ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 06, pp.51934-51939, June, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF MECLIZINE AND FOLIC ACID IN BULK DRUG AND PHARMACEUTICAL FORMULATIONS

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ARTICLE INFO

Article History:
Received 23rd March, 2017
Received in revised form
17th April, 2017
Accepted 18th May, 2017
Published online 20th June, 2017

Key words:

HPLC, Meclizine, Folic acid,
Validation method.

ABSTRACT

A simple, specific, accurate, precise stability indicating reversed-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the simultaneous determination of folic acid (FA) and meclizine hydrochloride (MEH). An isocratic separation of FA and MEH were achieved on C 18, 250 × 4.6mm ID, 5 μm particle size columns at column oven temperature 37^0C with a flow rate of 0.5mLmin ^{1} and using a diode array detector to monitor the detection at 254 nm. The mobile phase consisted of buffer: acetonitrile : trifluoro acetic acid at a ratio of 30 : 70 : 0.1 (v/v). The retention times of FA and MEH was found to be 5.25 and 10.14 min, respectively. Suitability, specificity, linearity, accuracy, precision, stability, and sensitivity of this method for the quantitative determination. The proposed method is reliable and robust and can be used as quality control tool for the estimation of these drugs in combined pharmaceutical solid dosage forms.

Citation: Naveen Kumar, G. S. and Srinivasa, U. 2017. "Development and validation of hplc method for simultaneous estimation of meclizine and folic acid in bulk drug and pharmaceutical formulations", *International Journal of Current Research*, 9, (06), 51934-51939.

INTRODUCTION

Meclizine HCl is piperazine 1-((4-chlorophenyl) phenylmethyl)-4-((3-methylphenyl) methyl)-dihydrochloride monohydrate is an antiemetic agent used in post-operative vomiting (The Merck index, 2001; British Pharmacopoeia, 2003). (Figure 1) a piperazine derivative and antihistamine with anti muscarinic and central sedative properties, mainly used for its antiemetic action and in the prevention and treatment of nausea and vomiting associated with a variety of conditions. Folic acid is chemically 4-(2-amino-4-hydroxypteridin-6-yl) methyl amino benzoyl-l-glutamic acid, part of the vitamin B group (vitamin B_9) is a water soluble vitamin (Figure 2). It is one of the most important coenzyme of the haemopoietic systems that control the generation of ferrohaeme (Indian Pharmacopoeia, 2011; Nafisa *et al*., 2014). Marketed tablet formulations of these agents play an important role in the treatment of persistent nausea and vomiting during pregnancy. The review of literature reveals that there were analytical methods of two drugs individually in pharmaceutical dosage forms and even in biological samples and few methods reported for either of one drug with combination of another drug. Few analytical methods like RP-HPLC, HPTLC, HPLC, Spectroscopic methods have been reported for the determination of meclizine and its combination with other drugs in pharmaceutical preparations. Estimation of folic acid in combination with other drugs by spectrophotometric methods and HPLC has been reported (Kumar *et al*., 2012; Hamid and Omera, 2014; Reddy Bari and Kaskhedikar, 1997; Shinde *et al*., 2016; Throat *et al*., 2015; Abera-Sturi *et al*., 2002; Rucha *et al*., 2015; Shivani and Tulja Rani, 2015; Pathak and Rajput, 2008; Sreeram *et al*., 2013; Arayne *et al*., 2008; Rama Krishna *et al*., 2014), but there is no work in the literature reported about the simultaneous determination of meclizine and folic acid by HPLC method. The present work describes the development and validation of stability indicating RP-HPLC method, which can quantify folic acid and meclizine simultaneously in pharmaceutical solid dosage form. Thus there is need to develop a simple rapid and economical method for routine analysis of meclizine and folic acid. The objective of present study was to develop and validate simple, sensitive, accurate, precise, rapid and economical method for estimation of meclizine and folic acid in bulk and in pharmaceutical formulations.

