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Journal of Pharmaceutical and Biomedical Analysis
31 (2003) 669–677
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

A flow injection sensor for simultaneous determination of sulfamethoxazole and trimethoprim by using Sephadex SP C-25 for continuous on-line separation and solid phase UV transduction

M.L. Fernández de Córdova, P. Ortega Barrales, G. Rodríguez Torné, A. Molina Díaz *

Department of Physical and Analytical Chemistry, Faculty of Experimental Sciences, University of Jaén, E-23071 Jaén, Spain

Received 4 May 2002; received in revised form 29 August 2002; accepted 21 October 2002

Abstract

A flow-through sensor based on integration of spectrophotometric detection and the different kinetics of retention/elution of analytes on a solid support is proposed for the simultaneous determination of sulfamethoxazole (SMZ) and trimethoprim (TMP). The solid support (Sephadex SP C-25) fills both, a microcolumn placed on-line and the sensing microzone. The intrinsic absorbance of both compounds is monitored directly on the solid phase at 269 nm and so, no derivatization step is required. Using two alternate solutions, 10^{-4} M hydrochloric acid and 0.20 M NaAc/HAc (pH 5.0) buffer, the sensor responds linearly in the measuring range of 50–250 and 10–70 μg ml^{-1} with detection limits of 9.5 and 0.6 μg ml^{-1} (500 μl of sample volume) for SMZ and TMP, respectively. The main advantages of the sensor are simplicity, rapidity and low reagents consumption. Its application to SMZ and TMP determination in synthetic samples and pharmaceutical preparations is demonstrated. The results obtained by the proposed method were compared with those obtained by a standard HPLC method.

Keywords: Optosensor; Flow injection; Spectrophotometry; Sulfamethoxazole; Trimethoprim; Pharmaceuticals

1. Introduction

Sulfamethoxazole (SMZ) or 5-methyl-3-sulfanylamidoisoxazole is a chemotherapeutics agent widely used as antibacterial drug. Its individual determination has been carried out by spectrophotometry [1,2] and by fluorimetry [3,4]. HPLC has also been proposed for determination of SMZ and its main metabolites in human plasma and urine [5]. The pharmaceuticals containing sulphonamides consist only of one drug or one sulphonamide associated with another drug, which increases the power of the sulphonamide. So, SMZ is usually combined with trimethoprim

* Corresponding author. Tel.: +34-953-012147; fax: +34-953-012141.
E-mail address: amolina@ujaen.es (A.M. Díaz).

0731-7085/03/$ - see front matter
doi:10.1016/S0731-7085(02)00685-4

(TMP) in a fixed proportion 5:1, respectively, this association being called cotrimoxazol.

TMP or 2,4-diamino-5-(3,4,5-trimethoxybenzyl)-pyrimidine is also a bacteriostatic drug. Its determination in pharmaceutical preparations has been usually carried out by spectrophotometric methods [6,7] and sometimes by electroanalytical methods [8,9]. Liquid chromatography [10–12] and gas chromatography [13] have also been proposed for its individual determination or together with its major metabolites of oxidation in different matrices.

The simultaneous determination of both analytes has been usually carried out by spectrophotometric methods with multicomponent analysis based on the use of second derivative and diode-array detection [14], first derivative and spectra ratio [15], PLS [16] and CLS [17].

In this paper, a flow-through sensor is proposed for the simultaneous determination of TMP and SMZ. The potentiality of flow-through optosensors in pharmaceutical analysis has recently been pointed out [18]. The possibility of determining not only one active principle [19,20] but two [21,22] and, sometimes more than two [23,24] has been demonstrated by using single continuous flow systems showing very interesting analytical features (rapidity, selectivity, sensitivity, low cost).

