ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
15 (1997) 453–459

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Flow-injection spectrophotometric determination of frusemide or sulphathiazole in pharmaceuticals

M.S. García, C. Sánchez-Pedreño*, M.I. Albero, V. Ródenas

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

Received for review 6 March 1996; revised manuscript received 29 May 1996

Abstract

Two sensitive and fast flow-injection spectrophotometric methods are proposed for the determination of frusemide or sulphathiazole based on the formation of coloured complexes between these compounds and Pd(II) at pH 5.0 and 55°C. Using the peak height as a quantitative parameter, frusemide or sulphathiazole was determined at 410 nm over the range 2.0×10^{-5}–4.0×10^{-4} M or 5.0×10^{-5}–3×10^{-4} M, respectively. The methods were applied to the determination of these sulphonamides in pharmaceuticals.

Keywords: Frusemide; Furosemide; Sulphathiazole; Flow injection; Spectrophotometry; Pharmaceuticals

1. Introduction

Frusemide (furosemide) (F) (4-chloro-*N*-furfuryl-5-sulphamoyl anthranilic acid) and sulphathiazole (S) (*N*-thiazol-2-ylsulphanilamide) belong to the group of sulphonamides, which are effective agents in the treatment of bacterial infections in humans. However, the most extensive use of frusemide is based on its powerful diuretic action, which is why it is used in patients with hypertension [1]. Sulphathiazole is widely used in veterinary practice for the treatment of various bacterial infections, e.g. in diseases of honey bees [2].

Various methods have been described for the determination of these sulphonamides. An important group of techniques comprises spectrophotometric methods without or with the addition of reagents to form derivatives of F [3–8] and S [9–13]. Other methods reported are fluorimetric for F [14,15], electroanalytica for F [16,17] and S [18–22] and gas chromatographic for F [23,24] and S [25]. Recently, several HPLC methods have been developed for F [26–38] and S [39,40]. Some of these methods suffer from interference from the tablet matrix, whereas others are not suitable for routine analysis because they need sophisticated instruments, not yet available in many control laboratories. An alternative, simple chemical procedure for the determination of frusemide or sulphathiazole in pure form and in pharmaceutical dosage forms is therefore necessary.

* Corresponding author.

PII S0731-7085(96)01874-2

Flow-injection analysis (FI) is characterized by its simplicity, speed and the use of inexpensive equipment; its results are accurate and precise and there are clear advantages because of the short time required for each assay. The usefulness of FI methods for routine analysis has been shown in a large number of determinations developed for clinical, pharmaceutical, food and environmental analyses. However, no studies have been reported on the determination of frusemide or sulphathiazole using FI techniques.

The objective of this work was the development of two simple, inexpensive and rapid FI methods for the routine determination of frusemide or sulphathiazole in pharmaceuticals. The proposed procedures are based on the visible absorption of the complexes formed between frusemide or sulphathiazole and Pd(II).

2. Experimental

2.1. Apparatus

The FI system consisted of a Gilson HP4 peristaltic pump (Worthington, OH, USA), an Omnifit injection valve (New York, USA), a Hellma 18 μl flow cell (Jamaica, NY, USA) and a Philips PU 8625 UV–visible spectrophotometer (Cambridge, UK) as the detector. Connecting tubing (0.5 mm bore) of polytetrafluoroethylene (PTFE) and various end-fittings and connectors (Omnifit) were used. A Colora Ultra-Thermostat V5 (Lorch, Würt, Germany) was used.

2.2. Reagents and solutions

All chemicals were of analytical-reagent grade and solutions were prepared with doubly distilled water.

2.2.1. Palladium dichloride standard solution (5×10^{-3} M)

A standard solution was prepared by dissolving 0.2216 g of Pd Cl_2 (Merck) in 5 ml of water, to which 0.5 ml of concentrated HCl had been added, and warming the mixture in a water-bath. The solution was cooled and diluted with water in a 250 ml calibrated flask.

