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Analytical Method Development and Validation for Simultaneous Estimation of Mefenamic Acid, Dicyclomine Hydrochloride and Pamabrom in Tablet Dosage Form

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ABSTRACT

A simple, linear, precise and accurate RP-HPLC method for simultaneous estimation of Mefenamic acid, Dicyclomine HCL, and Pamabrom in Tablet Dosage form was developed and validated. The chromatographic separation of the two drugs was achieved on Thermoscientific, BDS hypersil C18, 4.6 x 250 mm, 5µ column in an isocratic mode. The injection volume 20µl. The mobile phase consisting of Methanol: Water in the ratio of 85:15 v/v using orthophosphoric acid was delivered at a flow rate of 1ml/min and effluents were monitored at 240 nm. The retention time of Mefenamic acid, Dicyclomine HCl, and Pamabrom was found to be 3.392, 5.241 and 8.724 min respectively. Calibration curve was plots and correlation coefficients were 0.999, 0.998 and 0.999 of MEF, DIC and PAMA respectively. The method was also found to be robust. This method is precise, accurate and very simple to analysis Mefenamic acid, dicyclomine HCl and Pamabrom in tablets.

INTRODUCTION

Mefenamic acid is chemically 2-[(2,3-dimethyl phenyl) amino] benzoic acid, mol. formula $C_{15}H_{15}NO_2$, categorized in NSAID (Non-steroidal anti-inflammatory drug), Mefenamic acid is used for the treatment of rheumatoid arthritis, osteoarthritis, dysmenorrheal, and mild to moderate pain, inflammation, and fever and it is sparingly soluble in ether, slightly soluble in ethanol (95%) and in chloroform, practically insoluble in water. Dicyclomine Hydrochloride is chemically 2-(diethylamino)ethyl-1-cyclohexylcyclohexane-1-carboxylate), chemical formula $C_{19}H_{35}NO_2$, it is an anti-spasmodic (used to relieve spasm of involuntary muscle. For the treatment of Irritable Bowel Syndrome including adnominal pain. It is Freely soluble in ethanol (95%) & in chloroform, soluble in water, practically insoluble in ether. Pamabrom is chemically 2-amino-2-methylpropan-1-ol;8-bromo-1,3dimethyl-2,3,6,9-tetrahydro-1H-purine-2,6-dione. Chemical formula is $C_{11}H_{18}BrN_5O_3$. It is a diuretic drug. Pamabrom is used to treat bloating, swelling, feelings of fullness and other signs of water weight gain related to menstrual symptoms. It is soluble in water. This combination therapy was shown to be the antispasmodic agent (used for the menstrual pain) and analytical research and development of fixed-dose combination are found to be very interesting and challenging job, hence the development of method for Mefenamic acid, Dicyclomine Hydrochloride and Pamabrom in combination has been selected for the present study. A detailed literature survey shows that there exists literature concerning analytical method development and validation for drugs Paracetamol, Dicyclomine HCl and Pamabrom. The objective of this study is developing a new accurate precise and linear study RP-HPLC method for the simultaneous invalidating as per ICH guideline.

MATERIALS

Keplar Healthcare Pvt. Ltd (Ahmedabad, Gujarat, India) supplied Mefenamic acid working standard grade and its claimed purity was 99.2%. Dicyclomine HCl and Pamabrom working standard grade was supplied by same Keplar Healthcare Pvt. Ltd (Ahmedabad, Gujarat, India) and its claimed purity was 99.5% for both drugs. Water (HPLC grade), Methanol (HPLC grade), Spectrochem, Orthophosphoric Acid (HPLC grade) Merck, Rankem, AGILENT (1100) HPLC system column inertsil C8 (id 4.6 x 250 mm length.), Pump SP930 D solvent delivery system, detector UV 730 D UV-Visible detector, Rheodyne injector (20 µl capacity), syringe Hamilton (25 µl) and Chromatographic software CHEMSTATION.

Preparation of solution

Preparation of Standard Stock Solution of MEF

Accurately weighed quantity of MEF 100 mg was transferred into 25mL volumetric flask, was added 25mL of methanol then sonicated for 10 minutes. Final volume of solution was made up to mark with diluent to get stock solution containing 4 mg/mL of MEF in 25 mL volumetric flask, the resultant stock solution of MEF having strength of 4000µg/mL.

Preparation of working stock solution of MEF

A solution of 40µg/mL of MEF solution was prepared by diluting 0.1 mL of standard stock solution with diluents in 1 mL volumetric flask up to the mark.

