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Flow-injection spectrofluorimetric determination of flufenamic and mefenamic acid in pharmaceuticals

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Abstract

Two sensitive and rapid flow-injection (FI) spectrofluorimetric methods are proposed for the determination of flufenamic acid (FF) and mefenamic acid (MF), based on the formation of complexes of these compounds with Al(III) in an ethanolic medium. The calibration graphs resulting from the measurements of the fluorescence at $\lambda_{exc} = 351$ nm and $\lambda_{em} = 440$ nm, and $\lambda_{exc} = 355$ nm and $\lambda_{em} = 454$ nm for the complexes with FF and MF, respectively, are linear over the range 0.030–1.20 μg ml^{-1} for FF and 0.30–16.1 μg ml^{-1} for MF. The methods have been applied to the determination of these drugs in pharmaceutical preparations.

Keywords: Al(III); Flow-injection method; Flufenamic acid; Mefenamic acid; Pharmaceuticals; Spectrofluorimetry

1. Introduction

Flufenamic acid (FF), 2-[[3-(trifluoromethyl)-phenyl]-amino]-benzoic acid, and mefenamic acid (MF), 2-[(2,3-dimethylphenyl)amino]-benzoic acid, are non-steroidal anti-inflammatory and analgesic drugs used in rheumatoid arthritis, other painful musculoskeletal disorders and post-trauma inflammation. Several methods have been described for their determination based on titrimetric [1,2], polarographic [3], chromatographic [4–6], spectrophotometric [7–13] and fluorimetric [14–18] techniques.

There is a constant search for simple, reliable, automated and semi-automated methods for the rapid quantification of substances of therapeutic interest in pharmaceutical preparations. However, no flow-injection (FI) method for the determination of FF and MF has been described.

This paper proposes two simple, inexpensive and rapid FI methods for the routine determination of FF and MF in pharmaceuticals. The procedures are based on fluorescence measurements of the complex formed between each acid and Al(III).

2. Experimental

2.1. Apparatus

The FI system comprised a Gilson (Villiers Le Bell, France) Minipuls HP4 peristaltic pump with isoversinic flow tubes of 2 mm i.d. (Worthington, OH, USA), an Omnifit injection valve (New York, USA) a Hellma 176.052 QS flow cell

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(inner volume 25 mm^3) (Hellma, Jamaica, NY, USA) and a Hitachi (Tokyo, Japan) F-3010 spectrofluorimeter equipped with a plotter unit as the detector. Poly(tetrafluoroethylene) (PTFE) connecting tubing of 0.5 mm i.d., various end-fittings and PTFE standard T-pieces were used.

2.2. Reagents

All chemicals were of analytical-reagent grade and double-distilled water was used.

Aluminium chloride solution (3.7×10^{-2} M)

This reagent was prepared by dissolving 0.5000 g of anhydrous aluminium chloride (Merck) in 100 ml of ethanol (96% v/v) (Merck).

Mefenamic acid stock solution (6.7×10^{-4} M)

This solution was prepared by dissolving 0.0161 g of MF (Sigma, St. Louis, MO, USA) in 100 ml of ethanol (96% v/v) (Merck).

Flufenamic acid stock solution (4.3×10^{-4} M)

This solution was prepared by dissolving 0.0121 g of FF (Sigma) in 100 ml of ethanol (96% v/v) (Merck).

Working standard solutions of lower concentrations were freshly prepared by suitable dilution of the stock solutions with ethanol.

2.3. Dosage forms of mefenamic and flufenamic acids

Coslan Capsules

MF 250 mg and excipients (Parke-Davis Lab., Spain).

Coslan suppositories

MF 250 mg and excipients (Parke-Davis Lab., Spain).

Movilisin cream

FF 3 g, salicylic acid 2 g, mucopolysaccharide polysulphate 0.2 g and excipients to 100 g (Alfarma Lab., Spain).

2.4. Recommended procedures for calibration

The FI system is shown in Fig. 1. A 3.7×10^{-3} M Al(III) solution in ethanol was pumped at a rate of 1.2 ml min^{-1}. An 84 µl sample containing 0.030–1.20 µg ml^{-1} FF or 0.30–16.1 µg ml^{-1} MF was injected into the FI manifold and the fluorescence (λ_{ex} = 351 nm, λ_{em} = 440 nm for Al(III)–FF or λ_{ex} = 355 nm, λ_{em} = 454 nm for Al(III)–MF) was measured and recorded. Calibration graphs were prepared by plotting the fluorescence intensity of the peak versus FF or MF concentration.