The use of an on-line minicolumn in a flow-through solid phase spectroscopic system extends the analytical potential of these single systems [25,26]. When a cation exchanger gel is used as solid sensing zone, the cationic species can be analyzed based on the strong retention of one of them on the minicolumn while the another reaches the detection area following then the elution of the first analyte [27]. Nevertheless, when the analytes show electric charge of different sign, one of them could not be retained on the solid sensing support. This is an interesting situation when the latter is present at a very higher concentration than the former, because the solid support on the detection area can retain (and preconcentrate) the analyte at a lower concentration and, at the same time, does not allow the second analyte to be preconcentrated, but excluded from the resin beads. Therefore, although its concentration is higher, the detection zone is now active only in the interstices, so selectively decreasing the sensitivity of the analytical response towards this active principle [25] and allowing the simultaneous detection of both by means of two different principles: preconcentration of one of the analytes (retained on the beads) and selective measure of the absorbance of the another one when it flows among the interstitial solution through the beads.

The sensor here proposed is based on the use of Sephadex SP C-25 cation exchanger gel, which fills both an on-line minicolumn and the detection zone in the cell. TMP is retained on the minicolumn while SMZ is determined as it passes among the interstices of the sensing zone. Then, TMP is eluted from the minicolumn and its analytical signal developed when it is transitorily retained on the sensing beads as it passes through the cell.

2. Experimental

2.1. Chemicals

Analytical-reagent grade acetic acid, sodium acetate, hydrochloric acid, potassium monohydrogenphosphate, sodium chloride, sodium citrate and citric acid were obtained from PANREAC (Barcelona, Spain). Aqueous solutions were made with doubly distilled water. All experiments were carried out at room temperature.

SMZ (ALDRICH, Madrid, Spain) and TMP (ALDRICH) stock solutions of 300 and 100 μg ml^{-1}, respectively, were prepared by dissolving the appropriate amount of compound in 5% (v/v) ethanol/water solution. Working solutions were prepared fresh daily by dilution with doubly distilled water. These solutions are stable for at least 1 week in a refrigerator at 4–5 °C. TMP solutions must be protected from light.

The carrier/eluting solution $(C/E)_A$ used consisted of a 10^{-4} M hydrochloric acid solution (pH 4.0).

The eluting solution E_B was a 0.20 M sodium acetate (NaAc)/acetic acid (HAc) buffer solution at pH 5.0.

Sephadex SP C-25 (ALDRICH) ion-exchanger gel (40–120 μm; capacity: 4.3 meq g^{-1}) in the H^+ form was used as solid support. It was placed (as

an aqueous slurry) with the aid of a syringe inside a 1-mm Hellma 138-QS quartz flow-through cell (50 μl inner volume) with glass wool in the outlet to keep resin beads from moving. The microcolumn was also filled with this solid support.

2.2. Apparatus

All spectra, absorbance measurements and real-time data acquisition of flow injection peaks were collected with a VARIAN CARY 50 spectrophotometer. It was controlled by means of a 486 personal computer, fitted with the software package WIN UV for data acquisition and processing.

A liquid chromatograph Model HP 1050 (Hewlett-Packard Co., Arondale, PA), provided with a μBondapak C18 (3.9 × 300 mm) column (Waters Chromatography, Millford, MA) and a diode-array detector HP1040 M series II, was used for the reference method.

A four-channel Gilson Minipuls-3 (Villiers-Le-Ber, France) peristaltic pump with rate selector was used to generate the flow stream in the single manifold required for the system. The sample injection and the alternate selection of the carrier were carried out by using two four-way Rheodyne Model 5041 rotary valves with a single tube loop, one of them connected as a selection valve (SV). Other apparatus consisted of a Selecta (Barcelona, Spain) Model Ultrasons ultrasonic bath and a digital Crison (Barcelona, Spain) Model 2002 pH meter fitted with a glass/saturated calomel electrode assembly and a temperature probe. PTFE tubing of 0.8 mm. i.d. was also used.

2.3. Manifold and procedure

The simple optosensing FIA manifold used for the simultaneous determination of TMP and SMZ is shown in Fig. 1.

