A Simple Spectrofluorimetric Method for Determination of Mefenamic Acid in Pharmaceutical Preparation and Urine

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Received June 5, 2006

A simple, sensitive and rapid spectrofluorimetric method was developed for determination of mefenamic acid in pharmaceutical preparation and human urine. The procedure is based on the oxidation of mefenamic acid with cerium (IV) to produce cerium (III), and its fluorescence was monitored at 354 nm after excitation at 255 nm. The variables affecting oxidation of drug were studied and optimized. Under the experimental conditions used, the calibration graphs were linear over the range 0.03-1.5 mg L^{-1}. The limit of detection was 0.009 mg L^{-1} and the relative standard deviation for 5 replicate determinations of mefenamic acid at 1.0 mg L^{-1} concentration level was 1.72%. Good recoveries in the range of 102-107 and 102-109% were obtained for pharmaceutical preparation and human urine, respectively. The proposed method was applied to the determination of MF in one pharmaceutical preparation and human urine. The amounts of mefenamic acid found are very similar to those obtained by a standard method.

Key Words : Mefenamic acid, Spectrofluorimetry, Cerium, Urine

Introduction

Mefenamic acid {[2-(2,3-dimethylphenyl)amino]benzoic acid} (MF) is an anthranilic acid derivative, although its anti-inflammatory properties are considered to be minor. It is used specially in the treatment of rheumatoid arthritis and osteoarthritis and other muscular-skeletal diseases. MF has also been found effective to produce closure of patent ductus arteriosus in premature neonates.[1]

The wide use of fenamic acids has promoted extensive literature on their determination in dosage forms or biological fluids. Different methods such as titrimetry,[2] spectrophotometry,[3-5] luminescences,[6,7] electrophoresis[8,9] and chromatography[10-14] have been described in the literature for MF determination in different samples. Also, several spectrofluorimetric methods have been described for the determination of MF alone or in the mixture with others in pharmaceutical preparations or biological fluids.[15-18]

In the present work a new, simple and sensitive spectrofluorimetric method is described for the determination of MF in pharmaceutical preparation and human urine. The proposed method is based upon oxidation of MF with Ce(IV) and subsequent monitoring the fluorescence of the formed Ce(III) at 354 nm after excitation at 255 nm. This technique permits analysis and quantification of the MF in pharmaceutical preparation or human urine with simple and sensitive spectrofluorimetric method.

Experimental Section

Apparatus. All fluorescence measurements were made on a Shimadzu RF-5301 PC spectrofluorophotometer equipped with a 150 W Xenon lamp and using 1.00 cm quartz cells. Instrument excitation and emission slits both were adjusted to 3 nm.

Reagents. A 1000 mg L^{-1} solution of MF (obtained from Zahravi, Tabriz, Iran) was prepared by dissolving appropriate amount of MF in 2.0 mL sodium hydroxide (NaOH) solution (1.0 mol L^{-1}) and diluting to 25 mL with double distilled water and was kept in refrigerator. Working standard solutions were obtained by appropriate dilution of the stock standard solution. The Ce(IV) solution at concentration 5.0×10^{-3} mol L^{-1} was prepared from Ce(IV)-sulfate-tetrahydrat (E-Merck) in 0.2 mol L^{-1} sulphuric acid and was kept in the refrigerator at 4 °C for two week. A 1.0 mol L^{-1} NaOH and hydrochloric acid (HCl) solution and 10.0 mol L^{-1} H_2SO_4 solution were also prepared.

All other reagents were of analytical-reagent grade (E. Merck) and all solutions were prepared in doubly distilled water.

Recommended procedure for calibration. To a set of 10 mL volumetric flasks, increasing volumes from the working standard solution of the MF were quantitatively transferred, so as to contain the drug within the concentration range 0.03-1.5 mg L^{-1}. To each flask 0.1 mL Ce(IV) and 1.0 mL H_2SO_4 solution were added. Each flask was made up to volume with water and after standing for 10 min, the fluorescence intensity was measured at 354 nm with the excitation wavelength set at 255 nm against a blank prepared similarly.

Procedure for the MF capsule. The contents of ten capsules of MF (Razak, Tehran, Iran) were finely ground. An accurately weighed powdered sample containing 25 mg MF was transferred to a 100 mL volumetric flask, dissolved in 2.0 mL NaOH and the volume adjusted to the mark with distilled water. This solution was diluted quantitatively to yield concentration in the range of working standard

solution and then, proceed as described under procedure for calibration.