More dilute solutions were obtained by appropriate dilution with Britton–Robinson buffer.

2.2.2. Stock frusemide solution (2×10^{-3} M)

A stock standard solution was prepared by dissolving 0.0661 g of frusemide (Sigma, St. Louis, MO, USA) in 1 ml of 1 M NaOH and diluting to 100 ml with distilled water.

2.2.3. Stock sulphathiazole solution (1×10^{-3} M)

A stock standard solution was prepared by dissolving 0.0511 g of sulphathiazole (Sigma) in 1 ml of 1 M NaOH and diluting to 100 ml with water.

Working standard solutions were prepared daily by suitable dilution of the stock standard solution.

2.2.4. Britton–Robinson buffer solutions

These covered the pH range 4.0–8.0 and were prepared by adding suitable volumes of 1 M NaOH to 100 ml of 0.2 M phosphoric acid, acetic acid and boric acid and diluting to 1000 ml with distilled water.

2.3. Dosage forms

Diurolasa tablets (Casa, Spain) contained 40 mg of frusemide with lactose and other excipients, Seguril tablets (Hoechst Ibérica, Spain) contained 40 mg of frusemide with lactose and other excipients, Seguril injection (Hoechst Ibérica) contained 20 mg of frusemide with excipients and Bucodrin tablets (Fardi, Spain) contained 0.10 g of sulphathiazole with 0.002 g of ethacridine, 0.003 g of ephedrine ricinoleate, 1.8 g of sucrose and other excipients. Sulphathiazole powder was obtained from Andreu (Spain).

2.4. Procedure for calibration

Fig. 1 shows the FI system: 72 μl of furosemide or sulphathiazole were injected into an inert carrier stream, which then joined the reagent stream of 3×10^{-3} M $PdCl_2$ at pH 5.0. The peak height was measured at 410 nm. A calibration graph was prepared by plotting the absorbance of the peak (A) versus frusemide or sulphathiazole concentra-

tion over the ranges 2.0×10^{-5}–4.0×10^{-4} and 5.0×10^{-5}–3.0×10^{-4} M, respectively.

2.5. Procedure for the assay of pharmaceuticals

The average tablet weight was calculated from the contents of 10 tablets that had been finely powdered and weighed. A portion of this powder, equivalent to ca. 40 mg of frusemide or 50 mg of sulphathiazole, was accurately weighed. For sulphathiazole, a portion of 50 mg of the powder was weighed. The samples were shaken with 0.6 ml of 1 M NaOH for frusemide or 1 ml of 1 M NaOH for sulphathiazole. The mixture was then introduced into an ultrasonic bath for 5 min and diluted with water in a calibrated 100 ml flask. For Seguril injection the contents of one injection were dissolved in water in a 100 ml calibrated flask. An accurately measured volume (1–2 ml) of the corresponding solution was diluted with water in a calibrated 10 ml flask and the described procedures were applied.

The reference method applied for the determination of frusemide [41] was UV spectrophotometry at 271 nm in 0.1 M NaOH. For sulphathiazole, the reference method [42] was titration with sodium nitrite using amperometric detection.

3. Results and discussion

Preliminary studies showed that frusemide or sulphathiazole reacts with Pd(II) in moderately weak acid to produce coloured complexes. At pH 5.0 the frusemide–Pd(II) compound presents two absorption maxima at 410 and 520 nm and the Pd(II)–sulphathiazole compound one at 410 nm. Frusemide and sulphathiazole do not absorb at these wavelengths. Palladium(II) has a very low absorbance at 410 or 520 nm under the same experimental conditions. Studies carried out in media of different pH (4.0–8.0) showed that the absorbance of the complexes at the absorption maxima increases with pH up to 5.5 and decreases at higher pH.

