

Method development and validation of mefenamic acid, dicyclomine hydrochloride, and pamabrom of marketed formulation by ultraviolet

Gagandeep Pabla¹, Anshul Kumar², Pawan Porwal¹, Durgadas Anghore^{2*}

¹Department of Pharmaceutical Chemistry, ISF College of Pharmacy, Moga, Punjab, India, ²ISF College of Pharmacy, Ghal Kalan, Moga, Punjab 1420001, India

Correspondence:

Durgadas Anghore, Associate Professor, ISF College of Pharmacy, MOGA, Punjab, India.
Phone: +91-9736365252.
E-mail: dd_anghore@yahoo.com

How to cite this article: Gagandeep, Kumar A, Porwal P, Durgadas, Anghore. Method development and validation of mefenamic acid, dicyclomine hydrochloride, and pamabrom of marketed formulation by ultraviolet. *Pharmaspire* 2018;7:64-67.

Source of Support: Nil,

Conflicts of Interest: None declared.

ABSTRACT

The method has been developed and validated using the spectrophotometric technique for the simultaneous estimation of mefenamic acid (MEF), dicyclomine hydrochloride (DCL), and pamabrom (PABr) in bulk powder and in pharmaceutical formulations with a high degree of specificity, selectivity, and assurances. The first Vierdot's method was performed and absorption maxima of MEF, DCL, and PABr at 285, 214.6, and 279 nm, respectively. Calibration graphs were established in the range of 2–10 µg/mL for MEF and PABr and 200–1000 µg/mL for DCL. Dilution was prepared in 0.1 N sodium hydroxide.

Keywords: Dicyclomine, mefenamic acid, method validation, pamabrom, ultraviolet spectrophotometry

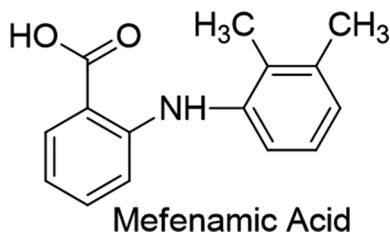
INTRODUCTION

For the discovery, development and manufacturing of pharmaceuticals, analytical method development plays an important role. Thus, to determine the amount or concentration of active pharmaceutical ingredient(s) in pure or pharmaceutical dosage form, analytical method development is a standardized laboratory procedure. Various analytical methods that are used by quality control laboratories to ensure the identity, purity, potency, and performance of drug products are high-performance liquid chromatography, ultraviolet (UV) spectrophotometry, high-performance thin-layer chromatography, titration, and fluorescence spectroscopy.^[1,2]

MEFENAMIC ACID (MEF)

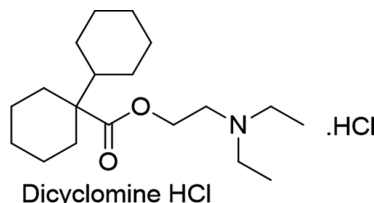
MEF is chemically 2-(2, 3-dimethylphenyl amino) benzoic acid. MEF is used for the short-term treatment of mild-to-moderate pain from various conditions. It is also used to decrease pain and blood loss from menstrual periods. MEF is known as a nonsteroidal anti-inflammatory drug (NSAID). MEF is a nonsteroidal drug with analgesic, anti-

inflammatory, and antipyretic activity. It inhibits prostaglandin synthesis and competes for binding at the prostaglandin receptor site.^[3]



DICYCLOMINE HYDROCHLORIDE (DCL)

Dicyclomine is chemically 2-(diethylamino) ethyl 1, 1'-bi (cyclohexyl)-1-carboxylate. It is an antispasmodic and anticholinergic (antimuscarinic) agent. Its action is achieved through a dual mechanism: A specific anticholinergic effect (antimuscarinic) at the acetylcholine-receptor sites, a direct effect on smooth muscle (musculotropic).^[4]