A 500-μl sample solution containing both analytes, 50–250 μg ml^{-1} of SMZ and 10–70 μg ml^{-1} of TMP, was injected by means of the rotary injection valve (IV) into the carrier C/E_A at a flow-rate of 1.40 ml min^{-1}. This carrier allowed the strong retention of TMP in the solid support filling the microcolumn (c) and the arrival of SMZ to the sensing zone. After developing the analytical

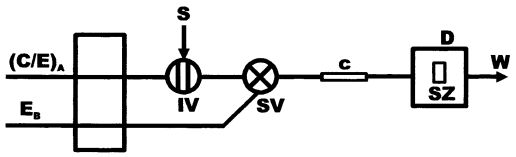

Fig. 1. Optosensing FI manifold used for the simultaneous determinations of SMZ and TMP $(C/E)_A$: 10^{-4} M hydrochloric acid solution (pH 4.0); E_B: 0.20 M sodium acetate/acetic acid buffer solution (pH = 5.0); S: sample; IV: injection valve; SV: selection valve; c: microcolumn; D: spectrophotometer; SZ: sensing zone; W: waste.

signal, the carrier itself eluted the analyte, so regenerating the ion-exchanger gel and returning the absorbance value to the baseline. After this, and by rotating the SV, the eluting solution E_B was passed through the system so allowing the elution of TMP from the microcolumn and its arrival to the detector for determination. This second solution itself allowed also to removed the analyte from the sensing zone.

The absorbance was measured continuously at a wavelength of 269 nm. When the analytical signal came back to its baseline, the next sample was injected and determined as before.

2.4. Procedure for the assay of dosage forms

Different commercial pharmaceutical formulations (tablets, capsules and suspensions) containing the mixture SMZ–TMP or only TMP (the only available pharmaceuticals in Spanish Pharmacopoeia), were employed to test the applicability of the proposed method. For tablets and capsules, a total of 10 U were ground to a very fine powder and homogenized. Sample solutions were prepared by weighing an appropriate amount of the powder, dissolving it in 12.5% (v/v) ethanol/water by using an ultrasonic bath and filtering off through a 0.45-μm pore size Millipore membrane filter. For suspensions, the contents of 5 U were mixed and a volume of suspension was accurately measured, dissolved in 12.5% (v/v) ethanol/water solution and treated as above indicated. In all of the cases, the filtrate was diluted appropriately before injecting in the flow system.

2.5. Reference method

The determination of SMZ and TMP by HPLC was used as a reference method [28]. Chromatography was performed using a mobile phase of 80% water, 20% acetonitrile and 0.1% triethylamine adjusted to pH 6.10 with glacial acetic acid. Flow-rate was set at 2.0 ml min^{-1}. The loop of the sample-injection value was 2.0 μl and the wavelength of measurement was 254 nm.

3. Results and discussion

3.1. Study of experimental variables

3.1.1. Absorption spectra and selection of solid support

In order to select the solid sensing zone, several types of ion-exchangers with different functional groups were tested: anionic exchangers on dextran (Sephadex QAE A-25, Sephadex DEAE A-25) and cationic exchangers on dextran (Sephadex SP C-25, Sephadex CM C-25). A non-polar sorbent (octadecyl silane C_{18}) was also tested. Dowex resins were not considered for the sorption test due to their high absorption in UV region. SMZ was very weakly retained on cationic resins and, however, its fixation on anionic resins was very strong for pH values in the basic zone. As before reported [29], the sorption of SMZ on an anionic solid support could be explained by the presence of a SO_2 group in its structure and the existence of a process of hydrolysis when working in an appropriate basic medium. On the other hand, TMP was not retained on the anionic resins tested but it was strongly retained on cationic ones, specially on Sephadex SP C-25 resin in acidic medium, which can be attributed to the protonation of the aromatic nitrogen atoms in its structure. So, Sephadex SP C-25 resin was selected for further experiments, although the support Sephadex QAE A-25 is the best for the individual determination of SMZ.

