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Simultaneous dissolution profiles of two drugs, sulfadiazine-trimethoprim and amitriptyline-perphenazine, in solid oral dosage forms by a FIA manifold provided with a single spectrophotometric detector

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Abstract

The simultaneous determination of two dissolution profiles with the aid of a Flow Injection Analysis assembly has been applied to: (a) sulfadiazine-trimethoprim in tablets and (b) amitriptyline-perphenazine in sugar coated pills. The selected combinations are drugs which have overlapping UV-vis spectra. The officially proposed procedure from the pharmacopoeias has been adapted for the FIA methodology and derivative spectrophotometry and zero crossing. Preliminary experiments on the suitability of the simultaneous determination of both drugs were performed. The empirical profiles were adjusted by regression analysis using different approaches. The 3-parameter plot method was finally selected as the most suitable for the sulfadiazine-trimethoprim and the 4-parameter equation plot for amitriptyline-perphenazine.

Keywords: FIA; Pharmaceuticals; Dissolution profile; In vitro availability tests; Sulfadiazine; Trimethoprim; Amitriptyline; Perphenazine

1. Introduction

The solution profile of a pharmaceutical formulation or the 'in vitro' availability is an established mandatory test in international pharmacopoeias [1–3]. Formerly recommended for oral formulations i.e. tablets, now procedures are published for tablets, sugar coated pills and patches. The test is not only valid for measuring the availability of the active principles, but it is also an assay for checking the reproducibility of the manufacturing process, bearing in mind that the results are due to not only chemical contents but also to the physical properties of the dosage like particle size or excipients, amongst others. The 'in vitro' equivalence between a generic formulation and a reference formulation is also established by comparing both dissolution profiles and through the calculation of numerical factors defined by European Agency for Drug Evaluation and the Federal Food and Drug Administration [4,5].

The officially recommended procedure in pharmacopoeias results in a solution profile of the whole formulation. The solution is periodically monitored by spectrophotometry at a fixed wavelength. The efforts focused to obtain the individual profile of

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one active principle in the formulation are interesting and at present are not included in pharmacopoeias. One relevant attempt to obtain individual profiles was performed with the aid of the Flow Injection Analysis (FIA) methodology. Koupparis and coworkers pioneered the application of FIA to these assays in a paper [5] in which paracetamol was oxidised by Fe (III) and the resulting Fe (II) spectrophotometrically monitored after being complexed by 2,4,6-trypyridyl-S-triazine. Other FIA spectrophotometric procedures have been also published [6–8]. Very few articles have been published dealing with detectors others than spectrophotometric ones [9–11].

This article deals with the simultaneous measurement of two individual dissolution profiles from two active principles present in the same pharmaceutical formulation by means of the FIA methodology. The solution vessel is coupled to a FIA manifold and provided with a UV-vis spectrophotometer detector. The photo-diode array spectrophotometer allowed us to record absorbance of the solution at several wavelengths. In a former paper [12], we presented for first time, the simultaneous determination of two 'individual' dissolution profiles using a single spectrophotometer in a FIA manifold; the procedure was applied to tablet pharmaceutical forms. For the present work two binary mixture of pharmaceuticals (sulfadiazine-trimethoprim and amitriptyline-perphenazine) with overlapping spectra were selected; the chosen mathematical method for solving this problem was zero crossing in the first derivative spectra. We also selected two different pharmaceutical forms to test the new procedure; tablets for the mixture sulfadiazine-trimethoprim, and, for first time, sugar coated pills containing amitriptyline and perphenazine. The 4-parameter equation plot is shown to be the most adequate for this kind of formulations.

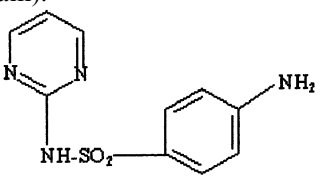
On the other hand, derivative spectrophotometry was introduced in the early 1950s by Hammond and Price [13–15] and has been widely used in the determination of pharmaceutical mixtures, both in batches [16–21] and using FIA [16,22,23]. However, at present it is widely used by a number of authors [24–27]; for example in clinical samples for mixtures [26] or for avoiding interferences [28]: for the individual determination of one metal in mixtures [29,30]; and, for other purposes like food additives [31,32] or pesticides [33].

