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DEVELOPMENT AND VALIDATION OF HPLC ANALYTICAL ASSAY METHOD FOR MEFENAMIC ACID TABLET (PONSTAN)

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ABSTRACT

Mefenamic acid is analgesic and anti-pyretic used to treat menstrual pain. A simple assay method by HPLC was developed and validated for mefenamic acid tablet (Ponstan). Analyses of mefenamic acid in a commercial tablet, Ponstan were performed using HPLC- Uv-Visbleat 275 nm on a reverse phase column Chromolith (RP-18e, 100 mm x 4.6 mm, 5 µm), a binary mobile phase; A: 0.1% formic acid in deionised water, B: 100% acetonitrile. The validation aspects were selectivity, linearity, precision, accuracy and quantification limit. Linearity, 5-250 mgL^{-1}, provided determination coefficients (R^2) of 0.9995, and proved precise since the RSD% was less than 5% for three replications analysis. The recoveries obtained ranged from 99% to 108%. In this study, the optimisation of mobile phase, flow rate, volume injection and wavelength were achieved through a statistical treatment. The retention time and drug content of mefenamic acid was 3.9 min and 97%, respectively. This method is precise, accurate and very simple to analysis mefenamic acid in tablets.

Key words: Pharmaceutical, Mefenamic acid, Ponstan tablet, HPLC assay method.

INTRODUCTION

Mefenamic acid is a non-steroidal anti-inflammatory drug used to treat pain, including menstrual pain. It is typically prescribed for oral administration. Mefenamic acid is marketed in the USA as Ponstel but commonly known in UK as Ponstan. Mefenamic acid has molecular formula $C_{15}H_{15}NO_2$ and molecular weight 241.29 g/mol. Chemically,

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mefenamic acid is 2-[(2,3-dimethyl phenyl) amino] benzoic acid as presented in Fig. 1. It is metabolized to 3-hydroxymethyl mefenamic acid and further oxidation to a 3-carboxy mefenamic acid may occur. The physical properties for mefenamic acid are white solid, melting temperature 230^{o}C and water solubility 20 ppm[1].

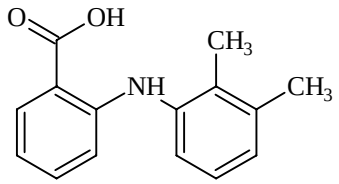

Fig. 1: Chemical structure of mefenamic acid

The assay of mefenamic acid in immediate release tablets is usually carried out by UV spectrophotometry as British Pharmacopoeia[2]. Literature survey revealed that several methods were used to analysis of mefenamic acid in tablets, human urine and human blood serum. These methods include Infrared, high performance liquid chromatography, capillary electrophoresis, spectrophotometry, potentiometric and colorimetric[3-9].

The aim of this study is performing very simple method in terms of mobile phase and program to analysis mefenamic acid in Ponstan tablets, compared with the provided standard method as British Pharmacopoeia.

EXPERIMENTAL

Materials and methods

Standard and chemical reagents

Drug standards for mefenamic acid (CAS : 61-68-7) were obtained from Sigma Aldrich (USA). Deionised distilled water (DIW) used was obtained from EASY Pure RODI (USA). HPLC grade methanol (MeOH) Merck (Germany), HPLC-grade acetonitrile (ACN) Merck (Germany) and formic acid (FA) Merck (Germany) were used.

Chromatographic conditions

Liquid chromatography was performed on Waters (Milford, MA 01757 USA) system equipped with a binary pump, an auto sampler system and a Uv-Visible detector. Chromatography was performed on a Chromolith® Performance RP-18e (4.6 × 100 mm, 5 µm) column in an oven at 35 ± 0.3^{0}C. All compounds were eluted off the column with a mobile phase consisting of (A) 0.1% formic acid in deionised water (DIW) and (B) 100% ACN at 1.0 mL min^{-1}. The elution started at 0% B and was then linearly increased to 100%

B over 4 min and then kept isocratic for 1 min. The injection volume was 20 µL. The retention time of mefenamic acid was around 3.9 min and the total run was 5 min.