Procedure for the human urine. Urine sample was obtained from an apparently healthy male volunteer who took single oral dose of 250 mg MF per capsule. Urine sample was collected for 4 h after administration of MF and stored in a refrigerator under 4 °C. Aliquots of this sample were centrifuged and 0.5 mL portion of clear section was used to determine MF by the proposed method. The sample was transferred to a centrifuge tube and the pH was adjusted to 2-3 with HCl. Then, the mixture was extracted with 2 × 6 mL of diethyl ether for 15 min. The organic layers were collected into a small conical flask and evaporated to dryness on a water bath. The drug residue was dissolved in 0.5 mL NaOH and 1.0 mL distilled water and transferred into a 10 mL volumetric flask and subjected to the above mentioned procedure. A blank value was determined by treating drug-free urine in the same way.

Results and Discussion

MF reacts with Ce(IV) in sulphuric acid medium and forms Ce(III). The excitation and emission spectra of Ce(III) are given in Figure 1 with maxima at 255 ± 3 and 354 ± 3 nm, respectively. Reaction conditions were studied and suitably adjusted as follows:

Effect of Ce(IV) concentration. The effect of Ce(IV) concentration on the fluorescence intensities was optimized as shown in the Figure 2. It was found that 0.5-1.0 × 10^{-4} mol L^{-1} from Ce(IV) led to the constant and maximum signals. At concentrations lower than this range the fluorescence intensity decreases due to insufficient concentration of Ce(IV) for oxidation, whereas higher amounts of Ce(IV) may probably quench the fluorescence and decrease the fluorescence intensity. A volume of 0.1 mL from this reagent (final concentration of 0.5 × 10^{-4} mol L^{-1}) was used in the rest of work.

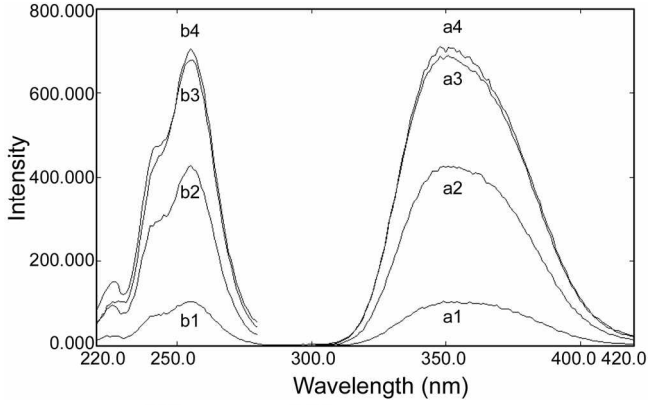

Figure 1. Emission and excitation spectra of Ce(III): a1 & b1 Emission and excitation of Ce(III) in reagents blank; a2 & b2: Emission and excitation of Ce(III) after oxidation with drug solution (0.6 mg L^{-1} MF); a3 & b3: Emission and excitation of Ce(III) after oxidation with standard solution of MF (1.0 mg L^{-1}); a4 & b4: Emission and excitation of Ce(III) after oxidation with MF (1.0 mg L^{-1}) extracted from spiked urine; 0.5 × 10^{-4} mol L^{-1} Ce(IV); 1.0 mol L^{-1} H_2SO_4.

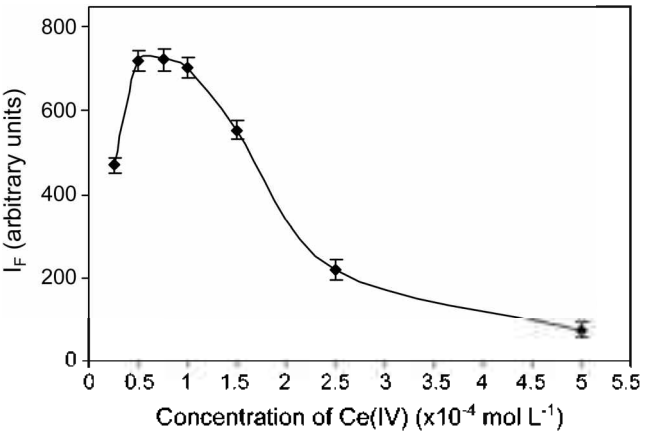

Figure 2. Effect of Ce(IV) concentration on the spectrofluorimetric responses: 1.0 mg L^{-1} MF; 1.0 mol L^{-1} H_2SO_4.

