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Short communication

Simultaneous dissolution profiles of two drugs in pharmaceutical formulations by an FIA manifold

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Abstract

This article deals with the simultaneous determination of dissolution profiles of two drugs with overlapped spectra, present in the same pharmaceutical formulation. The official procedure for the dissolution profile is adapted to the continuous-flow methodology; the dissolution vessel is connected to an FIA manifold, in which the sample aliquots from the dissolution vessel are treated in order to adjust to the suitable pH and dilution degree to be monitored. The resulting solution is injected into the carrier stream, an acetic acid–acetate buffer at pH 4.3 and forced to the flow-cell of the spectrophotometer. The simultaneous determination of both profiles is based on the first derivative spectra and the zero-crossing mathematical procedure. The empirical profile of the curve is adjusted by regression using different approaches; the three-parameter plot method is selected. The analytical errors, when the concentration of one drug is very low or very high, are also checked. A binary mixture in commercially available formulations of solid oral administration of sulphametoxazole and trimethoprim is presented. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: FIA; Pharmaceuticals; Dissolution profile; In vitro availability tests; Sulphametoxazole; Trimethoprim

1. Introduction

Testing of the dissolution rates of the active principles present in a pharmaceutical formulation, also known as 'in vitro' availability, is a required task in pharmaceutical manufacturing and quality control laboratories. Some years ago, it was included as a legal requisite and the procedures are standardised and published in a number of pharmacopoeias [1–3].

The assays are required with two main goals: to estimate the availability of the active principle and to check the stability (reproducibility) in the preparation procedure of one pharmaceutical formulation. These requirements resulted in a high number of works trying to automate the proce-

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dure—even some robots have been designed to solve the legal requirements.

The method recommended in pharmacopoeias is based on the spectrophotometric measurement of the resulting solution, the total absorbance due to all solved substances (drugs and excipients) is recorded; only insoluble products are separated on-line. To obtain a single dissolution profile of a formulation requires a careful control of different and very influential parameters, namely pH, temperature, stirring rate and ionic strength mimicking the bioavailability conditions. The resulting profile is also influenced by employed stuff in its manufacture; particle size, the active principle content and the binder composition [4].

A typical procedure for implementation of FIA dissolution tests has been reported in a number of papers [5,6], along with the resulting advantages. Their basic advantage is to obtain the individual profile of one drug present in the mixture. With the aid of an FIA assembly, it is an easy way to implement the required different chemical processes to obtain a single profile from the 'global' profile of the pharmaceutical formulation. The accuracy of the dissolution rates thus obtained is very similar to that of the 'classical' manual method recommended in pharmacopoeias. Both give highly consistent results.

Automation of the procedures for individual dissolution tests with the aid of FIA methodology employs spectrophotometry as the most used detection. Koupparis et al. [7], as pioneers in the application of FIA assemblies to those assays, described the solution profiles for paracetamol by oxidation with Fe(III); the released Fe(II) complexed with 2,4,6-trypyridyl-*S*-triazine was spectrophotometrically monitored. A spectrophotometric method was also applied to salicylic acid, salycilamide or methyl salicylate, in which a purple complex is formed with Fe(III) in acidic medium [8].

The ion chloride determination can also be used as an indirect determination of some active principles present as chlorhydrates and it is supposed that no other source of chloride is present in the formulation. The chloride ion releases SCN^- anions from the reagent $Hg(SCN)_2$ resulting in a red colour with Fe(III) [9]. This procedure has been exploited for obtaining dissolution profiles of sulphonamides and propanteline bromide [10].

Other detection methods have been used in the realisation of dissolution assays in FIA. For example, ondasetron was quantified by measuring, with the flame atomic absorption detector, the released Pb(II) from a solid-phase reactor filled with PbO_2 [11]. The immobilisation also has been used to determine the 'in vitro' availability of sulfadiazine which reacts with the released nitrite from a copperised cadmium column [12]. In an article dedicated to demonstrating that any detector is useful for the purpose of obtaining individual solution profiles with the aid of an FIA system, (besides spectrophotometric measurements) biamperometric determination for paracetamol, spectrofluorimetric for captopril and atomic absorption for glycine were used [5].