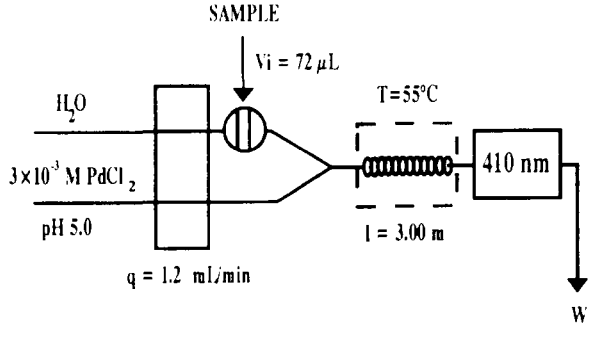

Fig. 1. FI manifolds for the determination of frusemide (F) and sulphathiazole (S).

It was found that at pH 5.0, the molar ratio of frusemide or sulphathiazole to palladium(II) in both complexes was 2–1. The apparent molar absorptivities (l mol^{-1} cm^{-1}) were for Pd(II)–F 3×10^3 and 1.5×10^3 at 410 and 520 nm, respectively, and for Pd(II)-S 6×10^2 at 410 nm. The absorbance was measured at 410 nm against reagent blanks in both cases in subsequent studies.

The reactions between Pd(II) and frusemide and sulphathiazole were used to develop two spectrophotometric–FI methods for determining these drugs.

Preliminary experiments under continuous-flow conditions were carried out to test the manifold configuration and the approximate ranges of the 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 water stream; this was chosen because injection of the sample into the reagent stream led to negative peaks. The acidity of the carrier–palladium(II) reagent solution was previously adjusted to pH 5.0 for both drugs. The reagents and the carrier stream of water were pumped at the same flow-rate to achieve effective mixing of the sample and reagent solutions. Palladium(II) reacted at 55°C with frusemide or sulphathiazole to produce coloured compounds and the absorbance was measured at 410 nm in the detector previously adjusted to zero with the Pd(II) carrier solution. The presence of frusemide or sulphathiazole caused an increase in the analytical signal that was proportional to its concentration.

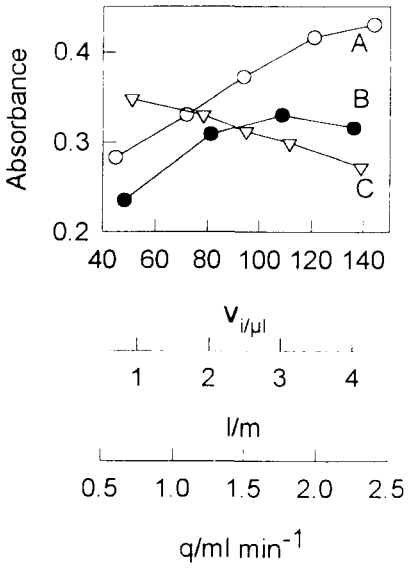

Fig. 2. Effects of (A) loop size, (B) reactor length and (C) flow-rate on the peak absorbance. Sample injected was 2×10^{-4} M frusemide.

The use of FI as an alternative to existing methods for the determination of frusemide or sul phathiazole is dependent on 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 Pd(II) concentration) and temperature were optimized by the univariate method in the continuous-flow procedure with a fixed concentration of the frusemide or sulphathiazole of 2×10^{-4} M.

3.1. Influence of FI variables

Fig. 2 shows the effect of the FI variables studied on the peak height for frusemide. An increase in loop size produces an increase in peak height (Fig. 2A). Similar results were obtained for sulphathiazole. A loop size of 72 µl was chosen in both cases, a sample volume at which sufficient sensitivity was obtained with little waste of sample.

The influence of reactor length was studied from the minimum distance possible between the injection valve and detector up to 4 m. The results (Fig. 2B) showed that the peak height increases with the reactor length up to 3 m, decreasing at greater lengths. A reactor length of 3 m (0.5 mm i.d.) was selected for frusemide or sulphathiazole, as this provided a high sampling frequency and reproducibility.