The method was validated in accordance with International Conference on Harmonization guidelines for validation of analytical procedures[10, 11].

Specificity and selectivity

These parameters were determined by comparing the chromatograms of the mefenamic acid standard, tablet drug Ponstan and methanol as a solvent.

Linearity

The linearity of an analytical procedure is its ability within a given range to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample[10,11]. The linearity was tested in the concentration range value of 0.5- 250 mgL^{-1}.

Accuracy

The accuracy was determined by adding different amounts of the standard to the solvent; 10, 20 and 50 mgL^{-1}. Then all concentrations were analyzed with triplicate injection to calculate the accuracy in terms of recovery.

Precision

The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). The repeatability was calculated as relative standard deviation (RSD) with three replications and three different concentrations during same day. Intermediate precision was studied by comparing the assays on two different days.

Limit of quantification (LOQ)

LOQ was determined as the concentration in which the ratio signal/noise was around 10 which found to be 0.5 mgL^{-1}.

Sample preparation

In this study, Ponstan as a brand name of mefenamic acid was analyzed under same optimal chromatographic conditions as shown in Table 1. Ten tablets of Ponstan each containing 500 mg of mefenamic acid were initially powdered. A quantity of powder equivalent to 10 mg of mefenamic acid was weighed and transferred to 10 mL volumetric

flask. The solution was placed on magnetic stirrer at 45^0C for 10 min, and then filtered through 0.2 µm membrane filter Nylon. A total volume of 90 mgL^{-1} Ponstan was prepared and 20 µL was injected, the chromatogram was hold up to 5 min.

Table 1: Optimal chromatographic conditions of Ponstan

Aspect	Description
Mobile phase	(A) 0.1% formic acid in deionised water (DIW) and (B) 100% acetonitrile (ACN)
HPLC Column	Chromolith® Performance RP-18e (4.6 × 100 mm, 5 µm)
Flow rate	1.0 mL^{-1}
Injection volume	20 µL
Retention time	3.9 min
Runtime	5 min

Optimization method

Four parameters were optimized to get better separation. These parameters were mobile phase, flow rate, wavelength and volume injection.

Statistical analysis

The statistical analysis were achieved for wavelength, volume injection and flow rate by using statistical analysis software (SPSS ANOVA Version 19, Duncan with P = 0.05).

RESULTS AND DISCUSSION

Analytical method development

The optimization of mobile phase, flow rate, wavelength and volume injection is considered very important to achieve good separation and peak area. In this study, we observed no significant difference between the results obtained with the mobile phase (100% ACN and ACN/DIW, 90 : 10). However, 100% ACN provide better separation and shorter time.

The mobile phase made up of 100% methanol produced too late peak with area lower than last two mobile phases, may be this is attributed to low polarity of methanol compared with acetonitrile.

In case of these three mobile phases (ACN/DIW, 60:40; ACN/DIW, 70 : 30; ACN/DIW, 50 : 50) no peak was obtained, may be this is attributed to the very low polarity of these mobile phases to be suitable for elution mefenamic acid. In one experiment done, we get peak for mefenamic acid using these mobile phases but need to increase run time till 10 min. The mobile phase chosen for analytical method validation was 100% ACN, presented a mobile phase holdup time of 3.9 min and good separation as shown in Table 2.

