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VALIDATED RP-HPLC METHOD AS A TOOL FOR SIMULTANEOUS ESTIMATION OF DROTAVERINE HCl AND MEFENAMIC ACID IN COMBINED TABLET DOSAGE FORM

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ABSTRACT

A simple, linear, accurate and precise RP-HPLC method has been developed for the simultaneous estimation of Drotaverine HCl and Mefenamic acid in tablet dosage form. The method was developed on HPLC Waters 2695 using BDS Hypersil C_8 Column (100 x 4.6 mm, 5 µ) and the mobile phase of potassium dihydrogen phosphate (pH-2): methanol. Isocratic elution with a flow rate of 1 mL/min was employed at 30^{o}C and the responses were measured at 244 nm by using Waters PDA 2998 detector. The retention times of Drotaverine HCl and Mefenamic acid were found to be 2.033 and 6.659 min. The method shows linearity in the range of 25-75 µg/mL and correlation coefficient was found to be 0.999 for both the drugs. The method was validated as per ICH guidelines and met all specifications including force degradation studies. Statistical studies revealed that the proposed method can be successfully applied for routine analysis of Drotaverine HCl and Mefenamic acid in tablet dosage forms.

Key words: Drotaverine HCl, Mefenamic acid, RP-HPLC, Validation.

INTRODUCTION

Drotaverine, an antispasmodic drug is structurally related to papaverine and is a selective phosphodiesterase IV inhibitor. It has antispasmodic activity due to inhibition of phosphodiesterase IV and is a anticholinergic antispasmodic[1,2]. Its IUPAC name is 1-(3, 4-diethoxybenzylidine)-6, 7-diethoxy-1, 2, 3, 4-tetrahydroisoquinoline, chemical formula is $C_{24}H_{31}NO_4$.HCl and molecular weight is 433.97 g/mol.

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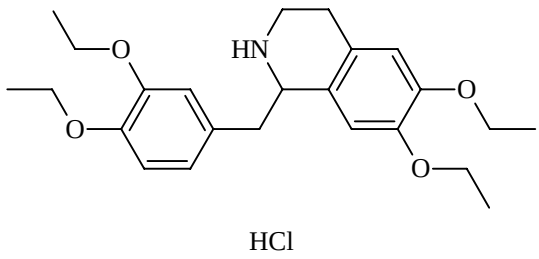

Fig. 1: Chemical structure of Drotaverine HCl

Mefenamic acid, an anthranallic acid derivative is a non-steroidal anti-inflammatory drug with extensive antiinflammatory, antipyretic and analgesic activity. The anti-inflammatory activity is mainly due to its prostaglandin synthetase inhibition. It is a COX-1 and COX-2 inhibitor, thus reducing the production of prostaglandins, which are implicated in pain and inflammation[2,3]. Systematic IUPAC name is 2-(2,3-dimethylphenyl)aminobenzoic acid, formula is $C_{15}H_{15}NO_2$ and molecular mass is 241.285 g/mol.

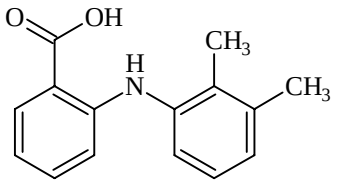

Fig. 2: Chemical structure of mefenamic acid

Literature review reveals that several methods are available for the estimation of Drotaverine HCl and Mefenamic acid for individual drugs as well as in combination. Various methods such as HPLC[4-8], spectrophotometry[9,10], HPTLC[11], and absorption ratio[12] methods have been reported. The aim of the present study is to develop a sensitive and new RP-HPLC method using a simple mobile phase and less solvent consumption, which is rapid for estimation of Drotaverine HCl and Mefenamic acid in tablet dosage forms and subsequent validation as per ICH guidelines.

EXPERIMENTAL

Materials and methods

Chemicals

All the chemicals and solvents used were AR grade and HPLC grade. These were

purchased from Rankem, New Delhi. Water (HPLC grade) was obtained from Milli-Q water purification system. Pure drugs of Drotaverine and Mefenamic acid were procured from Fourts Pharma, Chennai. Commercial tablets of Drotaverine HCl and Mefenamic acid were obtained from local pharmacy (Tavera-M, manufactured by Allkind Healthcare and marketed by Leeford Healthcare).

