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Analytical Method Development and Stability Indicating an RP-HPLC Method for Determination of Mebeverine HCl and Chlordiazepoxide

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Keywords: Mebeverine HCl, Chlordiazepoxide, method development, stability indicating, RP HPLC methods

ABSTRACT

The study describes method development and subsequent validation of an RP-HPLC method for simultaneous estimation MEB, CDZ, in bulk and combined tablet dosage forms. A gradient mobile phase system consisting of (520:320:160:0.7) buffer, methanol, acetonitrile, N-hexylamine. Using phosphate buffer (PH 4.5 adjusting with orthophosphoric acid). The chromatographic separation achieved on a column Inertsil C_8 (250×4.6mm, 5µm) flow rate 1.0 ml/min detection of wavelength 254nm. By using UV detector. Results the development method result Mebeverine HCl elute 4.62min Chlordiazepoxide elute 8.83min. Mebeverine HCl exhibit linearity in the range 1350µg/ml. while Chlordiazepoxide exhibit linearity in the range 20µg/ml. the precision exemplified by relative slandered deviation Mebeverine HCl 0.650%, and Chlordiazepoxide 0.769%, percentage mean recoveries were found to be in the range 97.99% to 101.95%. During accuracy studies. The limit of detection (LOD) Mebeverine HCl 14.021 µg/ml and Chlordiazepoxide 0.439 µg/ml was found to be respectful. The limit of quantitation was found to be Mebeverine HCl 42.88 µg/ml and Chlordiazepoxide 1.33 µg/ml respectively. Conclusion. The developed stability indicating RP-HPLC method was found to be simple, accurate, sensitive, precise, specific and rapid. This method can be applied for routine quantitative analysis of Mebeverine HCl hydrochloride and Chlordiazepoxide in bulk and pharmaceutical formulations like tablets. This method was also capable to separate the degradation product of both drugs hence it can be used to check the quality of the product after different storage condition and in stress degradation study.

INTRODUCTION

Mebeverine hydrochloride is chemically 3,4-dimethoxybenzene acid 4[ethyl[2- (4-methoxyphenyl)-1-methylethyl]amino]-butyl ester hydrochloride, mol formula $C_{25}H_{36}CINO_5$, mol weight 466.01, melting point 235-240c, very soluble in water, this belongs to a group of medicines called antispasmodics. This medicine is used to treat symptoms of irritable bowel syndrome (IBS). It acts as a musculotropic antispasmodic agent with a direct action on the smooth muscle of the gastrointestinal tract especially of the colon. Mebavarine is an act anticholinergic .it appears to work directly on smooth muscle within the gastrointestinal tract and may have an aesthetic effect, affect the calcium channels, and may affect muscarinic receptors. Chlordiazepoxide is 7-chloro-N-methyl–5–phenyl-3H-1, 4– benzodiazepine–2–amine 4–oxide (BZD) mol formula $C_{16}H_4CIN_3O$ mol weight 299.75, melting point 134-136c, very soluble in acetone, benzodiazepine class it is used to treat anxiety insomnia and withdrawal symptoms from alcohol and or drug abuse. BZD enhance the effect of the neurotransmitter gamma-amino butyric acid (GABA) at the (GABAa) receptor, produce hypnotic anxiolytic anticonvulsant and muscle relaxant properties. Fixed dose combination containing Mebeverine Hydrochloride (135mg) and Chlordiazepoxide (5mg) is available in tablet form in the market. This combination therapy was shown to be the anti spasmodic agent and analytical research and development of fixed-dose combination are found to be very interesting and challenging job, hence the development of stability indicating method for Mebeverine Hydrochloride and Chlordiazepoxide in combination has been selected for the present study. A detailed literature survey reveals that there exists .literature concerning analytical method development and validation for individual drugs MEB and CHL. while there is only literature reported on RP-HPLC method development for the simultaneous quantitative estimation of MEB and CHL in the pharmaceutical dosage form. Hence explored in developing a new accurate precise and linear study RP-HPLC method for the simultaneous in validating as per ICH guideline.

MATERIALS

Healthy Pharma Pvt. Ltd. (Maharashtra, India) supplied Mebeverine Hydrochloride- Working standard grade and its claimed purity was 99.92% and 99.82% respectively. Chlordiazepoxide – working standard grade was supplied by Shreeji Pharma international Pvt. Ltd. (Vadodara, India) and its claimed purity was 99.86%.Water (HPLC grade), Methanol (HPLC grade), Spectrochem, Ortho phosphoric Acid (HPLC grade), Merck, INDIA

N-hexylamine (HPLC grade), Merck, INDIA Acetonitrile (HPLC) Grade Rankem, INDIA, Shimadzu HPLC system column inertsil C8 (5 µm, 150 mm × 4.6 mm i.d.),Pump LC-20 AT solvent delivery system, detectorSPD-20A UV-Visible detector, Data processor Spin chrome CFR version 2.4.1.93Rheodyne injector, Manufacturer: Shimadzu, Japan, Analytical Balance (Max. 200 gm-Min. 0.0001 mg) Model: TE 2145Manufacturer: Sartorius, India. A double beam UV-Visible spectrophotometer having two matched cells with 1cm light path, Model: Pharmspec-1700 Manufacturer: Shimadzu, Japan, PH Meter Model: 7007 Manufacturer: Digi sun electronics, Hot air oven Model: PSM 03 Manufacturer: Thermo electrical co, Ultrasonicator Model: RC-SYSTEM MU-1700Supplier: Serve well instruments, Distillation Apparatus Model: VQMD 2.5L Manufacturer: Srinivas products.