The spectral characteristics of SMZ and TMP in homogeneous solution and sorbed on Sephadex SP C-25 were established by using as carrier a 0.0125 M sodium chloride solution at pH 2.0 in the manifold shown in Fig. 1, without microcolumn. The absorption spectra in solution show a maximum at 269 and 288 nm, for SMZ and TMP, respectively. When SMZ and TMP are sorbed on Sephadex SP C-25, these absorption maxima appear at 269 and 273 nm, respectively. So, there is a hypsochromic shift in the maximum of the spectrum of TMP from solution to solid phase, which can be attributed to the modification of its surrounding environment in the resin with respect to the solution. So, the spectral overlapping of both analytes, in solution and in solid phase, makes impossible the simultaneous determination of both by conventional spectrophotometry. As a result of the retention of the analytes on the resin, the analytical signals obtained for SMZ and TMP were increased approximately 1.5 and ten times, respectively, in relation to those ones obtained by using a conventional flow injection system under the same working conditions. The sorption of SMZ on the solid support originates a light increase in sensitivity, as it is scarcely retained on the resin and it is determined practically by measuring the absorbance of the interstitial solution among the resin beads. This is a very important fact because it allows the simultaneous determination of both analytes (with only one injection of sample) which are always associated in pharmaceuticals in a relation 5:1 (SMZ:TMP), respectively. Therefore, the solid phase states a differential behavior of the analytes as they pass through it in the detection zone itself. This differential behavior in the retention–elution process of both analytes on the solid support is the key of the working of this biparameter flow-through sensing device. On the other hand, it is necessary to point out that the sensitivity in the determination of SMZ is appropriate for pharmaceuticals samples.

By placing a pre-column before the flow cell, filled with the same support in the flow cell, TMP was hold while SMZ was detected. This was possible because SMZ has a faster kinetics in the retention–elution process through the active solid support, and it was measured firstly. Then, with an appropriate eluting solution, TMP was carried to the cell and measured.

3.1.2. Influence of the carrier solution and use of a microcolumn

The effect of pH value of the carrier solution on the fixation of both analytes on the solid support was studied by passing through the manifold of Fig. 1 but without microcolumn various carrier solutions, constituted of doubly distilled water or 0.0125 M sodium chloride with the appropriate concentration of NaOH or HCl (pH ranging from 1 to 11). The analytes were injected separately. For all the carrier solutions tested, SMZ was retained and eluted by the carrier itself, and the maximum absorbance signal was obtained for pH values between 2 and 6 (Fig. 2). When sodium chloride solutions were passed through the system, TMP was also retained only transitorily on the resin and the maximum absorbance was obtained in the same pH range that for SMZ (pH from 2 to 6) (Fig. 2). However, when the carrier was only water at pH values above 2, TMP was strongly retained on the solid support and the posterior use of an eluting solution was necessary in order to desorb it from the resin (for pH values below 2 TMP was retained and eluted by the carrier itself). From these results, a 10^{-4} M hydrochloric acid solution was selected as the appropriate carrier/eluting solution for the determination of SMZ. Since the different kinetics in the retention–elution process of SMZ and TMP, the use of a microcolumn, filled with the same solid support that in the flow cell (Sephadex SP C-25) and placed just before it, allowed the sequential arrival of both analytes to the sensing zone. TMP was strongly retained in the microcolumn while SMZ passed through it and reached the sensing zone.

The appropriate solution to elute TMP from the microcolumn and carry it to the sensing zone for its determination, was studied by injecting a mixture of SMZ and TMP in the manifold of Fig. 1 with a microcolumn of 20 mm of length. The eluting solutions tested were constituted of sodium chloride or sodium acetate/acetic acid, citric acid/potassium monohydrogenphosphate and sodium citrate/citric acid buffer solutions at pH 4 and 5 (in order to not change the compactation of the resin and not to alter the baseline). A 0.2 M HAc/NaAc (pH 5.0) eluting solution was found to be the most appropriate for the determination of TMP.

3.1.3. Influence of the pH value of the sample

The sample pH value did not influence the analytical signal when its value was maintained in the 2.5–6 range and, hence, there is no need to adjust the sample pH as the pH value for the sample solutions were always between this range.

