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ORIGINAL ARTICLE **OPEN ACCESS**

Simultaneous Estimation and Validation of Meclizine Hydrochloride and Caffeine in Bulk and Tablet Dosage Form By RP-HPLC

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ABSTRACT
A Simple, selective, rapid, sensitive and validated RP-HPLC (High Performance Liquid Chromatography) method has been developed for separation and analysis of meclizine hydrochloride and caffeine in bulk and in tablet dosage form. Separation was achieved on a reversed-phase Cosmosil C18, (250×4.6ID, 5µ) using a mobile phase Methanol: Water (65:35 v/v) at flow rate of 0.9 ml/min. The pH was adjusted to pH 3 by the addition ortho phosphoric acid (OPA). The samples were detected using a UV detector at 225 nm. The chromatographic conditions yield good separation between drugs with retention time (RT) 3.846 for meclizine hydrochloride and 5.825 for caffeine. Proposed method was validated according to ICH guidelines, which include linearity, precision, accuracy, robustness. The result obtained were within the acceptance criteria as per ICH guidelines.
Keywords: *HPLC, meclizine hydrochloride, caffeine, Methanol, water, Reverse Phase Chromatography, Validation.*

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INTRODUCTION

Analytical chemistry is the science of obtaining, processing, and communicating information about the composition and structure of matter. In other words, it is the art and science of determining what matter is and how much of it exists. Analytical chemistry plays an important role in nearly all aspect of chemistry for example agriculture, clinical, environmental, forensic, manufacturing, metallurgical, and pharmaceutical chemistry. The scientific approach which the analytical chemist applies consist of series of steps which includes understanding and defining the goal of analysis; nature of the sample; literature search; plan of action and execution.[1,2]

At present several analytical methods are available for analyzing analytes viz. spectroscopic and chromatographic. Spectroscopic method includes UV-visible, infrared, mass, NMR, absorbance spectroscopy while chromatographic methods include high performance liquid chromatography (HPLC), high-performance thin-layer chromatography (HPTLC), gas chromatography (GC), super-critical chromatography, gel permeation chromatography methods etc. Quality of manufactured drug in tablets, solution and emulsion form must be carefully controlled in pharmaceutical industry otherwise the drug can itself affect the therapeutic value. [3,4]

When completely unknown sample is presented to an analyst, the first requirement is usually to ascertain what substances are present in it. The solution of such problem lies within the provenance of Qualitative analysis. In both types of analysis the required information is obtain by measuring physical and chemical properties that are characteristically related to the components of interest. [5,6]

MATERIAL AND METHODS

Instrument Used:

HPLC: HPLC 3000 series P-3000-M reciprocating (UV Visible Detector)

Active Pharmaceutical Ingredients Used:

Meclizine hydrochloride

Caffeine

EXPERIMENTAL WORK

High Performance Liquid Chromatography (HPLC) Method for Analysis of Meclizine hydrochloride and Caffeine:

Ultraviolet (UV) spectroscopy: (Selection of analytical wavelength)