This article deals for first time with the simultaneous recording of two dissolution profiles from two active principles present in the same pharmaceutical formulation with the aid of an FIA manifold provided with a single detector, the UV-vis spectrophotometer. This goal required a photo-diode array spectrophotometer, allowing us to record the absorbance of the solution at several wavelength values. When the two studied pharmaceuticals have no completely overlapped spectra, selecting two wavelength (usually at the maximum absorbance) values can solve the problem. For the present work, a binary mixture of pharmaceuticals (sulphametoxazole and trimethoprim) with overlapped spectra was selected and the proposed mathematical method was the zero crossing in the first derivative spectrum.

The simultaneous determination of two pharmaceuticals with overlapped spectra has been solved by different mathematical approaches; one is the derivative spectrophotometry [13–19]. Derivative spectrophotometry is an effective way for analysing mixtures, particularly with the quick recording of the diode array spectrophotometers; thus, the FIA-diode array spectrophotometers seem a useful couple for solving such mixtures as those found in pharmaceutical formulations [20].

2. Experimental

2.1. Reagents and apparatus

2.1.1. Reagents

Used reagents were all analytical unless stated: acetic acid, sodium acetate, sodium hydroxide, ammonia, ammonium chloride, ethanol, sodium phosphate and citric acid; all from Panreac (Barcelona, Spain). Sulphametoxazole and trimethoprim were from Guinama (Valencia, Spain).

2.1.2. Flow-assembly

An UV-vis photo-diode array spectrophotometer (from Hewlett-Packard, model HP8452) were provided with a flow-cell (from Hellma) of 1 cm light-path and an inner volume (18 μl) was used as a detector. Flow assemblies were provided with a six-port rotary valve (from Rheodyne, model 5021) and peristaltic pumps (from Gilson, model Minipuls-2). All tubing was made of PTFE with internal diameter 0.8 mm (from Omnifit) and methacrylate merging devices of the 'arrow tip' type. In several preliminary assays, a glass-mixing chamber or a column (4.6 cm length and 0.5 cm diameter), filled with glass beads 0.5 mm diameter as inert reactor, were included. Sample aliquots from a dissolution vessel were periodically inserted into the carrier stream, an acetic–acetate buffer solution at pH 4.29, which forced the sample to the flow-cell of the detector. The finally proposed FIA manifold is depicted in Fig. 1.

2.2. Sample preparation

For batch procedures, six tablets were taken and powdered in an agate mortar and pestle; the required amount was weighed to prepare for stock solutions. Those stock solutions were prepared after filtering to avoid insoluble excipients and levelled to the mark with the required medium. The studied commercially available formulations were (a) Abactrim (from Roche S.A.), label claim: 400 mg sulphametoxazole and 80 mg trimethoprim; and (b) Bronco Bactifor (from Andrómaco) with a manufacturer claim of 400 mg sulphametoxazole and 80 mg trimethoprim.

For obtaining the solution profiles, a tablet was placed in the tip of the mechanical stirrer rod (in a platinum basket) and introduced into a 0.1 mol l^{-1} HCl at 37 °C, rotation speed of 75 rpm and time interval of 60 min. The tip of the PTFE tubing introduced in the solution vessel was provided with a filter to avoid the pass of insoluble excipients.

2.3. Procedures

Preliminary experiments in batch were performed with two goals: (a) to confirm the procedure for the simultaneous determination of both

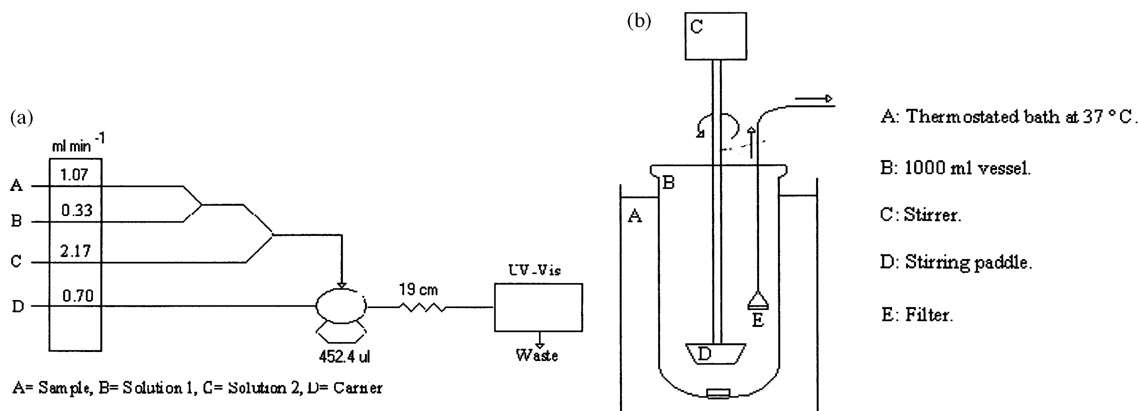


Fig. 1. (a) FIA assembly connected to a dissolution standard vessel to obtain dissolution profiles of Abactrim and Bronco Bactifor (sulphametoxazole/trimethoprim). FIA and chemical conditions are also shown. (b) Dissolution test vessel (USP Pharmacopoeia).