Preparation of Stock solution:

For Mebeverine HCl (1350µg/ml):

An accurately weighed quantity of Mebeverine HCl working/reference standard about 135 mg was transferred into 100 ml volumetric flask and makeup to mark with methanol (1350µg/ml).

For Chlordiazepoxide (200µg/ml)

An accurately weighed quantity of Chlordiazepoxide working/reference standard about 20 mg was transferred into 100 ml volumetric flask and makeup to mark with methanol (20µg/ml).

The combined standard solution I of Mebeverine HCl (270 µg/ml) and Chlordiazepoxide (10 µg/ml) Pipette out 20ml of standard stock solution I and 5ml of standard stock solution II into the 100ml volumetric flask with mobile phase

Sample preparation (50 µg/ml):

Weigh five tablets and transfer into 250ml of volumetric flask, add 180ml of methanol and sonicate 45min and cool to room temperature. Add 2ml of 2M Hydrochloric acid and immediately make up with water. Further, dilute 5ml into 50ml with mobile phase.

Chromatographic conditions:

The finalized HPLC system specifications are shown in below table

Finalised HPLC system specification

Column	Inertsil C_8 (250×4.6mm, 5µm)
Mobile phase	Buffer : Methanol : ACN : n-hexylamine(520 :320 :160:0.7)
pH of the mobile phase	4.5 using ortho phosphosphoric acid
Wavelength	254nm
AUFS	0.1000
Pressure	180kgf

System Suitability

Combined standard solutions of Mebeverine HCl (270 µg/ml) and Chlordiazepoxide (10 µg/ml) were prepared and analyzed six times. Chromatograms were studied for different parameters such as tailing factor, resolution and theoretical plates to see that whether they comply with the recommended limit or out of recommended limit.

Method validation of RP-HPLC Method

Accuracy

To study the accuracy, 10 tablets were weighed and powered. Analysis of the same was carried out as mentioned in section 4.3.7. Recovery studies were carried out by standard addition method by adding the known amount of Mebeverine HCl and Chlordiazepoxide separately to the pre analyzed sample at three different concentration levels i.e. 80%, 100%, and 120% of assay concentration and percent recoveries were calculated.

Precision

The precision of an analytical method.

Repeatability

The precision of an analytical procedure is the degree of agreement among the individual test result when the procedure is the applied repeatedly to multiple sampling of a homogenous sample. The sample solution of Mebeverine and Chlordiazepoxide prepared by following concentration Mebeverine and Chlordiazepoxide (270mcg and 10mcg).

Reproducibility

Sample and standard solution were prepared and analyzed by Analyst 1 and Analyst 2, separately. The values obtained were evaluated using F-test and t-test to verify their reproducibility.

Linearity and Range

The concentration ranges of 135-405 µg/ml for Mebeverine and 5-15 µg/ml for Chlordiazepoxide were prepared and analyzed. From the data, linearity and range were the determination

Limit of Detection and Limit of Quantitation

Detection limit and quantitation limit were determined based on the standard deviation of y-intercepts of six calibration curves and the average slope of six calibration curves.

$$LOD = 3.3 \times \frac{\text{Standard Deviation of y} - \text{Intercepts of Six Calibration Curves}}{\text{Average Slope of Six Calibration Curves}}$$

$$LOQ = 10 \times \frac{\text{Standard Deviation of y} - \text{Intercepts of Six Calibration Curves}}{\text{Average Slope of Six Calibration Curves}}$$

The effect of the change in the pH of mobile phase and flow rate on the retention time, tailing factor, theoretical plates and resolution were studied. Combined standard solutions of Mebeverine HCl (270 µg/ml) and Chlordiazepoxide (10 µg/ml) were prepared and analyzed at different pH (4.3, 4.5, 4.8) of the mobile phase and at the different flow rate (1.0, 1.20, 1.4 ml/min).

Forced degradation studies of tablets:

For acid degradation:

Weighed 5 and transferred into three sets of 250 ml round bottom flasks. About 20 ml of HCl of different strengths (0.1N) was the addition of diluents to the volume all flasks and refluxed on the heated mantle for 3 hr at 50 ºC and after 3 hour cool to room temp and add 0.1M NaOH For neutralizing. Further, dilute 5ml into50ml proceed same as sample and placebo with active.

For basic degradation:

Weighed 5 and transferred into three sets of 250 ml volumetric flasks. About 20 ml of 1M NaOH and an addition of diluent to the volume all flasks and refluxed on a heated mantle for 3 hr at 50 ºC and after 3 hours cool to room temp and add 0.1M HCl For neutralizing. Further, dilute 5ml into50ml proceed same as sample and placebo with active

For peroxide degradation:

Weighed 5 and transferred into three sets of 250 ml volumetric flasks. About 20 ml of H_2O_2 of different strengths (3%) was added to all flasks and refluxed on a heated mantle for 1 hr at 60 ºC.

For Thermal degradation

Before doing the analysis tablets expose in the hot air oven at 105 ºC for 24houes. Weigh 5 Tablets and transferred into 250ml volumetric flask continue same Assay procedure.

For Thermal/Humidity degradation:

Before doing the analysis, tablets should be store in the stability chamber at 40ºC/75%RH for one week. Weigh 5 Tablets and transferred into 250ml volumetric flask continue same Assay procedure.