Access this article online

Website: www.isfcppharmaspire.com

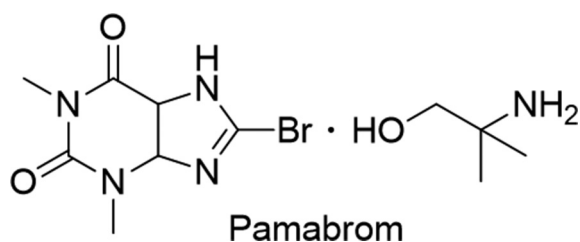
P-ISSN: 2321-4732

E-ISSN: XXXX-XXXX

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

PAMABROM (PABR)

PABr is chemically 2-amino-2-methylpropanol 8-bromothioephyllylate. It is a xanthine derivative and it might increase the renal blood flow by virtue of their cardiac stimulant property and vasodilator action which promotes filtration of fluid by the glomeruli and also produce diuresis by diminishing the tubular reabsorption of water. Interference in tubular reabsorption of Na^+ and Cl^- perhaps by acting on the enzyme concerned with the transport of these ions.^[5-7] Physical property has been described in Table 1.



EXPERIMENTAL

Chemicals and reagents

Marketed formulation of Twagic spas Manufactured Date: April 2017, Expiry Date: March 2019, Manufactured by Akums Drugs and Pharmaceuticals Ltd. and marketed by Kepler Healthcare Pvt. Ltd. was procured from Kepler Healthcare Pvt. Ltd. Ahmedabad, Gujarat, India. Acetonitrile, methanol, and water, were purchased from Rankem (New Delhi, India).

Instrumentation

UV-visible double beam spectrophotometer Perkin-Elmer Lambda-35 was used for all spectrophotometric measurements, having slit width of 1 nm, installed with UV Winlab and UV Winlab data processor and UV probe (2.31) software. Ultra-bath sonicator (PCI analytics 3.5 L capacity) was used for proper mixing and sonication of stock solutions. FTIR spectrometer (Thermo Nicolet iS10, Corp., USA) with computer loaded with EZ Omnic software, Analytical Balance (Mettler Toledo, AB204-S/FACT), and Water purification systems ELIX 03 (MILLIPORE, USA).

Standard solution

Standard laboratory solution was prepared using 0.1 N sodium hydroxide. Weighed accurately 25 mg of standard drug for each of MEF, DCL, and PABr then transfer in 25 ml of volumetric flask and make up the volume up to mark. Stock solution used to prepare desired concentration range as per sample calibration range. The final standard laboratory mixture dilution contained 10 $\mu\text{g}/\text{mL}$ of MEF, 10 $\mu\text{g}/\text{mL}$ of PABr, and 100 $\mu\text{g}/\text{mL}$ of DCL which was used for UV spectroscopy scanning. Weight 25 mg of standard drug of each MEF, DCL, and PABr then transfer in 25 ml of volumetric flask and make up the volume. The final standard laboratory mixture dilution contained 100 $\mu\text{g}/\text{mL}$.

Sample preparation

A total of 20 tablets were weighed and finely powdered. The equivalent weight was calculated and according to the average weight, required drug was taken in volumetric flask and MEF (10 $\mu\text{g}/\text{mL}$), PABr (10 $\mu\text{g}/\text{mL}$), DCL (100 $\mu\text{g}/\text{mL}$) were prepared. In spiking method, required amounts of MEF, DCL, and PABr (APIs) were added. The resultant solution was filtered through Whatman filter paper number 41. Then, further dilutions were made in 0.1 N sodium hydroxide and absorbance was measured and analyzed. Stock solutions used to prepare desired concentration range as per sample calibration range of 2–24 $\mu\text{g}/\text{mL}$ of MEF, 2–24 of PABr, and 200–1000 of DCL.

RESULTS AND DISCUSSION

Vierdot's method

In this method, calibration curves were obtained of all the three drugs and the absorbance of MEF, DCL, and PABr was determined at 200–400 nm. The λ_{max} was found at 285, 214.6, and 279 nm, respectively. The linearity curves were plotted for quantitative analysis of MEF, DCL, and PABr. Linearity ranges were observed in concentration range of 2–10 $\mu\text{g}/\text{mL}$ of MEF, 2–10 of PABr, and 200–1000 of DCL. The results of calibration curves were plotted and shown in Figure 1 and Table 2. From these calibration curves, slope, intercept, and correlation coefficient were obtained. Thus, regression equations were calculated. These equations were used for simultaneous estimation of MEF, DCL, and PABr in standard laboratory mixture and marketed formulation. The limit of detection and limit of quantification have been calculated as per ICH guidelines. The intraday and interday were carried out and relative standards deviation was calculated. The method was found to be precise due to low values of the percentage relative standard deviation shown in Table 2 and assay of market formulation contain MEF, PABr and DCL were shown in Table 3. The Overlain Spectra of MEF, DCL, PABr and Mixture shown in Figures 2 and 3.