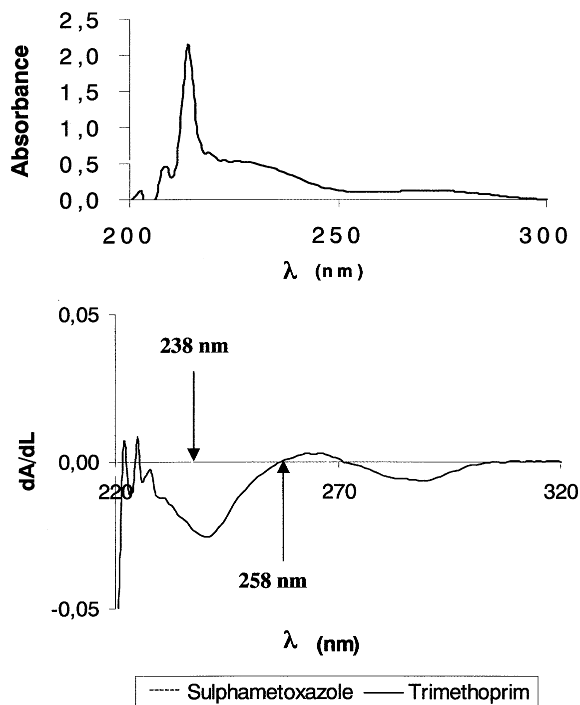


Fig. 2. Spectra and first derivative of the two pharmaceuticals.

pharmaceuticals; and (b) to establish the quality of analytical results (analytical errors) at low and high concentrations of the drug. A third required goal was to know what happens with the concentration ratio sulfamethoxazole and trimethoprim (both drugs present a different solution rate) to obtain derivative spectra results.

Once finished with these assays, the suitable assembled FIA was designed to adapt the method in static to the continuous-flow and to perform the dissolution test. Once the most appropriate assembly was selected, all chemical parameters were optimised. Finally, the dissolution profiles were obtained and the obtained results were adjusted by regression.

2.3.1. FIA procedures

Aliquots from the solution vessel in HCl medium were aspirated through the channel A and merged with the NaOH (channel B) solution to partially neutralise the acidity. The resulting mixture merged with the acetic–acetate buffer (at pH 4.29) solution. With the pH adjusted, the resulting sample mixture was inserted into the carrier stream formed by the same buffer and flowed through channel C. The carrier stream (channel D) led the sample to the flow-cell of the detector and then absorbances were simultaneously recorded at λ 258.0 and 272.0 for sulfamethoxazole and trimethoprim, respectively.

3. Results and discussion

Tests were carried out to assay the simultaneous determination of the binary mixture of sulphametoxazole and trimethoprim. Spectra of individual solutions were recorded from 190.0 to 390.0 nm and the corresponding first derivative to find the zero crossings. Tests at different pH values over the range 1–12 were performed; pH values on the 4–5 interval were selected. Fig. 2 depicts the zero crossing values at the final selected pH 4.29 with the acetic acid–sodium acetate buffered solution, were found at 238.0 and 268.0 nm for sulphametoxazole and at 258.0 and 272.0 for trimethoprim.

Then, determinations were performed at 258.0 and 238.0 nm, for sulphametoxazole and trimethoprim, respectively. The linearity interval for sulphametoxazole 0.24–24 mg l^{-1} fitted the equation $y = 0.0021x + 0.0006$, with a correlation coefficient of 0.9991; for trimethoprim were 0.1–12 mg l^{-1} and equation $y = -0.0035x + 0.0007$ correlation coefficient 0.9992, where y, means the absorbance and x, the drug concentration in mg l^{-1}.