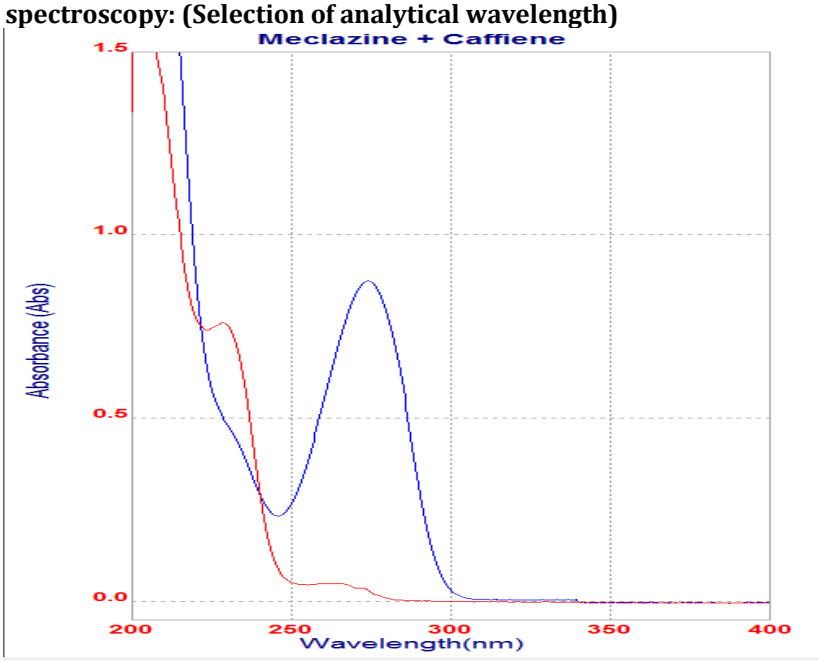

Figure No. 1. Wavelength of Meclizine hydrochloride and Caffeine (225nm)

Selection of mobile phase:

Mixtures of different solvents were tried after several permutation and combination, it was found that the Methanol: Water gives satisfactory results. The pH was adjusted to pH 3 by the addition ortho phosphoric acid (OPA).

Development and Optimization of RP – HPLC Method for Meclizine hydrochloride Caffeine: (RP-HPLC Method development):

After several trials following optimized condition is selected

Sample Name: Trial (Optimized)

Wavelength: 225nm

Mobile Phase: Methanol:Water (65:35)

Sample volume: 20µl

Flow rate: 0.9 ml/min

Pressure:9-10Mpa

Run time: 8.16min

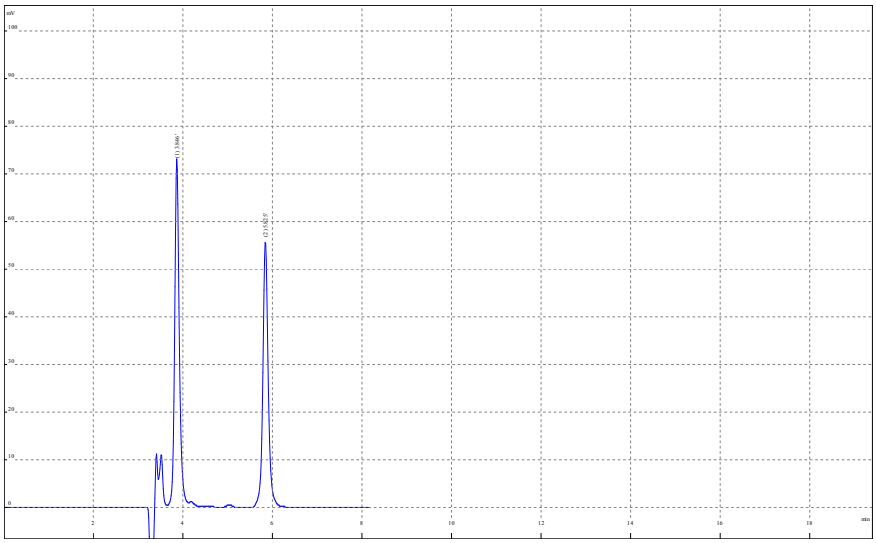

Figure 2: Chromatogram for Optimized Trial

Name	RT(min)	Area	Resolution	Theoretical plate	Asymmetry factor
Meclizine	3.846	9669978	2.05	8936	1.21
Caffeine	5.825	716355	0.00	8694	1.10

Observation: Peaks are eluted properly and good in shape, method was selected.