Apparatus

Separation was carried out using Waters HPLC equipped with PDA detector 2998 and autosampler injector. Data collection and processing was performed by Empower software. Analysis was carried out at 244 nm using BDS Hypersil C_8 column (100 x 4.6 mm internal diameter and particle size 5 µ).

Preparation of mobile phase

It was prepared by dissolving 13.6 g of potassium dihydrogen ortho phosphate in 1000 mL of water in a volumetric flask and pH adjusted to 2 with ortho phosphoric acid. Then 700 mL of the above solution and 300 mL of methanol were mixed to get 70 : 30 (v/v), filtered through Whatman filter paper and sonicated.

Preparation of standard solution

Accurately weighed quantities of Drotaverine HCl (8 mg) and Mefenamic acid (250 mg) were transferred to 100 mL volumetric flask. Then it was adjusted to final volume. From this, 5 mL was diluted to 50 mL with water to get a final concentration range of 80 µg/mL for Drotaverine HCl and 250 µg/mL for Mefenamic acid.

Preparation of sample solution

20 commercial tablets (Tavera M) were weighed, powdered and the powder equivalent to average weight (equivalent to 80 mg of Drotaverine HCl and 250 mg of Mefenamic acid) was transferred to 100 mL of volumetric flask. Then further dilutions were made with the diluent to get 80 µg/mL and 250 µg/mL solutions, which were mixed, filtered through Whatman filter paper and sonicated.

Optimisation of the method[13]

Well defined peaks were obtained by intensively changing the experimental parameters including columns, mobile phase composition, flow rate, temperature while keeping all others constant until the optimized conditions were obtained.

Table 1: Optimized chromatographic conditions

Parameter	Condition
Mobile phase	KH_2PO_4 : Methanol (70 : 30 % v/v)
Diluent	Water
Flow rate	1 mL/min
Column	BDS Hypersil C_8 (100 x 4.6 mm, 5 µ)
Column temperature	30ºC
Wavelength	244 nm
Injection volume	10 µL
Run time	8 min

Analysis of the tablet formulation

Twenty commercial tablets were weighed, powdered and powder equivalent to average weight was transferred to 100 mL volumetric flask. Further dilutions were made to get the final concentration. Then 10 µL of sample solution was injected into the HPLC under the same optimized conditions, the chromatogram was recorded and amount of the drug content in the sample was calculated.

Validation of the proposed method[14]

System suitability

System suitability was carried out on freshly prepared standard solutions by injecting 6 replicates with 8 min interval. The results obtained were recorded and compared with the acceptance criteria of the parameters like retention time, theoretical plates, tailing, % RSD, resolution and standard deviation.

Specificity

Purity of the sample was evaluated by checking the blank and placebo interference with our analyte. Placebo solution, diluent and the sample solution were injected to observe for any possible interference at the Rt of the analyte peaks.

Accuracy

The procedure was performed for accuracy determination in 50%, 100% and 150% concentration solutions by preparing six replicates for lower and higher concentrations and triplicate at mean concentration and injected to determine the recovery percentage.

Precision

Precision was carried out as method precision and intraday precision. Six replicates of the 100% sample solution were prepared and injected. The same procedure was carried out for the same stock solution after keeping aside for sometime. The % assay and % RSD were calculated, which must be within the acceptance criteria.

Linearity

Linearity was performed at the five concentration levels 50%, 75%, 100%, 125% and 150% of the standard solution. Each of these dilutions were prepared and injected into the column thereby obtaining the corresponding chromatograms. The area of the peaks were noted and the linearity was observed at this concentration range by plotting a graph between concentration vs peak area. Basing on the calibration curve, the correlation coefficient was calculated, which must be within the acceptance criteria.

LOD and LOQ

According to the S/N ratio of standard injection, the LOD and LOQ values are determined, which are considered as the limit of sensitivity. LOQ is 3 times the LOD value. These are calculated by using the following formulae.

$$LOD = 3.3\ (SD)/S \quad \ldots(1)$$

$$LOQ = 10\ (SD)S \quad \ldots(2)$$

Robustness

To show the method to be robust, small changes in the flow rate and temperature was done in the optimized conditions for the sample solution and evaluating whether the results were not affected by these alterations.

Ruggedness

Ruggedness was performed by using six replicates of the sample solution which were prepared and analysed by different analysts, columns and on different days. Results of % RSD always show < 2%, which shows that the method was rugged.

