ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
17 (1998) 267–273

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Flow-injection spectrophotometric determination of diclofenac sodium in pharmaceuticals and urine samples

M. Soledad García, M. Isabel Albero, Concepción Sánchez-Pedreño *, José Molina

Department of Analytical Chemistry, Faculty of Chemistry, University of Murcia, 30071 Murcia, Spain

Received 8 April 1997; received in revised form 23 July 1997

Abstract

A sensitive and fast flow-injection spectrophotometric method for the determination of diclofenac sodium based on the formation of coloured compound with Ce(IV)-3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) in H_2SO_4 3×10^{-2} M medium is proposed. Using the peak height as a quantitative parameter diclofenac was determined at 580 nm over the range 0.20–8.0 μg ml^{-1}. The proposed method was successfully applied to the determination of diclofenac in pharmaceuticals and urine samples.

Keywords: Diclofenac–MBTH–Ce(IV); Flow injection; Spectrophotometry; Pharmaceuticals; Urine

1. Introduction

Diclofenac (2-[2,6-dichlorophenyl)amino-benzeneacetic acid monosodium salt) DCF is a non-steroidal anti-inflammatory drug (NSAID) advocated for use in painful and inflammatory rheumatic and certain non-rheumatic conditions. It is available in a number of administration forms which can be given orally, rectally or intramuscularly. The drug has a relatively short elimination half-life, which limits the potential for drug accumulation. As an analgesic it has a fast onset and long duration of action. Extensive clinical experience has been gained with diclofenac, clearly establishing its safety profile. It is well tolerated compared with other NSAIDs and rarely produces gastrointestinal ulceration or other serious side effects. Thus, diclofenac can be considered as one of the few NSAIDs of 'first choice' in the treatment of acute and chronic painful and inflammatory conditions [1].

The literature reveals several methods for the determination of diclofenac in human urine and in pharmaceutical preparations. Among these methods there are chromatographic: GLC [2] HPLC [3–7], GC [8–11], TLC [12–15], spectrophotometric [16–25], fluorometric [26], NMR [27], anodic-stripping voltammetric [28], ISE [29] and capillary electrophoresis [30]. Some of these methods are not suitable for routine analysis because they need sophisticated instruments, not yet available in many control laboratories.

Flow injection analysis (FI) is an easy and inexpensive way to automate analytical determinations and can be applied in several situations to

* Corresponding author. Fax: +34 68 835418.

0731-7085/98/$19.00
PII S0731-7085(97)00195-7

reduce reagent consumption and increase the repeatability, selectivity and accuracy of the determinations. However, only one method to determination of DCF has used flow injection technique [31].

The objective of this work was the development of a simple, inexpensive and rapid FI method for the routine determination of diclofenac in pharmaceuticals and urine. The proposed method involves the use of 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) in the presence of Ce(IV) as the chromogenic reagent for diclofenac.

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 18-μl flow cell (Hellma, Jamaica, NY, USA) and a Philips PV 8675 v/s spectrophotometer (Cambridge, UK) 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 the solutions were prepared with double-distilled water.

2.2.1. Diclofenac sodium stock solution (1000 μg ml^{-1})

This solution was prepared by dissolving 100 mg of diclofenac sodium salt, (2-[2,6-dichlorophenyl)amino]-benzeneacetic monosodium salt) (Sigma) in 100 ml of water.

2.2.2. MBTH stock solution (2000 μg ml^{-1})

This reagent was prepared by dissolving 200 mg of 3-methyl-2-benzothiazolinone hydrazone hydrochloride (Sigma-Aldrich Química. Madrid, Spain) in 100 ml of water. The stock solution was kept at 4°C and is stable at least during 2 weeks.

Diclofenac and MBTH working solutions of lower concentrations were freshly prepared by suitable dilution.

2.2.3. Ceric ammonium nitrate solution (8×10^{-4} M) in H_2SO_4 3.0×10^{-2} M

This reagent was prepared by dissolving 0.2193 g of ceric ammonium nitrate (Sigma) in 1.7 ml of 9 M sulphuric acid and diluting to 500 ml with water.