Optimized Chromatographic Conditions:

Table No. 1. Optimized Chromatographic Condition

Parameter/ conditions	Description/Values
Column name	Cosmosil C18, (250×4.6ID,5µ)
Wavelength	225 nm
Flow rate	0.9 ml/min
Injection volume	20µL
Column Temperature	Ambient
Run time	8.16 min
Retention time	Meclizine hydrochloride: 3.846 Caffeine: 5.825
Mobile Phase	Methanol: Water (65:35)

Application of proposed method for analysis of marketed formulation:

Table No.2. Assay Result

Sr. No.	Name of Drug	Concentration (µg/ml)	Area of Standard	Area of Sample	% Assay (w/v)
1	Meclizine	15	734056	6883838	99.95%
2	Caffeine	12	565152	5547909	99.50%

Validation of method for analysis of Meclizine hydrochloride and Caffeine:

System Suitability:

HPLC system was optimized as per the chromatographic conditions. 20 µl of standard solutions of drugs were injected in triplicate into the chromatographic system. The chromatograms were recorded and measure the response for the major peak. System suitability parameter such as retention time, theoretical plate and asymmetry factor. Then the %RSD of all parameter was found to be not more than 1.5%. [7]

Linearity:

Table No. 3. Data of Linearity for Meclizine hydrochloride and Caffeine

Conc. Of Meclizine HCL (µg/ml)	Area	Conc. Of Caffeine (µg/ml)	Area
10	794517	10	675519
20	989739	20	876809
30	1203614	30	1093506
40	1434663	40	1303300
50	1639108	50	1535976

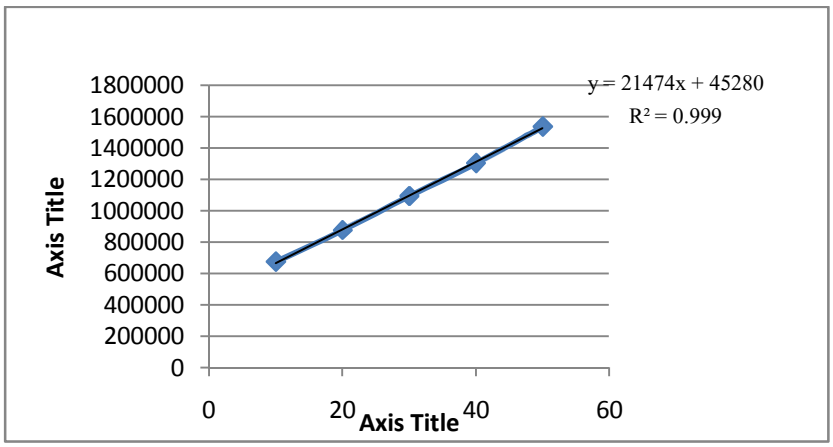

Figure No. 3. Chromatogram for linearity of Meclizine

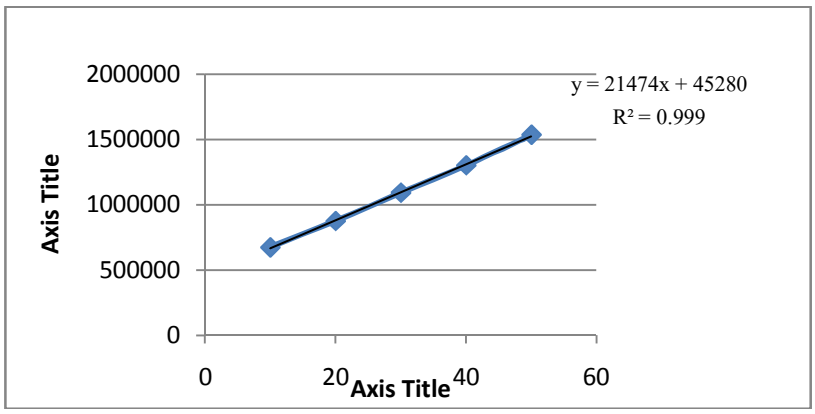

Figure No. 4. Chromatogram for linearity of caffeine

From the linearity study of Meclizine hydrochloride and Caffeine was found to be linear in the concentration ranges from 10-50µg/ml. Correlation coefficient value (R2) found to be 0.99 for Meclizine hydrochloride and Caffeine. [8]

Accuracy: (% Recovery :)

