AJPRHC

Research Article

SIMULTANEOUS ESTIMATION OF MECLIZINE HYDROCHLORIDE AND NICOTINIC ACID IN PHARMACEUTICAL DOSAGE FORM BY RP-HPLC METHOD

SUDHEER REDDY M[*1], KRISHNA KARTHIK P[2], VINOD KUMAR K[3]

Division of Pharmaceutical Analysis and Quality Assurance, Center for Pharmaceutical Research (CPR), Raghavendra Institute of Pharmaceutical Education and Research (RIPER), K.R. Palli cross, Chiyyedu (Post), Anantapur, A.P.-515721.

*Corresponding author

E-mail: molagavallisudheer@gmail.com

This paper is available online at www.jprhc.in

ABSTRACT

A simple, selective, rapid, precise and economical reverse phase high performance liquid chromatographic method has been developed for the simultaneous estimation of meclizine hydrochloride and nicotinic Acid from pharmaceutical formulation using C_{18} (25 cm x 4.6 mm i.d., 5 µ) column with a mobile phase consisting of Methanol: water (adjusted to pH 3.0 using orthophosphoric acid) in the ratio of 80:20 v/v. The detection wavelength was carried out at 231 nm. The linear regression analysis data for the linearity plot showed good linear relationship with correlation coefficient value for Meclizine Hydrochloride and Nicotinic Acid were R^2=0.9991 and R^2=0.9996 in the concentration range of 10-70 µg/ml, 5-35 µg/ml respectively. The validation of method was carried out utilizing ICH-guidelines. The proposed method can be used for the estimation of these drugs in combined dosage forms.

Key words: RP-HPLC, Meclizine Hydrochloride, Nicotinic Acid.

INTRODUCTION

Meclizine Hydrochloride [MEC] (Figure 1), chemically 1-(p-chloro-alpha-phenyl, benzyl)- 4- (m-methyl-benzyl)-piperazine dihydro chloride monohydrate**.** It is an antihistamine, considered to be antiemetic it is used as an anti vertigo or antiemetic agent, specifically in the prevention and treatment of nausea, vomiting and dizziness associate with motion sickness. It works by blocking a chemical messenger in the brain, it helps to reduce or prevent vomiting and dizziness caused by motion sickness. It also used for vertigo caused by certain inner ear problems. Meclizine should be taken with caution in the elderly because of increased risk of confusion and amnesia. The drug is safe in pregnancy. Figure 1.

Nicotinic acid [NA] (pyridine-3-carboxylic acid) is a B group vitamin. It is one of the oldest known agent for the treatment of dyslipidemia. It favorably modifies the major lipid and lipoprotein classes that are recognized as independent risk factors for atherosclerosis and coronary heart disease. It can be used as a therapeutic option for cardiovascular risk reduction in atherogenic dyslipidemia. Chronic administration of Nicotinic acid reduces whole body insulin sensitivity via elevation of circulating fatty acid and accumulation of skeletal muscle lipid. Nicotinic acid and its derivatives can be used as peripheral vasodilators. Figure 2.

Several analytical methods based on UV [1], Spectroflourimetry [9], RP-HPLC [3-5], HPTLC [2], Electrophoresis [7-8] and LC-MS [6] was reported for the determination of metformin. Although literature survey reveals that various methods were reported for Meclizine hydrochloride and Nicotinic Acid both for single estimation and in combination with others drugs, but no estimation method has been reported for the analysis of these drugs in combination.

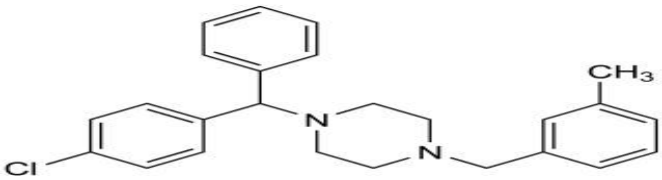

Fig. 1 Meclizine hydrochloride

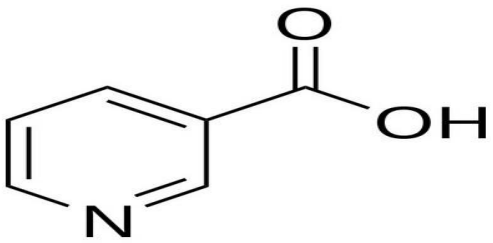

Fig. 2 Nicotinic Acid

EXPERIMENTAL

HPLC instrumentation and conditions

HPLC system (Agilent HPLC Model-1200 with Ezchromeelite Software) containing C_{18} (Agilent, 250 x 4.6 mm. 5 µ) column with UV- PDA detection. LABINDIA-3000^{+} UV-Visible double beam spectrophotometer with a fixed slit width 1nm and 1cm matched quartz cells was used for all the spectral measurements.