The accuracy of the selected procedure for obtaining the dissolution profiles was formerly tested; it required the calculation of the analytical errors in the determination of each drug at low (which corresponds with the first stages of the test) and at high (final part of the test) concentrations. Several mixtures were prepared containing the following sulphametoxazole/trimethoprim concentration ratios: 1:0, 0:1, 2:1 and 5:1. The prepared concentrations were in the linear range from 4–24 and 1–9 for sulphametoxazole and trimethoprim, respectively. Relative errors (%) were calculated versus the added amount and all comprised in the range 1.9–4.2; two cases ex-

cepted with calculated errors 5.6 and 6.1. To confirm the accuracy of the procedure, two formulations were analysed and errors calculated against the label claim; Abactrim and Bronco Bactifor, average relative errors (three replicates) 4.1 and 5.5, respectively.

The differences in solubility among the two drugs can be an influential parameter affecting the results. This led us to test if the numerical ratio of the derivative absorbances was constant when the concentration ratio was also kept constant, bearing in mind if differences in solubility during the performance of the dissolution assay are reflected in measurements. Tested ratio sulphametoxazole/trimethoprim 5/1 as in formulations and the calculated signal ratio were in the range from 3.188 to 3.321; average 3.246 and R.S.D. 0.3%.

Then, it was decided that batch assays did not merit further research and the required FIA assembly was designed on the basis of the official recommendations in which the solution must be performed in 0.1 l mol^{-1} HCl and the measuring solution at pH 4.29 with the aid of the acetic acid/sodium acetate buffer; and as the dissolution period increased, the higher the concentrations of the drug and the corresponding measurement to be performed in-line avoiding absorbances in the range of too high photometric error; which means in-line addition of the corresponding dilution. The obtained empirical results after optimisation assays are presented in Fig. 3.

3.1. Dissolution tests from commercially available tablets

Several preliminary assays were carried out with Abactrim to check the absorbance values when the dissolution was completed with the goal of optimising the sample dilution. A filter unit was added to the tip of the tubing. To find the optimal conditions, the following sets of empirical conditions were tested. Any of the reported assays required a previous determination of the residence time interval (interval from sample insertion to appearance of the peak vertex) were empirically obtained.

3.1.1. Assay 1

Chemical parameters; carrier, buffer solution; sample, two different media 0.1 mol l^{-1} HCl and 0.1 mol l^{-1} NaOH; and for diluting the solution from the solution vessel a channel with the buffered solution. FIA parameters: flow-rates (in ml min^{-1}) carrier: 1.59, NaOH solution: 1.33, sample 1.58, diluting solution: 2.5. Other FIA variables: sample loop: 90 cm (0.8 mm) and distance injection from valve to flow-cell: 19 cm (0.8 mm). Spectra were recorded after a residence time of 25 s. Absorbances at high concentrations were too high, resulting in deviations of the wavelength maximum due to experimental errors which resulted in distorted curves of the corresponding dissolution profile. Because of this, assays with increased sample dilution were proposed as described in the following assay series.

3.1.2. Assay 2

Same experimental conditions as in the assay 1 except the flow-rates. Flow-rate (in ml min^{-1}): carrier 1.13, NaOH 0.97, sample solution: 1.07 and diluting solution: 2.17. Spectrophotometric measurements were recorded after 39 s as residence time. The series resulted in the same type of errors as reported in assay 1. Higher sample dispersion was required; in flow methods, the easier way to dilute a solution is performed by varying the flow-rates of the merging solutions (sample, NaOH and buffer in this assembly).

3.1.3. Assay 3

As in the assay 2 except: 0.04 mol l^{-1} NaOH, NaOH and sample flow rate, 0.50 and 1.07 ml min^{-1}, respectively. Residence time also 39 s. With this set of parameters errors were clearly minors (deviation of the wavelength of maximum absorbance was minor) resulting in a slightly distorted dissolution profile for trimethoprim.

3.1.4. Assay 4

Assay 3 was changed by using the flow rates 0.7 and 0.33 ml min^{-1} for carrier and NaOH, respectively; and the absorbance measurements

obtained 31 s of being injected the sample aliquot. Maxima absorbance values were < 1.2 resulting in satisfactory dissolution profiles.

Next work was performed to obtain several dissolution profiles with the commercial formulations (Abactrim and Bronco Bactifor) to test the reproducibility.