Table No. 4. Data of Accuracy for Meclizine

Conc.	Conc. (µg/ml)	Area	Standard Deviation		Accuracy	Precision
			Mean	SD	%SD	%RSD
1	10 10 10	783869 782064 782563	782832	932.0820779	0.1190654	
2	30 30 30	1216473 1205763 1210223	1210819.667	5379.872985	0.4443166	0.213709063
3	50 50 50	1633865 1634376 1635210	1634483.667	678.933232	0.0415381	

Table No. 5. Data of Accuracy for Caffeine

Conc.	Conc. (µg/ml)	Area	Standard Deviation		Accuracy	Precision
			Mean	SD	%SD	%RSD
1	10 10 10	680212 672048 671550	674603.3333	4863.625945	0.7209609	
2	30 30 30	1097604 1087779 1090201	1091861.333	5118.611758	0.4687969	0.278689491
3	50 50 50	1528518 1533287 1529512	1530439	2516.016097	0.1643983	

D. Robustness:

Effect of change of wavelength:

Sample Name: Composition2

Wavelength: 227nm

Mobile Phase: Methanol:Water (65:35)

Sample volume: 20µl

Flow rate: 0.9 ml/min
Pressure:9-10Mpa
Run time: 8.13min

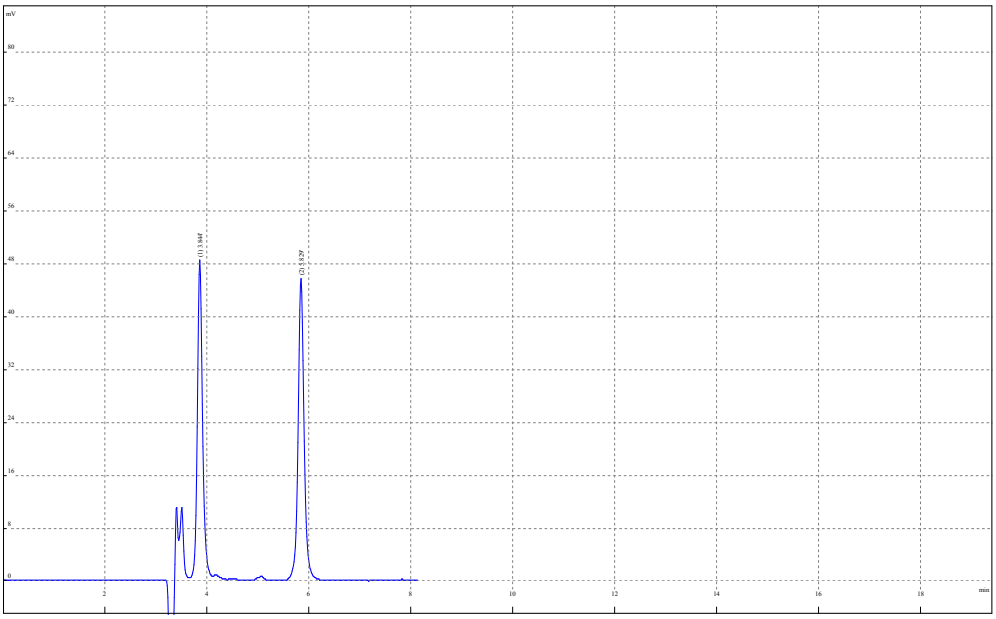

Figure 5.: Chromatogram for effect of change of wavelength

Time	Area	Resolution	Th. Plate	Asymmetry
3.844	989960	2.11	8310	1.19
5.829	868713	0.00	7679	1.07

Sample Name: Composition2
Wavelength: 223nm
Mobile Phase: Methanol:Water (65:35)
Sample volume: 20µl
Flow rate: 0.9 ml/min
Pressure:9-10Mpa
Run time: 8.12min