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions:

The mixed solution of Mebeverine HCl (270 µg/ml), Chlordiazepoxide (10 µg/ml) was prepared and injected into the HPLC system. The solution was analysed using different mobile phases like Buffer: Methanol: Acetonitrile: n-hexylamine (52:43:5:0.7) pH 4.5 in C8 Column, Buffer: Methanol: Acetonitrile: n-hexylamine (52:32:10:0.7) in 1.2ml flow pH 4.5 in C18 Column, Buffer: Methanol: Acetonitrile: n-hexylamine (64:10:28:0.7) pH 4.5 in C8 Column. Buffer: Methanol: Acetonitrile: n-hexylamine (52:32:16:0.7) in pH 4.5 C8 column in flow 1.2 it was found the good result, we confirmed and choosing that chromatographic condition for Mebeverine HCl and Chlordiazepoxide tablets.

Selection of Analytical Wavelength

The mixed solution of Mebeverine HCl (270 µg/ml), Chlordiazepoxide (10 µg/ml) MP were scanned in the UV region of 400 to 190 nm using mobile phase as blank and the overlain spectra were recorded. It was observed that all the three drugs observed prominently at 254 nm, hence this wavelength was used for the measurement of absorption.

CONCLUSION

The developed stability indicating RP-HPLC method was found to be simple, accurate, sensitive, precise, specific and rapid. This method can be applied for routine quantitative analysis of Mebeverine HCl hydrochloride and Chlordiazepoxide in bulk and pharmaceutical formulations like tablets. This method was also capable to separate the degradation product of both drugs hence it can be used to check the quality of the product after different storage condition and in stress degradation study.

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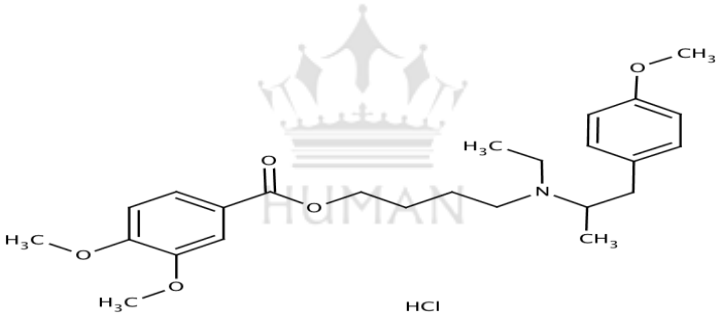


Fig. 1: Structure of Mebeverine hydrochloride

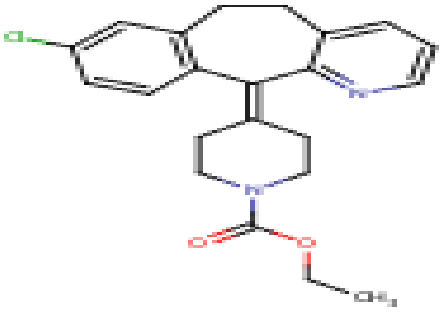


Fig. 2: Structure of Chlordiazepoxide

SYSTEM SUITABILITY

Results of System Suitability Parameters

Analyse	Retention Time* (min)	Tailing Factor* (T)	Theoretical Plates* (N)	Resolution* (R)
MEBEVERINE HCl	4.65	1.1	4537	--
CHLORDIAZEPOXIDE	8.84	1.3	3890	4.942
Required limits	--	T < 2	N > 2000	R >2

Mobile Phase Composition (Buffer: ACN :MeOH: n-hexylamine) %v/v	pH	Column	Flow rate ml/min	Drug	R_t (min)	Tailing Factor	Theoretical Plates	Theoretical Plates/250 mm	Resolution
52:32:16:0.7	4.5	C_8	1	Mebeverine HCl	2.7	1.118	7753	31012	--
				Chlordiazepoxide	2.5	0.510	2595	10380	1.586
52:32:16:0.7	3	C_8	1	Mebeverine Hcl	2.6	1.756	8569	20782	1.586
				Clordiazeboxide	1.8	0.567	7825	50652	1.965
52:32:16:0.7	2	C_8	1	Mebeverine HCl	2.8	1.765	6352	56324	1.689
				chlordiazeboxide	2.8	0.715	7526	45636	1.626
52:32:16:0.7	7	C_8	1	Mebeverine Hcl	3.1	1.474	7324	29295	1.4526
				chlordiazepoxide	3.5	1.654	7145	28579	1.373

Mobile Phase Composition (Buffer :ACN :MeOH: n-hexylamine) %v/v	PH	Column	Flow rate min/ml	Drug	RT	Tailing Factor	Theoretical plate	Resolution
52:32:16:0.7	4.5	C8	1.2	Mebeverine HCl	4,.65	1.2	4564	4.58
				Chlordiazepoxide	8.84	1.4	3456	
52:43:5:0.7	4.5	C8	1	Mebeverine HCl	7.5	1.6	2378	0.9
				Chlordiazepoxide	5.5	1.7	3424	
52:32:16:0.7	4.5	C18	1.2	Mebeverine HCl	0.7	1.5	3009	12.8
				Chlordiazepoxide	21.74	1.8	2135	
72:12:16:0.7	4.5	C8	1.2	Chlordiazepoxide	1.4	0.9	2546	-
64:10:28:0.7	4.5	C8	1.2	Mebeverine Hcl	18.8	1.3	3567	-
52:32:16	7	C8	1.2	Mebeverine Hcl	8.1	1.3	3876	3.1
				Chlordiazepoxide	10.6	1.8	2890	