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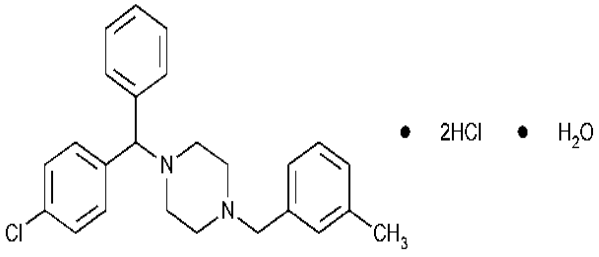

Figure 1. Structure of Meclizine

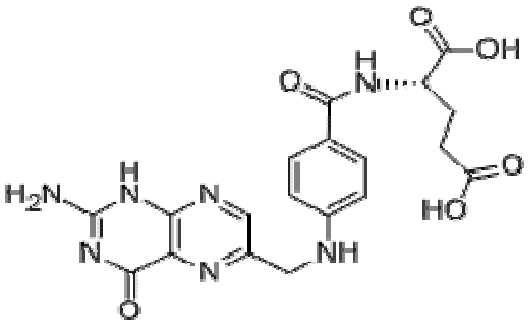

Figure 2. Structure of folic acid

MATERIALS AND METHODS

Instruments

(a) HPLC- LC100 UV– Detector, Model LC-100 Systronics.
(b) Electronic Balance- Shimadzu.

Reagents and Chemicals

Meclizine Hydrochloride and folic acid reference standard were kindly provided by Hetero Pharmaceuticals, Hyderabad for providing gift sample of working standard. Methanol of analytical grade was used as a solvent. All chemicals and reagents used were of analytical reagent grade. The brand name of marketed combined tablet formulation is PNV PLUS containing Meclizine Hydrochloride 25 mg and Folic acid 2.5 mg manufactured by Yash Pharma. Lab. Pvt. Ltd. Acetonitrile HPLC grade (Scharlau), anhydrous sodiumdihydrogen phosphate (Scharlau), phosphoric acid AR grade, (Merck) and trifluoroacetic acid (Fisher Scientific) were usedfor analytical purposes. Ultrapure water was used to prepare the mobile phase. Ultrapure water was prepared by using LabconcoWater Pro PS purification system.

Instrument and Chromatographic Condition

Chromatographic separation was achieved by using systronics LC-100 high-performance liquid chromatography, equipped with degasser PGU-20A 5, variable wavelength programmable diode array detector UV, auto sampler SIL-20 AC HT, and column oven CTO-10 A5 VP. Pronto SILC 18, 250 × 4.6mm ID, 5 μm particle size was used as the stationary phase. The column temperature was kept at 37^0C, and the mobile phase flow rate was maintained at 0.5mL min 1.The detection was monitored at 254 nm.The injection volume was 20 μL, and the run time was 13 min for each injection.

Mobile Phase. A mixture of buffer, acetonitrile, and trifluoroacetic acid (TFA) at a ratio of 30: 70: 0.1 (v/v) was prepared. The resulting solution was sonicated for 5 min using ultrasonic bath, and finally the mixture was filtered using 0.2 μm membrane filter.

Preparation of Buffer for Mobile Phase. 12 g of anhydrous sodium dihydrogen phosphate was dissolved in 900 mL of ultrapure water. Then the pH was adjusted to 3.0 with orthophosphoric acid and volume up to 1000mL with ultrapure water and sonicated for 5min using ultrasonic bath then filtered through 0.2 μm membrane filter.

Diluting Solution. A mixture of buffer, acetonitrile, and trifluoroacetic acid (TFA) at a ratio of 35: 65: 0.1 (v/v) was used as the diluents.

Standard Preparation (at Nominal Concentration). FA and MEH working standards were accurately weighed and were transferred into a clean and dry 100 mL standard volumetric flask and dissolved to prepare 0.50 mgmL ^{1} and0.25 mgmL ^{1} concentrations of FA and MEH stock solution, respectively, with the diluting solution and finally volume up to the mark. The solution was sonicated for 5 min using ultrasonic bath and then filtered through 0.2 μm disk filter.

Sample Preparation. Twenty tablets (PNV PLUS tablet) were crushed and then powdered finely. To prepare assay sample solution, powdered sample equivalent to 2.5mg folic acid and 25mg meclizine hydrochloride was weighed accurately and taken into 100mL volumetric flask. About 40mL of diluting solution was added and shaken thoroughly to extract the drug from the excipients and then sonicated for 5min to complete dissolution of drug. The solution was allowed to cool at room temperature and then volume up to the mark. The solution was filtered through whatman filter paper (no. 42) and then finally filtered through 0.2 μm disk filter.