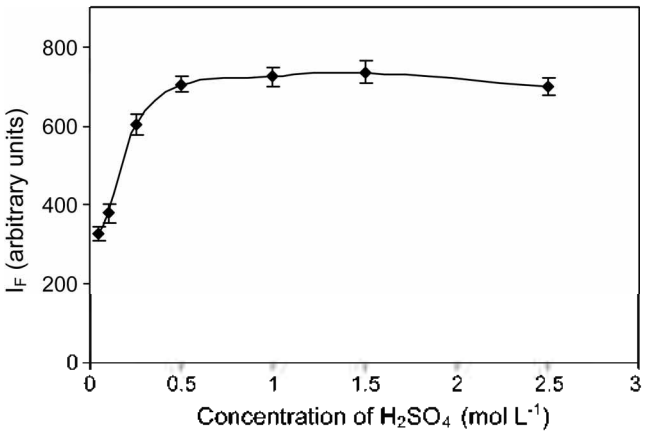

Figure 3. Effect of H_2SO_4 concentration on the spectrofluorimetric responses: 1.0 mg L^{-1} MF; 0.5 × 10^{-4} mol L^{-1} Ce(IV).

Effect of H_2SO_4 concentration. The effect of H_2SO_4 concentration on the fluorescence intensities was studied and found that concentrations above 0.5 mol L^{-1} from H_2SO_4 resulted to the constant and maximum signals (Figure 3). A volume of 1.0 mL from 10 mol L^{-1} H_2SO_4 (final concentration of 1.0 mol L^{-1}) was selected for other experiments.

Effect of temperature and time. In comparison with other methods using Ce(IV) as oxidant, the reaction is rapid in ambient temperature without needing to heating. Whereas, heating in high temperatures for several minutes has been reported in other methods for determination of macrolide antibiotics[19] and psychoactive drugs.[20] The effect of equilibration time on fluorescence intensities was investigated and the results showed that an equilibration time of 10 min are adequate to obtain maximum fluorescence signals.

Characteristics of the method. Calibration graphs obtained under the experimental conditions specified in the procedure were linear over the range 0.03-1.5 mg L^{-1} of MF. The relative standard deviation (RSD) obtained for 5 replicate determinations of 1.0 mg L^{-1} of MF was 1.72%. The

limit of detection (LOD) calculated as three times the standard deviation of the blank signals was 0.009 mg L^{-1} which was sufficiently low as to be valuable for detecting of MF in different biological fluids and is comparable or better than those reported in other fluorimetric methods used for determination of MF.[15-18] In addition, the LOD and linear dynamic range (LDR) of our method is comparable or better than other methods using Ce(IV) as oxidant for pharmaceutical compounds.[19,20]

Interference study. The influence of frequently encountered excipients and additives on the proposed method was studied by adding different amounts of possible interferents to sample containing 1.0 mg L^{-1} of MF. The tolerance limit was taken as the concentration causing an error of not more than 5% in the determination of the drug. No interference was observed from the presence of lactose, glucose, citrate, saccharose, starch, talk, magnesium stearate in the ratios commonly used in pharmaceutical preparations.

The validation and application of the method.

i) Application to commercial formulation: The proposed method was successfully applied to the analysis of MF in its pharmaceutical dosage form (250 mg per capsule) and the results are shown in Table 1. The data in this table show that the MF content measured by the proposed method was in excellent agreement with those obtained by the manual reference British Pharmacopoeia method,[21] which involves the direct titration of MF with NaOH in an ethanolic medium. A comparison using t-test at 95% confidence interval demonstrates that there is not significant difference among the achieved results using these two methods.[22] The accuracy of the proposed method was further tested by performing recovery experiments on solutions prepared from MF formulation. The results are summarized in Table 2

Table 1. Determination of MF in pharmaceutical dosage form (250 mg per capsule)

Method	*MF content (mg per capsule)	
Proposed method	255.2 ± 4.43	**t = 2.49 (2.78)
Standard method	249.7 ± 3.12	**F = 2.02 (19)

*Average of three determinations ± standard deviation. **Figures between parenthesis are the tabulated t and F values at $p = 0.05$ (22)

Table 2. Results of recoveries of spiked samples

Sample	MF added (mg L^{-1})	*MF found (mg L^{-1})	Recovery (%)
0.5 mg L^{-1} MF solution (prepared from drug formulation)			
	0.1	0.602 ± 0.010	102
	0.2	0.714 ± 0.013	107
	0.5	1.025 ± 0.018	105
Human urine (0.5 mL)			
	0.2	0.212 ± 0.004	106
	0.5	0.510 ± 0.009	102
	1.0	1.090 ± 0.019	109

*Average of three determinations ± standard deviation

and recoveries ranged from 102-107%. These recoveries along with coincidence of excitation and emission spectra of the drug to those of standard solution of MF (see Figure 1), indicate that no significant matrix effect was observed in the proposed procedure.

ii) Application to human urine: Drug-free urine sample obtained from healthy volunteer was used for recovery experiments. Aliquots of 0.5 mL of urine sample was spiked with MF at concentrations of 0.2, 0.5 and 1.0 mg L^{-1} and recovery experiments were conducted as well for these samples. The results are summarized in Table 2 and recoveries ranged from 102-109%. For the practical application of method, urine sample was collected for 4 h after a single oral administration of 250 mg of MF to one volunteer. A 0.5 mL portion of sample was used for determination of MF and the concentration of MF in urine was found to be 3.72 ± 0.06 mg L^{-1}. The coincidence of excitation and emission spectra of MF extracted from spiked urine to those of standard solution of MF in Figure 1 is the clear reason for the extraction of MF from urine without interference from other species.