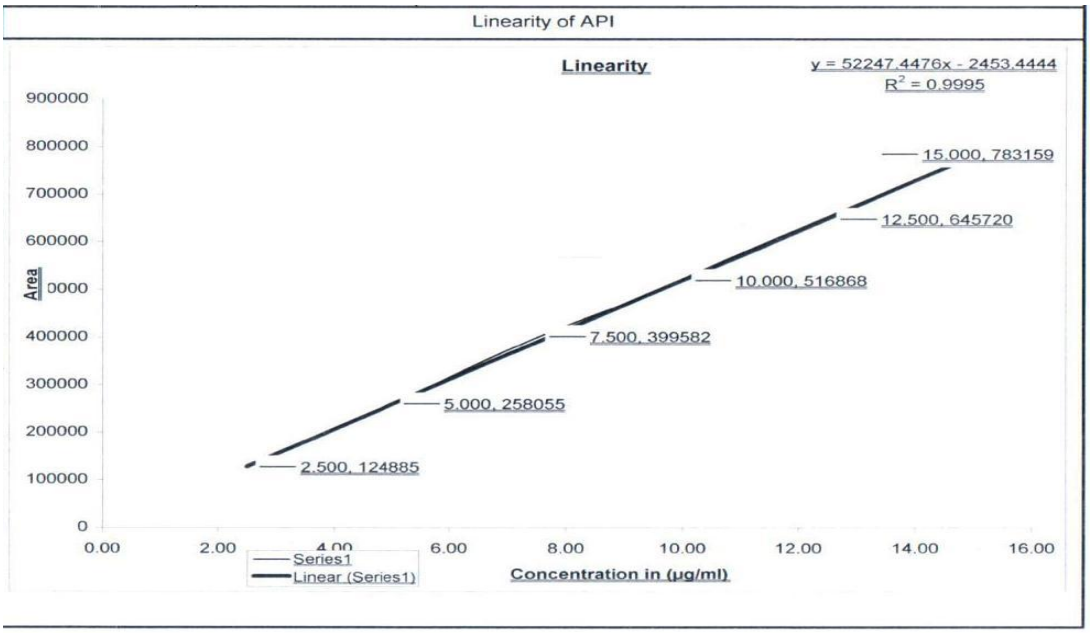
ACCURACY Results of Accuracy by RP-HPLC Method

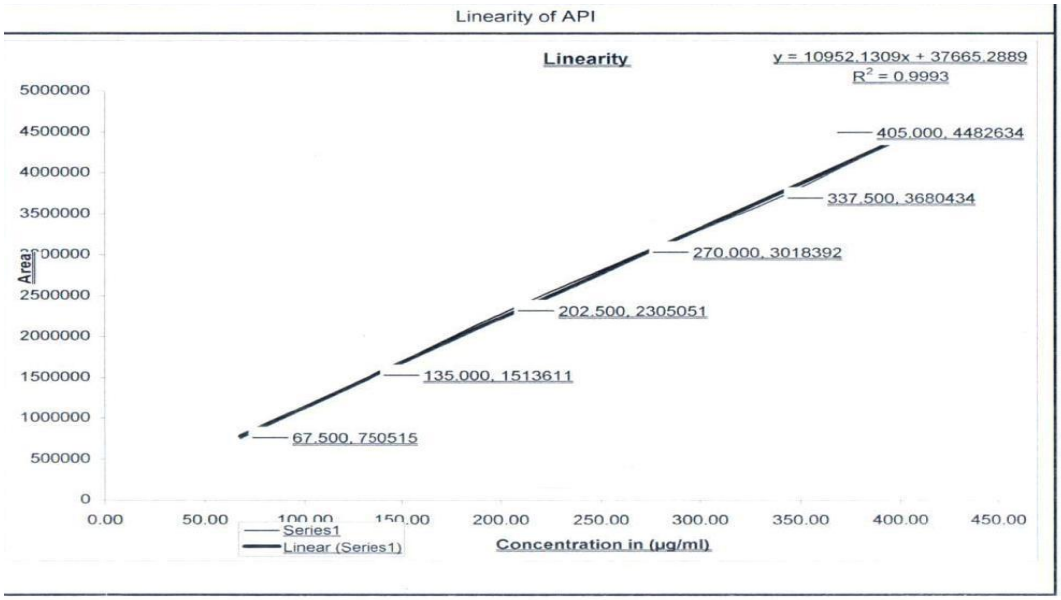
Level of % Recovery	Sr. No.	Label claim (µg/tab)		Concentration (µg)		Amount of Standard Drug Added (µg)		Total Amount Recovered (µg)		%Recovery	
		MEB	CHL	MEB	CHL	MEB	CHL	MEB	CHL	MEB	CHL
80%	1	135	5	216.0	8	540	20	529.18	20.12	97.9975	100.626
	2	135	5	216.0	8	540	20	535.47	19.66	99.1605	99.8329
	3	135	5	216.0	8	540	20	550.57	19.87	101.9583	99.3736
100%	1	135	5	270	10	675	25	680.200	24.54	100.7705	98.1904
	2	135	5	270	10	675	25	662.313	25.068	98.12058	100.2722
	3	135	5	270	10	675	25	680.823	24.987	100.8627	99.9498
120%	1	135	5	324	12	810	30	817.84	30.18	100.9681	100.626
	2	135	5	324	12	810	30	798.32	29.94	98.5596	99.8329
	3	135	5	324	12	810	30	806.33	29.81	99.5473	99.3736

Statistical Validation Data for Accuracy

Level of % Recovery	Mean* (% Recovery)		±SD		%RSD	
	MEB	CHL	MEB	CHL	MEB	CHL
80%	99.94	99.94	2.04	0.63	2.03	0.63
100%	99.47	99.95	1.56	1.12	1.57	1.13
120%	99.94	99.93	1.21	0.62	1.21	0.64