Method Validation Parameters

System Suitability. To assess system suitability of the method, the repeatability, theoretical plates, tailing factor, and retention time of six replicate injections of standard FA and MEH of concentrations 0.50 mgmL ^{1} and 0.25mgmL ^{1}, respectively, were used, and the percent relative standard deviation (%RSD) values were calculated in each case.

Linearity. The linearity of the method was determined at five different concentration levels (80%, 90%, 100%, 110%, and 120%) ranging from 0.395 to 0.592mgmL ^{1} of FA and0.203-0.304 mgmL ^{1}of MEH, respectively. The linearity was evaluated by peak area versus concentration, which was calculated by the least-square regression analysis, and the respective regression equation was computed.

Specificity. The specificity of the developed RP- HPLC method for the determination of FA and MEH in bulk drug and pharmaceutical preparation (PNVPLUS tablet) was investigated by chromatographic analysis of the following.

Accuracy. Accuracy was carried out for drug-matrix solutions. Accuracy parameter was determined by the recovery test, which consisted of adding known amounts of FA and MEH in to the placebo sample solutions. This test was conducted by three different concentrations (80, 100, and120%) of test sample in three replicate sample preparations, and the percent recoveries (mean ± %RSD of three replicates) of FA and MEH

in drug-matrix form were calculated. The accuracy was also evaluated by linear regression analysis and computed.

Precision. Precision of the method was studied by analysis of three replicates of standard solution in three different concentrations (80, 100, and 120%). It was demonstrated by repeatability (intraday precision) and intermediate precision (interday precision) of standard solutions. The results were expressed as %RSD of the measurements.

Stability of Solution. The solution stability is tested by allowing the prepared drug matrix tested sample to stand exposed to room light and ambient room temperature for three consecutive days. The sample is to be assayed daily and compared to freshly prepare standard solutions.

Sensitivity. For sensitivity study the limit of detection (LOD) and limit of quantitation (LOQ) were estimated by determination of signal-noise ratio. The LOD (α = 3.3) and LOQ (α = 10) of the proposed method were calculated using the following equation:

$$A = \alpha \times C \times NS, \quad (1)$$

Where A is LOD or LOQ, C is the concentration in ppm, and N/S is the signal-noise ratio.

Ruggedness. Ruggedness of the current method was determined by analyzing six assay sample solutions of PNV PLUS tablet by different instrument, column, and two analysts in the same laboratory to check the reproducibility of the test result.

Robustness. To determine the robustness of the current method, the effect of flow rate was studied at 0.48 and0.52mLmin 1 instead of 0.5mLmin 1. The effect of column temperature was studied at 35 and 39^{0}C instead of 37^{0}C. The effect of mobile phase composition was assessed at (buffer: ACN = 31.3: 68.7, v/v) and (buffer: ACN = 28.7: 71.3, v/v) instead of (buffer: ACN = 30:70, v/v). The effect of wavelength change was studied at 252 nm and 256 nm instead of at 254 nm.

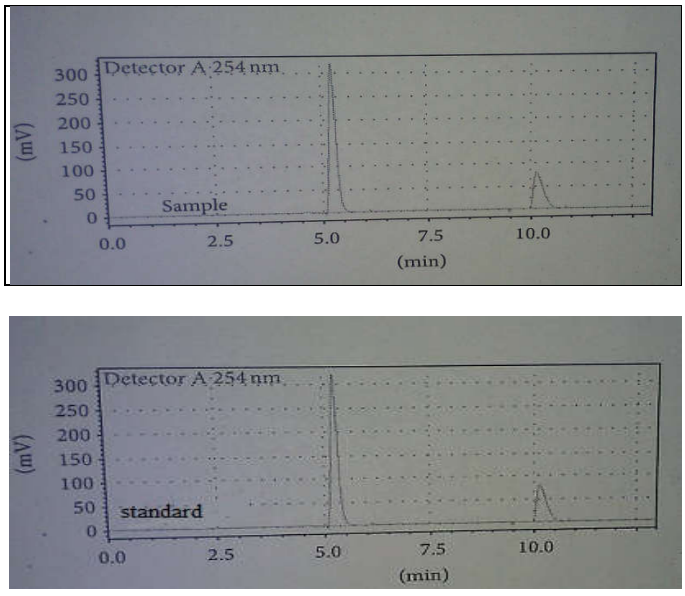

Figure 3. RP-HPLC chromatogram of sample, and standard of folic acid and meclizine HCl

RESULTS AND DISCUSSION

Method Validation

System Suitability. The results (Mean ± %RSD of six replicates) of the chromatographic parameters (Table 1) indicate the good performance of the system.