2. Experimental

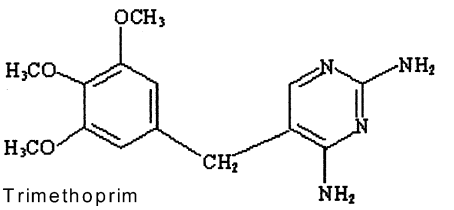
2.1. Reagents and apparatus

2.1.1. Reagents

Pharmaceuticals sulfadiazine, trimethoprim, amitriptyline and perphenazine all from Guinama (Valencia, Spain).



Sulphadiazine



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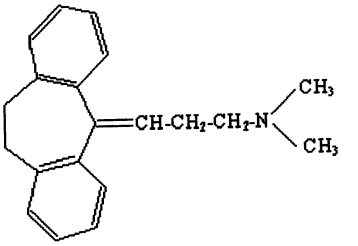
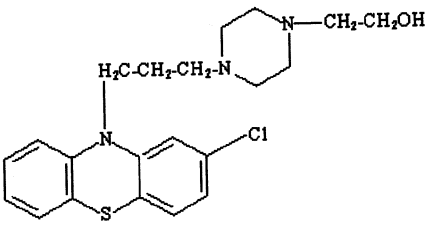


Amitriptyline

Perphenazine



All reagents used were of analytical standard unless stated: sodium hydroxide, ammonia, ammonium chloride, ethanol, sodium phosphate and citric acid; wee from Panreac (Barcelona, Spain).

2.1.2. Flow-assembly

The finally proposed FIA manifolds and the solution assembly are depicted in Fig. 1. These assemblies were connected to an Hewlett Packard, model HP8452, UV-vis photo-diode array spectrophotometer which contained a Hellma flow-cell (1 cm light-path and inner volume 18 μl); a Rheodyne, model 5021, 6-port rotary valve; and, a Gilson, model Minipuls-2, peristaltic pump. All tubing was from Omnifit and made of PTFE with internal diameter 0.8 mm. Methacrylate merging devices of the 'arrow tip' type, home made, were also required as flow connectors in the assemblies. In preliminary assays were included a glass mixing chamber or a column (4.6 cm length and 0.5 cm diameter) filled with glass beds 0.5 mm diameter as inert reactor.

Sample aliquots from the dissolution vessel filled with 0.1 mol l^{-1} HCl were periodically inserted into the carrier stream leading the sample to the flow-cell of the detector. The medium required for the solution monitoring which was different from the recommended media in the solution vessel and the required media was prepared 'in situ' with the aid of the flow assembly.