LINEARITY





PRECISION

Results of Method Precision of AMH for RP-HPLC Method

No.Sample Prep	LC in (mg)		Amount in mg		Amount in %	
	MEB	CHL	MEB	CHL	MEB	CHL
01	135	5	133.51	5.00	98.9	100.0
02	135	5	133.65	5.00	99.0	99.9
03	135	5	134.46	4.94	99.6	98.7
04	135	5	134.24	4.97	98.7	99.3
05	135	5	134.86	4.98	99.9	99.5
06	135	5	134.43	4.90	98.1	98.0
Mean Value			133.69	4.96	99.3	99.2
STDev			0.869	0.038	0.644	0.760
%RSD			0.650	0.769	0.650	0.769

1 Intermediate Process

Results of Intermediate Precision of AMH for RP-HPLC Method

No.Sample Prep	LC in (mg)		Amount in mg		Amount in %	
	MEB	CHL	MEB	CHL	MEB	CHL
01	135	5	134.25	5.01	98.7	100.1
02	135	5	133.78	4.99	99.1	99.8
03	135	5	135.00	4.98	100.0	99.6
04	135	5	133.28	4.95	98.8	98.9
05	135	5	133.78	4.94	99.1	98.8
06	135	5	134.59	4.90	99.7	98.1
Mean Value			133.96	4.96	99.2	99.2
STDev			0.692	0.037	0.512	0.746
%RSD			0.516	0.752	0.516	0.752

Result of Robustness for Variation in pH

PH	Analyse	Retention Time* (min)	Tailing Factor (T)	Theoretical Plates (N)	Resolution(R)
4.1	Mebeverine HCl	4.6	4500	1.1	-
	Chlordiazepoxide	8.8	3876	1.3	4.56
4.3	Mebeverine HCl	4.4	4497	1.2	-
	Chlordiazepoxide	8.2	3789	1.4	4.41
4.7	Mebeverine HCl	4.7	4400	1.3	-
	Chlordiazepoxide	9.0	3789	1.5	4.43

* % RSD was found to be less than 3 for each drug; #Mean of 3 Estimations

The result of Robustness for Variation in Flow Rate (ml/min)

Flow rate	Analyse	Retention Time* (min)	Tailing Factor (T)	Theoretical Plates (N)	Resolution(R)
1.1	Mebeverine HCl	5.0	4456	1.2	-
	Chlordiazepoxide	9.1	3876	1.1	4.50
1.3	Mebeverine HCl	4.8	4697	1.4	-
	Chlordiazepoxide	8.9	3789	1.3	4.49
1.7	Mebeverine HCl	4.3	4610	1.1	-
	Chlordiazepoxide	8.0	3679	1.5	4.46

* % RSD was found to be less than 3 for each drug; #Mean of 3 Estimations

FORCED DEGRADATION STUDY

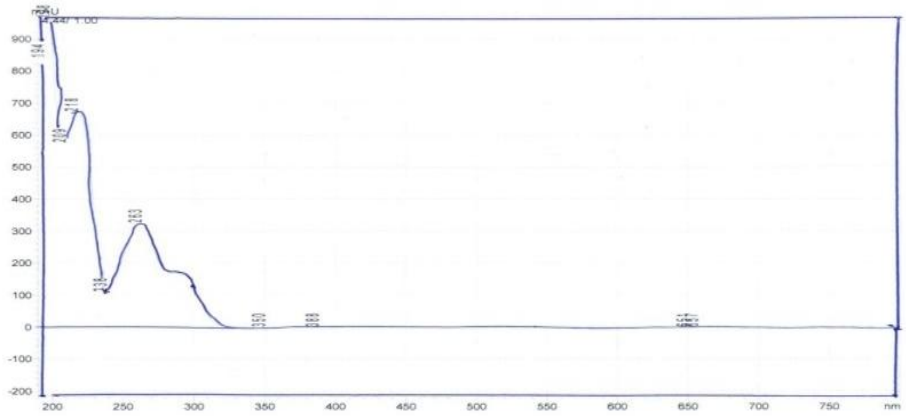
Results of forced degradation study of a mixture of standard drugs by the proposed RP-HPLC method

Stress condition/Strength duration	**Drugs**	**% Assay of drugs after degradation**	**% Degradation**
ACID/0.1M Hcl/3Hr	MEB	99.58	0.42
	CHL	99.86	0.14
BASIC/1M NaOH/3Hr	MEB	102.93	0
	CHL	97.95	2.05
OXIDATIVE/H2O2/1Hr	MEB	99.04	0.06
	CHL	85.81	14.59
THERMAL/105 C/24Hr	MEB	81.39	18.61
	CHL	79.42	20.48
THERMAL/HUMIDITY/40C/75%RH /24Hr	MEB	84.70	15.30
	CHL	81.36	18.64

Results of forced degradation study of tablet formulation by the proposed RP-HPLC method

