative standard deviation of Assay values between two analysts should be not more than 2.0%.

Table 11: Results for Ruggedness.

Guaiphensine	%Assay	Ambroxol Hcl	%Assay	Loratadine	%Assay
Analyst 01	99.41	**Analyst 01**	100.02	**Analyst 01**	99.41
Analyst 02	100.02	**Anaylst 02**	99.21	**Anaylst 02**	100.02
%RSD	0.45%	**%RSD**	0.311%	**%RSD**	0.45%

Discussion: From the observation the %RSD between two analysts Assay values not greater than 2.0%, hence the method was rugged.

Stability indicating forced degradation studies.

Tab-12: stability studies of GUAI, AMB &LOR.

Deg parameters	**Deg time**	**Guaifensine**			**Ambroxol Hcl**			**Loratadine**		
		Peak area of degraded product	Peak area of Std	% deg	Peak area of degraded product	Peak area of standard	% deg	Peak area of degraded product	Peak area of standard	% deg
Acid degradation (0.1 N Hcl)	3 hr	493.836	548.118	9.9	1949.450	2164.007	9.9	456.360	522.492	12.6
Base degradation (0.1N NaoH)	3 hr	502.845	548.118	8.2	1993.305	2164.007	7.8	479.399	522.492	8.2
Thermal degradation	3 hr	527.851	548.118	3.6	2052.678	2164.007	5.1	500.124	522.492	4.2
Photolytic degradation	3 hr	510.344	548.118	6.8	1990.471	2164.007	8.0	471.478	522.492	9.7

Acceptance criteria: % degradation should NMT- 28%

Discussion: The percentage degradation calculated was within the limits. Thus the method developed is stable & the drug determination is not effected by shelf life or storage of drug.

Conclusion

The Newly Developed Analytical Method For The Simultaneous Estimation Of LORATADINE, AMBROXOL HYDROCHLORIDE & GUAIPHENESIN Was Found To Be Simple, Precise, Accurate And High Resolution And Shorter Retention Time Makes This Method More Acceptable And Cost Effective.

Since All The Acceptance Criteria Of The Parameters Selected For Validation Are Satisfied, The Method Is Validated & Also The Method Developed Is Found To Be Stable Under Forced Degradation Studies & is not effected by storage or shelf life of the drug. The Developed Method Was Found To Be Accurate, Reliable & is found to be a promising approach that can be employed for estimation of Lorataidne, Ambroxol Hydrochloride and Guaiphenesin in Bulk Drug and Pharmaceutical Liquid Dosage form.

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