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 the

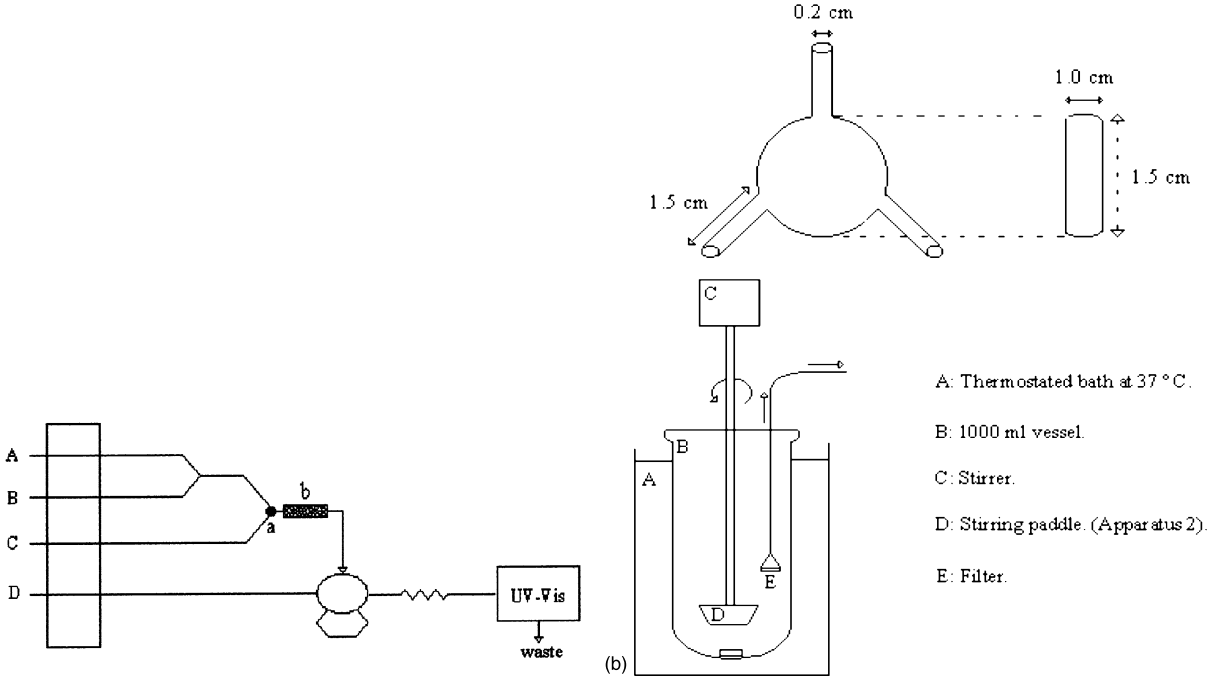


Fig. 1. FIA assembly connected to dissolution standard vessel to obtain dissolution profiles: 1(a) Manifold for the mixture sulfadiazine-trimethoprim: A, sample solution; B, sodium hydroxide; C, mixture of the ammonia-ammonium buffer at pH 10 and ethanol; D, carrier stream formed by the mixture as in the C channel. a, mixing chamber; b, inert solid-phase reactor; and, W, waste. A similar assembly as 1(a) is proposed for the mixture amitriptyline-perphenazine, with the following parameters: A, sample solution; B, mixture 10% of citric acid and $NaHPO_4$ at pH 5.0 plus 20% ethanol; and, C, carrier stream formed by the same mixture as channel B. a and W like Fig. 1a. Mixing chamber (a) absent. 1(b)- Top, mixing chamber (a in the assembly) with its dimensions; front (left) and lateral (right) view. Bottom, dissolution test vessel as proposed in the USP Pharmacopoeia.

stock solutions. These stock solutions were prepared after filtering to remove insoluble excipients and making up to volume to mark with the required medium. The commercially available formulations studied contained the following amounts of active principles:

1. Mixture sulfadiazine-trimethoprim in Triglobe (from Astra) in the ratio 5.0:1.1 containing 820 and 180 mg of sulfadiazine and trimethoprim, respectively per tablet.
2. Amitriptyline-perphenazine: Mutabase (from Schering-Plough), declared, amitriptyline 10 mg and perphenazine 2 mg in a sugar coated pill.

Solution profiles were prepared by placing the formulation into a platinum basket (tablet) nesting in the tip of metallic rod and close to the bottom of the dissolution vessel; or for the sugar coated pill, the formulation was left in the bottom of the vessel. The rod is a mechanical axe rotating at 75 rpm; see USP Pharmacopoeia [1] (apparatus 2) and Farmacopea Española [3] for details. The dissolution medium was 0.1 mol l^{-1} HCl and the temperature was maintained at 37 °C up to the maximum time interval of 60 min. A filter unit was placed in the tip of the PTFE tubing for aspirating the resulting vessel solution to remove insoluble excipients.

2.3. Procedures

Preliminary experiments in batch mode were performed with two goals: (a) to establish and confirm the procedure for the simultaneous determination of both pharmaceuticals; and, (b) to establish the quality of analytical results (analytical errors) at low and high concentrations of the drug Third goal was to know the influence of the concentration ratio on the analytical results (drugs present in a formulation can present different dissolution rates) and to obtain derivative spectra figures. The first goal is linked to the search for the best pH to analyse both drugs bearing in mind two points; first the influence of the pH in the spectra and, to obtain the best sensitivity (and limit of detection) when passing from zero order derivative to first, or other higher order.

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

3. Results and discussion

3.1. Mixture sulfadiazine-trimethoprim

Preliminary experimental data for processing were obtained from the paper of Berzas and coworkers [34] for in batch determination of the contents of both drugs in pharmaceutical formulations. According to these authors the sample was dissolved at pH 10 in a mixture of 10% 0.5 mol l^{-1} ammonia-ammonium buffer and 20% ethanol; the zero crossings of the first derivative spectra were observed at 248.5 and 242.25 nm for sulfadiazine and 288.0 and 258.8 nm for trimethoprim. Suitable wavelengths for analyses were 288.0 and 248.5 nm for sulfadiazine and trimethoprim, respectively. First experiments were aimed at confirming these values; spectra of individual solutions were recorded from 190.0 to 390.0 nm for the solutions. Fig. 2 depicts the spectra at pH 10 and first derivatives in the suitability selected wavelengths from our empirical results were 288.0 and 248.5 nm for sulfadiazine and trimethoprim, respectively can be observed.