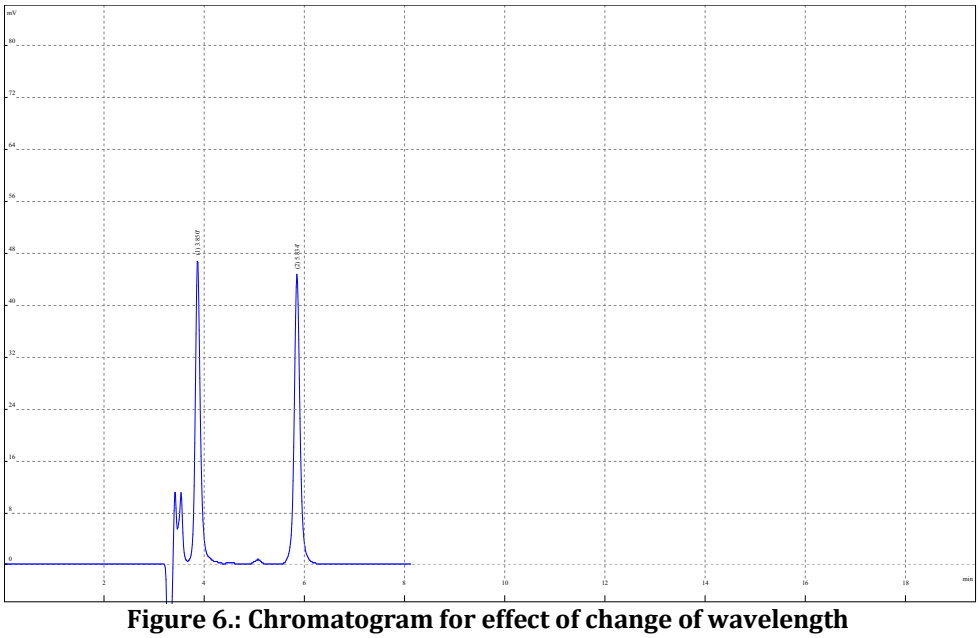

Figure 6.: Chromatogram for effect of change of wavelength

Time	Area	Resolution	Th. Plate	Asymmetry
3.850	995032	2.54	8636	1.20
5.834	871986	0.00	8086	1.07

Table No. 6. Result of change in wavelength for Meclizine hydrochloride

Conc.	Conc.	Area	Mean	SD	%SD
1	20	989960	990186	4737.05	0.47839952
	20	995032			
	20	985566			

Table No. 7. Result of change in wavelength for Caffeine

Conc.	Conc.	Area	Mean	SD	%SD
1	20	868713	871908	3156.23	0.36199121
	20	871986			
	20	875024			

Effect of change of flow rate:
Sample Name: Composition2
Wavelength: 225nm
Mobile Phase: Methanol:Water (65:35)
Sample volume: 20µl
Flow rate:1.1 ml/min
Pressure:9-10Mpa
Run time: 7.11min