The effect of flow-rate on peak height was studied over the range 0.5–2.5 ml min^{-1} for both compounds. The results obtained for frusemide are shown in Fig. 2C. An increase in the flow-rate resulted in a small decrease in the analytical signal. For both frusemide or sulphathiazole a flow-rate of 1.2 ml min^{-1} was selected as a compromise between sensitivity and sampling rate.

3.2. Influence of reagent concentration and temperature

Based on preliminary spectrophotometric studies, it is advisable to use a weakly acidic medium for the formation of the compounds between frusemide or sulphathiazole and Pd(II). The influence of pH on peak height was studied in the range 4.0–7.0 (by using Britton–Robinson buffers). Fig. 3A shows the results obtained for frusemide. Maximum and constant absorbance values were obtained in the pH range 4.8–5.3 for frusemide and 4.4–5.5 for sulphathiazole. The pH selected was 5.0 in both cases.

The influence of palladium(II) concentration was studied in the range 10^{-3}–4×10^{-3} M. Fig.

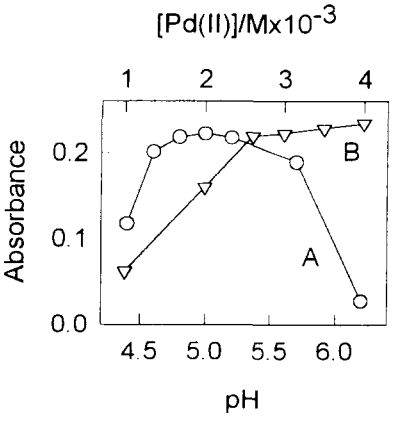

Fig. 3. Effects of (A) pH and (B) Pd(II) concentration on the peak absorbance. Sample injected was 2×10^{-4} M frusemide.

Table 1
Data for calibration graphs ($n = 10$) for frusemide and sulphathiazole using the proposed FI methods

Parameter	Frusemide	Sulphathiazole
Linear range (mol l^{-1})	2×10^{-5}–4×10^{-4}	5×10^{-5}–3×10^{-4}
Slope (A mol^{-1})	1655	453
SE of slope (±)	3.0	3.2
Intercept	8.1×10^{-4}	3.8×10^{-4}
SE of intercept (±)	4.5×10^{-4}	5.1×10^{-4}
Correlation coefficient	0.9999	0.9995

3B shows that for frusemide, constant and maximum absorbance values were obtained with Pd(II) concentrations higher than 2.5×10^{-3} M. A concentration of 3.0×10^{-3} M $PdCl_2$ was selected for both frusemide and sulphathiazole.

Under the selected conditions, the effect of temperature was studied with 2×10^{-4} M frusemide or sulphathiazole between 45 and 65°C. Higher temperatures produced small bubbles in the FI system. It was observed that a temperature of 55°C was suitable for both procedures.

3.3. Features of the proposed methods

With the described manifold and under the selected experimental conditions (Fig. 1), a linear relationship between the frusemide or sulphathiazole concentration and the absorbance peak was obtained. The data for the calibration graphs obtained are shown in Table 1.

The RSD values for the determination of frusemide or sulphathiazole at 2×10^{-4} M (confidence level 95%, $n = 10$) were 0.3% and 0.9%, respectively.

The limits of detection (signal-to-noise ratio = 3) were 5.5×10^{-6} M for frusemide and 1.4×10^{-5} M for sulphathiazole.

The flow system selected provided good sensitivity and a sampling frequency of 50 samples h^{-1}.

3.4. Study of interferences from other substances

The effects of foreign species on both compounds were studied. Since the aim of this work was the determination of these compounds in pharmaceuticals, the effects of common excipients and additives were carefully examined. The results for the determination of 2×10^{-4} M frusemide or sulphathiazole are given in Table 2. The tolerance limit was taken as the concentration causing an error of not more than ±3% in the determination of each of the drugs. No interference was observed from the presence of ethacridine or ephedrine ricinoleate in the amounts commonly contained in the pharmaceutical preparations of sulphathiazole assayed. As can be seen, the proposed methods are sufficiently selective.