Linearity curve

The linearity curves were plotted for quantitative analysis of MEF, DCL and PABr. Linearity ranges were observed in concentration range of 2–10 $\mu\text{g}/\text{mL}$ of MEF, 2–10 of PABr and 200–1000 of DCL.

CONCLUSION

UV spectrophotometer method was developed and validated for the simultaneous determination of MEF, DCL, and PABr in

Table 1: Resulting parameter of MEF, PABr, and DCL

MEF		PABr		DCL	
Conc.	abs	Conc.	abs	Conc.	abs
2	0.097	2	0.100	200	0.032
4	0.215	4	0.178	400	0.201
6	0.341	6	0.263	600	0.450
8	0.441	8	0.337	800	0.627
10	0.566	10	0.402	1000	0.830

DCL: Dicyclomine hydrochloride, MEF: Mefenamic acid, PABr: Pamabrom

Table 2: Resulting parameters of Vierdot's method

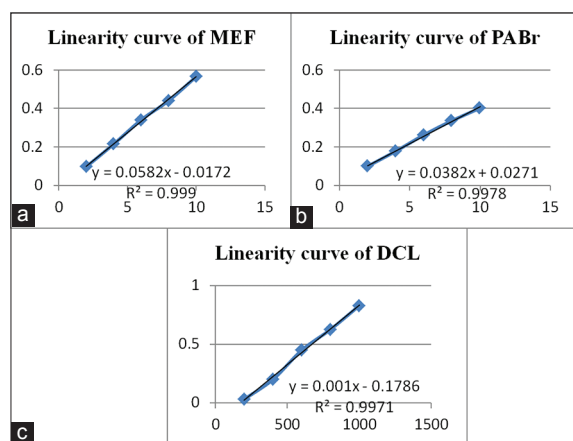
Validation parameter	MEF	PABr	DCL
Absorption maxima, λ_{\max} (nm)	285	279	214.6
Linearity range ($\mu\text{g/mL}$)	2–10	2–10	200–1000
Coefficient of determination (R^2)	0.999	0.997	0.997
Regression equation (y)	$y=0.0582x-0.0172$	$y=0.0382x+0.0271$	$y=0.001x-0.1786$
Slope (b)	0.0582	0.0382	0.001
Intercept (a)	-0.0172	+0.0271	-0.1786
Limit of detection ($\mu\text{g/mL}$)	0.30	0.44	51.08
Limit of quantification ($\mu\text{g/mL}$)	1.02	1.48	170.28
Precision (%RSD)	Intraday=0.55 Interday=0.57	Intraday=0.47 Interday=0.53	Intraday=0.74 Interday=0.76

UV: Ultraviolet, DCL: Dicyclomine hydrochloride, MEF: Mefenamic acid, PABr: Pamabrom

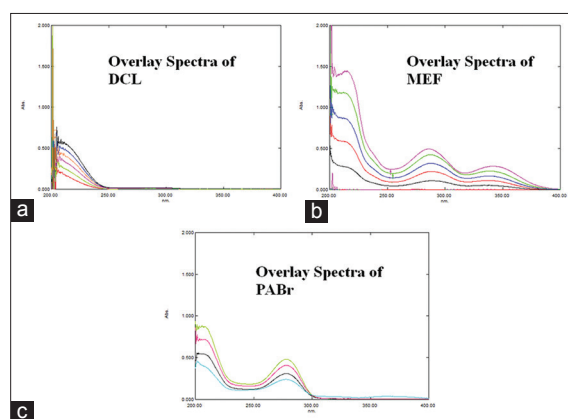
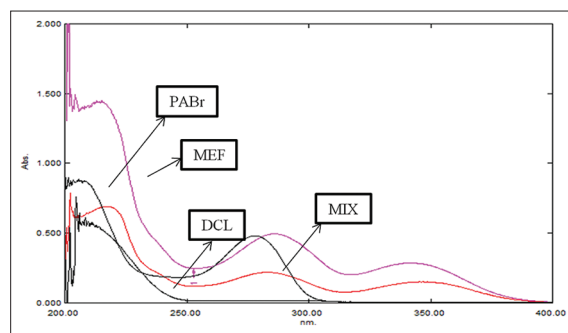
Table 3: Result of assay using UV spectrophotometry