Linearity. The peak area was dynamic-linear in the concentration ranges of 0.395–0.592mgml ^{1} for FA and 0.203–0.304mgml ^{1} for MEH, respectively. Highly significant correlation coefficient ($R2$) demonstrated the linearity of the method (Table 2).

Table 1. Chromatographic characteristics of system suitability study

Parameters	Value (Mean ± %RSD)*	
	FA	MEH
Peak area	3485193 ± 0.08	1347970 ± 0.08
Tailing factors	1.29 ± 1.06	1.53 ± 0.45
Retention time	5.25 ± 0.02	10.14 ± 0.04
Theoretical plates	5198 ± 0.10	8127 ± 0.08

*Mean and %RSD of six replicate.

Table 2. Parameters of regression analysis

Parameters	FA	MEH
Linearity range (mgmL−1)	0.395–0.592	0.203 - 0.304
Correlation coefficient	0.999	1.000
% Y intercept	1.61	0.17

*Mean and %RSD of six replicate.

Specificity. The chromatograms of blank, placebo, test sample, and standard were used to justify the specificity of target analyte. The method was specific since excipients in the formulation did not interfere in the estimation of FA and MEH (Figure 3). The samples submitted to acidic and alkaline condition showed significant alteration in the peak area, and also there was no detectable degradation peak(s). Similarly, during oxidative and reductive hydrolysis study, degradation peak(s) was not found. In every case the peak purity was 99.99%.The results acquired from peak purity tool confirmed that the active components' peak response was pure proving no other substances in the same retention time. Percent of degradation n is mainly 3.78% for FA during oxidation study as well as 4.64% for MEH in acidic condition (Table 3).

Accuracy. The results were expressed as percent recoveries of the particular components in the samples. The overall results of percent recoveries (mean ± %RSD) of drug-matrix solutions are indicating good accuracy of the proposed RP-HPLC method (Table 3). Correlation coefficient $R2$= 0.999and 0.999 established excellent accuracy for the active ingredients FA and MEH, respectively.

Precision. The values of %RSD for intraday and inter day variation were found very well and within 2% limit, indicating that the current method is repeatable (Table 4).

Stability of Solution. In the stability study, the retention time remained unchanged till third day, but peak area of FA and MEH deviated at third day by more than 2.0% from initial. This indicates that both solutions were stable for at least 48

Table 3. Accuracy studies of FA and MEH in drug-matrix solutions

	Amount added ($mgmL^{-1}$)	Peak area	Amount recovered ($mgmL^{-1}$)	%Recovery	%Recovery (mean ± %RSD)	Over all (mean ± %RSD)
FA	0.395	2804505	0.392	99.24	100.85 ± 1.38	100.32 ± 1.06
	0.395	2805301	0.402	101.65		
	0.395	2804670	0.402	101.6		
	0.494	3481689	0.497	100.71	100.57 ± 0.33	
	0.494	3487433	0.495	100.20		
	0.494	3485039	0.498	100.81		
	0.592	4181905	0.595	100.51	99.55 ± 1.02	
	0.592	4181507	0.590	99.66		
	0.592	4183455	0.583	98.48		
MEH	0.203	1084674	0.207	101.88	101.76 ± 0.17	100.81 ± 1.01
	0.203	1084350	0.206	101.64		
	0.203	1085947	0.207	101.88		
	0.252	1345809	0.253	100.38	100.00 ± 0.33	
	0.252	1342589	0.252	99.81		
	0.252	1348349	0.252	99.81		
	0.304	1624589	0.308	101.41	100.63 ± 1.21	
	0.304	1623470	0.308	101.25		
	0.304	1625347	0.302	99.22		