Force degradation studies[15]

Acid degradation

1 mL from the prepared sample solution was transferred into 25 mL volumetric flask. To the flask, 10 mL of 1 N HCl was added and the solution was sonicated for 30 min. The

final volume was made with the diluent and injected into the HPLC.

Base degradation

1 mL from the prepared sample solution was transferred into 25 mL volumetric flask. To the flask, 10 mL of 1 N NaOH was added and the solution was sonicated for 30 min. The final volume was made with the diluent and injected into the HPLC.

Oxidative degradation

1 mL from the prepared sample solution was transferred into 25 mL volumetric flask. To the flask, 1 mL of 1% H_2O_2 was added and the solution was sonicated for 30 min. The final volume was made with the diluents and injected into the HPLC.

Photolytic degradation

The sample was kept in the sunlight for < 24 hours and further dilutions were made to get the final volume with the diluents. It is then injected into HPLC.

RESULTS AND DISCUSSION

System suitability

All the parameters retention time, peak tailing, % RSD, theoretical plates and USP resolution were within the acceptance criteria indicating the system suitability.

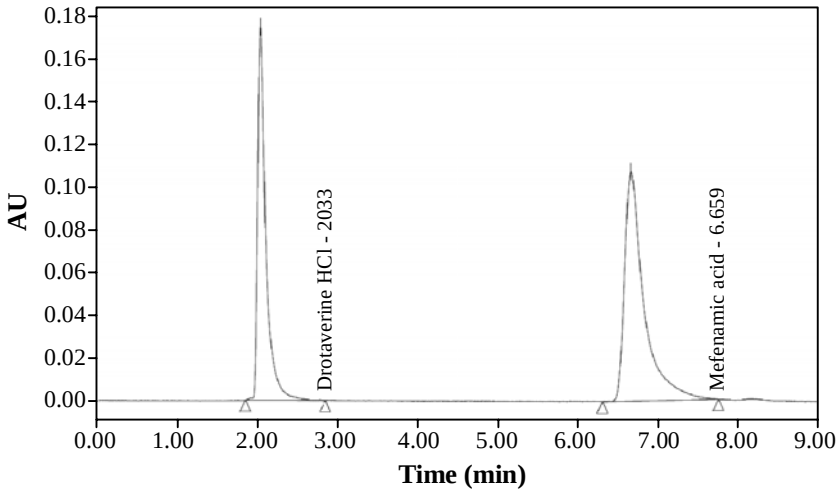

Fig. 3: Chromatogram of standard Drotaverine HCL (DRO) and Mefenamic acid (MEF) with structure of analytes

Table 2: System suitability parameters

Parameters	DRO	MEF
Retention time	2.033	6.659
Peak tailing	1.111	1.277
Theoretical plates	3504	4454
% RSD	0.1	0.1
USP Resolution	15.664	

Specificity

No interference peaks were observed at the Rt of the analyte peaks, which shows the method was specific.

Table 3: Specificity data

Interference	DRO	MEF
Analyte	2.033	6.659
Placebo	Not detected	Not detected
Blank	Not detected	Not detected

Accuracy

For both the drugs, the % recovery and % RSD are within the limits (97-103%, < 2), which shows the method was accurate.

Table 4: Accuracy data

Concentration level (%)	Mean recovery (%)		% RSD	
	DRO	MEF	DRO	MEF
50	100.52	102.45	0.1	0.3
100	100.63	102.55	0.2	0.1
150	100.47	102.38	0.2	0.3

Precision

In method precision and intraday precision, the % assay and % RSD values are within the limits (97-103%, < 2) for both the drugs.

Table 5: Precision data

Injection number	% Assay (method)		%Assay (intraday)	
	DRO	MEF	DRO	MEF
1	99.5	101.3	99.3	98.4
2	99.5	101	99.5	99.5
3	100.4	101.5	98.5	99
4	100.1	102.5	99.8	99.8
5	100.5	101.3	99.5	100.5
6	99.5	101.4	98.7	100.2
Average	99.7	101.41	99.2	99.56
% RSD	0.35	0.15	0.3	0.15

Linearity

The graph shows linearity and the correlation coefficient was found to be 0.999 for both the drugs. Linearity was assessed by plotting concentration vs area graph.