3.2. Regression analysis of the obtained plots

3.2.1. First fit

To check the reproducibility of the resulting profiles were fit to a sixth degree polynomial equation and their comparative study was based on the calculation of the average of the

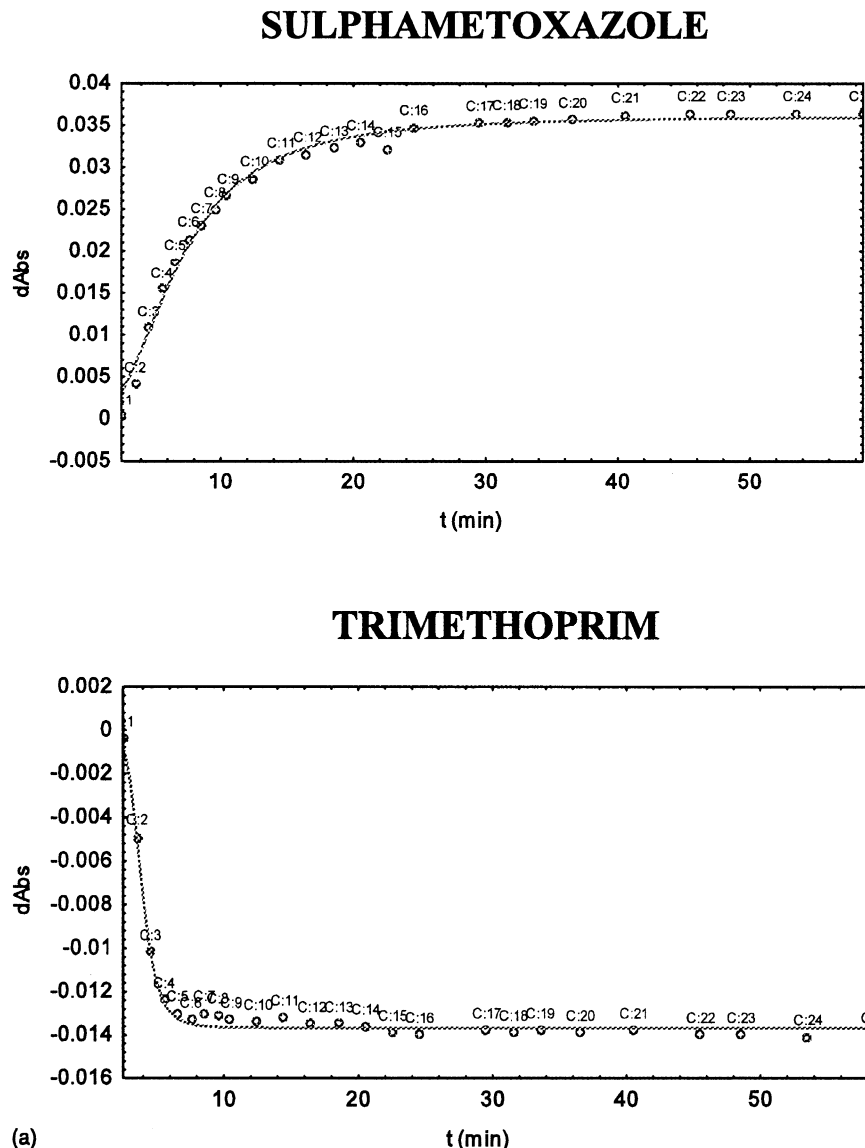
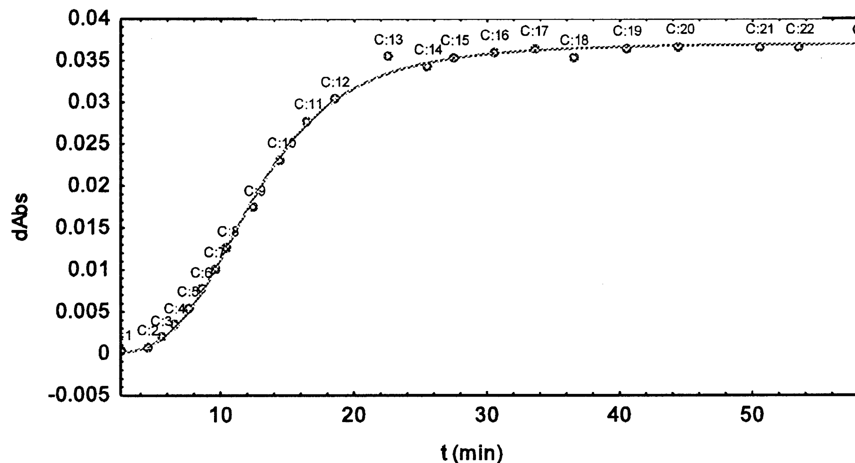


Fig. 3. Dissolution test curves obtained for Abactrim and Bronco Bactifor.

