FIA and batch simultaneous determination of sulfamethoxazole and trimethoprim in pharmaceutical formulations by derivative spectrophotometry

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Abstract

The simultaneous determination of sulfamethoxazole and trimethoprim was performed in batch and continuous-flow procedures (FIA) using derivative spectrophotometry. The first order derivative was selected and the vawelength for monitoring sulfamethoxazole and trimethoprim were 258 and 238 nm, respectively. The FIA-calibration graph was linear over the ranges 0.5 – 15 and 0.1 – 30 mg l^{-1} with a detection limit of 0.05 and 0.15 mg l^{-1} for monitoring sulfamethoxazole and trimethoprim, respectively. The sample throughput was 31 h^{-1} for both active principles. The procedure was applied to the determination of both drugs in different pharmaceutical formulations.

Key-words

Spectrophotometry, pharmaceuticals, FIA, continuous-flow, sulfamethoxazole and trimethoprim.

1. INTRODUCTION

Derivative spectrophotometry was introduced in the early fifties by Hammond and Price [1]. However it was only from few years ago is widely exploited due to development of microcomputer technology. Derivative UV-vis spectrophotometry results in increase in the versatility of the techniques for solving mixtures: to eliminate the effect of turbidity in the sample matrix, to correct the background noise, and, to improve some details of the spectra. Even previous separation steps can be avoided.

Many papers concerning derivative spectrophotometry have been published; namely, simultaneous determination of inorganic compounds [2 – 4], that of amino acids and proteins [5 – 7] in biochemical samples; and, in analytical determination of organic compounds in the environmental field [8/9] and simultaneous determination of active principles in pharmaceutical formulations; so in batch [10 – 12] as in continuous-flow methodologies like FIA [13/14]. Recently the couple FIA-derivative spectrophotometry have been applied to pharmaceutical field for simultaneous recording of two dissolution profiles [15/16]. From all of those papers can be deduced the accuracy of results is mostly depending on the selection of spectrum interval and on the derivative mathematical degree.

Merging diode array spectrophotometry with flow injection analysis (FIA) methodology following adds the following advantages to the above reported characteristics of derivative spectrophotometry: the quickness, low consumption of chemicals and higher reproducibility.

This paper deals with an automated simultaneous determination of sulfamethoxazole and trimethoprim in pharmaceutical formulations by an FIA procedure using aqueous solutions. The first derivative is proposed and the analytical determination of mixtures is applied both in batch and in continuous-flow methodology with the mathematical approach known as zero crossings.. Recently the mixture sulfamethoxazole - trimethoprim has been spectrophotometrically analyzed in batch mode using absolute ethanol medium [16]. This couple was selected in the present study due to the wide use of the mixture; even the spanish formulary reports over hundred twenty commercially available formulations.

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2. Experimental

2.1 Reagents:

Aqueous solutions of sulfamethoxazole and trimethoprim (Guinama) were prepared in pure water (reverse osmosis and further de-ionisation (Sybron/Barnstead Nanopure II). Other used reagents of analytical grade were acetic acid, sodium acetate, hydrochloride acid and sodium hydroxide.

Measurements were made in sample solutions containing each 20 v/v % acetic-acetate buffer (pH 4.29). Buffer solution was prepared by mixing 70 ml of 2 mol l^{-1} acetic acid and 30 ml of 2 mol l^{-1} sodium acetate and then by adding appropriate amount of water (one litter).

For the capsules, the powder in the capsules was homogenised in the agate mortar.

The usual trimethoprim/sulfamethoxazole in pharmaceutical formulations is close to mass ratio 1/5. The applied method for the determination of both active principles (in batch and in continuous-flow) was the derivative spectrophotometry combined with the zero crossings mathematical procedure.

For the batch procedures the sample was dissolved in the buffer solution and then spectra were recorded over the range 190 - 390 nm (integration time 0.5 s). From the first derivative of the spectra, the suitable vawelength for the determination was found to be 258 and 238 nm for sulfamethoxazole and

