Pergamon

Journal of Pharmaceutical & Biomedical Analysis, Vol. 12, No. 3, pp. 343–346, 1994

0731–7085/94 $6.00 + 0.00

Determination of diclofenac sodium, famotidine and ketorolac tromethamine by flow injection analysis using dichloronitrophenol

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Abstract: Diclofenac sodium, famotidine and ketorolac tromethamine were determined by flow injection analysis (FIA) with spectrophotometric detection. The sample solutions (5–50 μg ml^{-1} of diclofenac sodium, 10–80 μg ml^{-1} of famotidine and 10–120 μg ml^{-1} of ketorolac tromethamine) in methanol were injected into a flow system containing 0.01% (w/v) of 2,4,dichloro-6-nitrophenol (DCNP) in methanol. The colour produced due to the formation of a charge transfer complex was measured with a spectrophotometric detector set at 450 nm. A sampling rate of 40 per hour was achieved with high reproducibility of measurements (RSD below 1.6%). The FIA method was applied to the determination of diclofenac sodium, famotidine and ketorolac tromethamine in pharmaceutical formulations.

Keywords: *Diclofenac sodium; famotidine; ketorolac tromethamine; dichloronitrophenol; charge transfer complex; flow injection analysis.*

Introduction

Diclofenac sodium, chemically 2-[(2,6-dichlorophenyl)amino]phenyl-acetic acid monosodium salt, is a drug possessing anti-inflammatory and analgesic activities. Methods reported for determination of the drug in formulation include spectrophotometry [1–6], GC [7] and HPLC [8].

Famotidine is a new H_2 antagonist and chemically it is 3-[[[2-[(aminoiminomethyl)-amino]-4-thiazolyl]methyl]thio]-*N*-(amino-sulphonyl) propanimidamide. The drug is official in the USP XXII [9] which specifies non-aqueous titration for the assay of raw material and a HPLC method for tablet analysis. The other methods reported for the estimation of the drug include colorimetry [10–12] and HPLC [13–16].

Ketorolac tromethamine is a new drug possessing analgesic and anti-inflammatory activities and chemically it is the tromethamine salt of (±)-5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid. HPLC [17, 18] and spectrophotometric methods [19] have been reported for the analysis of the drug in plasma and tablet formulations.

In recent years, flow injection analysis (FIA) has proved to be a relatively inexpensive and useful analytical technique with applications in various fields [20]. In spite of limited selectivity, the FIA technique has been widely used in pharmaceutical analysis [21] because of short start-up time, simplicity of instrumentation and high sampling rate. The technique is suitable for batch type analysis where a few to hundreds of samples are to be analysed.

This paper describes the applicability of FIA for the determination of diclofenac sodium, famotidine and ketorolac tromethamine in their formulations using spectrophotometric detection.

Experimental

Reagents

All reagents were of analytical grade. Diclofenac sodium, famotidine and ketorolac tromethamine were obtained from Quantum Chemical Co. Ltd (Taiwan), Cheminor Drugs Ltd (India) and Lupin Labs Ltd (India), respectively. Tablets and injectable preparations were obtained from local manufacturers. A 0.02% (w/v) solution of 2,4-dichloro-6-nitrophenol (DCNP) was prepared by dissolving 100 mg of the reagent in 500 ml of methanol. This reagent should be protected from light and used within 2 days.

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Stock solutions of diclofenac sodium (0.5 mg ml^{-1}), famotidine (1 mg ml^{-1}) and ketorolac tromethamine (1 mg ml^{-1}) were prepared in methanol. Aliquots of stock solutions were suitably diluted with methanol to provide concentrations in the range of 5–50 μg ml^{-1} for diclofenac sodium, 10–80 μg ml^{-1} for famotidine and 10–120 μg ml^{-1} for ketorolac tromethamine.

Apparatus and procedure

The FIA system employed in this study was assembled (Fig. 1) using the following components: plunger pumps (Model LC-6A), UV-vis detector (Model SPD-6AV) and computing integrator (Model CR-3A) from Shimadzu Corporation (Japan) and six-bore injector (Model 7125) from Rheodyne Inc. (USA). The reaction coil, back pressure coil and connecting tubes used were made of stainless steel.