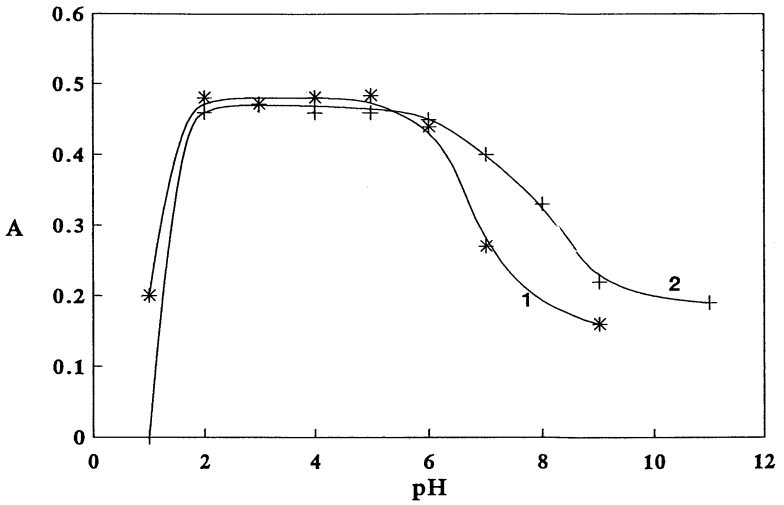

Fig. 2. Influence of pH 1. SMZ; 2. TMP. [SMZ] = 200 μg ml^{-1}; [TMP] = 20 μg ml^{-1}; [NaCl] = 0.0125 M; V = 200 μl.

3.1.4. Level of resin in the flow cell

The level of the resin into the cell has to be the necessary one to fill it up to a sufficient height, thus permitting the light beam to pass completely through the solid layer. Higher levels would imply that the support zone where the species of interest is sorbed would fall outside the detection area and so, a lower and wider signal would be obtained; with lower levels, the light beam would pass through the solution completely or partially and, consequently, a decrease in the signal would be obtained. So, the top of the resin is kept as close as possible to the light beam, this latter being completely covered by the resin. In our system it was found that the optimum height of the resin into the flow cell was 15 mm measured from the bottom of the cell.

3.1.5. Amount of resin in the microcolumn

The microcolumn used was made in glass with 1 mm of internal diameter. The influence of the length of resin in the microcolumn was tested for values between 0 and 22 mm. The optimum length of the solid support was found to be 9 mm as the separation of the analytes was completed from this value (Fig. 3). Values above this one allowed a complete separation but originated lower sampling frequencies.

3.1.6. Influence of FIA variables

The influence of the flow-rate on the absorbance was studied for different values (0.80–1.70 ml min^{-1}) using the same concentration of the analytes. For values below 1.4 ml min^{-1} the increase in flow-rate resulted in decreasing peak heights and residence times, as expected. However, higher flow-rate values originated a very weak increasing in absorbance values and in residence times, which can be attributed to the compactation of the solid support, both in the microcolumn and in the flow cell. A flow-rate of 1.4 ml min^{-1} was selected for further experiments.

The effect of the sample volume on sensitivity was studied by varying the length of the sample loop injected and consequently the sample volume, from 200 to 2020 μl, using the same concentration of SMZ and TMP, 100 and 15 μg ml^{-1}, respectively. It was found that the increase of the sample volume injected resulted in an increase of absorbance. In the case of SMZ, this increase in sensitivity is not linear for sample volumes higher than 500 μl. For TMP, the sensitivity increases linearly with the sample volume up to a value of 1300 μl (according to a stronger retention). A sample volume of 500 μl was chosen for the calibration of the sensor.

As no derivatization step is necessary, the length of the transport system between the injection valve and the microcolumn and between the microcolumn and the flow cell was the minimum allowing the units to be connected (about 10 cm). It minimizes the dispersion and allows a higher sampling frequency.

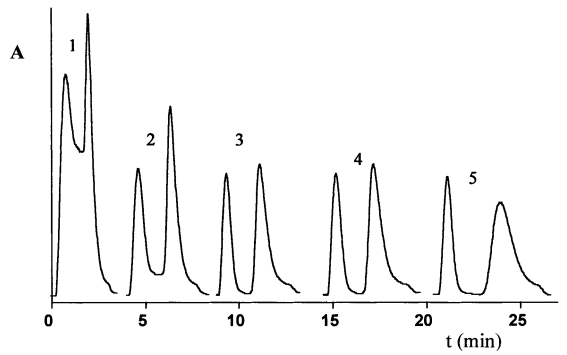

Fig. 3. Influence of the amount of resin in the microcolumn. 1, Without microcolumn; 2, 0.4 cm; 3, 0.9 cm; 4, 1.6 cm; 5, 2.2 cm. [SMZ] = 200 μg ml^{-1}; [TMP] = 60 μg ml^{-1}; V = 500 μl.