SULPHAMETOXAZOLE



TRIMETHOPRIM

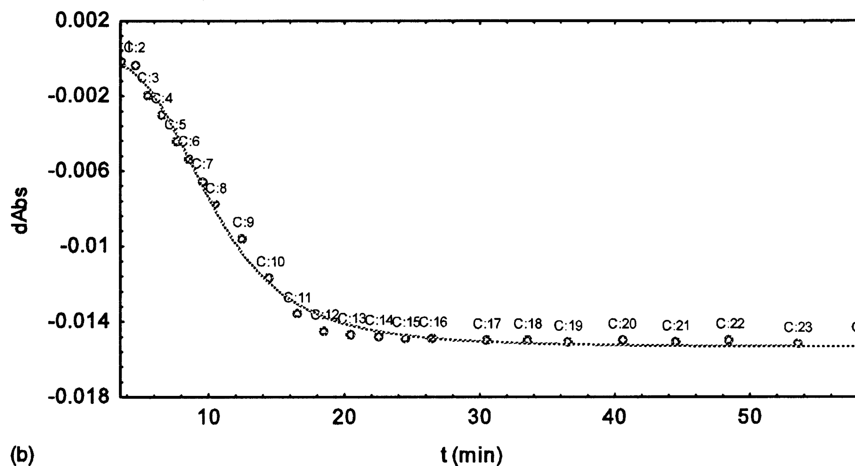


Fig. 3. (*Continued*)

R.S.D. (in percent) for each coefficient. See Tables 1–6.

The first problem was to decide the most significant coefficients to compare results. Searching for a better significance of the profiles a linear regression of each climbing interval, see Table 3 for Abactrim and Table 6 for Bronco Bactifor.

Information on the reproducibility studies was not obtained from comparing the polynomial equations from repeated series of assays and a different type of fitting equation was studied; a mathematical relationship among the most significant parameters allowed a better characterisation of the obtained equations.

3.2.2. Second fit

A new regression was studied with the aid of the 3-parameters equation [21]. This equation has been proposed for the mathematical fitting of the hyperbolic type plots, such as those obtained in enzy-

Table 1
Polynomial regression for sulphametoxazole

Degree	6	5	4	3	2	1	0	R^2
Assay 1	5.0E−11	1.0E−8	8.0E−7	4.0E−5	0.0009	0.0109	0.0218	0.998
Assay 2	5.0E−11	1.0E−8	8.0E−7	4.0E−5	0.0008	0.0103	0.0217	0.996
Assay 3	4.0E−11	9.0E−9	8.0E−7	4.0E−5	0.0009	0.0106	0.0210	0.996
Assay 4	6.0E−11	1.0E−8	1.0E−6	5.0E−5	0.0010	0.0120	0.0256	0.996
R.S.D. (%)	16.33	5.13	11.76	11.76	9.07	6.77	9.23	

Average of the R.S.D.: 10.00%.

Table 2
Polynomial regression for trimethoprim

Degree	6	5	4	3	2	1	0	R^2
Assay 1	8.0E−11	2.0E−8	1.0E−6	5.0E−5	0.0009	0.0085	0.0136	0.936
Assay 2	8.0E−11	2.0E−8	1.0E−6	4.0E−5	0.0009	0.0081	0.0138	0.910
Assay 3	8.0E−11	2.0E−8	1.0E−6	4.0E−5	0.0009	0.0079	0.0126	0.943
Assay 4	9.0E−11	2.0E−8	1.0E−6	5.0E−5	0.001	0.0092	0.0163	0.943
R.S.D. (%)	6.06	0	0	12.83	5.41	6.81	11.18	

Average of R.S.D.: 6.04%.