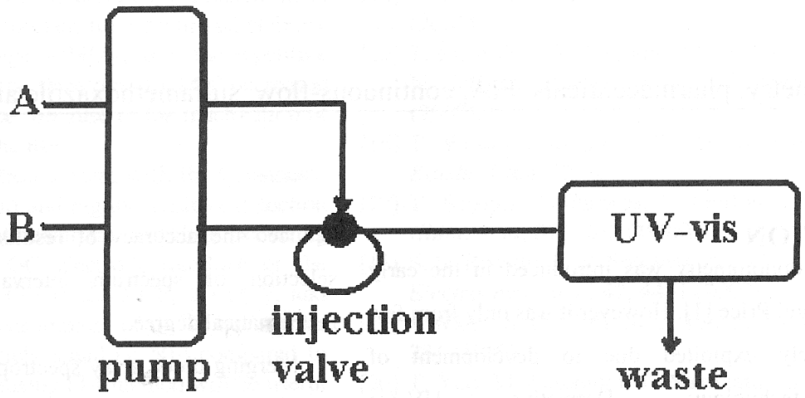

Fig 1. Optimised FIA manifold. Residence time for the sample, 20 s; A sample dissolved in HAc/Ac^- buffer pH 4.29; B, HAc/Ac^- buffer pH 4.29; Flow rate in A and B, 1.75 ml/min; Injected sample volume, 533.6 µl; Valve-detector distance, 27 cm.

2.2 Apparatus and FIA assembly

A simple mono-channel FIA assembly was employed (see Figure 1). The sample solution was injected by an injection valve (model 5.051 from Rheodyne); the flow was obtained with the aid of a Gilson (model Minipuls 2) peristaltic pump. The PTFE tubing was of 0.8 mm id. and the absorbances were measured by means of a Hewlet Packard 8.452 spectrophotometer, provided with a flow-cell (from Hellma) of 18 µl inner volume and 10 mm light-path length. The same spectrophotometer provided with a cell 10 mm light-path length was used for batch procedures.

2.3 Procedures

Commercially available samples (tablets) were powdered by an agate mortar and pestle (6) and then the required amount of the powder was weighed. The powder was directly dissolved in the selected medium.

trimethoprim, respectively.

FIA outputs were obtained at 20 s (slightly over the 19 s of the average residence time), with an integration time interval of 0.5 s, and recording the spectra over the range 190 - 390 nm.

The contents of the analysed pharmaceutical formulations according label claim were: **SEPTRIN** (Medeva Pharma, tablets); sulphamethoxazole, 400 mg; and, trimetoprim, 80 mg. **SEPTRIN** (Medeva Pharma, Suspension, content by ml); sulphamethoxazole, 40 mg; trimetoprim 8 mg; and, excipients, sucrose, ethanol 96 % (0.33 % v/v) and Glycerol and others excipients. **BRONQUI-MUCIL** (Uriach, capsules), sulphamethoxazole, 400 mg; trimetoprim, 80 mg; and, brovanexin hydrochloride, 25 mg.

Presented results are the average of five replicates. Errors were calculated against the values obtained with the officially recommended [18] procedures for trimetoprim and sulfamethoxazole. These methods were: a) for sulfamethoxazole:

a potentiometric titration in aqueous medium with presence of acetic and hydrochloride acids using as a titrant as solution of $NaNO_2$ and the electrodes SCE and platinum were used as reference and indicating electrodes. B) for trimethoprim: the titrimetric procedure was in non-aqueous medium (acetic and anhydride acetic) against perchloric acid as titrant; in this case glass electrode was used as indicating electrode.

3. Results and Discussion

3.1 Influence of the media

Fist of all the UV-vis spectra of the selected drugs (sulfamethoxazole and trimethoprim) were recorded; spectra were daily recorded for testing the stability of the drug aqueous solutions up to two weeks. No variations in spectra were observed.

spectra were obtained up to third order. After obtaining the derivative spectra attention was paid to pH interval 4.0 – 9.0.

Further assays consisted in a potentiometric adjust of the pH into this range with the aid of the buffer mixtures tartaric acid – sodium tartrate or acetic acid – sodium acetate. Former buffer changed the spectrum of sulfamethoxazole without advantages; on contrary latter buffer resulted in higher outputs.

Spectra were obtained at different pH values with the acetic – acetate buffer and are depicted in Figure 2. We selected the medium for further work at pH 4.29. First derivative spectra at this pH resulted in two zero crossings for each drug. Those values are: 272 and 258 nm for sulfamethoxazole; and, 238 and 268 nm for trimethoprim.