Table 4. Intra-day and inter-day precision of the method

	Sample Conc.(%)	Peak area (day 1)	Peak Area (Mean ± %RSD)	Peak area (day 2)	Peak Area (Mean ± %RSD)	Overall (Mean ± %RSD)
FA	80	2802394	2803476 ± 0.04	2804064	2804395 ± 0.01	2803937 ± 0.03
		2803487		2804585		
		2804551		2804530		
	100	3484272	3485861 ± 0.05	3485123	3486142 ± 0.0	3486001 ± 0.04
		3487553		3487737		
		3485765		3485572		
	120	4181758	4182542 ± 0.04	4180235	4181749 ± 0.04	4182145 ± 0.04
		4181439		4081346		
		4184431		4283672		
MEH	80	1084379	1084597 ± 0.02	1084421	1084530 ± 0.01	1084565 ± 0.02
		1084567		1084667		
		1084856		1084503		
	100	1346371	1346558 ± 0.06	1345206	1346558 ± 0.06	1346120 ± 0.046
		1345755		1345394		
		1346371		1346446		
	120	1347546	1624784 ± 0.08	1623168	1623586 ± 0.04	1624185 ± 0.07
		1625633		1623243		
		1623342		1623165		
		1625377		1624346		

Table 5. Stability of analytical sample solution

Day	Room Temperature (°C)	% Recovery* FA	MEH
1	25 ± 2	100.92	100.49
2	25 ± 2	100.17	99.68
3	25 ± 2	96.69	99.00

*Mean of three replicates.

Table 6. Ruggedness of the method

Amt of FA ($mg\ tab^{-1}$)	Amt of MEH ($mg\ tab^{-1}$)	Analyst 1, instrument 1, column 1 Amt found FA ($mg\ tab^{-1}$) (Mean ± %RSD)*	Amt found MEH ($mg\ tab^{-1}$) (Mean ± %RSD)*	Analyst 2, instrument 2, column 2 Amt found FA ($mg\ tab^{-1}$ (Mean ± %RSD)*	Amt found MEH ($mg\ tab^{-1}$) Mean ± %RSD)*
2.5	50	2.53 ± 0.03	50.94 ± 0.04	2.55 ± 0.04	50.97 ± 0.03

*Mean of six replicates.

Table 7. Robustness of the method

Parameters	Actual Variance	Amount added ($mgmL^{-1}$)		%Recovery (Mean ± %RSD)*	
		FA	MEH	FA	MEH
Flow Rate	0.48ml min^{-1}	0.25	0.5	100.55 ± 0.33	100.04 ± 0.04
	0.52ml min^{-1}	0.25	0.5	100.12 ± 0.02	100.18 ± 0.07
Organic (%)	68.7	0.25	0.5	99.95 ± 0.22	100.15 ± 0.04
in mobile phase	71.3	0.25	0.5	99.75 ± 0.51	100.18 ± 0.07
Detector	252 nm	0.25	0.5	100.00 ± 0.15	99.81 ± 0.19
Wavelength	256 nm	0.25	0.5	100.00 ± 0.11	99.53 ± 0.37
Column	39^0C	0.25	0.5	100.17 ± 0.20	100.08 ± 0.11
Temperature	35^0C	0.25	0.5	100.41 ± 0.46	100.07 ± 0.05

*Mean of three replicates

hours, which was sufficient to complete the analytical procedure (Table 5).

Sensitivity. The LOD and LOQ by the proposed method were found for FA1.90 ppm and 5.74 ppm as well as for MEH 3.75 ppm and 11.35 ppm, respectively.

Ruggedness. The results (% of Recovery ± RSD) of six assay samples are indicating the ruggedness of the current method (Table 6).

Robustness. The effects of robustness study under different altered conditions of this proposed method are satisfactory (Table 7). The mean recovery and %RSD of analyzed sample indicate that the current method is robust.

Conclusion

The developed RP-HPLC method for the simultaneous determination of folic acid and meclizine hydrochloride is simple, precise, accurate, and reproducible and highly sensitive. The developed method was validated based on ICH guidelines (21). Hence, this method can be routinely used for the simultaneous determination of folic acid and meclizine hydrochloride in pure and pharmaceutical formulations.

Acknowledgment

The authors are thankful to The Management, Sarada Vilas College of Pharmacy for their kind help and providing necessary facilities.

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