2.2.4. Sulphuric acid (9 M)

This was prepared by dilution of the concentrated acid.

2.2.5. Dosage form of diclofenac

(1) Luase tablet (Alfarma Lab., Spain): 50 mg diclofenac sodium and excipient to complete the total weight of the tablet. (2) Dolotren tablet (FAES Lab., Spain) 50 mg of diclofenac sodium and excipient to complete the total weight of the tablet. (3) Voltaren tablet (Geigy Lab., Spain) 50 mg diclofenac sodium, lactose and other excipient to complete the total weight of the tablet. (4) Voltaren ampoules (Geigy Lab., Spain) 75 mg diclofenac sodium, 120 mg bencilic alcohol, sulphite and other excipient to complete the total volume (3 ml) of the vial. (5) Voltaren suppositories (Geigy Lab., Spain) 100 mg diclofenac sodium and excipient to complete the total weight of the suppositories.

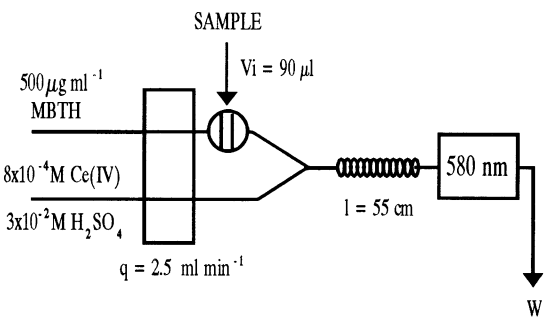


Fig. 1. FI manifold for the determination of diclofenac.

2.3. FI procedures

Fig. 1 shows the flow-injection system: 90 μl of diclofenac solution were injected into an 500 μg ml^{-1} MBTH stream, which then joined the 8.0×10^{-4} M Ce(IV) in 3.0×10^{-2} M H_2SO_4 stream. The absorbance was measured at 580 nm. A calibration graph was prepared by plotting the maximum absorbance of peak versus diclofenac concentration over the range 0.20–8.0 μg ml^{-1}.

2.4. Determination of diclofenac in pharmaceutical samples

2.4.1. Tablets

Three tablets were accurately weighed by separate, finely powdered and shaken with 20 ml of distilled water, the solutions were introduced into an ultrasonic bath for 10 min, and diluted with water in a 1000 ml calibrated flask. Suitable aliquots of these solutions were diluted 1:50 and injected through a Millipore filter into the manifold described in the procedure for calibration.

2.4.2. Ampoules

The content of the three ampoules separately were dissolved in 1000 ml of water in a calibrated flask. Suitable aliquots of these solutions were diluted 1:125 with distilled water and injected into the FI system described.

2.4.3. Suppositories

Three suppositories were weighed separately, cut into small pieces and transferred to a small porcelain dish, 15 ml of distilled water were added and the mixture was heated at 40–45°C during 5 min, the aqueous phase was separated and the treatment was applied five times. The aqueous solutions were mixed and finally diluted to 100 ml with water in a calibrated flask. Suitable aliquots of these solutions were diluted 1:1000 and injected in the FI system described.

2.5. Determination of diclofenac in human urine samples

Urine samples before the ingestion of DCF (blank) were collected and calibration graphs were prepared by adding suitable volumes, 0–500 μl of 100 μg ml^{-1} DCF to 100 μl of human blank urine samples which were diluted to 10 ml with water. Aliquots of these solutions were injected in the FI manifold described and the recommended procedure was applied. The recovery of DCF was obtained by adding different known amounts, 1.0–2.5 μg ml^{-1}, of DCF to blank urine samples, which were then treated in the same way as the calibration standards.