Further work was performed to obtain empirically the linear range interval and calibration graphs and to test the experimental errors in the analytical procedure. Sulfadiazine: 1–35 mg l^{-1}; $Y = -0.0009$. X–0.0003, $R^2 = 0.9990$. Trimethoprim; 1–30 mg l^{-1}; $Y = -0.00037$. X–0.0004, $R^2 = 0.9995$, where Y is the absorbance and X the concentration of the drug in mg l^{-1}). Then synthetic and commercial samples were analysed and relative errors versus the added amount or the label claim calculated, respectively. All analytical results obtained were within the accepted analytical error range, as in the reported paper

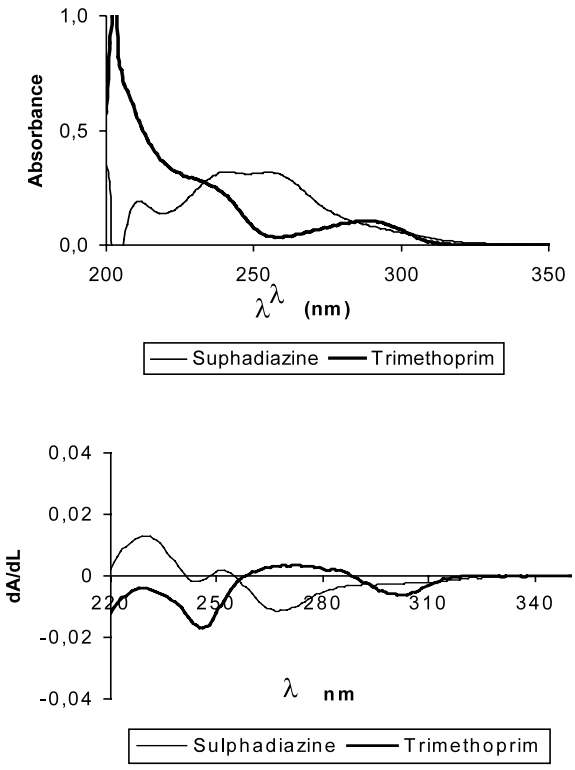


Fig. 2. Spectra and first derivative of the sulfadiazine and trimethoprim. Buffer, 0,5 mol l^{-1} NH_3/NH_4^+ (10) and 20% ethanol.

[33]. Finally synthetic samples were prepared maintaining the concentration ratio of both drugs (5:1 sulfadiazine/trimethoprim, as in commercial formulations) but in different total amounts (4, 12, 16, 20, 24 mg l^{-1} of each drug) to check the stability of the ratio of analytical outputs. Outputs ratios obtained were in the range 0.0676–0.0709, average 0.06934 and relative standard deviation (S.D.) 0.1%.

Next the suitable FIA manifold was constructed with the following goals: (a) met with the official recommendations that the dissolution must be performed in 0.1 l mol l^{-1} HCl at 37.0 °C; (b) the solution measured should be at pH 10 with the pH being adjusted with the ammonium/ammonia buffer; and, (c) as the dissolution period increases the concentrations of the drug increased and the corresponding measurement must be performed in-line avoiding excessive absorbances causing photometric error by on-line addition diluent. Using synthetic solutions and by sample injection (usual FIA mode), spectra were obtained with the aid of the FIA assembly after establishing suitable residence times for each parameter tested. Finally, the conditions selected were: 0.02 mol l^{-1} NaOH flowing at 2.0 ml min^{-1}; other flow-rates were (ml min^{-1}): sample, 0.4; buffer solution, 9.0; and, carrier, 2.0. Residence time 19 s and pH in effluents, 9.9.

3.2. Dissolution tests from commercially available oral dosage formulations

Several preliminary assays were carried out coupling the FIA assembly and the dissolution vessel, 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.

The first attempt with the chemical and FIA parameters as reported above resulted in highly distorted curves of the corresponding dissolution profile. Probably this was due to inefficient mixing of the solutions; a mixing chamber was included at junction point a (Fig. 1(a, b)).

Then the same experimental conditions were tested as in the previous paragraph excepting the mixing chamber; smaller distortions were observed; an increased sample dilution seemed necessary. The NaOH solution (channel B) was not required and it was substituted by the same buffer mixture as for channels C and D.