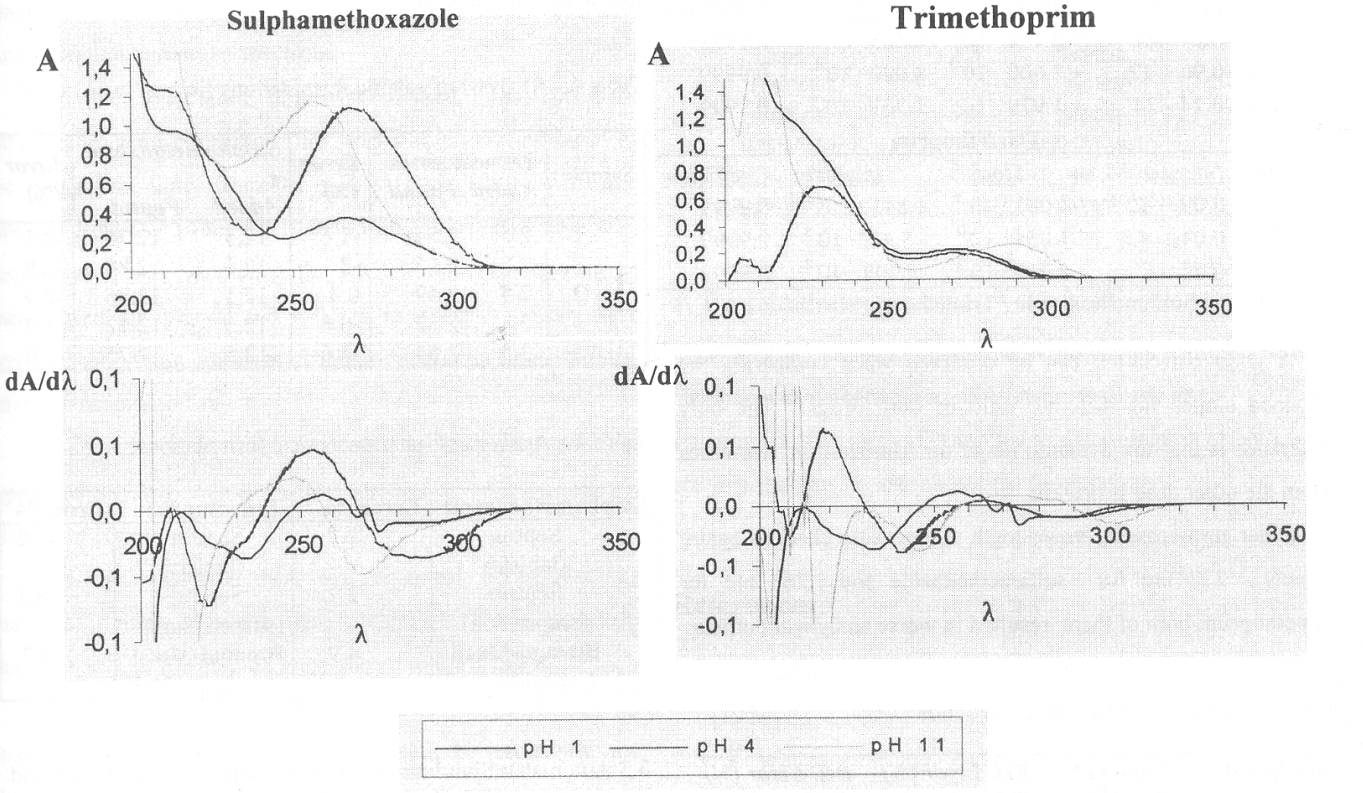

Fig. 2. Spectra and derivative spectra (first order) from sulfamethoxazole and trimethoprim at different pH values. Spectra at pH 3.0, 5.0, 7.0, 9.0 are very similar to the spectrum at pH 4.0; and, spectrum at pH 13.0 with the one from pH 11.0

Firstly, to study the simultaneous determination of both drugs in a mixture, the solutions The different solutions of both drugs, sulfamethoxazole and trimethoprim, were prepared by adjusting the pH with HCl or NaOH (potentiometric adjust) over the pH range 1 - 11; and, the spectra were recorded form 190 to 390 nm; see Figure 2.. From the measured spectra the derivative

3.2 Analytical applications in batch

Calibration graphs were obtained with the pure drug solutions and with the mixture by using the first derivative spectra. For the pure drug the observed dynamic range and the fitted equations were: a) trimethoprim at 238 nm over the range 1 – 10 mg l^{-1}; the first derivative fitted with the following linear equation, dA/dx = - 0.0038 C – 0.0004; (correlation coefficient 0.9999); and, b) for sulfamethoxazole the: zero crossing at 258

nm resulted in a linear dynamic range from 0.2 to 24 mg l^{-1}; and the linear equation dA/dx = 0.0021 C + 0.0003 (correlation coefficient, 0.9990).