3. Results and discussion

3.1. Preliminary studies

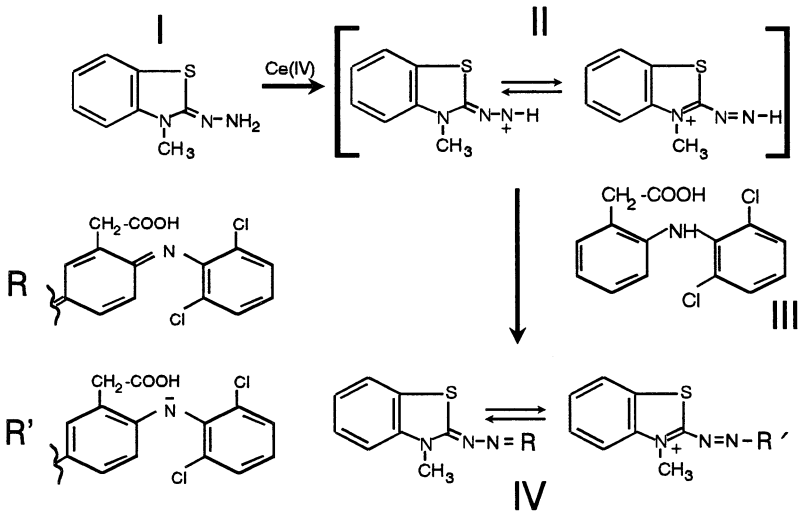
Diclofenac sodium containing a diphenylamine group reacts with MBTH in presence of an oxidizing agent to form a coloured oxidative coupling product. Preliminary studies showed that in diluted sulphuric acid medium (1.5×10^{-2} M) and employing Ce(IV) as oxidant, the product formed presented an absorption maximum at 580 nm. Mixtures of MBTH and oxidant (reagent blank) or DCF and oxidant show low or no absorbance at this wavelength. The absorbance was measured at 580 nm in all subsequent studies.

Under the reaction conditions MBTH (**I**) loses two electrons and one proton on oxidation forming an electrophilic intermediate (**II**) which has been postulated to be the active coupling specie. The intermediate reacts with diclofenac (**III**) by electrophilic attack on the most nucleophilic site on the aromatic ring of the diclofenac, p-position to secondary amino group, and the resulting intermediate is spontaneously oxidized with Ce(IV) to form the coloured specie (**IV**) [17] (Scheme 1).

The above reaction between diclofenac–MBTH–Ce(IV) is used in this work to develop an spectrophotometric-FI method for determining the antiinflammatory agent in its pure form, in pharmaceuticals and in human urine.

3.2. Flow system

Preliminary experiments under continuous-flow conditions were carried out to test the manifold configuration and the approximate ranges of the



Scheme 1. Reaction between diclofenac–MBTH–Ce(IV).

tested parameters. The design of the manifold selected is shown in Fig. 1. A two-channel FI assembly was adopted in which the sample was injected into the MBTH stream. The acidity of the carrier–Ce(IV) reagent solution was previously adjusted to 3.0×10^{-2} M in H_2SO_4. Ce(IV) reacted with MBTH and DCF to produce a coloured compound and the absorbance was measured at 580 nm in the detector previously adjusted to zero with the blank carrier solution. The presence of diclofenac caused an increase in the analytical signal that was proportional to its concentration.

The use of FI as an alternative to existing methods for the determination of DCF is dependent on the optimization of the system to achieve maximum peak height with a short residence time and minimum dispersion. As a consequence, different FI variables (sample volume, reaction coil length and flow-rate), chemical variables (acidity and concentration of MBTH and Ce(IV) were optimized by the univariate method in the continuous-flow procedure with a fixed concentration of 2.0 μg ml^{-1} DCF.

3.2.1. Influence of the FI variables

The influence of the inner diameter of the tubes in the range 0.3–0.8 mm was studied, and an inner diameter of 0.5 mm was selected because a better analytical signal is obtained.

The effect of the sample injection volume, the reactor length and flow rate on the peak height was studied with a reagent concentration of 500 μg ml^{-1} MBTH, 8×10^{-4} M Ce(IV) in 3.0×10^{-2} M in H_2SO_4.

The volume of sample injected was varied from 55 to 155 μl by changing the length of sample loop in the injection valve. The absorbances increased slightly with increasing loop size up to 90 μl, above which they were practically constant. The volume injected was selected at 90 μl.