On others words NaOH was replaced by the buffer and the new flow-rate (of channel B) was 5.4 ml min^{-1}. With this set of parameters errors were clearly suppressed resulting in suitable dissolution profiles for sulfadiazine and trimethoprim.

Work was then performed to obtain several dissolution profiles with the commercial formulation Triglobe. Results (four replicates) can be seen in Table 1 and Fig. 3.

Table 1
Empirical results (four assays) from dissolution test for Top, Sufadiazine in triglobe (First derivative, λ 288 nm) and Bottom, Trimethoprim (first derivative, λ 258 nm)

Assay 1 t (min)	DAbs/dλ	Assay 2 t (min)	DAbs/dλ	Assay 3 t (min)	dAbs/dλ	Assay 4 t (min)	dAbs/dλ
Sufadiazine in triglobe (*First derivative*, λ *288 nm*)							
2.32	−0.0003	2.32	5E-5	2.32	5.80E-5	2.32	−0.0008
4.32	−0.0008	4.32	−0.0004	4.32	−0.0002	4.32	−0.0019
6.32	−0.0028	6.32	−0.0042	6.32	−0.0009	7.32	−0.0033
8.32	−0.0060	8.32	−0.0058	8.32	−0.0041	8.32	−0.0038
10.32	−0.0070	11.32	−0.0092	11.32	−0.0062	10.32	−0.0052
12.32	−0.0094	12.32	−0.0103	12.32	−0.0062	12.32	−0.0058
14.32	−0.0104	14.32	−0.0100	14.32	−0.0073	14.32	−0.0068
16.32	−0.0107	16.32	−0.0105	16.32	−0.0080	16.32	−0.0076
18.32	−0.0120	20.32	−0.0106	18.32	−0.0087	18.32	−0.0076
20.32	−0.0110	22.32	−0.0113	20.32	−0.0095	20.32	–
22.32	−0.0130	26.32	−0.0118	23.32	−0.0105	22.32	−0.0089
24.32	−0.0127	28.32	−0.0123	24.32	−0.0109	24.32	−0.0092
26.32	−0.0121	30.32	−0.0133	26.32	−0.0111	26.32	−0.0098
28.32	−0.0122	33.32	−0.0123	28.32	−0.0117	28.32	−0.0107
30.32	−0.0132	36.32	−0.0124	30.32	−0.0121	30.32	−0.0110
33.32	−0.0131	39.32	−0.0129	33.32	−0.0128	33.32	−0.0113
36.32	−0.0144	42.32	−0.0132	36.32	−0.0126	37.32	−0.0119
39.32	−0.0143	45.32	−0.0129	39.32	−0.0132	39.32	−0.0118
42.32	−0.0149	48.32	−0.0129	42.32	−0.0140	42.32	−0.0115
45.32	−0.0147	52.32	−0.0120	45.32	−0.0146	45.32	−0.0120
49.32	−0.0127	56.32	−0.0141	48.32	−0.0147	48.32	−0.0114
52.32	−0.0132	60.32	−0.0141	52.32	−0.0147	51.32	−0.0119
56.32	−0.0134	–	–	56.32	−0.0151	56.32	−0.0119
60.32	−0.0134	–	–	60.32	−0.0155	60.32	−0.0119
Trimethoprim (*first derivative*, λ *258 nm*)							
2.32	0.0002	2.32	3E-5	2.32	−0.0003	2.32	−0.0002
4.32	−0.0005	4.32	−0.0018	4.32	−0.0007	4.32	−0.0025
6.32	−0.0066	6.32	−0.0079	6.32	−0.0029	7.32	−0.0061
8.32	−0.0097	8.32	−0.0111	8.32	−0.0053	8.32	−0.0066
10.32	−0.0102	11.32	−0.0123	11.32	−0.0069	10.32	−0.0072
12.32	−0.0112	12.32	−0.0126	12.32	−0.0080	12.32	−0.0082
14.32	−0.0125	14.32	−0.0139	14.32	−0.0086	14.32	−0.0086
16.32	−0.0121	16.32	−0.0108	16.32	−0.0097	16.32	−0.0097
18.32	−0.0136	20.32	−0.0147	18.32	−0.0098	18.32	−0.0094
20.32	−0.0125	22.32	−0.0126	20.32	−0.0107	20.32	−0.0098
22.32	−0.0152	26.32	−0.0122	23.32	−0.0117	22.32	−0.0115
24.32	–	28.32	−0.0150	24.32	–	24.32	−0.0118
26.32	−0.0145	30.32	−0.0145	26.32	−0.0105	26.32	−0.0123
28.32	–	33.32	−0.0125	28.32	−0.0129	28.32	–
30.32	−0.0138	36.32	−0.0137	30.32	−0.0121	30.32	−0.0130
33.32	−0.0129	39.32	−0.0115	33.32	–	33.32	−0.0140
36.32	−0.0116	42.32	−0.0145	36.32	−0.0163	37.32	−0.0141
39.32	–	45.32	−0.0142	39.32	−0.0143	39.32	−0.0140
42.32	−0.0102	48.32	−0.0132	42.32	−0.0140	42.32	−0.0126
45.32	−0.0098	52.32	−0.0129	45.32	−0.0150	45.32	−0.0138
49.32	−0.0104	56.32	–	48.32	−0.0144	48.32	−0.0141
52.32	−0.0106	60.32	–	52.32	−0.0180	51.32	−0.0143
56.32	−0.0124	–	–	56.32	−0.0174	56.32	−0.0141
60.32	−0.0092	–	–	60.32	−0.0158	60.32	−0.0150