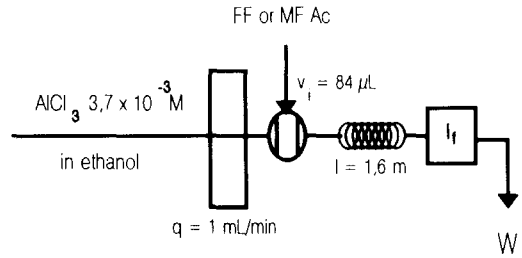

Fig. 1. FI manifold for the determination of flufenamic and mefenamic acid.

2.5. Procedure for the assay of dosage forms

Capsules

The contents of at least 10 capsules were weighed and mixed. An amount of capsule powder equivalent to 250 mg of MF was accurately weighed, dissolved in ethanol (96% v/v) and any remaining residue removed by filtration. The clear solution was diluted to 250 ml with ethanol in a calibrated flask. Aliquots of 1 ml were diluted to 250 ml with ethanol and the described procedure was applied.

Suppositories

At least ten suppositories were weighed, cut into small pieces and transferred to a small porcelain dish. These pieces were melted by stirring in a water-bath at 40 °C until homogeneous and the mixture was cooled until solidified; weighed portions equivalent to 250 mg of MF were transferred into a beaker and the procedure described for capsules was carried out.

Cream

An amount of cream (60–70 mg) was accurately weighed, dissolved in 25 ml of ethanol (96% v/v) at 30 °C and diluted with ethanol to 200 ml in a calibrated flask. Aliquots of 2 ml were diluted to 100 ml with ethanol and the described procedure was applied.

3. Results and discussion

FF and MF are derivatives of anthranilic acid and form complexes with many metals, such as copper, zinc, aluminium and iron [19]. Some of these reactions have been used for the determination of the drugs: Zommer-Urbanska and Bojarowicz [8] developed a spectrophotometric method for the determination of MF with Fe(III); Aboul Khier and co-workers reported spectrophotometric methods for the determination of FF and MF with Cu(II) [9] or Fe(III) [10]; Hattori et al. [15] determined FF with Al(III) by spectrofluorimetry.

In an ethanolic medium, Al(III) reacts with FF and MF to produce fluorescent complexes with maximum excitation at 351 nm and 355 nm, respectively, and maximum emission at 440 nm and 454 nm. Neither FF nor MF emits fluorescence in an ethanolic medium at the concentrations studied in this work.

The reaction between Al(III) and FF or MF acid was adopted in order to develop two spectrofluorimetric FI methods for determining the drugs.

The design of the manifold shown in Fig. 1 is simple. The sample is injected into a stream of Al(III) chloride dissolved in ethanol. Al(III) forms the FF–Al(III) or MF–Al(III) complex and the fluorescence is measured at the λ_{em} and λ_{ex} cited. In the absence of the drug (blank) a very small and constant noise signal was obtained. The presence of the drug caused a great increase in the analytical signals obtained, which were proportional to the drug concentrations.

The use of FI as an alternative to existing methods for the determination of FF and MF is dependent on the optimization of this system to achieve maximum peak height with low residence time and minimum dispersion. As a consequence, several experiments were conducted in order to establish the best experimental conditions to operate the FI manifold. All variables were selected by the univariate method.

Fig. 2 shows the effect of the loop size, reactor length and flow-rate on fluorescence for Al(III)–FF. The volume of sample injected was varied from 55 to 130 µl by changing the length of the sample loop in the injection valve. The peak height increased linearly with increasing loop size (Fig. 2, curve A). A loop size of 84 µl was chosen as a compromise between high sensitivity and low sample consumption.

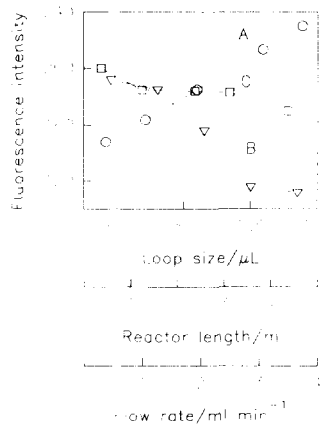

Fig. 2. Effect of (A) loop size, (B) reactor length and (C) pumping rate on the peak height. Sample injected: 0.24 µg ml^{-1} flufenamic acid.