The influence of reactor length was studied from the minimum distance possible between the injection valve and the detector up to 110 cm. The results showed that the peak height decreases as reactor length increases up to 75 cm. A 55-cm reactor was selected.

The effect of flow rate on peak height was studied over the range 0.75–5.25 ml min^{-1}. Constant and maxima values of absorbance were obtained in the range 2.0–3.0 ml min^{-1}. A flow rate of 2.5 ml min^{-1} was selected.

3.2.2. Influence of reagent concentration

The influence of H_2SO_4 and Ce(IV) concentration was studied in the range 1.0×10^{-2}–10^{-1} M

and 2×10^{-4}–10^{-3} M, respectively, with a fixed concentration of 2.0 μg ml^{-1} DCF and 500 μg ml^{-1} MBTH. Constant and maximum values of absorbance are obtained in a concentration range of 2.0×10^{-2}–3.5×10^{-2} M for H_2SO_4. The absorbance increased with increasing Ce(IV) concentration up to 7×10^{-4} above which they were constant. Concentrations of 3.0×10^{-2} M H_2SO_4 and 8×10^{-4} M Ce(IV) were selected.

The influence of MBTH concentration was studied in the selected experimental conditions in the range 100–2000 μg ml^{-1}. Constant and maximum absorbance values were obtained up to 400 μg ml^{-1}. A concentration of 500 μg ml^{-1} was selected.

3.2.3. Features of analytical methods

With the described manifold and under the selected experimental conditions of 8×10^{-4} M Ce(IV) in 3.0×10^{-2} M H_2SO_4 and 500 μg ml^{-1} of MBTH, calibration graphs linear between 0.20–8.0 μg ml^{-1} were obtained. The regression equation found was $A = 5.48 \times 10^{-2} \pm 2.91 \times 10^{-4}$ [DCF] $+ 4.28 \times 10^{-3} \pm 1.06 \times 10^{-3}$, where [DCF] is expressed in μg ml^{-1} with a correlation coefficient of 0.9999. The variation coefficient for ten determinations of 1.0 μg ml^{-1} was $\pm 0.87\%$. The limit of detection and quantification calculated according to the recommendations of IUPAC [32] was 0.023 and 0.077 μg ml^{-1} of DCF. The sampling frequency was 70 samples h^{-1}.

3.3. Applications

In order to demonstrate the applicability of the proposed method to the determination of DCF the method was applied to the analysis of DCF in various samples of pharmaceutical preparations and human urine.

3.3.1. Pharmaceutical samples

The influence of commonly used excipients and additives in pharmaceutical dosage forms of DCF was studied in the determination of 2.0 μg ml^{-1} of DCF. No interference was observed from the presence of lactose, glucose, saccharose, starch, ethanol, bencilic alcohol, sulphite, glycerine, saccharine or propylene glycol with the proposed method in the ratios commonly used in pharmaceutical preparations of DCF.

The results obtained for the determination of DCF in pharmaceutical preparations are summarized in Table 1 and show that the DCF contents measured by the proposed method were in excellent agreement with the labelled contents. Since no method for the assay of DCF has been reported in any of the international pharmacopoeias, the UV spectrophotometric method at 273 nm [33] has been utilized for comparing the results of the proposed method in pharmaceutical preparations. For all the formulations examined the results obtained by the reference and FI methods were compared by applying the F-test and t-test at the 95% confidence level. The calculated F and t values did not exceed the theoretical ($F_{4,4} = 9.605$, $t = 2.78$), which indicates that there is no significant difference between the two methods with respect to precision and accuracy, in the determinations of DCF.

Recovery studies were also carried out on samples of the pharmaceuticals assayed that contained different concentrations of DCF, 0.97–1.01 μg ml^{-1}, to which known amounts of DCF, 0.40, 0.80 or 1.20 μg ml^{-1} had been added. Every sample was analyzed by quintuplicate. In all cases quantitative recoveries between 97.08 and 102.03% were obtained.