Conclusions

The proposed method represent a promising approach in the area of pharmaceutical monitoring with low cost, high speed, simplicity and sensitivity and therefore can be recommended for the routine analysis of the drug in quality control laboratories. The proposed method show good accuracy and repeatability for determination of drug in pharmaceutical dosage form and biological fluid. The sensitivity of the proposed method is comparable or better than other fluorimetric methods with wider linear range.[15-18] In addition, the LOD and LDR of our method is comparable or better than other methods using Ce(IV) as oxidant for pharmaceutical compounds.[19,20] This method uses distilled water as solvent where the standard method use ethanol as solvent. On the other hand, other fluorimetric methods use toxic chloroform[17,18] as solvent or micellar media[16] for producing of fluorescence. So, the proposed method is more economic and safe.

Acknowledgements. This work has been carried out under the financial support of Research Office of Tabriz University of Medical Sciences.

References

1. Espinosa-Mansilla, A.; Muñoz de la Peña, A.; Cañada-Cañada, F.; González-Gómez, D. *Anal. Biochem.* **2005**, *37*, 275.
2. Çakýrer, O.; Kýlýç, E.; Atakol, O.; Kenar, A. *J. Pharm. Biomed. Anal.* **1999**, *20*, 19.
3. Maron, M.; Wright, G. *J. Pharm. Biomed. Anal.* **1990**, *8*, 101.
4. Aboul Khier, A.; El-Sadek, M.; Baraka, M. *Analyst* **1987**, *112*, 1399.
5. Dinç, E.; Yücesoy, C.; Onur, F. *J. Pharm. Biomed. Anal.* **2002**, *28*, 1091.
6. Arnaud, N.; Georges, J. *Anal. Chim. Acta* **2003**, *467*, 149.
7. Aly, F. A.; Al-Tamimi, S. A.; Alwarthan, A. A. *Anal. Chim. Acta* **2000**, *416*, 87.

8. Pérez-Ruiz, T.; Martínez-Lozano, C.; Sanz, A.; Bravo, E. *J. Chromatogr. B* **1998**, *708*, 249.
9. Polášek, M.; Pospíšilová, M.; Urbánek, M. *J. Pharm. Biomed. Anal.* **2000**, *23*, 135.
10. Hilton, M. J.; Thomas, K. V. *J. Chromatogr. A* **2003**, *1015*, 129.
11. Rouini, M. R.; Asadipour, A.; Hoseinzadeh, Y.; Aghdasi, F. *J. Chromatogr. B* **2004**, *800*, 189.
12. Sun, Y.; Takabe, K.; Kido, H.; Nakashima, M. N.; Nakashima, K. *J. Pharm. Biomed. Anal.* **2003**, *30*, 1611.
13. Mikami, E.; Goto, T.; Ohno, T.; Matsumoto, H.; Inagaki, K.; Ishihara, H.; Nishida, M. *J. Chromatogr. B* **2000**, *744*, 81.
14. Hirai, T.; Matsumoto, S.; Kishi, I. *J. Chromatogr. B* **1997**, *692*, 375.
15. Albero, M. I.; Sanchez-Pedreño, C.; Garcia, M. S. *J. Pharm. Biomed. Anal.* **1995**, *13*, 1113.
16. Sabry, S. M. *Anal. Chim. Acta* **1998**, *367*, 41.
17. Capitán-Vallvey, L. F.; Navas, N.; Consonni, M. V.; Todeschini, R. *Talanta* **2000**, *52*, 1069.
18. Pérez Ruiz, T.; Martínez Lozano, C.; Tomás, V.; Carpena, J. *Talanta* **1998**, *47*, 537.
19. Khashaba, P. Y. *J. Pharm. Biomed. Anal.* **2002**, *27*, 923.
20. Mohamed, F. A.; Mohamed, H. A.; Hussein, S. A.; Ahmed, S. A. *J. Pharm. Biomed. Anal.* **2005**, *39*, 139.
21. *British Pharmacopoeia*; HM Stationary Office, 1998.
22. Miller, J. C.; Miller J. N. *Statistics for Analytical Chemistry*; Wiley: New York, 1984.