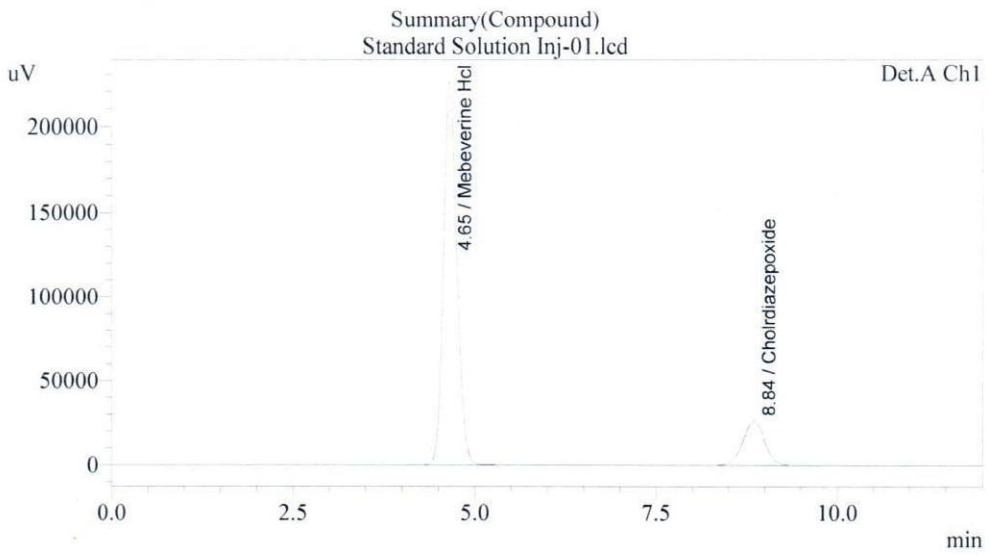
Stress condition/Strength duration	Drugs	% Assay of drugs after degradation	% Degradation
ACID/0.1M Hcl/3Hr	MEB	99.07	0.93
	CHL	98.31	1.69
BASIC/1M NaOH/3Hr	MEB	99.62	0.38
	CHL	97.93	2.37
OXIDATIVE/H2O2/1Hr	MEB	101.45	0
	CHL	85.95	14.05
THERMAL/105 C/24Hr	MEB	78.08	21.92
	CHL	79.90	20.10
THERMAL/HUMIDITY/40C/75%RH /24Hr	MEB	82.99	17.01
	CHL	79.42	20.58

Selection of Analytical Wavelength

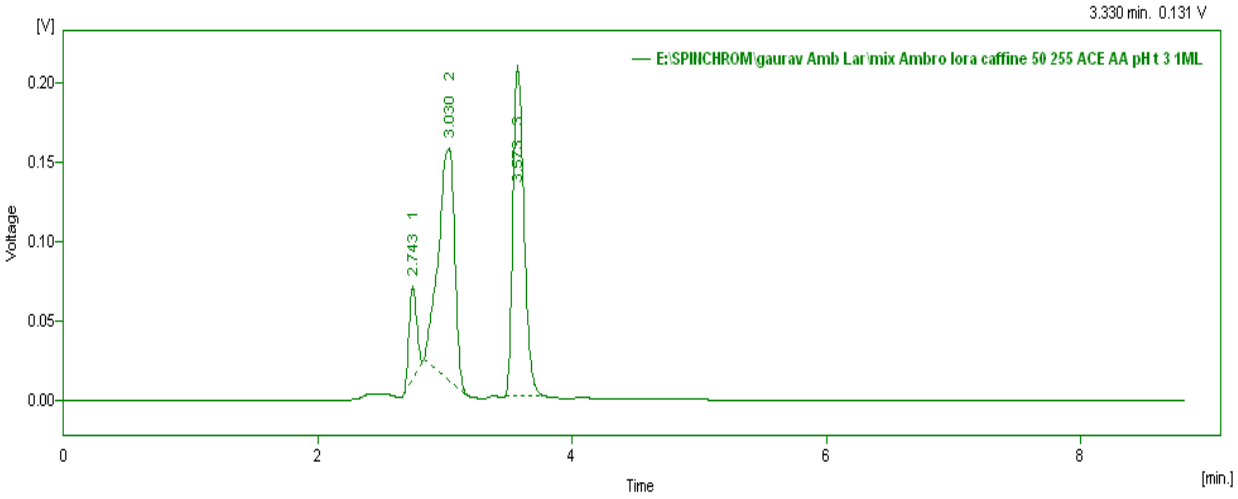


The mixed solution of Mebeverine HCl (270 µg/ml), Chlordiazepoxide (10 µg/ml) MP were scanned in the UV region of 400 to 190 nm using mobile phase as blank and the overlain spectra were recorded. It was observed that all the three drugs observed prominently at 254 nm, hence this wavelength was used for the measurement of absorption.

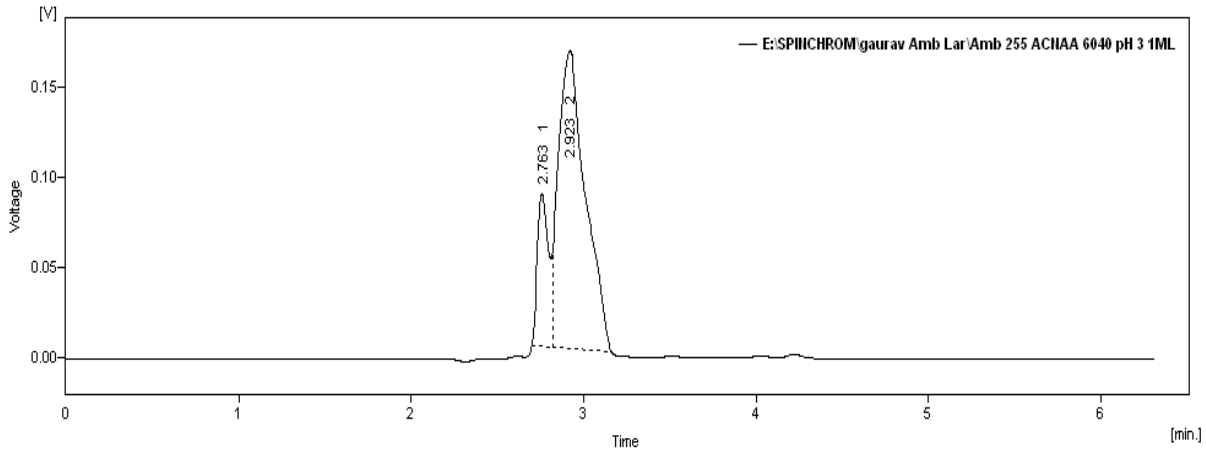
Chromatogram of Mebeverine HCl and Chlordiazepoxide in the standard buffer: Acetonitrile: methanol: n-hexylamine (52:32:16:0.7, pH 4.5) at Flow Rate 1.2ml/min, at 254 nm on C_8column