Drug	Label claim (mg)	Vierdot's method	
		Amount (mg)	Estimation %
MEF	500	480.27	96.0544
PABr	25	10.325	103.253%
DCL	10	26.451	105.803%

UV: Ultraviolet, DCL: Dicyclomine hydrochloride, MEF: Mefenamic acid, PABr: Pamabrom

**Figure 1:** Linearity graph in 0.1 N sodium hydroxide (a) mefenamic acid, (b) pamabrom, and (c) dicyclomine hydrochloride

pharmaceutical dosage forms. The method was found to be simple, precise, and rapid. The assay results were found by three methods in fair agreement. In UV spectrophotometer, Vierdot's method was used for selective determination of MEF, DCL, and PABr in the presences of pharmaceutical dosage forms or any variety of matrices. The derivative method serves as alternative method for the determination of MEF, DCL, and PABr in commercial samples. A comparative study of the use UV spectrophotometer method for the resolution of ternary mixture of MEF, DCL, and PABr has been accomplished. The results of Vierdot's method have significant resolution. UV spectrophotometer method was less expensive and do not require sophisticated instruments or any specific condition. As per literature survey, no analytical method has been developed for this particular combination (MEF, dicyclomine HCl, and PABr) of drugs by UV method. The present study was to develop sensitive, simple, rapid, economical, precise, and accurate UV method to determine mefenamic acid, dicyclomine HCl, and PABr in marketed tablet formulation.

**Figure 2:** Overlay graphs of linearity (a) dicyclomine hydrochloride, (b) mefenamic acid, (c) pamabrom**Figure 3:** Overlay spectra of, dicyclomine hydrochloride, mefenamic acid, pamabrom, and mixture

REFERENCES

- Patel MM, Bhuvra SD, Patel HD, Mori KN. An overview of the recent developments in analytical methodologies for determination of proton pump inhibitors in bulk drugs, pharmaceuticals and biological matrices. *Eurasian J Anal Chem* 2014;9:25-48.
- Mittal M, Upadhyay Y, Anghore DD, Kumar A, Rawal RR. Simultaneous estimation of acebrophylline, montelukast, and levocetirizine dihydrochloride in marketed formulation by high-performance liquid chromatography method. *Pharmaspire* 2018;10:23-8.
- Colombo R, Ferreira CR, Lanza RV. Removal of mefenamic acid from aqueous solutions by oxidative process: Optimization through experimental design and HPLC/UV analysis. *J Environ Manage* 2016;167:206-13.

4. Prajapati D, Raj H. Simultaneous estimation of mefenamic acid and dicyclomine hydrochloride by RPHPLC method. *Int J Pharm Biosci* 2012;3:611-25.
5. Abdelaleem EA, Naguib I, Hassan ES. HPTLC and RP-HPLC methods for simultaneous determination of paracetamol and pamabrom in presence of their potential impurities. *J Pharm Biomed Anal* 2015;114:22-7.
6. Mittal M, Upadhyay Y, Anghore DD, Rawal RR. Simultaneous estimation of acebrophylline, montelukast and levocetirizine dihydrochloride in marketed formulation by UV-spectroscopy. *World J Pharm Pharm Sci* 2016;5:1274-84.
7. Kumar N, Anghore DD, Rawal RK, Pandey A. RP-HPLC and UV method development for simultaneous estimation of doxofylline, montelukast and levocetirizine dihydrochloride in pharmaceutical dosages form. *Anal Chem Lett (Taylor and Francis)* 2018;8:195-204.