3.2. Figures of merit

In the above optimum conditions, calibration graphs were prepared from the results of triplicate 500 μl injections of the corresponding mixtures of TMP and SMZ standard solutions. The analytical figures of merit are given in Table 1. Data were fitted by standard least-squares treatment. Detection limits were estimated on the basis of the three times the standard deviation of the blank. Reproducibility was established by comparing the responses of ten independent determinations of solutions containing 125 and 25 μg ml^{-1} of SMZ and TMP, respectively.

Table 1
Analytical figures of merit V: 500 μl

Parameter	SMZ	TMP
Intercept $\pm\sigma$	-0.098 ± 0.010	-0.004 ± 0.006
Slope $\pm\sigma$ (ml μg^{-1})	0.004 ± 0.005	0.014 ± 0.001
Linear dynamic range ($\mu g\ ml^{-1}$)	50–250	10–70
Correlation coefficient (r)	0.9992	0.9992
Detection limit ($\mu g\ ml^{-1}$)	9.5	0.6
Quantification limit ($\mu g\ ml^{-1}$)	32	2.0
R.S.D. (%) ($n=10$)	0.4	1.4
Thoughput (h^{-1})	14	14

3.3. Study of foreign species

In this study, other foreign species were added to the sample solution (containing both analytes) and their effects on the absorbance signals were investigated. Results are given in Table 2. The study was carried out with 200 and 40 $\mu g\ ml^{-1}$ of SMZ and TMP, respectively. Potential interfering species tested were among those usually accompanying the analytes in pharmaceutical preparations. The effect of each species was considered as an interference when caused a relative error in the signal of $\pm3\%$ in comparison with the signal obtained for each analyte in absence of the species, respectively. Some of the tested species did not cause interference even at the highest tested level because they are not sorbed on the solid sensing supports (e.g. lactose and saccharose). There is an evident difference between the tolerance values to the presence of foreign species in the determination of both compounds, as in all the cases the tolerance level is much lower for the determination of SMZ than for TMP. It can be due to the weak retention of SMZ on the solid support and so, to its determination practically in the interstitial solution between the beads of it. While TMP is strongly retained on the resin and so separated from the matrix (so increasing the selectivity of its determination), SMZ is determined practically in homogeneous solution in the presence of other species that are not retained on the solid support owing to its neutral or anionic character at the working pH value.

3.4. Applications

The proposed sensor was validated by applying it to the determination of SMZ and TMP in pharmaceutical preparations containing the two analytes together or only TMP. The pretreatment and procedure described under Section 2.4 were used in each instance. The results obtained show a good agreement with the composition values indicated by the suppliers and with those obtained by HPLC (Table 3). After the concentration of both analytes was determined, a recovery study was carried out by adding the respective analyte by triplicate at four different concentration levels for Septrin (tablets) and Bronquimucil (suspension) formulations. The concentration levels tested were 25, 50, 100 and 150 $\mu g\ ml^{-1}$ and 10, 20, 30 and 40 $\mu g\ ml^{-1}$ for SMZ and TMP, respectively. Mean recovery values ranged from 99.7 to 100.5% were obtained. These good results prove the applicability of the proposed sensor.

Moreover, some synthetic binary mixtures of both analytes were prepared from stock solutions in relations varying from 1:5 to 1:1 w:w

Table 2
Study of interferences [SMZ] = 200 $\mu g\ ml^{-1}$; [TMP] = 40 $\mu g\ ml^{-1}$

Foreign species	Tolerance level ($\mu g\ ml^{-1}$ interfering species)/($\mu g\ ml^{-1}$ SMZ)	Tolerance level ($\mu g\ ml^{-1}$ interfering species)/($\mu g\ ml^{-1}$ TMP)
Saccharose	3	>120[a]
Saccharin	0.05	2.5
Lactose	10	>120[a]
Benzoic acid	0.04	>30[a]
Sodium citrate	0.05	>35[a]

[a] Maximum ratio tested.