Table 2: The optimization of mobile phase on mefenamic acid analysis

Effect of mobile phase		
Mobile phase (B)	**Rt (min) ± SD, n = 4**	**Mean area ± SD, n = 4**
100% CAN	3.908 ± 0.022	2658430 ± 82108.75
ACN/DIW (90 : 10)	4.136 ± 0.026	2600964 ± 41152.11
ACN/DIW (90 : 10)	4.136 ± 0.026	2600964 ± 41152.11
ACN/DIW (60 : 40)	No peak	No peak
ACN/DIW (70 : 30)	No peak	No peak
ACN/DIW (50 : 50)	No peak	No peak
100% MeOH	4.651 ± 0.039	1787543 ± 93651.84

Flow rate was optimised with (0.8, 1.0, 1.5 and 2 mL/min). At 0.8 mL/min, there is no peak appeared in the chromatogram with 3 replications. This is attributed to the insufficient flow rate to elute mefenamic acid through the column. However, a significant difference was observed among all the rest flow rates. Based on the results obtained, 1 mL/min showed the best results in terms of peak area and retention time. An optimization on the flow rates mefenamic acid analysis shown in Table 3.

Table 3: The optimization of flow rate on mefenamic acid analysis

Effect of flow rate		
Flow rate (mL/min)	**RT (min) ± SD, n = 3**	**Peak area ($\mu V.S^{-1}$) ± SD**
1	3.906 ± 0.027	2684118 ± 78442.8
0.8	No peak	No peak
1.5	2.322 ± 0.010	2022083 ± 172058.1
2	1.725 ± 0.023302	1331381 ± 50930.6

In case of wavelength, there is no significant difference among the three wavelengths as shown in Table 4. While, volume injection appeared significant difference between 5 and 20 µL, this is related to the amount of analyte passed through the column. Table 5 was presented the results of optimization of wavelength on mefenamic acid analysis.

Table 4: The optimization of wavelength on mefenamic acid analysis

Effect of wavelength		
Wavelength (nm)	**Rt (min) ± SD**	**Peak Area (µV/s) ± SD**
285	3.8395 ± 0.05102	14229773 ± 630344.5
275	3.85325 ± 0.034316	15243020 ± 728019.2
260	3.85975 ± 0.055229	13999256 ± 572856.1

Table 5: The optimization of injection volume on mefenamic acid analysis

Effect on injection volume		
Volume Injection	**R_t (min) ± SD**	**Peak area (µV/s) ± SD**
5	3.908667 ± 0.034933	194823.3 ± 34957.63
10	3.902667 ± 0.052272	269591 ± 16103.19
20	3.907667 ± 0.04884	359033 ± 15353.61

Analytical method validation

Linearity

The linear regression equation obtained by the proposed method was y = 26202 x + 48145.7, where y represents the integrated peak area in the chromatogram, and x represents mefenamic acid concentration in mg/L. The correlation coefficient obtained of 0.9995 demonstrates the good quality of the calibration curve.

Accuracy

Accuracy is one of the most important parameters of an analytical methodology and it can be expressed as the percent recovery of known amounts of drug added to a sample. The recoveries were determined by adding known amounts of the mefenamic acid standard (10, 20 and 50 mgL^{-1}) to the solvent (0 mgL^{-1}).

The results presented in Table 6 refer to the average of three assays for each concentration. The results are in good agreement with acceptable values for the validation of

an analytical procedure, which is recovery achieved 80-110 %. Ponstan solution was presented good recoveries and agreement with standards of method validation[10,11] as shown in Table 7.

Table 6: An average of three assays for each samples concentrations

Concentration (mgL^{-1})	Mean (n = 4)	RSD	Recovery%
10	10.3	3.2	103
20	19.8	4.1	99
50	52.6	5.3	105

Table 7: Summary of recoveries and agreement with standards of method validation

Concentration ponstan (500 mgL^{-1})	Actual concentration (mgL^{-1})	Recovery %
90	92.5	102
90	88.0	98
90	82.0	91
Average	87.5	97
Standard deviation (ppm)	5.2	5.5
Relative standard deviation (%)	5.9	5.6

In Fig. 2, a mefenamic acid chromatogram obtained under chromatographic conditions shows a single well defined peak of mefenamic acid, with a 1.25 and 1.5 for tailing factor and asymmetry, respectively.