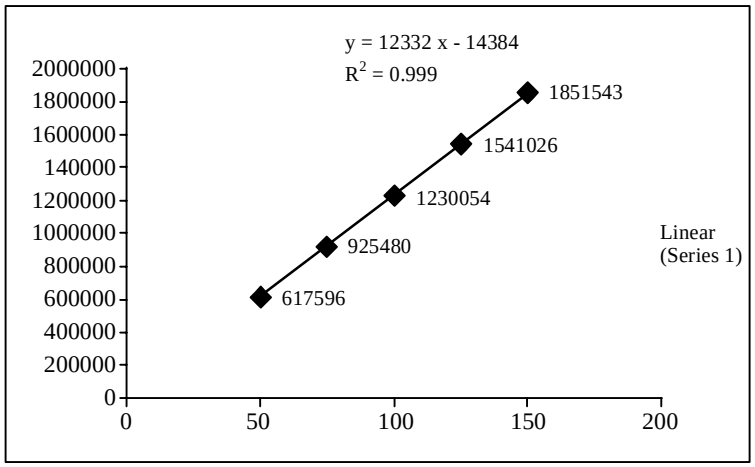

Fig. 4: Linearity curve of Drotaverine HCl

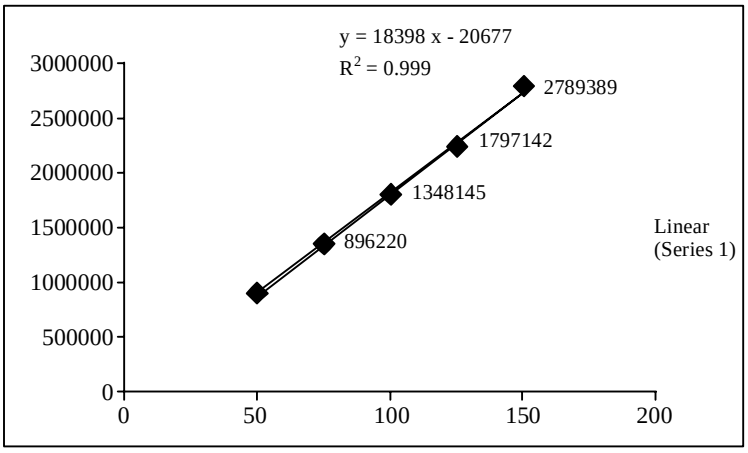

Fig. 5: Linearity curve of Mefenamic acid

Table 6: Linearity data

Concentration %	Area		µg/mL	
	DRO	MEF	DRO	MEF
50	617596	896220	25	25
75	925480	1348145	37.5	37.5
100	1230054	1797142	50	50
125	1541026	2243987	62.5	62.5
150	1851543	2789389	75	75

LOD and LOQ

For both the drugs, the minimum concentration at which the analyte can be detected and quantified was determined, which shows the limit of sensitivity.

Table 7: LOD and LOQ data

Values	DRO	MEF
LOD	0.21 µg/mL	0.25 µg/mL
LOQ	0.59 µg/mL	0.72 µg/mL

Robustness

Statistical analysis shows that there is no significant change in the results showing that the method was robust.

Table 8: Robustness data

Parameters	Optimized	Variation	Retention time		Plate count		Tailing	
			DRO	MEF	DRO	MEF	DRO	MEF
Flow rate	1 mL/min	0.8	2.538	8.321	2726	7743	1.720	1.107
		1.2	1.648	5.403	2668	5334	1.522	1.522
Temperature	30ºC	25	1.998	6.627	2549	4740	1.814	1.919
		35	1.996	6.576	2664	5366	1.742	1.563

Forced degradation studies

In all the cases, the net degradation is between 1-50% and the developed method effectively separated the degradation products from the standard peak.

Table 9: Force degradation studies data

Degradation conditions	Retention time		% Degradation	
	DRO	MEF	DRO	MEF
Acid	2.001	6.556	10	28
Base	1.999	6.546	9	30
Peroxide	1.996	6.537	32	33
Light	1.994	6.524	6	7

CONCLUSION

The present study describes a linear, precise and accurate RP-HPLC method, which was validated as per ICH guidelines. The method was rapid and very simple without any interference of excipient peaks. Hence, the above said method can be adopted for routine analysis in the estimation of Drotaverine HCl and Mefenamic acid from tablet dosage form. The method was also cost effective with respect to solvent consumption.

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