Chemicals and reagents

Analytically pure MEC and NA were kindly provided by Hetero Labs Ltd, Hyderabad as gift samples. Analytical grade methanol was purchased from Merck & Co. Glassware's used in each procedure were soaked overnight in a mixture of chromic acid and sulphuric acid rinsed thoroughly with double distilled water and dried in hot air oven. Water (HPLC grade) was purchased from Merck, India. Triple distilled water is used for all purpose.

Chromatographic conditions

The mobile phase consisted of Methanol and water in ratio Methanol: Water (80:20), and pH was adjusted to 3.0 with OPA. The contents of the mobile phase were filtered before use through a 0.45µ membrane and degassed for 10 min. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1.0 ml/ min and the injection volume was 20 µl. The column temperature was maintained at ambient temperature. The eluents were monitored at 231 nm. The result of the optimized chromatogram was shown in Figure3 and Table1.

Preparation of standard stock solutions

Accurately weighed 10 mg of MEC and NA standard were transferred to separate 10 ml volumetric flask and dissolved in 10 ml Methanol. The flasks were shaken and volume was made up to the mark with Methanol to give solutions containing 1000 µg/ml MEC and 1000 µg/ml NA. From this solution 1ml was transferred to volumetric flask of 100 ml capacity. Volume was made up to the mark to give a solution containing 100 µg/ml MEC and 100 µg/ ml NA.

Calibration of standards

The standard calibration curve was constructed for Nicotinic Acid and Meclizine Hydrochloride. Different volumes of stock solutions of each were accurately transferred in to 10 ml volumetric flasks and diluted to mark to yield a concentration range of 5-35 µg/ml solutions of Nicotinic Acid and 10-70 µg/ml solutions of Meclizine hydrochloride. The calibration line was obtained by plotting the peak area against concentration of drug.

Sample preparation

Twenty tablets of marketed formulation PNV tab (Yash Pharma, Mumbai, India) containing MEC 25 mg and NA 50 mg formulation were weighed, and finely powdered. Tablet powder equivalent to 100 mg PIO with

relevant quantities of MET was weighed and transferred to a 100 ml volumetric flask, extracted for 30mins with water and volume was made up to 100 ml with diluent. 0.25 ml of above solution was taken in 10 ml volumetric flask and volume was made up to 10 ml with mobile phase, and final solution was filtered through 0.45 µ syringe filter and it was analysed. The results of the assay were shown in Table 4.

Method validation

The proposed method has been extensively validated in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness and reproducibility. The accuracy was expressed in terms of percent recovery of the known amount of the standard drugs added to the known amount of the pharmaceutical dosage forms. The precision (Coefficient of Variation - C.V.) was expressed with respect to the repeatability, intra-day and inter-day variation in the expected drug concentrations. After validation, the developed methods have been applied to pharmaceutical dosage form.

System Suitability Criteria

It is defined as tests to measure the method that can generate result of acceptable accuracy and precision. The system suitability was carried out after the method development and validation have been completed. The system suitability was assessed by five replicate analyses of the drugs at concentrations of 20 µg/ml of MEC and 30 µg/ml of NA and for this, parameters like plate number (n), tailing factor, HETP, peak asymmetry of samples were measured, and shown in table 2.