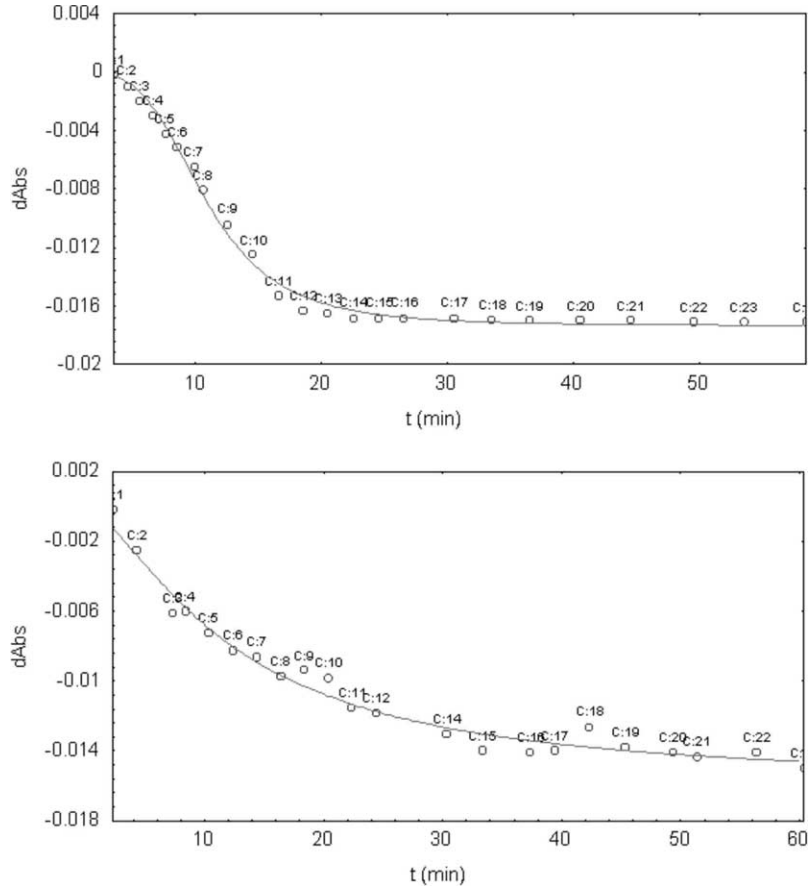


Fig. 3. Dissolution test curves obtained for the mixture sulfadiazine (top) and trimethoprim in Triglobe

3.3. Regression analysis of the obtained plots

To check the reproducibility of the resulting profiles we check to fit the empirical results to a 6th° polynomial equations and their comparative study was based on the calculation of the average of the rsd (in %) for each coefficient.

As no good fits were obtained a new regression was studied with the aid of the so called 3-parameters equation or Higuchi equation [35]. This equation has been proposed for the mathematical fitting of hyperbolic type plots like those obtained in enzymatic reactions with a Michaelian kinetic for body-antibody reactions.

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

The depicted parameter 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 corresponding to the slope of the climbing interval of the profile. Computerised calculations were performed with the aid of the program 'STATISTICA' working in windows, (Copyright Statsoft. Inc 1993) and the results can be seen in Table 2.