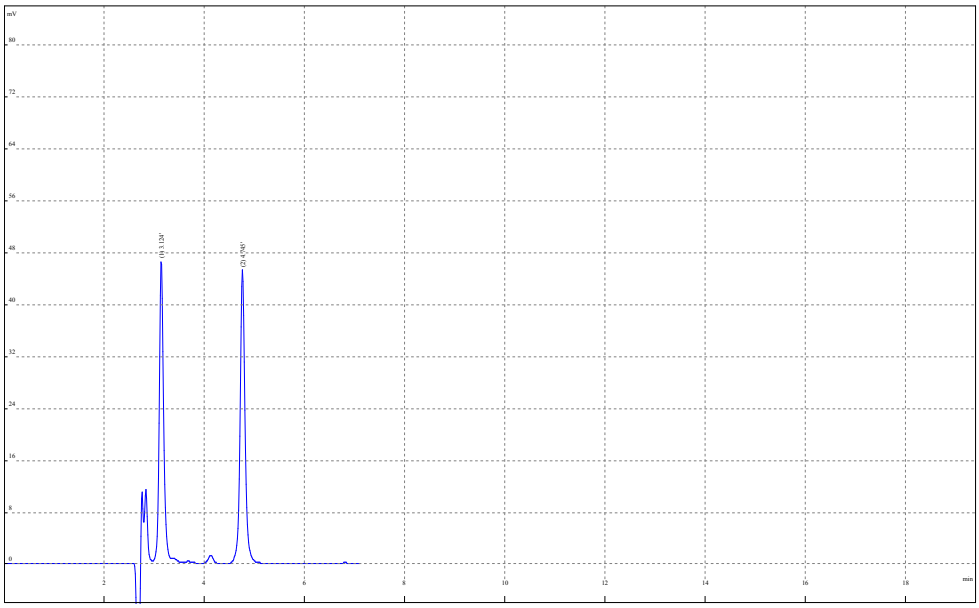

Figure 7: Chromatogram for effect of change of flow rate

Time	Area	Resolution	Th. Plate	Asymmetry
3.124	987635	2.15	8122	1.19
4.745	866664	0.00	7861	1.09

Sample Name: Composition2
Wavelength: 225nm
Mobile Phase: Methanol:Water (65:35)
Sample volume: 20µl
Flow rate: 0.7 ml/min
Pressure:9-10Mpa
Run time: 10.13min

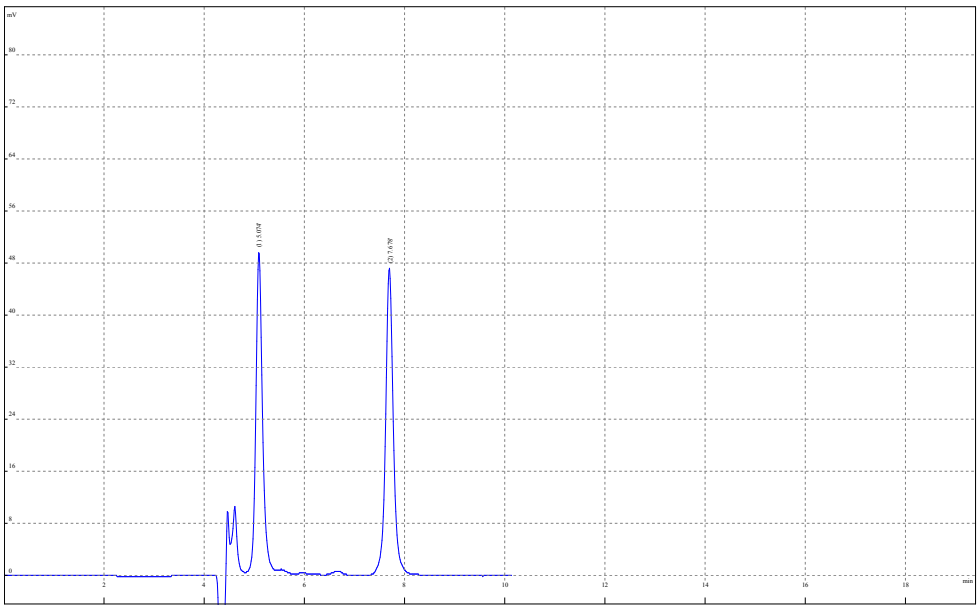

Figure 8.: Chromatogram for effect of change of flow rate

Time	Area	Resolution	Th. Plate	Asymmetry
4.928	983838	2.18	8821	1.19
5.792	875709	0.00	8226	0.94

Table No. 8. Result of change in flow rate for Meclizine hydrochloride

Conc.	Conc.	Area	Mean	SD	%SD
	20	987635			
1	20	983838	985649	1904.59	0.19323169
	20	985473			

Table No. 3.9. Result of change in flow rate for Caffeine

Conc.	Conc.	Area	Mean	SD	%SD
	20	866664			
1	20	875709	872465	5035.29	0.57713387
	20	875021			

The results of robustness were found to be satisfactory within range. %RSD of change in wavelength was found 0.47% foe Meclizine hydrochloride and 0.36% for Caffeine. %RSD of change in flow rate was found 0.19% for Meclizine hydrochloride and 0.57 % for Caffeine. [9,10]

Limit of Detection (LOD):

For Meclizine hydrochloride:

LOD= 3.3X0.213/0.999

= 0.70 µg/ml

For caffeine:

LOD= 3.3X0.278/0.999

= 0.91µg/ml

LOD was found to be 0.70µg/ml for Meclizine hydrochloride and 0.91µg/ml for Caffeine.