RESULTS

Method Validation parameters

Method was validated as per ICH (Q2) guidelines with respect to linearity, accuracy, precision, specificity, and robustness, limit of detection and limit of quantification.

a) Specificity

Commonly used excipients (starch, microcrystalline cellulose and magnesium stearate) were spiked into a pre weighed quantity of drugs. Specificity of the method was shown by quantifying the analyte of interest in the presence of matrix and other components. Blank injections have shown no peaks at retention time of 3.3 min and 5.5 min, the proposed method was specific for the detection of NA and MEC respectively. The selectivity of the method was performed by injecting the solution after the degradation. The degradants formed during solution stability study were well separated from the analyte peak after 20 hrs of sample preparations. Thus the method can be applied to evaluate the stability of the solution.

b) Linearity

Appropriate volume of aliquot from NA and MEC standard stock solution was transferred to volumetric flask of 10 ml capacity. The volume was adjusted to the mark with methanol to give a solution containing 5-30 µg/ml NA and 10-70 µg/ml MEC. The slope, Y-intercept and correlation coefficient were calculated. The regression line relating standard concentrations of drug using regression analysis, the calibration curves were linear in the studied range and equations of the regression analysis were obtained: $Y=143611x+63798$; $R^2=0.9996$ for NA and $Y=92198x+13890$; $R^2=0.9991$ for MEC respectively. The mean and correlation coefficient of standard curves (n=3) were calculated. The represented data was shown in below figure 4,6 and Table 3.

c) Accuracy

Accuracy was assessed by determination of the recovery of the method by addition of standard drug to the pre-quantified placebo preparation at 3 different concentration levels 80%, 100% and 120%, taking into consideration percentage purity of added bulk drug samples. Each concentration was analyzed 3 times and average recoveries were measured. Results of assay and recovery were presented in the table 5.

d) Precision

The repeatability was evaluated by assaying 6 times of sample solution prepared for assay determination. The intraday and interday precision study of MEC and NA was carried out by estimating same concentration of NA (20 µg./ml) and MEC (30 µg/ml), 6 times on the same day and on 3 different days (first, second, third) and the results are reported in terms of C.V. The results are shown in Table VIa and VIb .Table 6.

e) Robustness

The robustness of the method was evaluated by analyzing the system suitability standards and evaluating system suitability parameter data after varying the HPLC pump flow rate (±0.1 ml) and organic solvent content (±2ml) and Wavelength(±0.2) .The alterations caused a significant change in peak area R.S.D (%), USP tailing factor and retention times. Results of robustness studies are shown in Table 7.

f) **LOD and LOQ**

LOD and LOQ were calculated from the formula 3.3 x (σ/S) and 10 x (σ/S), respectively where, σ is standard deviation of intercept and S is the mean of slope. The LOD and LOQ can also be determined by S/N. The value for LOD should be 3-5 whilst for LOQ 10-15. The results are presented in Table 8.

g) Solution stability and Mobile phase stability

The stability of NA and MEC in solution was determined by leaving test solutions of the sample and reference standard in tightly capped volumetric flasks at room temperature for 3 days during which they were assayed at 12 hr intervals. Stability of mobile phase was determined by analysis of freshly prepared sample solutions at 12 hr intervals for 48 hrs and comparing the results with those obtained from freshly prepared reference standard solutions. The mobile phase was prepared at the beginning of the study period and not changed during the experiment. The % assay of the results was calculated for both the mobile phase and solution-stability experiments.

Table 1: Optimized Chromatographic conditions of Nicotinic Acid and Meclizine hydrochloride on C_{18} (Column)

S.No	Parameters	Optimised Condition
1	Mobile Phase Optimized	80 MeOH : 20 H_2O 0.2%TEA, pH 3.0 adj., with OPA
2	Flow Rate (mL/ min)	1
3	Run Time (min)	10
4	Column Temperature ^{O}C	23
5	Volume of Injection (µL)	20
6	Detection Wavelength (nm)	231
7	Retention time Rt	NA -3.3 min, MEC-5.5min

Table 2: System suitability parameters for Meclizine Hydrochloride and Nicotinic Acid

Parameter	Values obtained (n = 6)		Acceptance Criteria
	Nicotinic Acid	Meclizine Hydrochloride	
Plate Count	9871 ± 62	6019 ± 87	>4000
Tailing Factor	1.094 ± 0.018	1.582 ± 0.022	≤ 2.0
Capacity factor	0.64	2.24	0.5<k<20
HETP	0.00253	0.00415	----
R_t	3.3 min	5.5 min	-----