Preparation of Standard Stock Solution of DIC

Accurately weighed quantity of DIC 2 mg was transferred into 25mL volumetric flask, was added 25mL of methanol then sonicated for 10 minutes. Final volume of solution was made up to mark with diluent to get stock solution containing 80mg/mL of DIC in 25 mL volumetric flask, the resultant stock solution of DIC having strength of 80µg/mL.

Preparation of working stock solution of DIC

A solution of 0.8 µg/mL of DIC solution was prepared by diluting 0.1 mL of standard stock solution with diluents in 10 mL volumetric flask up to the mark.

Preparation of standard stock solution of PAMA

Accurately weighed quantity of PAMA 5 mg was transferred into 25mL volumetric flask, was added 25 mL of diluents then sonicated for 10 minutes. Final volume of solutions was made up to mark with diluent to get stock solution containing 200mg/mL of PAMA in 25mL volumetric flask, the resultant stock solution having strength of 200 µg/mL.

Preparation of working stock solution of PAMA

A solution of 2 µg/mL of PAMA solution was prepared by diluting 0.1 mL of standard stock solution with diluents in 10 mL volumetric flask up to the mark.

Preparation of Sample Solution

Twenty tablets were weighed. The powder from twenty tablets were collected and weighed. The Powder equivalent to 500 mg of MEF, 20 mg of DIC and 25mg PAMA. Total weight of 20 tablets was 18.478 gm. Average powder weight 0.9239gms per tablet. Equivalent weight for 100 mg was 184.28 mg in 25 ml methanol.

Chromatographic Condition

Optimized condition was found to be as follows

Finalized HPLC system specification

Column	Thermoscientific, BDS hypersil C18, 4.6 x 250 mm, 5µ
Low Rate	1.0 mL/min.
Mobile Phase	METHANOL: 0.1 % OPA WATER 85 : 15
Detection	240 nm
Injection Volume	20 µl
Run time	10 Minute
Diluent	Mobile Phase

Accuracy

Accuracy of the method was determined by recovery experiments. To the formulation, the reference standards of the drug were added at the level of 80%, 100%, 120%. The recovery studies were carried out three times and the percentage recovery and percentage relative standard deviation of the recovery were calculated.

Precision

Intra-day precision

Combined standard solutions containing mixture of 40 ppm, 12 ppm and 200 ppm of MEF, 0.8ppm, 2.4ppm and 4ppm of DIC and 0.2ppm, 06 ppm and 10ppm of PAMA were analyzed 3 times on the three different days. The % R.S.D for the MEFA, DIC and PAMA was calculated.

Interday precision

Combined standard solutions containing mixture of 50 ppm, 150 ppm and 250ppm of MEF, 0.8ppm, 2.4ppm and 4ppm of DIC and 50 ppm, 150 ppm and 250ppm of PAMA were analyzed 3 times on the three different days. The % R.S.D for the MEF, DIC and PAMA was calculated.

LOD and LOQ

The LOD and LOQ were estimated from the set of 5 calibration curves used to determine method linearity. It may be calculated as

$$LOD = 3.3 \times (SD / Slope)$$

$$LOQ = 10 \times (SD / Slope)$$

Where, SD = the standard deviation of Y- intercept of 5 calibration curves.

Slope = the mean slope of the 5 calibration curves. Result of LOD and LOQ shows below

Parameter	**MEF**	**DIC**	**PAMA**
S.D. of Intercept	67.45	5.75	13.5
Slope of Calibration Curve	40.77	89.64	107
LOD (ppm)	0.54	0.022	0.14
LOQ (ppm)	1.64	0.069	0.45

Robustness

Change following parameters, one by one and observe their effect on system suitability test and assay.

- Change mobile phase composition by ± 1.0mL of organic solvent.
- Change Wavelength ± 1nm