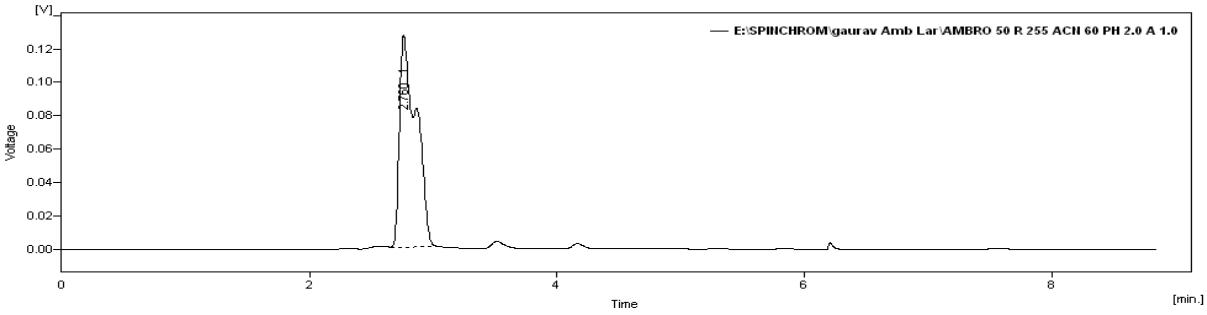
Chromatogram of Mebeverine HCl and Chlordiazepoxide Sample for buffer: Acetonitrile: methanol: n-hexylamine (52:32:16:0.7, pH 4.5) at Flow Rate 1 ml/min, at 254 nm on C_8 column



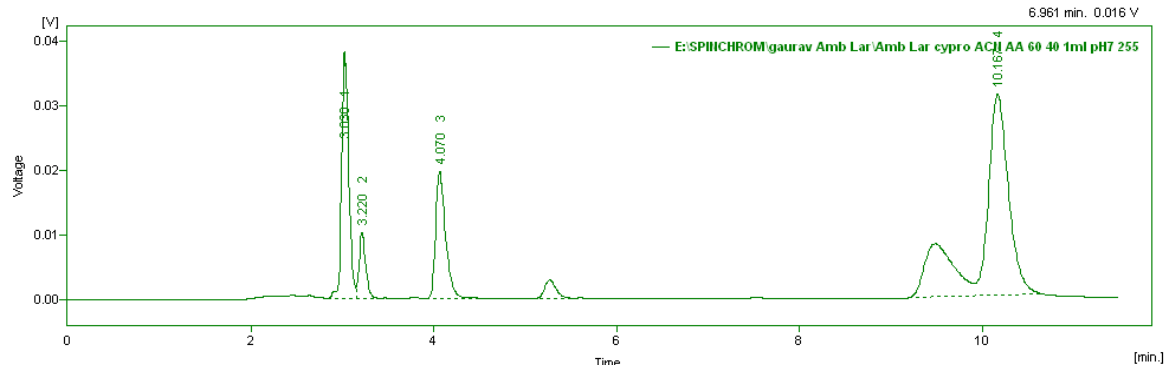
Chromatogram of Mebeverine Hcl and Chlordiazepoxide in buffer: Acetonitrile: methanol: n-hexylamaine (52:32:16:0.7, pH 7.0) at Flow Rate 1 ml/min, at 254 nm on C_8 column.



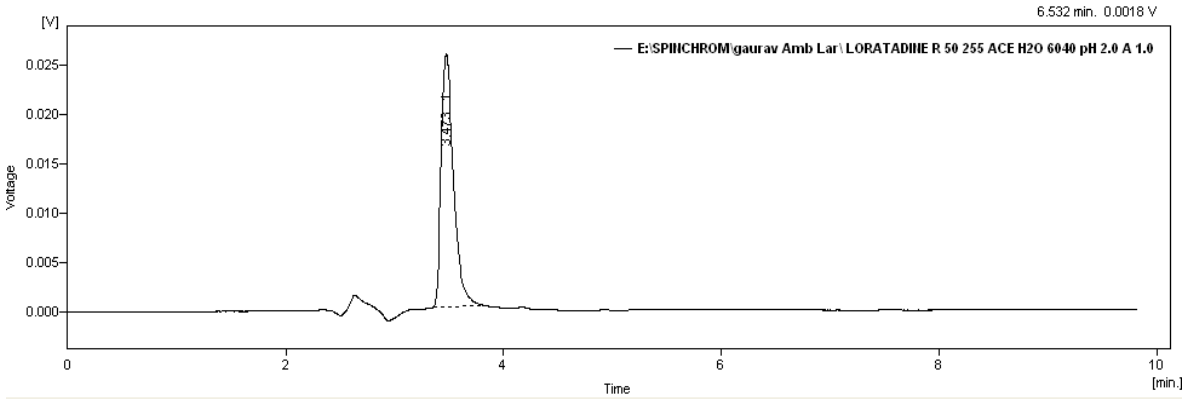
Chromatogram of Mebeverine Hcl in buffer: Acetonitrile: methanol: n-hexylamaine (52:32:16:0.2, pH 3.0) at Flow Rate 1 ml/min, at 254 nm on C_8 column.