Table 3: Calibration of Meclizine Hydrochloride and Nicotinic Acid

Concentration of Nicotinic Acid (µg/ml)	Peak Area mean± SD (n=3) of Nicotinic Acid	Concentration of Meclizine Hydrochloride (µg/ml)	Peak Area mean± SD (n=3) of Meclizine Hydrochloride	% RSD	
				NA	MEC
10	634176 ± 5685	5	514042 ± 4112	0.9753	0.8956
20	1351955 ±1487	10	925385 ± 1388	1.1214	1.5968
30	2113120 ±1479	15	1383993 ± 6919	0.7315	0.5265
40	2818764 ±3664	20	1833394 ± 20167	1.3217	1.1561
50	3569235 ±1784	25	2299671 ± 29895	0.5014	1.3725
60	4259189 ±6388	30	2760404 ± 19322	1.5705	0.7549
70	4912484 ±3929	35	3288036 ± 29592	0.8236	0.9045

Table 4: Assay report of formulation

S. No.	Brand name	Content	Peak Area mean ± S.D	Assay	% RSD
1	PVB	20mg-Nicotinic Acid	2818764 ±38450	100.35%	1.3641
		40mg-Meclizine HCl	1833394 ± 16817	98.67%	0.9173

Table 5: Recovery Report of Meclizine Hydrochloride and Nicotinic Acid

Drug	Amount taken (µg/ml)	Recovery Level	Amount of Drug Added	Amount Found (µg/ml) Mean± S.D	% RSD	% Recovery
NA	20	80%	16	35.38 ± 0.462	1.3064	98.27
		100%	20	40.23 ± 0.351	0.8739	100.59
		120%	36	44.72 ± 1.029	1.6847	101.64
MEC	40	80%	32	71.84 ± 0.851	1.1856	99.78
		100%	40	80.17 ± 1.156	0.9268	100.21
		120%	48	87.85 ± 0.633	0.7216	99.4

Table 6: Precision

Intra-day and Inter-day Precision

VIa: Intra-day Precision Data for Meclizine Hydrochloride and Nicotinic Acid

S. No	Conc. (µg/ml) of MEC	Peak Area mean ± S.D (n=3) of MEC	Conc. (µg/ml) of NA	Peak Area mean ± S.D (n=3) of NA	% RSD	
					MEC	NA
1	10	1296867 ± 14524	20	965872 ± 11976	1.1281	1.2494
2	20	2837674 ± 24403	40	1764786 ± 13412	0.8672	0.7696
3	35	4927676 ± 63464	70	3276071 ± 22604	0.7427	0.6929

VIb: Inter-day Precision Data of Meclizine Hydrochloride and Nicotinic Acid

S. No	Conc. (µg/ml) of MEC	Peak Area mean ± S.D (n=3) of MEC	Conc. (µg/ml) of NA	Peak Area mean ± S.D (n=3) of NA	%RSD	
					MEC	NA
1	10	1284762 ± 20813	20	984654 ± 14375	1.6285	1.4692
2	20	2856782 ± 38280	40	1782594 ± 14617	1.3469	0.8269
3	35	4946762 ± 44026	70	3289168 ± 30918	0.8957	0.9438

Table 7: Robustness studies of Meclizine Hydrochloride and Nicotinic Acid

S. No	Parameter	Modification	Retention time		Asymmetry	
			NA	MEC	NA	MEC
1	Flow rate	0.9 mL/ min	3.7 min	6.3 min	0.9965	1.1235
		1.0 mL/ min	3.3 min	5.5 min	0.9025	1.1416
		1.1 mL/ min	3.0 min	5.1 min	0.9437	1.1354
2	Mobile phase Composition ($MeOH:H_2O$)	78 : 22	3.3 min	6.1 min	0.6895	1.4693
		80 : 20	3.3 min	5.5 min	0.9025	1.1416
		82 : 18	3.3 min	5.2 min	0.7972	2.3340
3	Wavelength	229	3.3 min	5.7 min	1.0440	1.1450
		231	3.3 min	5.5 min	1.0440	1.1450
		233	3.3 min	5.5 min	1.0440	1.1450