3.5. Applications

The proposed FI methods were applied to the determination of frusemide and sulphathiazole in various pharmaceuticals. Interference from the sample matrix was not a problem. Table 3 shows the results obtained by applying the standard methods of the British Pharmacopoeia [41,42] and the proposed FI methods. For all the formulations assayed the results obtained by the reference and FI methods were compared by applying the F-test and the paired t-test at the 95% confidence level. In all cases, the calculated F and t values did not exceed the theoretical values ($F_{4,4} = 9.60$, $t = 4.30$); this indicates that there is no significant

Table 2
Effects of various foreign species on the determination of 2×10^{-4} M frusemide and sulphathiazole

Foreign species	Maximum molar ratio tolerated	
	[Species]/[frusemide]	[Species]/[sulphathiazole]
Glucose, sucrose	60	20
Lactose, galactose	60	20
Mannitol	50	15
Saccharin	10	3
Starch	5	2
Caffeine	2	0.5

Table 3
Determination of frusemide and sulphathiazole in pharmaceutical preparations

Sample	Component	Concentration		
		Labelled	Reference method [a]	FI method [a]
Diurolasa tablets	Frusemide	40 [b]	39.92 ± 0.32 [b]	40.16 ± 0.28 [b]
Seguril tablets		40 [b]	40.92 ± 0.35 [b]	40.76 ± 0.29 [b]
Seguril injection		20 [c]	20.04 ± 0.15 [c]	20.02 ± 0.10 [c]
Bucodrin tablets	Sulphathiazole	100 [b]	99.60 ± 1.50 [b]	99.49 ± 1.20 [b]
Sulphathiazole powder		100	100.74 ± 1.20	100.83 ± 1.10

[a] Mean of five determinations ± SD.
[b] mg per tablet.
[c] mg per vial.

difference between the reference and proposed methods with respect to precision and accuracy in the determination of frusemide or sulphathiazole in pharmaceuticals. Recovery studies were also carried out on samples to which known amounts of frusemide or sulphathiazole had been added. In all cases quantitative recoveries between 98.9 and 100.9% for F and between 99.0 and 100.9% for S were obtained.

4. Conclusions

The proposed FI methods for the determination of frusemide and sulphathiazole showed good accuracy and reproducibility and were faster and simpler than most of the methods reported for the determination of these compounds in pharmaceuticals.

The FI methods proposed are useful for the quality control of frusemide and sulphathiazole in pharmaceutical dosage forms since there is no interference from the common additives and excipients that might be found in commercial preparations.

Acknowledgement

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

References

[1] A. Goodman, L.S. Goodman, T.W. Hall and F. Murad, Las Bases Farmacológicas de la Terapéutica, Panamericana, Madrid, 7th edn., 1984, pp. 853, 1047.
[2] L.P. Barry and G.M. MacEacherm, J. Assoc. Off. Anal. Chem., 66 (1983) 4–7.
[3] British Pharmacopoeia 1988, HM Stationery Office, London, 1988, p. 262.
[4] H. Salem, M. El-Maamli, M. El-Sadek and A. Kheir, Spectrosc. Lett., 24 (1991) 451–470.
[5] C. Sastry, T. Prasad, B. Sastry and E. Rao, Analyst, 113 (1988) 255–258.
[6] C. Sastry, M. Suryanarayana and A. Tipirneni, Talanta, 36 (1989) 491–494.
[7] S. Agatonovic, D. Zivanovic, D. Radulovic and D. Pecanac, J. Pharm. Biomed. Anal., 8 (1990) 483–486.
[8] E. Casassas and I. Fabregass, Anal. Chim. Acta, 106 (1979) 151–154.
[9] J. Berzas-Nevado, F. Salinas, I. De Orbe and L. Capitan, J. Pharm. Biomed. Anal., 9 (1991) 117–122.
[10] Y. Agrawal, S. Menon and R. Giridhar, Anal. Lett., 20 (1987) 829–837.
[11] C. Sastry and B. Rao, J. Indian Chem. Soc., 59 (1983) 1107–1110.
[12] L. Capitan-Vallvey, J. Berzas, M. Valencia and I. De Orbe, Mikrochim. Acta, 111 (1993) 223–229.
[13] S. Filipeva, L. Strelets, V. Petrenko and V. Buryak, Farmatsiya, 36 (1987) 39–42.
[14] A. Foney, B. Kimpel, A. Blair and R. Cutler, Clin. Chem., 20 (1974) 152–158.
[15] I. Shuckla, O. Rai and S. Ahmad, J. Inst. Chem. (India), 62 (1990) 125–128.
[16] S. Agarwal and M. Walash, Indian J. Pharm., 34 (1972) 109–111.
[17] K. Nikolic and K. Velasevic, J. Pharm. Belg., 44 (1989) 387–390.