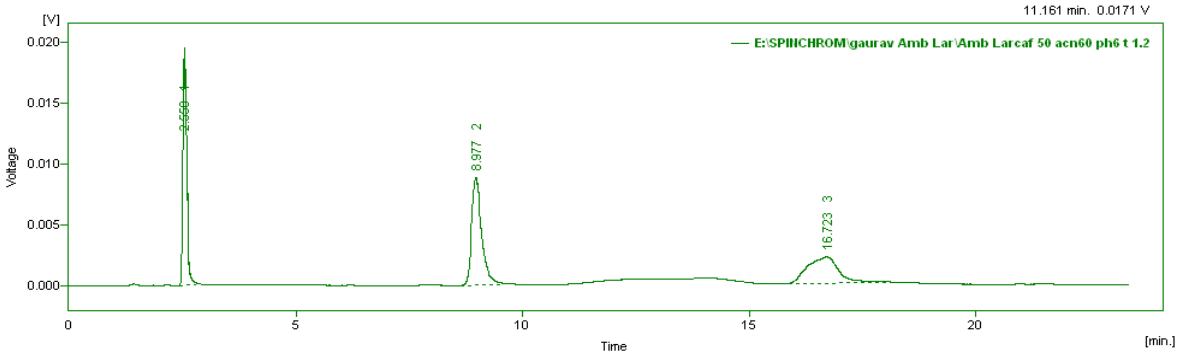
Chromatogram of Mebeverine Hcl in buffer: Acetonitrile: methanol: n-hexylamaine (52:32:16:0.7, pH 2.0) at Flow Rate 1 ml/min, at 254 nm on C_8 column.



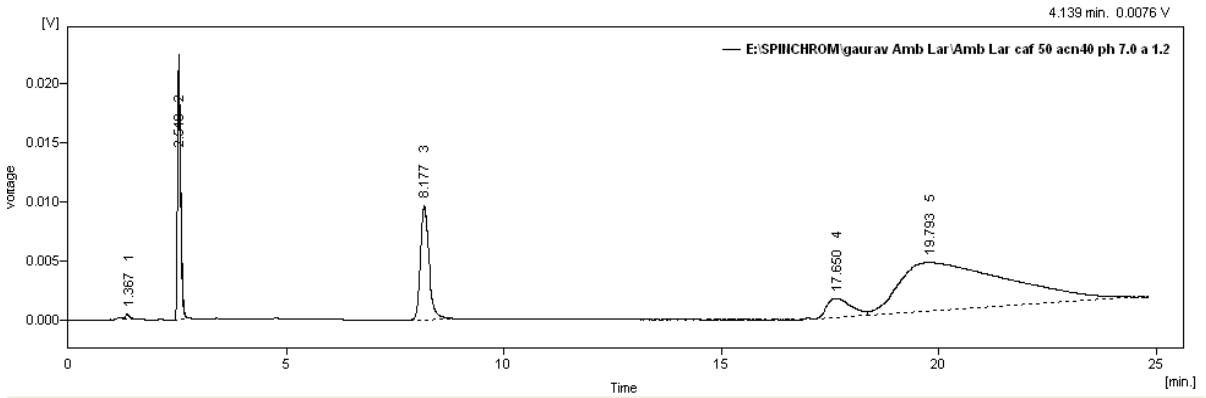
Chromatogram of Mebeverine Hcl in buffer: Acetonitrile: methanol: n-hexylamaine (52:43:5:0.7, pH 2.0) at Flow Rate 1 ml/min, at 254 nm on C_8 column.



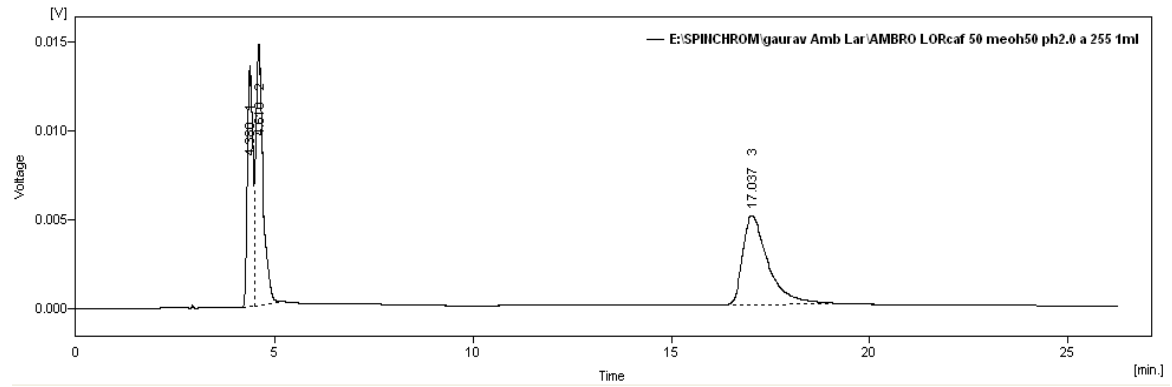
Chromatogram of Chlordiazepoxide Hcl in buffer: Acetonitrile: methanol: n-hexylamaine (72:12:16:0.7, pH 2.0) at Flow Rate 1 ml/min, at 254 nm on C_8 column



Chromatogram of Mebeverine HCl and Chlordiazepoxide Blank peaks in the buffer: Acetonitrile: methanol (52:32:16:0.7, pH 7.0) at Flow Rate 1 ml/min, at 254 nm on C_8 column



Chromatogram of Mebeverine HCl and Chlordiazepoxide Blank peaks in the buffer: Acetonitrile: methanol (72:12:16:0.7, pH 7.0) at Flow Rate 1 ml/min, at 254 nm on C_8 column



Chromatogram of Mebeverine HCl and Chlordiazepoxide Blank peaks in buffer: Acetonitrile: methanol (72:12:16:0.7, pH 7.0) at Flow Rate 1 ml/min, at 254 nm on C_{18} column