Table 3
Obtained linear equations in the regression analysis of climbing intervals from the Abactrim profiles

	Sulphametoxazole			Trimethoprim		
	Slope	Intercept	R	Slope	Intercept	R
Assay 1	0.0064	0.0160	0.997	0.0056	0.0134	0.996
Assay 2	0.0052	0.0134	0.998	0.0049	0.0121	0.997
Assay 3	0.0060	0.0148	0.997	0.0056	0.0135	0.996
Assay 4	0.0058	0.0155	0.998	0.0053	0.0135	0.997
R.S.D. (%)	8.55	7.57		6.20	5.22	

Table 4
Polynomial regression for sulphametoxazole

Degree	6	5	4	3	2	1	0	R^2
Assay 1	9.0E−11	2.0E−8	1.0E−6	6.0E−5	0.001	0.0055	0.0097	0.998
Assay 2	8.0E−11	2.0E−8	1.0E−6	5.0E−5	0.001	0.0056	0.0101	0.999
Assay 3	9.0E−11	2.0E−9	1.0E−6	5.0E−5	0.0008	0.0030	0.0021	0.999
Assay 4	9.0E−11	2.0E−8	2.0E−6	7.0E−5	0.001	0.0089	0.0204	0.999
R.S.D. (%)	5.71	0.00	4.00	16.70	10.50	42.1	71.00	

Average of R.S.D.: 26.6%.

Table 5
Polynomial regression for trimethoprim

Degree	6	5	4	3	2	1	0	R^2
Assay 1	4.0E−11	8.0E−9	6.0E−7	2.0E−5	0.0003	0.0006	0.0006	0.997
Assay 2	4.0E−11	7.0E−9	5.0E−7	2.0E−5	0.0003	0.0005	0.0009	0.998
Assay 3	4.0E−11	7.0E−9	5.0E−7	2.0E−5	0.0002	0.0003	0.0018	0.997
Assay 4	5.0E−11	1.0E−8	7.0E−7	3.0E−5	0.0003	0.0018	0.0023	0.998
R.S.D. (%)	11.80	17.70	16.70	22.20	27.20	84.80	56.20	

Average of R.S.D.: 33.8%.

Table 6
Obtained linear equations in the regression analysis of climbing intervals from the Bronco Bactifor profiles

	Sulphametoxazole			Trimethoprim		
	Slope	Intercept	R	Slope	Intercept	R
Assay 1	0.0024	0.0124	0.998	0.0011	0.0042	0.996
Assay 2	0.0024	0.0130	0.997	0.0011	0.0043	0.995
Assay 3	0.0027	0.0111	0.991	0.0012	0.0032	0.989
Assay 4	0.0026	0.0147	0.981	0.0012	0.0046	0.997
R.S.D. (%)	5.94	11.70		5.02	14.90	

Table 7
Three-parameter equation plots for Abactrim Triglobe (sulphadiazine/trimethoprim)

	Sulphametoxazole				Trimethoprim			
	a	b	c	R	a	b	c	R
Assay 1	0.0362	6.7162	2.3808	0.995	−0.0136	3.8604	6.5368	0.995
Assay 2	0.0355	6.0251	2.2941	0.988	−0.0140	3.5768	6.9344	0.991
Assay 3	0.0377	5.7013	2.7803	0.993	−0.0147	3.8564	6.8238	0.996
Assay 4	0.0343	5.9836	2.951	0.995	−0.0145	3.8974	7.3639	0.999
R.S.D. (%)	3.95	7.06	12.10	0.33	3.43	3.91	4.96	0.33

The different parameters are compared using the R.S.D.

matic reactions with a Michaelian kinetic of the kinetic processes body–antibody.

$$V_2 = a/(1 + (b/V_1)^{**}c)$$

The depicted parameter meanings are: (a) signal figure (first absorbance derivative) when the total solution is finished; (b) half-maximum signal or the signal at half-time of the required interval for total dissolution; and (c) the exponent related with the slope of the climbing interval of the profile. Computerised calculations were performed with the aid of the program 'Statistica' working in windows, (©Statsoft Inc., 1993) and the results can be seen in Tables 7 and 8.

With the 3-parameters equation, the parameters are easy to compare and the curves fit better to the plot than the polynomial equations, so the total curve as the linear climbing interval. Three are the possible reasons to explain those better fittings: (a) it allows a kinetic meaning of the numerical parameters; (b) equations are easy to compare; and (c) best correlation coefficients were obtained.