[18] A. Fogg and J. Lewis, Analyst, 111 (1986) 1443–1444.
[19] A. Fogg, A. Rahim, H. Yusaff, J. Moreira and R. Zhao, Anal. Proc., 32 (1995) 95–97.
[20] H. Hopkala, Acta Pol. Pharm., 46 (1989) 359–364.
[21] M. Bufatina, I. Abdullin and G. Budnikov, Zh. Anal. Khim., 46 (1991) 993–998.
[22] British Pharmacopoeia 1988, HM Stationery Office, London, 1988, p. 548.
[23] B. Lindstroem and M. Molander, J. Chromatogr., 101 (1974) 219–221.
[24] A. Lisi, G. Trout and R. Kazlauskas, J. Chromatogr., 563 (1991) 257–270.
[25] J. Matusik, R. Sternal, C. Barnes and J. Sphon, J. Assoc. Off. Anal. Chem., 73 (1990) 529–533.
[26] L. Lovett, G. Nygard, P. Dure and S. Khalil, J. Liq. Chromatogr., 8 (1985) 1611–1628.
[27] S. Guermouche, M. Guermouche, M. Mausouri and L. Abed, J. Pharm. Biomed. Anal., 3 (1985) 453–458.
[28] T.C. Pinkerton, J.A. Perry and J.D. Rateike, J. Chromatogr., 367 (1986) 412–418.
[29] S. Sood, V. Green and Z. Norton, Ther. Drug. Monit., 9 (1987) 484–488.
[30] J. Posluszny and R. Weinberger, Anal. Chem., 60 (1988) 1953–1958.
[31] W. Radeck and M. Heller, J. Chromatogr., 497 (1989) 367–370.
[32] F. Russel, Y. Tan, J. Van Meijel, F. Gribnau and C. Van Ginneken, J. Chromatogr., 496 (1989) 234–241.
[33] M. Sangy, P. Menwly, A. Munafo and L. Rivier, J. Chromatogr., 564 (1991) 567–578.
[34] C. Gaitonde and P. Jayade, Indian Drugs, 28 (1991) 242–244.
[35] H. Reeuwijk, U. Tjaden and J. Van der Greef, J. Chromatogr., 575 (1992) 269–274.
[36] A. Matsura, T. Nagayama and T. Kitagwa, J. Chromatogr., 617 (1993) 339–343.
[37] T. Vrel, M. Van den Biggelaar-Mastea and C. Verwey-van Wissen, J. Chromatogr., 655 (1994) 53–62.
[38] V. Shinde, N. Tendolkar and B. Desai, Indian Drugs, 31 (1994) 273–276.
[39] T. Galeano, A. Guiberteau and F. Salinas, Anal. Lett., 23 (1990) 607–616.
[40] I. Kanion, H. Tsoukali and P. Epivatianos, Spectrosc. Lett., 26 (1993) 969–973.
[41] British Pharmacopoeia 1988, HM Stationery Office, London, 1988, p. 949.
[42] British Pharmacopoeia 1988, HM Stationery Office, London, 1988, p. 1007.