Table 8: LOD and LOQ data

S.No	Parameter	NA	MEC
1	LOD(µg/ml)	0.025	0.299
2	LOQ(µg/ml)	0.083	0.909

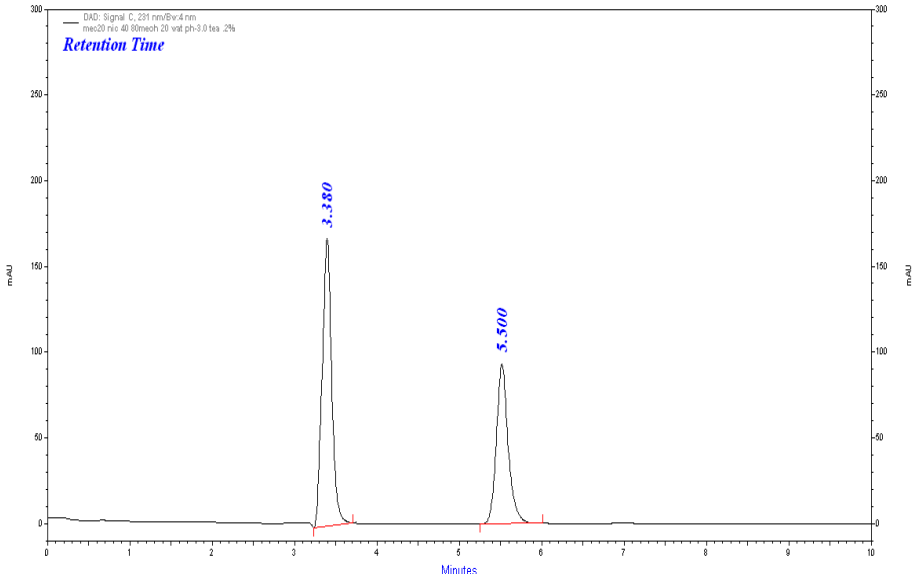

Fig. 3 Optimized chromatogram of Nicotinic Acid and Meclizine Hydrochloride

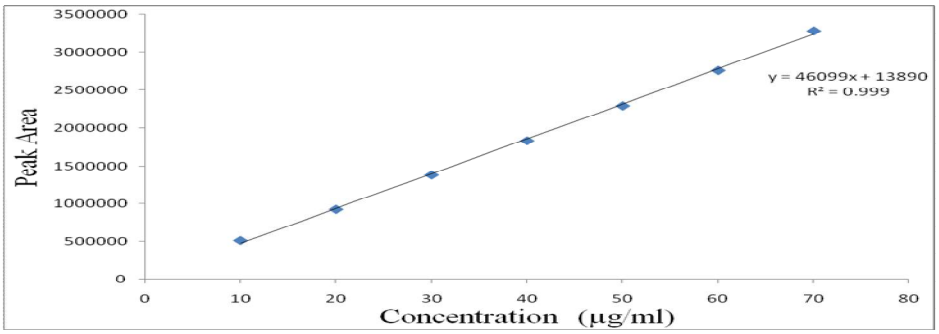

Fig. 4 Linearity of Meclizine Hydrochloride

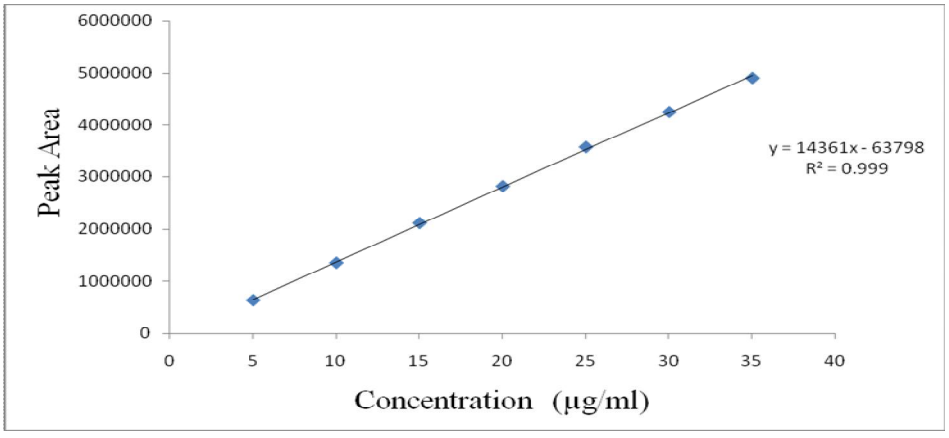

Fig. 5 Linearity of Nicotinic Acid

CONCLUSION

The proposed method gave good resolution between MEC and NA within short analysis time (10 min). Solution stability studies showed that the active pharmaceutical ingredients remained stable for 24 hrs at room temperature. The changes in flow rate, Wavelength, composition of mobile phase affected the retention times of the drugs, confirming that the method is not robust for change in Wavelength and mobile phase composition. High percentage recovery of drug shows the method is free from interference of excipients present in the formulation. Thus the proposed method is simple, rapid, sensitive, specific, accurate, and precise and does not involve complicated sample preparation procedures. The use of C_{18} column in the present work has shown better elution of analytes with good resolution, improved plate count, capacity factor. So the C_{18} column can be used to achieve high specificity in shorter time of analysis of Meclizine Hydrochloride and Nicotinic Acid as per ICH Q2 (R2) guidelines.

ACKNOWLEDGEMENT

The authors would like to thank Hetero Labs, Hyderabad for providing a gift sample of standard Meclizine Hydrochloride and Nicotinic Acid. The authors would like to thank Y. Padmanabha Reddy, M.Pharm., Ph.D. FIC. Principal, Raghavendra Institute of Pharmaceutical Education and Research for providing necessary facilities to carry out the work.

REFERENCES

1 Jaiprakash N, Sangshetti, ZahidZaheer, Mohammed Aqeel, Dehghan MHG. Validated Spectrophotometric Method for Simultaneous Estimation of Atorvastatin and Nicotinic acid in Combined (Pharmaceutical) Dosage form. Int J Pharma Tech Res 2012;4:999-1003