Limit of Quantitation:

For Meclizine hydrochloride:

LOQ= 10X0.213/0.999

= 2.13 µg/ml

For caffeine:

LOQ= 10X0.278/0.999

= 2.78µg/ml

LOQ was found to be 2.13µg/ml for Meclizine hydrochloride and 2.78µg/ml for Caffeine.

RESULT AND DISCUSSION

Linearity: From the linearity study of Meclizine hydrochloride and Caffeine was found to be linear in the concentration ranges from 10-50µg/ml. Correlation coefficient value (R2) found to be 0.9999 for Meclizine hydrochloride and Caffeine.

Accuracy: From the results shown in the accuracy table it was found that recovery value of pure drugs were between 98.0 % to 102% which indicates that the method is accurate and also reveals that commonly used excipients and additives present in the pharmaceutical formulations were not interfering in the proposed methods. Mean recovery was found 99.36% for Meclizine hydrochloride and 99.71% for Caffeine.

The relative standard deviation values for repeatability and intermediate precision studies were less than 2%. %RSD of repeatability was 0.27% for Meclizine hydrochloride and 0.33% for Caffeine. %RSD of intermediate precision was 0.24% for Meclizine hydrochloride and 0.30% for Caffeine.

Robustness: The results of robustness were found to be satisfactory within range. %RSD of change in wavelength was found 0.47% foe Meclizine hydrochloride and 0.36% for Caffeine. %RSD of change in flow rate was found 0.19% for Meclizine hydrochloride and 0.57 % for Caffeine.

LOD: LOD was found to be 0.70µg/ml for Meclizine hydrochloride and 0.91µg/ml for Caffeine

LOQ: LOQ was found to be 2.13µg/ml for Meclizine hydrochloride and 2.78µg/ml for Caffeine.

Table No.10. Result for validation parameter

Validation Parameter		Acceptance criteria	Results	
			Meclizine hydrochloride	Caffeine
Linearity		Correlation coefficient NLT 0.9990	0.9999	0.9999
Accuracy (%Recovery)		98 – 102 %	99.36 %	99.71 %
Precision	Repeatability	%RSD NMT 2.0	0.27 %	0.33 %
	Intermediate	%RSD NMT 2.0	0.24 %	0.30 %
Robustness	Change in wavelength	%RSD NMT 2.0	0.47 %	0.36 %
	Change in flow rate	%RSD NMT 2.0	0.19 %	0.57 %
Limit of detection (µg/ml)			0.70	0.91
Limit of quantitation (µg/ml)			2.13	2.78

Determination of Meclizine hydrochloride and Caffeine were estimated by RP-HPLC using Methanol: Water (65:35) at pH 3 adjusted with ortho phosphoric acid (OPA) with the flow rate of 0.9 ml/min. column used Cosmosil C18, (250×4.6ID,5µ) as a stationary phase. The retention time were found to be 3.846 for Meclizine hydrochloride and 5.625 for Caffeine and peak was observed at 225 nm which was selected as a wavelength for quantitative estimation. After development of the method it was validated for linearity, accuracy, precision, robustness studies according to ICH guidelines**.** The system suitability parameter also reveals that the values within the specified limit for the proposed method [11,32].

CONCLUSION

The developed RP-HPLC method was specific, simple, precise, accurate and robust, for the detection of Meclizine hydrochloride and caffeine in bulkand table dosage form.

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