Table 3
Determination of SMZ and TMP in pharmaceuticals

Pharmaceutical[a]	Labeled (mg)		Found (mg ± S.D.)[b]			
	SMZ	TMP	SMZ		TMP	
			Proposed method	HPLC	Proposed method	HPLC
Septrin (tablets)	400[c]	80[c]	399 ± 1	400 ± 2	79.9 ± 0.4	81.1 ± 0.3
Septrin (suspension)	40[d]	8[d]	40.2 ± 0.2	39.9 ± 0.3	7.97 ± 0.03[f]	7.95 ± 0.02
Bronquidiazina (suspension)	400[e]	80[e]	402 ± 2	404 ± 2	79.6 ± 0.5[f]	79.9 ± 0.4
Bronquimucil (tablets)	400[c]	80[c]	399 ± 2	397 ± 1	79.8 ± 0.4	80.1 ± 0.3
Bronquimucil (suspension)	40[d]	8[d]	39.7 ± 0.2	40.1 ± 0.2	8.0 ± 0.1	8.2 ± 0.1
Tediprima (tablets)	–	160[c]	–	–	159 ± 1	157 ± 1

[a] Septrin (tablets, from Medera Pharma, Madrid, Spain) containing SMZ 400 mg, TMP 80 mg and excip. q.s. Septrin (suspension, from Medera Pharma) containing SMZ 40 mg, TMP 8 mg, saccharose 500 mg, saccharin 1 mg and excip. q.s. per ml. Bronquidiazina (suspension, from Faes, Bilbao, Spain) containing SMZ 400 mg, TMP 80 mg, bromhexine hydrochloride 4 mg, sodium benzonate 250 mg, Balsam Tolu 325 mg and saccharin sodium 15 mg per 7.5 ml. Bronquimucil (tablets, from Uriach-Biochorm, Barcelona, Spain) containing SMZ 400 mg, TMP 80 mg, brovanhexine 25 mg and excip. q.s. Bronquimucil (suspension, from Uriach-Biochorm) containing SMZ 40 mg, TMP 8 mg, brovanhexine 2.5 mg, saccharin sodium 2.4 mg and saccharose 0.5 mg ml^{-1}. Tediprima (tablets, from Estedi, Barcelona, Spain) containing TMP 160 mg and excip. q.s.
[b] Data are average of three determinations.
[c] Per unit.
[d] Per ml.
[e] Per 7.5 ml of suspension.

(TMP:SMZ) and were resolved by the proposed method. Although mixtures containing a higher relation SMZ:TMP or an amount of TMP higher than that of SMZ could also have been analyzed by the proposed sensor they were not tested as they are not used in pharmaceuticals. Table 4 shows that the results obtained are very close to a 100% recovery in all cases.

4. Conclusions

A single flow injection sensor is developed for the determination of a binary mixture of SMZ and TMP. The use of a cation-exchanger in the flow-cell allows the sensitive and selective determination of the species, which is found at a lower concentration. On the other hand, the determination of

Table 4
Determination of SMZ and TMP in synthetic mixtures

Mixture		Ratio [TMP]:[SMZ]	Recovery ± R.S.D. (%)[a]	
TMP (μg ml^{-1})	SMZ (μg ml^{-1})		TMP	SMZ
40	200	1:5	99.7 ± 0.3	100.1 ± 0.6
40	160	1:4	100.2 ± 0.4	99.9 ± 0.1
50	100	1:2	99.8 ± 0.2	100.3 ± 0.5
50	50	1:1	100.4 ± 0.3	99.6 ± 0.5

[a] Data are average of three determinations.

the another analyte, which is found at a higher concentration is possible from the interstitial solution passing through the ion exchanger beads. Both analytes are separated on-line by means of a precolumn, filled with the same solid support in the flow-cell, which allows the sequential arrival of the analytes to the detection zone. This makes possible their simultaneous determination with only one sample injection and without any previous derivatization step.

Due to the spectral overlapping, the spectrophotometric methods reported for the simultaneous determination of SMZ and TMP usually employ multicomponent analysis. Therefore, the main advantages of the proposed method compared with those ones are its selectivity, rapidity and simplicity. Compared with HPLC methods, the proposed method is rapid and inexpensive.

Acknowledgements

The authors are grateful to the Ministerio de Educación y Cultura de España, Dirección de Enseñanza Superior e Investigación Científica (Project No. PB98-0301), for financial support.

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