2 Pravish kumar tiwari, Padmakarsathe. Development and validation of HPTLC Method for Niacin and Simvastatin in Binary Combination. Adv Biosci Biotechnology 2010;1:131-135

3 DevikaGS, Sudhakar M , Venkateshwara raoJ. Development and Validation of RP- HPLC Method for Simultaneous Determination of Niacin (extended release) and Lovastatin in Oral Solid Dosage form. Orient J Chem 2012;2:887-893

4 Suma BV, Kannan K, Madhavan V, Chandini RN. Simultaneous Estimation and Validation of Atorvastatin Calcium and Nicotinic Acid in Combined Tablet Dosage form by RP HPLC Method. Int J Pharm 2012;4:975-983

5 Tokunaga H, Okada S, Kimura T. Determination of Nicotinic Acid in Injections by High Performance Liquid Chromatography. Eisei Shikenjo Hokoku 1989;107:108-112

6 Pfuhl P, Karcheru, Haringn, Baumeister A , Tawab MA, Schubert–Zsilavecz M. Simultaneous Determination of Niacin, Niacinamide and Nicotinuric Acid in Human Plasma. J Pharm Biomed Anal 2005;36:1045-1052

7 Zarzycki PK, Kawalski P, Nowakowska J, Lamparczyk H. High Performance Liquid Chromatographic and Capillary Electrophoretic Determination of Free Nicotinic Acid In Human Plasma and Separation of Its Metabolites By Capillary Electrophoresis. J Chomatogr A 1995;709:203-208

8 Windahl KL, Trenerry VC, Ward CM. The Determination of Niacin in Selected Foods by Capillary Electrophoresis and High Performance Liquid Chromato Graphy Acid Extraction. Food Chem 1999;65:263-270

9 Tsuruta Y, Kohashi K, Ishida S, Ohkura Y. Determination of Nicotinic Acid In Serum By High Performance Liquid Chromatography With Fluorescence Detection. J Chromatogr 1984;30:309-315

10 ICH-Q2A. Text on Validation of Analytical Procedures. March 1995.

11 Validation of Analytical Procedures: Text and Methodology (Q2B), ICH Harmonised Tripartite Guideline.

12 General Chapter 1225, Validation of Compendial methods, United States Pharmacopeia 30, National Formulary 25, Rockville, Md., USA, The United States Pharmacopoeial Convention,Inc., (2007).