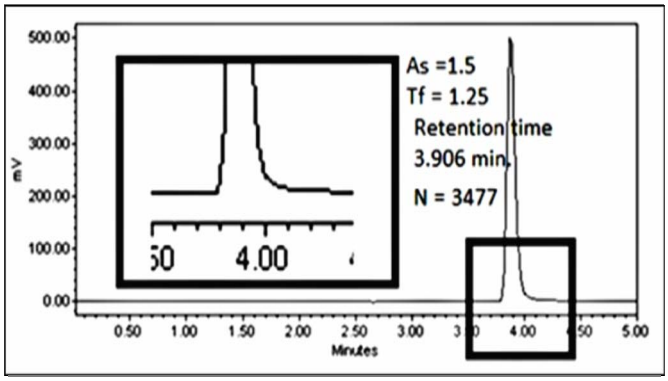

Fig. 2: Mefenamic acid chromatogram by HPLC-UV

Selectivity

Comparison of the chromatograms obtained for the solvent, mefenamic acid standard and mefenamic acid as tablet (Ponstan) revealed no significant interference, using same chromatographic conditions for all samples. Fig. 3 is referring to the selective method for the analyte concerned.

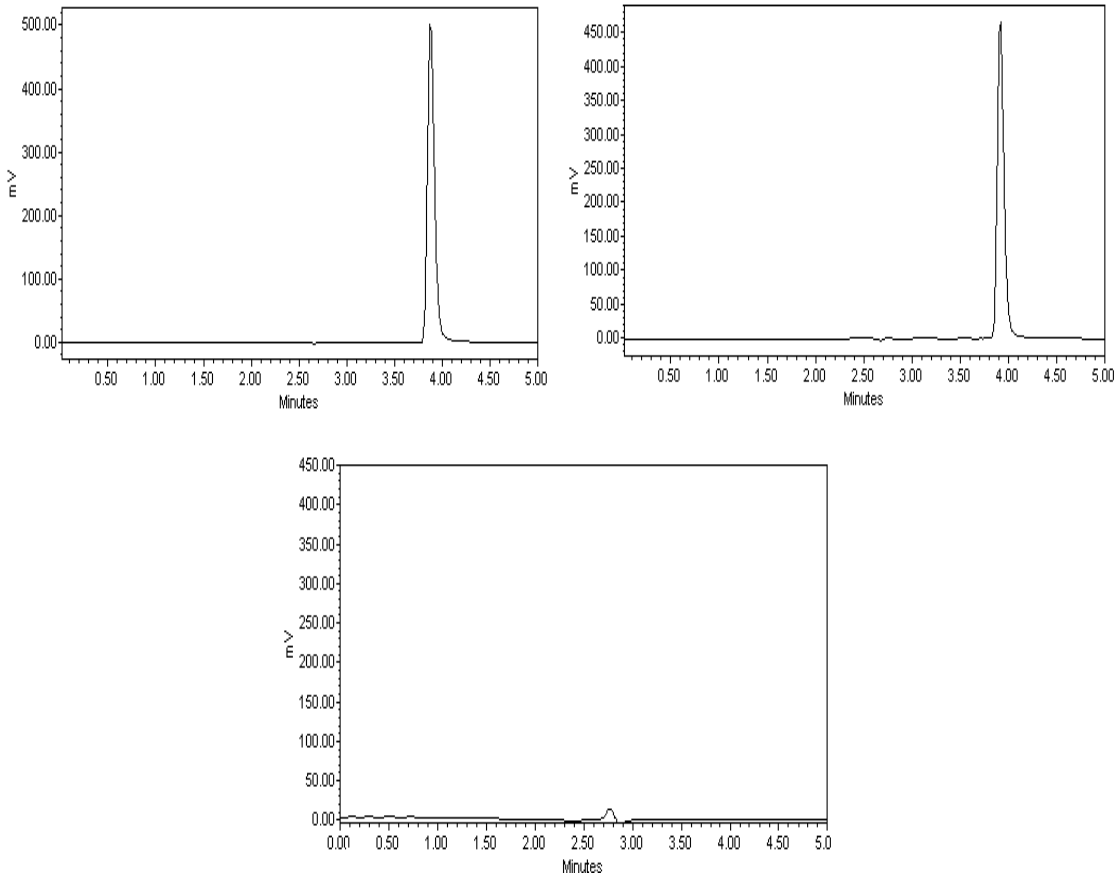

Fig. 3: Selective method of mefenamic acid analysis
(A: Standard solution B: Ponstan tablet solution; C: Blank)

Precision

The precision of the method was evaluated based on the results of the analysis of three samples with three replications for each one at day 1 and the results from intermediate precision from other three samples at day 2. The values were compared with the standards[10,11], thus all values demonstrated good results as shown in Table 8.

Table 8: Precision of method development on mefenamic acid analysis

Conc. (mg/L)	Repeatability		Intermediate Precision	
	R_t ± SD	Peak area ± SD	R_t ± SD	Peak area ± SD
10	3.891 ± 0.04	318740.3 ± 9173.6	3.886 ± 0.04	326706 ± 12257.24
75	3.898 ± 0.03	234394 ± 101749.2	3.895 ± 0.03	2361164 ± 91151.32
250	3.892 ± 0.03	657630 ± 271127.3	3.883 ± 0.03	6586437 ± 186902.2

Statistical analysis

The most significant results were obtained, flow rate in both retention time and peak area. The significance was 0.072 in case of wavelength, means only two wavelengths were significantly different; 260 nm and 275 nm in terms of peak area, so there is no significantly difference between 260 nm and 285 nm, as well as between 285 nm and 275 nm. At all wavelengths no significantly difference occurred with relation time because the retention time independent of wavelength. The wavelength 275 nm was selected as the best based on its highest peak.

Table 9: Statistical analysis results of developed method

Statistical analysis for wave length							