Change flow rate ±0.1mL/min

Results of Robustness Parameter shows below

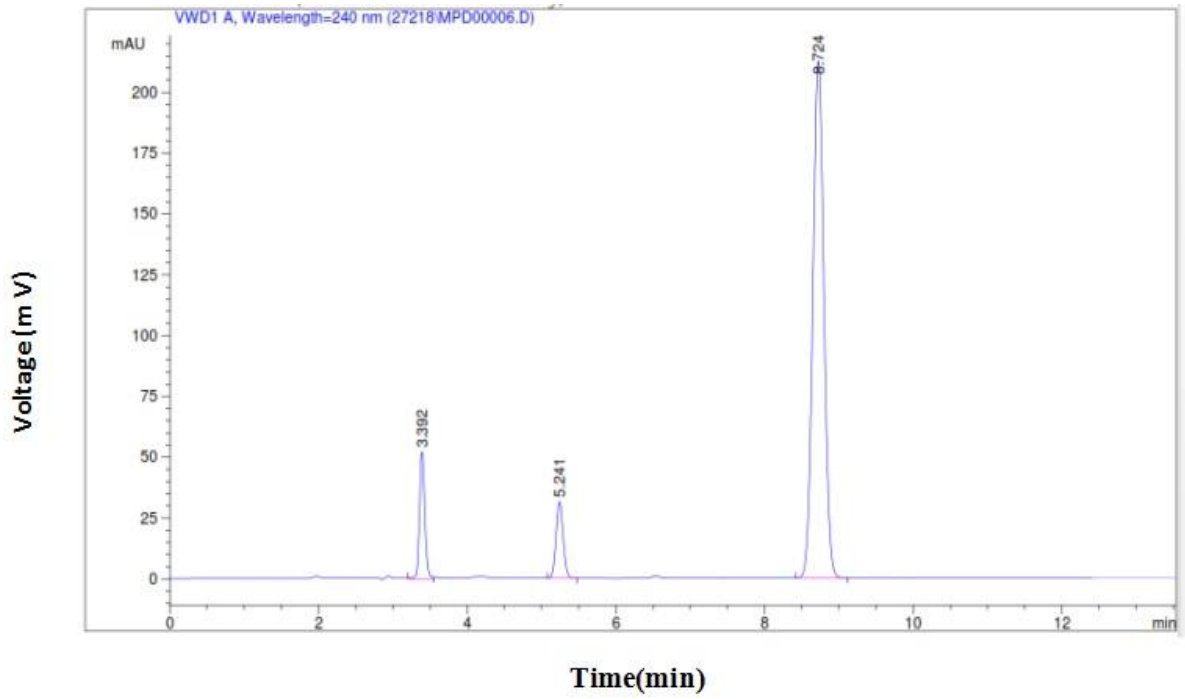
Condition	Peak area mean*			S.D			%R.S.D.		
	MEF	DIC	PAMA	MEF	DIC	PAMA	MEF	DIC	PAMA
Change in the Mobile Phase Composition (± 1 mL organic Phase)									
Change in +1mlorganic phase and	6979.0	382.1	1101.6	0.33	0.32	1.59	1.02	0.09	0.14
Change in -1 ml organic phase(84:16:v/v)	8395.13	357.01	1108.88	0.23	1.33	0.28	0.01	0.07	0.03
Change in the Wavelength by ± 1 nm									
Change in the + 1 nm and	7917.6	382.1	1019.2	6.69	0.33	0.03	0.08	0.09	0.00
Change in the – 1 nm	8625.45	357.01	1121.66	4.65	0.23	0.08	3.54	0.07	0.01
Change Flow rate (±0.1 mL/min)									
Change in the + 0.1mL/min F.R. and	9354.56	418.22	1230.69	1.10	0.69	1.07	0.01	0.16	0.09
Change in the - 0.1mL/min F.R.	7644.09	342.15	1005.09	1.58	0.76	0.42	0.02	0.22	0.04

RESULTS AND DISCUSSION

Optimization of method

Optimization of chromatographic condition, the effect of different chromatographic variables such as composition of mobile phase, pH of mobile phase, flow rate and column studied. The resulting chromatograms were recorded and obtained chromatographic parameters such as asymmetric factors, resolution and theoretical plate were selected for estimation. By using the C18 column the best resolution peak shape, without excessive tailing, were obtained the effect of both mobile phase composition, flow rate were studied.

Chromatogram of sample MEF, DIC and PAMA by using optimized method.



CONCLUSION

HPLC is the most widely used laboratory technique for separation identification and quantification of components of liquid and gaseous mixtures. Solid mixtures are also analyzed by first converting them to a liquid or gaseous state, using suitable sample preparation techniques.

- A novel RP- HPLC method has been developed for the simultaneous estimation of Mefenamic acid, Dicyclomine Hydrochloride and Pamabrom marketed formulations.

- The method gave good resolution for the all drugs with a short analysis time below 6 minutes. The developed method was validated. It was found to be simple, precise and accurate.

- The good % recovery in tablet forms suggests that the excipients present in the dosage forms have no interference in the determination. The % RSD was also less than 2 % showing high degree of precision of the proposed method.

- The proposed method can be used for routine analysis Mefenamic acid, Dicyclomine Hydrochloride and Pamabrom combined dosage form. It can be also used in the quality control in bulk manufacturing.

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