The calibration graph for mixtures was obtained in two different ways; first with a constant concentration of sulfamethoxazole 8.00 mg l^{-1} and varying the trimethoprim over the range ratio sulfamethoxazole / trimethoprim 1/0, 2/1 and 5/1. Results are depicted in the following Table 1. A second experiment consisted in preparing mixtures containing the reverse ratios (concentration of trimethoprim 4.00 mg l^{-1}). Results from those measurements are listed in the same table (at bottom).

Table 1. Numerical parameters of the calibration equations (Concentrations in mg l^{-1})

Sulphamethoxazole				
*Ratio**	*Interval*	*Slope*	*Intercept*	*Coefficient*
1/0	0.24 - 24	$+2.162 \cdot 10^{-3}$	$2.225 \cdot 10^{-4}$	0.9998
2/1	0.96 – 12	$+2.600\ 10^{-3}$	$8.000\ 10^{-5}$	0.9999
5/1	0.24 - 24	$+1.979 \cdot 10^{-3}$	$1.361 \cdot 10^{-4}$	0.9999
Trimethoprim				
*Ratio**	*Interval*	*Slope*	*Intercept*	*Coefficient*
0/1	0.09_6 - 12	$-4.081 \cdot 10^{-3}$	$1.843 \cdot 10^{-4}$	0.9999
5/1	0.04_8 - 4.8	$-4.054 \cdot 10^{-3}$	$-5.485 \cdot 10^{-5}$	0.9999
2/1	0.48 – 6	$-4.200\ 10^{-3}$	$-2.000\ 10^{-4}$	0.9999

*ratio as Sulfamethoxazole / Trimethoprim

A good correlation can be observed when comparing the obtained slopes for different ratio of both drugs. The only difference is that the dynamic range for trimethoprim decreases when the other drug is present.

Other zero-crossing vawelength values were checked again; namely, 272 nm for sulfamethoxazole and 268 nm for trimethoprim; both of them resulted in worse analytical outputs.

Table 2. Influence of foreign compounds

Interferent	*Content* (mg l^{-1})*	*Rel. Error (%) Trimethoprim*	*Rel. Error (%) Sulfamethoxazole*
Guaifenesin	1.0 / 1.0	69.3	12.9
Phenylephrine	1.0 / 1.0	4.6	2.1
Theophyllyne	0.1 / 0.1	4.6	2.1
Bromhexine	1.0 / 2.4	18	12.3
Sucrose	100 / 2000	1.9	0.3
Lactose	25 / 1000	0.8	2.7
Fructose	50 / 100	50	1.9
Glucose	50 / 500	0.6	4.2
Na sacharin	1.0 / 5.0	96	3.4
Sorbitol	25 / 5000	-1.6	-2.0

The influence of foreign substances which can be found in pharmaceutical formulations containing sulfamethoxazole and trimethoprim was tested by preparing solutions containing 12 mg l^{-1} sulfamethoxazole or 2,4 mg l^{-1} trimethoprim and different amounts of the foreign substance. The outputs were compared with the solution containing the same amount of the mixture sulfamethoxazole - trimethoprim.

The results are listed in Table 2. The content was the amount of foreign compound in mg l^{-1}, into the solution of sulfamethoxazole and trimethoprim, respectively. Guaifenesin and Bromhexine were the most serious interferents.

The intra-day reproducibility was checked by obtaining independent calibration graphs in different days (number of replicates 5); the standard deviations of the calculated slopes was : 0.00017 for trimethoprim and 0.00005 for sulfamethoxazoleThe method was tested in several samples: a) galenic formulations prepared in the lab according to (17). Results depicted in Table 3; and, b) commercially available pharmaceutical formulations (Table 4).

Table 3.- Analysis of galenic formulations (in mg)

Sample	*Trimethoprim*		*Error (%)*	*Sulphomethoxazole*		*Error (%)*
	Added	*Found*		*Added*	*Found*	
1	2.5	2.54	-1.6	12.5	12.76	-2.1
2	2.5	2.55	-2	12.5	12.86	-2.9
3	2.5	2.49	0.4	12.5	12.86	-2.9
4	2.5	2.52	-0.8	12.5	12.72	-1.7
5	2.5	2.54	-1.6	12.5	12.75	-2.0

Table 4.- Analysis of pharmaceutical formulations

Sulphamethoxazole	Error %	Trimetoprim	Error %
Septrin (tablets)	1.7	Septrin (tablets)	-5.2
Septrin (suspension)	2.2	Septrin (suspension)	-8.3
Bronqui-Mucil (capsules)	4.9	Bronqui-Mucil (capsules)	2.7

3.3 Automated Simultaneous Determination by a FIA manifold

The FIA assembly for the spectrophotometric determination of sulfamethoxazole and trimethoprim should be very simple provided it vas not required chemical derivation of the sample; only pH adjust and carry the sample to the detector flow-cell.