3.3.2. Human urine samples

For the determination of DCF in human urine a previous study on the dilution of the human urine samples was carried out. Several samples

Table 1
Determination of diclofenac in pharmaceuticals

Samples	Labelled	Diclofenac content	
		Reference method*	FI-method*
Dolotren	50[a]	49.25 ± 0.37[a]	49.51 ± 0.66[a]
Luase	50[a]	48.23 ± 0.41[a]	48.60 ± 0.77[a]
Voltaren	50[a]	49.95 ± 0.14[a]	49.45 ± 0.50[a]
Voltaren	75[b]	—	75.19 ± 0.16[b]
Voltaren	100[c]	99.58 ± 0.05[c]	100.78 ± 0.36[c]

* Average of five determinations ± S.D.
[a] mg $tablet^{-1}$; [b] mg $ampoule^{-1}$; [c] mg $suppository^{-1}$.

from different individuals before the ingestion of DCF (blank) were collected and analyzed by the described FI procedure after appropriate dilution. No analytical signal was obtained with dilutions 1:100.

Different amounts of DCF were added to blank urine samples and injected by triplicate to test the linearity by applying the procedure described in Section 2. The calibration graphs were found to be linear from 0.25–5.00 μg ml^{-1} (25–500 μg ml^{-1} in the original urine sample). The regression equation was $A = 1.41 \times 10^{-2} \pm 1.86 \times 10^{-4}$ [DCF] $+ 7.26 \times 10^{-3} \pm 3.43 \times 10^{-4}$, where A is the absorbance peak and the DCF concentration is expressed in μg ml^{-1}; the correlation coefficient was 0.9998. The quantification limit was 0.11μg ml^{-1}. A study on the reproducibility of the methods for 1.0 μg ml^{-1} of DCF added to the same urine samples showed that the variation coefficient of the absorbance was $\pm 1.60\%$ ($n = 10$).

Diclofenac is rapidly and efficiently absorbed after conventional oral, rectal, or intramuscular administration and it is eliminated principally by hepatic metabolism and subsequent urinary excretion. The normal dose for adults is 150 mg day^{-1}. In the urine passed during 24 h, between 15–30% of the dose administered is excreted as unchanged diclofenac[33]. The concentrations of DCF in this urine are found within the range of DCF determination by the FI-spectrophotometric method proposed.

The influence of 4′-hydroxidiclofenac was also included in this study since it has been reported [33] that is the major metabolite of diclofenac in urine. This compound was tolerated until tenfold.

The method was validated by applying the standard addition method. Different diluted urine samples, to which known amounts (1.0–2.5 μg ml^{-1}) of DCF had been added were analyzed by the proposed method. In all cases recoveries around 100% were obtained (Table 2).

Table 2
Recovery of DCF from human urine samples

DCF (μg ml^{-1})		% Recovery ± S.D.*
Added	Found*	
1.00	0.9956	99.56 ± 4.49
1.50	1.5142	100.95 ± 3.42
2.00	1.9862	99.31 ± 4.31
2.50	2.5049	100.19 ± 2.06

* Average of five determinations.

4. Conclusions

The FI-spectrophotometric method proposed for the determination of diclofenac has the known advantages of flow injection analysis techniques: simplicity, speed, the use of inexpensive equipment and the accuracy of its results and yielded a more practical method of DCF determination as compared to the manual procedures described in the bibliography. The new proposed method is more sensitive than the only one FI method reported for this drug.

This method is suitable for the analysis of DCF in pharmaceuticals, as there are no interferences from the excipients normally found in commercial preparations. The FI procedure developed for DCF allows its determination in human urine samples in the physiological concentration range obtained after the usual therapeutic dose of DCF has been administered.

Acknowledgements

The authors are grateful to the DGICYT (Project PB 93-1139) for financial support.

References

[1] P.A. Todd, E.M. Sorkin, Drugs 35 (1988) 244–285.
[2] A. Schweiser, J.V. Willis, D.B. Jack, M.J. Kendall, J. Chromatogr. 195 (1980) 421–424.
[3] J. Gobdillon, S. gauron, J.P. Metayer, J. Chromatogr. 338 (1985) 151–159.
[4] N. Beaulieu, E. Lovering, J. Lefrancois, H. Ong, J. Assoc. Off. Anal. Chem. 73 (1990) 698–701.
[5] D. Lansdorp, T.J. Janssen, P.J. Guelen, T.B. Vree, J. Chromatogr. 528 (1990) 487–494.
[6] H.L. Rau, A.R. Anoor, P.G. Rao, Indian Drugs 28 (1991) 285–286.