Table 8
Three-parameters equation plots for Bronco Bactifor

	Sulphametoxazole				Trimethoprim			
	a	b	c	*R*	a	b	c	*R*
Assay 1	0.0371	12.473	3.750	0.999	−0.0138	9.670	3.680	0.998
Assay 2	0.0405	13.250	3.675	0.999	−0.0153	10.195	3.591	0.998
Assay 3	0.0439	11.535	3.457	0.999	−0.0166	8.721	3.260	0.997
Assay 4	0.0446	13.824	4.179	0.999	−0.0173	10.755	3.719	0.997
R.S.D. (%)	8.33	7.77	8.03	0.00	9.67	8.79	12.49	0.12

The different parameters are compared using the R.S.D.

4. Conclusions

It is described, for the first time, how to obtain simultaneously dissolution profiles of two active principles present in the same pharmaceutical formulation. Using an FIA assembly provided with only one detector carries out the whole process. There are a number of published papers dealing with the solution profile of a single compound in a pharmaceutical formulation so in FIA, or even in the emerging continuous-flow multicommutation methodology [22,23]; but as far as the authors are aware, none deals with the simultaneous determination of solution profiles of two drugs.

The discussion of the suitable regression procedure for the obtained curves is also included. The 3-parameters equation seems to be more suitable for this purpose.

The procedure is simple and robust and allows the determination of solution profiles of two pharmaceuticals present in the same formulation, even when both spectra are overlapped which probably means a general applicability to any binary mixture.

References

[1] United States Pharmacopoeia (USPXXI) (National Formulary), p. 1291, 1421.
[2] British Pharmacopoeia, Her Majesty's Stationary Office, London, 1993, Vol. I, p. 547, 586.
[3] Real Farmacopea Española, Ministerio de Sanidad y Consumo, Madrid, 1997, p. 1601, 1720.
[4] J. Martínez Calatayud, Flow Injection Analysis of Pharmaceuticals. Automation in the Laboratory, Taylor and Francis, Cambridge, 1996.
[5] J.L. López Paz, J. Martínez Calatayud, Pharm. Technol. Eur. 10 (1998) 16.
[6] P. Solich, C.K. Polydorou, M.A. Koupparis, C.A. Efstathiou, Biomed. Chromatogr. 13 (1999) 113.
[7] M. Koupparis, P. Macheras, L. Tsaprounis, Int. J. Pharm. 27 (1985) 349.
[8] M.A. Koupparis, P.I. Anagnostopoulou, J. Pharm. Biomed. Anal. 6 (1988) 35.
[9] M. Koupparis, E.G. Sarantonis, J. Pharm. Sci. 75 (1985) 800.
[10] M. Koupparis, P. Macheras, C. Reppas, Int. J. Pharm. 20 (1984) 325.
[11] L. Lahuerta Zamora, J. Martínez Calatayud, Anal. Chim. Acta 300 (1995) 143.
[12] A. Mellado Romero, C. Gómez Benito, J. Martínez Calatayud, Anal. Chim. Acta 308 (1995) 451.
[13] S. Görög, Ultraviolet—Visible Spectrophotometry in Pharmaceutical Analysis, CRC Press, Boca Raton, FL, 1995.
[14] C. Bosch-Ojeda, F. Sanchez-Rojas, J.M. Cano Pavón, Talanta 42 (1995) 1195.
[15] A. Sachan, P. Trivedi, Indian Drugs 35 (1998) 762.
[16] Y.C. de Micalizzi, N.B. Pappano, N.B. Debattista, Talanta 47 (1998) 525.
[17] F. Salinas, A. Espinosa Mansilla, J.J. Berzas Nevado, Anal. Chim. Acta 233 (1990) 289.
[18] C.V.N. Prasad, A. Gautam, V. Bharadwaj, P. Parimoo, Talanta 44 (1997) 917.
[19] J.J. Berzas Nevado, J. Rodríguez Flores, C. Guiberteau-Cabanillas, M.J. Villasenor-Llerena, A. Contento-Salcedo, Talanta 45 (1998) 933.
[20] G.A. Rivas, S. Laredo Ortiz, J. Martínez, Calatayud Anal. Lett. 29 (1996) 2115.
[21] J. Swanbrick, J.C. Boylai (Eds.), Encyclopaedia of Pharmaceutical Technology, Marcel Dekker Inc, New York, 1992, pp. 121–167.
[22] L.F. Gouveia, J.L.F. Costa Lima, J.A.G. Morais, Anal. Chim. Acta 366 (1998) 271.
[23] R.A.S. Lapa, J.L.F. Costa Lima, J.L.M. Santos, Anal. Chim. Acta 419 (2000) 17.