The flow-system optimisation was performed by bearing in mind the following points: a) The maximum output in the FIA peak should be long enough to obtain reproducible spectra; b) High sensitivity, which means high transient outputs; and, c) a suitable sample throughput. The optimisation was performed with the aid of the univariate method by continuous recording of

the absorbance of a solution containing sulfamethoxazole (12.5 mg l^{-1}) and trimethoprim (2.5 mg l^{-1}). Parameters studied, tested interval and finally selected value were respectively: sample volume , 7 – 125.5 cm length of external loop, 452 µl; carrier flow-rate, 250 – 500 pump arbitrary units, 1.6 ml min^{-1}; and, distance from the injection-valve to detector flow-cell, 17.7 – 125.5 cm, 27 cm.

When FIA parameters were optimised the further work to be establish was to determine the chemical parameters the residence time (time from injection to peak maximum) and the time interval in which the transient signal (peak) kept the maximum, variation of the output minor than ± 0.01.

The experience consisted in recording two series of peaks (different concentrations). This allowed to record the spectra at twenty seconds from the injection. and over the vawelength range 190 - 390 nm, being the analytical signal the corresponding derivative spectrum.

Then the integration time was studied. Two series of experiments were performed; first was studied the influence of the integration time, from 0.1 y 1 s (cycle 0.1 s) with one solution 2 mg l^{-1} / 10 mg l^{-1} (trimetoprim and sulphametoxazole, respectively), and calculating the RSD. From this experiments was chosen an interval 0.3 - 0.7 s as optimum. Then were prepared calibration graphs at different integration time, into the selected range. See Table 5.

The slope increased when increasing the integration time but reproducibility was worse. A time interval of 0.5 s was chosen as the best compromise.

With the selected FIA and chemical parameters the analytical figures of merit were obtained. These analytical characteristics of the method are depicted in the Table 6.

Finally the method was also applied to analysis of several pharmaceutical formulations and comparing the results with the obtained with the officially recommended procedure as stated in the batch section.

Table 5. Calibration graphs at different integration time.

Integr. Time	Equation	Correl. Coef	RSD (%)
0.3 s	$3.6\ 10^{-4} - 3.51\ 10^{-3}\ C$ (1)	0.99997	$2.33\ 10^{-2}$
	$10.6\ 10^{-4} + 1.96\ 10^{-3}\ C$ (2)	0.99966	$1.62\ 10^{-2}$
0.5 s	$5.3\ 10^{-4} - 3.63\ 10^{-3}\ C$ (1)	0.9998	$2.41\ 10^{-2}$
	$8.1\ 10^{-4} + 2.02\ 10^{-3}\ C$ (2)	0.9999	$1.80\ 10^{-2}$
0.7 s	$7\ 10^{-4} - 3.658\ 10^{-3}\ C$ (1)	0.99996	$3.43\ 10^{-2}$
	$4.2\ 10^{-4} + 2.037\ 10^{-3}\ C$ (2)	0.99978	$2.99\ 10^{-2}$

Table 6. Analytical figures of merit.

	Sulfamethoxazole	*Trimethoprim*
Linearity range (mg l^{-1})	0.5-15.0	0.1-3.0
RSD (% ; n, 20)	3.4	5.0
Sample through, (h^{-1})	31	31
LOD (mg l^{-1})	0.05	0.15

Table 7. Analysis of pharmaceutical formulations.

Sulphamethoazole	*Error (%)*	*Trimetoprim*	*Error (%)*
Septrin (tablets)	2.8	Septrin (tables)	-4.8
Septrin (suspension)	3.0	Septrin (suspension)	-4.9
Bronqui-Mucil (capsules)	1.5	Bronqui-Mucil (capsules)	-3.3

4. Conclusions

A simple FIA-spectrophotometric procedure is presented for simultaneous determination of sulphametoxazole and trimetoprim. The method is applied to determination of both active principles in pharmaceutical formulations.

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