The influence of reactor length was studied from the minimum possible distance between the injection valve and the detector up to 5 m. The results (Fig. 2, curve B) show that the fluorescence decreases for lengths up to 3.5 m, after which it remains constant. A 1.6-m reactor (i.d. 0.5 mm) was selected as it provided a high analytical signal and low residence time.

The effect of flow-rate on fluorescence was studied over the range 0.25–3.5 ml min^{-1}. Fig. 2, curve C shows that between 1 and 2.5 ml min^{-1} this variable had no influence on the fluorescence. A flow-rate of 1 ml min^{-1} was selected as a compromise between reproducibility and sampling rate.

The flow system selected provided good sensitivity and a sampling frequency of 90 samples h^{-1}.

The influence of Al(III) concentration was studied in the range 3.7×10^{-4}–7×10^{-3} M with a fixed concentration of 0.24 µg ml^{-1} FF acid. Constant and maximum peak heights were obtained when an Al(III) concentration higher than 1.8×10^{-3} M was used. A concentration of Al(III) of 3.7×10^{-3} M was selected.

Studies carried out with 2.47 µg ml^{-1} MF acid gave similar results and the same values for each variable were selected to determine FF and MF.

3.1. Characteristics of the methods

Under the conditions outlined above, a series of standard solutions was injected in triplicate to test the linearity of the calibration graphs. The analytical results obtained are shown in Table 1. The limit of quantification, defined as

Table 1
Analytical results for the determination of flufenamic and mefenamic acids

	FF	MF
Linear range ($\mu g\ ml^{-1}$)	0.020–1.20	0.30–16.1
Regression equation ($n = 12$)	$I_F = 2.72 + 755.1 C_{FF}$	$I_F = 7.81 + 29.2 C_{MF}$
Correlation coefficient	0.9993	0.9995
Standard error of slope (%)	4.47	0.15
Standard error of intercept (%)	0.21	0.84

Table 2
Determination of flufenamic and mefenamic acids in pharmaceuticals

Sample	Labelled	Reference method [a]	Proposed method [a]
FF			
Cream	3 [b]	2.86 ± 0.3 [b]	2.88 ± 0.1 [b]
MF			
Capsules	250 [c]	249.7 ± 0.3 [c]	247.5 ± 0.12 [c]
Suppositories	250 [d]	248.5 ± 0.4 [d]	249.9 ± 0.65 [d]

[a] Average of five determinations ±SD. [b] g (100 g)$^{-1}$. [c] mg per capsule. [d] mg per suppository.

the concentration of FF or MF for which the fluorescence intensity was ten times the standard deviation of the blank [19], was 0.008 or 0.18 $\mu g\ ml^{-1}$, respectively. The RSD for the determination of FF and MF at the 0.12 or 3.2 $\mu g\ ml^{-1}$ level ($p = 0.05$, $n = 10$) was 1.26 and 1.30%, respectively.

3.2. Study of interference from other substances

The influence of frequently encountered excipients, additives and manufacturing impurities on the proposed methods was studied. 2,3-Dimethylaniline, paracetamol and diclofenac sodium did not interfere. No interference was observed in the presence of lactose, glucose, saccharose, starch or magnesium stearate, in the ratios commonly used in pharmaceutical preparations of FF or MF.

3.3. Practical application of the methods

In order to establish the validity of the proposed procedures for the determination of the two anti-inflammatory drugs, the methods were applied to the analysis of FF or MF in samples of various pharmaceutical preparations.

The data in Table 2 show that the FF and MF contents measured by the proposed FI procedures were in excellent agreement with those obtained by the manual reference British Pharmacopoeia method [1] which involves the direct titration of the acids with sodium hydroxide in an ethanolic medium. The accuracy of the proposed method was tested by performing recovery experiments on solutions prepared from FF and MF formulations. A mean recovery of 99% was obtained; the range was 98–101%.

4. Conclusions

Taking into account the characteristics of the FI technique with spectrofluorimetric detection used in this work, the methods proposed for the determination of FF and MF are simple, sensitive and more rapid than the manual procedures previously reported. The proposed methods show good accuracy and reproducibility, comparable with the other methods described.

The methods have been applied to the determination of FF or MF in pharmaceuticals, since there is no interference from excipients, additives or active ingredients that might be found in different commercial formulations.

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