[7] A. Avgerinos, T. Karidas, S. Malamataris, J. Chromatogr. 619 (1993) 324–329.
[8] I.V. Rayanam, G.K. Pillai, Indian Drugs 25 (1988) 191–194.
[9] G.K. Pillai, I.V. Rayanam, Indian Drugs 26 (1989) 579–580.
[10] A. Sioufi, F. Pommier, J. Godbillon, J. Chromatogr. 571 (1991) 87–100.
[11] R.T. Sane, S.R. Surve, M.G. Grangrade, V.V. Bapat, N.L. Chonkar, Indian Drugs 30 (1993) 66–72.
[12] C. Sarbu, J. Chromatogr. 367 (1986) 286–288.
[13] C. Sarbu, C. Marutoiu, M. Vlassa, Chromatographia 21 (1986) 599–600.
[14] V.M. Shinde, N.M. Tendolkar, B.s. Desai, J. Planar Chromatogr. Mod. TLC 7 (1994) 50–53.
[15] S.W. Sun, H. Fabre, H. Maillols, J. Liq. Chromatogr. 17 (1994) 2495–2509.
[16] R.T. Sane, R.S. Samant, V.G. Nayak, Indian Drugs 24 (1986) 161–162.
[17] C.S. Sastry, A.R.M. Rao, T.N.V. Prasad, Anal. Lett. 20 (1987) 349–359.
[18] C.S.P. Sastry, A.S.R.P. Tipirneni, M.V. Suryanarayana, Analyst 114 (1989) 513–515.
[19] C.S.P. Sastry, A.S.R.P. Tipirneni, M.V. Suryanarayana, Microchem. J. 39 (1989) 277–282.
[20] Y.K. Agrawal, K. Shivramchandra, J. Pharm. Biomed. Anal. 9 (1991) 97–100.
[21] S. Agatonovic-Kustrin, L. Zinanovic, D. Radulovic, M. Vasiljeav, Analyst 116 (1991) 753–756.
[22] H. Fabre, S.W. Sun, B. Mandrou, H. Maillols, Analyst 118 (1993) 1061–1064.
[23] B.V. Kamath, K. Shivram, Anal. Lett. 26 (1993) 903–911.
[24] J.C. Botello, G. Pérez-Caballero, Talanta 42 (1995) 105–108.
[25] M.S. Bhatia, S.R. Dhaneshwar, Indian Drugs 32 (1995) 446–450.
[26] L.A. Carreira, M. Rizk, Y. El-Shabrawy, N.A. Zakhair, S.S. Tonbar, J. Pharm. Biomed. Anal. 13 (1995) 1331–1337.
[27] S.A. Abdel Fattah, S.Z. El-Khateeb, S.A. Abdel Razeg, M.S. Tawakkol, Spectrosc. Lett. 21 (1988) 533–539.
[28] W.S. Wang, D.R. Jin, S.Y. Cui, X.Z. Pu, C.Y. Pu, Yaowu Fenxi Zashi 15 (1995) 40–41.
[29] S.S.M. Hassan, R.M. Abdel-Axix, M.S. Abdel Samad, Analyst 119 (1994) 1993–1996.
[30] M.G. Donato, W. Baeyens, W. Van den Bossche, P. Sandra, J. Pharm. Biomed. Anal. 12 (1994) 21–26.
[31] B.V. Kamath, K. Shivram, A.C. Shah, J. Pharm. Biomed. Anal. 12 (1994) 343–346.
[32] G.L. Long, J.D. Winefordner, Anal. Chem. 55 (1983) 712.
[33] A. Moffat, J.V. Jackson, M.S. Moss, B. Widdop (Eds.), Clarke's Isolation and Identification of Drugs, 2nd ed., The Pharmaceutical Press, London, 1986, p. 533.