The carrier solvent methanol was delivered with the plunger pump (A) at a flow rate of 0.6 ml min^{-1} and mixed with the DCNP reagent delivered through the second plunger pump (B) at a flow rate of 0.8 ml min^{-1}. The sample solution (40 μl) was injected manually into the carrier stream. The sample and the DCNP reagent were allowed to mix in the reaction coil (2 m, 0.8 mm i.d.) and the absorbance was measured at 450 nm. The area of the absorbance peaks was recorded by using the computing integrator.

The calibration graph for each drug was constructed by injecting 40 μl of the standard solutions in triplicate at a rate of one injection per 90 s. The output (peak) of the spectrophotometer was recorded on the chart recorder. When the baseline of the peak was reached the next sample was injected. The areas of the absorbance peaks were used for the quantification.

Analysis of tablets and injectables

For diclofenac sodium tablet analysis an accurately weighed portion of the tablet powder equivalent to 50 mg of the drug was transferred to a 50 ml volumetric flask containing 30 ml methanol. The powder was completely disintegrated by means of a mechanical shaker. The solution was made up to volume with methanol and filtered through Whatman No. 40 filter paper. Two millilitres of the filtrate were further diluted to 50 ml with methanol. For diclofenac sodium injections, a volume equivalent to 50 mg of the drug was diluted to 50 ml with methanol. Two millilitres of this solution were further diluted to 50 ml with the same solvent.

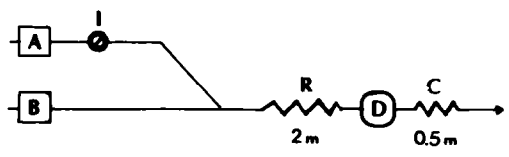

Figure 1
Analytical manifold used for the flow injection determination of diclofenac sodium, famotidine and ketorolac tromethamine. A and B, pumps; I, injector; R, reaction coil (0.8 mm i.d.); D, detector (450 nm); C, back pressure coil (0.25 mm i.d.).

For famotidine tablet analysis, a weight of powdered tablets equivalent to 30 mg of the drug was transferred to a 50 ml volumetric flask. The contents were shaken with 30 ml of methanol for 5 min, diluted to volume with the same solvent and filtered. Five millilitres of filtrate were further diluted to 50 ml with methanol.

For ketorolac tromethamine tablet analysis, a weight of powdered tablets equivalent to 30 mg of the drug was shaken with 30 ml methanol for 5 min in a 50 ml volumetric flask. The solution was made up to volume with the same solvent and filtered. Five millilitres of the filtrate were further diluted to 50 ml with methanol.

The sample solutions were injected in triplicate and the amount of the drug corresponding to the peak area was found from the calibration graph. The amount of the drug in the formulation was calculated using the dilution factor.

Results and Discussion

Diclofenac sodium, famotidine and ketorolac tromethamine were found to react readily with the reagent DCNP in methanol to yield an intense yellow colour. An electronic absorption, additional to the absorption of the components was observed. This may be ascribed to an intermolecular charge transfer (CT) transition involving electron transfer from the donor to the acceptor [22]. Due to the presence of a nitro and two chloro substituents, DCNP is expected to act as a π-acceptor. This is supported by the fact that no such CT band is observed in the region when the nitro group in DCNP is replaced by an amino group. The spectral data of the drug–DCNP complexes are given elsewhere [5, 12, 19].

This colour reaction is sensitive, fairly rapid to develop in the presence of excess reagent and utilizes a single solvent, and can be adapted for the FIA of these drugs.

Optimization of parameters

The experimental conditions such as reagent concentration, flow rate and the reaction coil length were optimized for maximum sensitivity, linearity and reproducibility. A concentration of 0.02% (w/v) DCNP in methanol was found to be optimum since at higher concentrations of the reagent, the background colour increased, and at lower concentrations the intensity of the colour formed was relatively less. Total flow rate was optimized to 1.4 ml min^{-1} since at higher flow rates, sensitivity was less. An increase in the reaction coil length above 2 m showed a decrease in peak response. Sample sizes above 40 µl showed negative absorption peaks at lower concentrations of the drug and also deviation from linearity. When a 40 µl sample loop was used, the calibration graphs were linear in the range 5–50 µg ml^{-1} for diclofenac sodium, 10–80 µg ml^{-1} for famotidine and 10–120 µg ml^{-1} for ketorolac tromethamine.