Wa. I	**N**	**Subset for alpha = 0.05, peak area (Sig = 0.072)**			**Subset for alpha = 0.05, retention time (Sig. = 0.58)**		
		1	**2**	**3**	**1**	**2**	**3**
260	3	13863661.33			3.8827		
285	3	14414555.33	14414555.33		3.8437		
275	3		15432426.66		3.8483		
Statistical analysis for flow rate							
Flow rate mL/min	N	Subset for alpha = 0.05, peak area (Sig = 0.00)			Subset for alpha = 0.05, retention time (Sig. = 0.00)		
		1	2	3	1	2	3
2	3	665690.66			1.7250		
1.5	3		2022083.33			2.3223	
1	3			2625909.00			3.8997

Cont…

Statistical analysis for volume injection							
Volume injection	**N**	**Subset for alpha = 0.05, peak area (Sig = 0.005)**			**Subset for alpha = 0.05, retention time (Sig. = 0.573)**		
		1	**2**	**3**	**1**	**2**	**3**
5	3	123034.66			3.943		
10	3		269591.0000		3.903		
20	3		376146.0000		3.883		

Flow rate was statistically treated and exhibited very high significantly difference in terms of peak area and retention time. The flow rate 1 mL/min was selected as the best because of its highest peak area and sharpness of peak compared with others.

In case of volume injection, no significantly difference between 10 and 20 µL in terms of peak area, while both 10 and 20 were significantly difference with 5. The 20 was selected to be the best volume injection, this is related to its highest peak area. Retention time was not affected by volume injection so no significantly difference. Table 9 shows the statistical analysis using ANOVA, Duncan P-0.05.

CONCLUSION

The results show that the HPLC method presented here can be considered suitable for the analytical determination of mefenamic acid in tablets, being linear in the concentration range used, high selectivity and specificity, high precision and adequate accuracy at the concentrations studied. Statistical analysis give significant differences at particularly optimize aspect especially flow rate.

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