Typical absorption peaks obtained for ketorolac tromethamine in a linearity study are shown in Fig. 2. Similar absorption peaks were obtained for the other two drugs. Average peak areas were used for the construction of calibration graphs in all three cases. The linearity range and correlation coefficient values are shown in Table 1.

To establish the practicality of the method, marketed products of diclofenac sodium

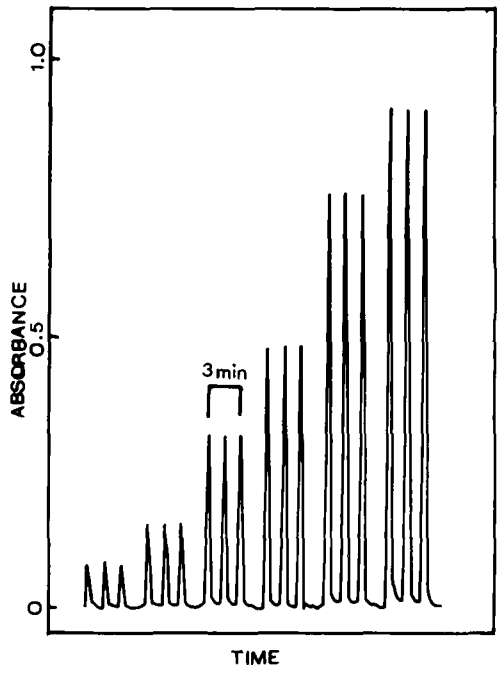

Figure 2
Typical FIA absorbance peaks used for the calibration graph of ketorolac tromethamine.

Table 1
Results of a linearity study in the FIA determination of diclofenac sodium, famotidine and ketorolac tromethamine

Drug	Linearity range (µg ml^{-1})	Intercept	Correlation coefficient
Diclofenac sodium	5–50	0.0412	0.9994
Famotidine	10–80	0.0364	0.9992
Ketorolac tromethamine	10–120	0.0324	0.9996

Table 2
Determination of diclofenac sodium (in tablets and injectables) famotidine (in tablets) and ketorolac tromethamine (in tablets) by the developed method compared with HPLC methods

		% found (± SD)*	
Dosage form	Label claim	FIA method	HPLC method
Diclofenac sodium tablet	50 mg	98.24 (1.54)	98.16 (0.32)
Diclofenac sodium injection	25 mg (per ml)	97.92 (1.48)	97.86 (0.46)
Famotidine tablet	40 mg	99.18 (1.44)	99.26 (0.28)
Ketorolac tromethamine tablet	10 mg	98.36 (1.44)	98.32 (0.36)

*Mean of six determinations.

(tablets and injectables), famotidine (tablets) and ketorolac tromethamine (tablets) were analysed by the proposed method and also by HPLC methods [8, 9, 18]. The results obtained from the FIA method are comparable with those obtained by HPLC methods, as shown in Table 2.

For recovery studies known amounts of the drugs were added to the respective sample solutions which had been analysed earlier. The recovery of the drug was in the range 98.31–100.6% for diclofenac sodium, 98.64–100.5% for famotidine and 98.23–99.86% for ketorolac tromethamine. Common excipients found in the tablet and injectable preparations did not interfere. However, interference was observed in the estimation of diclofenac sodium in presence of paracetamol. In such cases, diclofenac was quantitatively extracted from an acidified solution with chloroform. After evaporation of the chloroform, the residue was dissolved in methanol and analysed.

Conclusion

The proposed FIA method for the determination of diclofenac sodium, famotidine and ketorolac tromethamine is rapid, sensitive and accurate. The method is simple since it involves a one-step reaction and utilizes a single solvent. The reagent consumption is low and interference from common excipients is limited. The method can be applied to the routine analysis of diclofenac sodium, famotidine and ketorolac tromethamine and their formulations.

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[Received for review 14 April 1993; revised manuscript received 17 August 1993]