The Higuchi equation allows an easy comparison of the parameters and the curves fit better to this equation than the polynomial equations, so the total curve empirically obtained as the first linear climbing range of the curve. These better fits can be explained as the follows: (a) the Higuchi equation allows a kinetic meaning to be

attributed to numerical parameters; (b) equations are easy to compare; and (c) better correlation coefficients were obtained.

3.4. Mixture amitriptyline–perphenazine

This combination has antidepressive properties and this mixture is formulated as sugar coated pills, This means the time for dissolving the external cover should be considered.

Preliminary assays using the batch mode were performed with the empirical parameters according to the paper published elsewhere by García and co-workers [35]; the sample was solved in a solution mixture containing: 10% of the buffer citric acid/$NaHPO_4$ at pH 5 (49 ml of 0.1 M cítric acid plus 100–49 ml of 0.2 M $NaHPO_4$) 10 and 20% ethanol. Spectra were recorded over the wavelength range 190.0–300.0 nm. First and second derivative spectra were also recorded; (see Figs. 4 and 5) zero crossings were at 255 nm for amitriptyline (first derivative) and 252 nm for perphenazine (second derivative) 256 and 254 nm are the reported values in [36,37].

Then linear intervals were obtained with the corresponding equations with the above wavelengths and derivative spectra. Results obtained were (drug, wavelength (order of derivative) concentrations, equation and correlation coefficient):

Table 2
Three-parameters equation plots for Sulfadiazine (top) and Trimethoprim

	a	b	c	R
Sulfadiazine				
Assay 1	−0.0138	9.8857	2.7151	0.988
Assay 2	−0.0153	10.1915	3.5905	0.985
Assay 3	−0.0166	17.1525	1.8407	0.994
Ensayo 4	−0.0173	10.7549	3.7194	0.993
SRD (%)	9.76	28.81	29.43	0.429
Trimethoprim				
Assay 1	−0.0121	6.3851	5.3435	0.930
Assay 2	−0.0131	5.9576	5.1169	0.959
Assay 3	−0.0190	17.5723	1.5244	0.980
Assay 4	−0.0159	12.2769	1.4846	0.990
SRD (%)	20.37	52.13	63.93	2.74

The different parameters are compared using the relative standard deviation (S.D.).

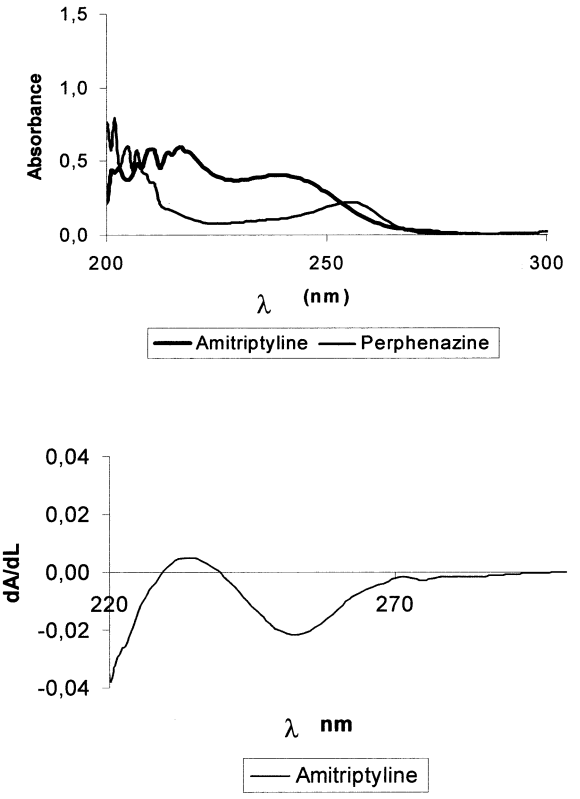


Fig. 4. Spectra and first derivative spectra of the two pharmaceuticals amitriptyline and perphenazine. Buffer, 0.1 mol l^{-1} citric acid and 0.2 mol l^{-1} NaH_2PO_4 (10) and 20% ethanol.

(a) Amitriptyline, λ 255.0 nm (1st derivative); 1, 10, 15, 20, 30 and 35 mg l^{-1} $Y = -0.0021x + 0.0001$, 1.000; and, (b) Perphenazine, λ 252.0 nm (2nd derivative). 1, 3, 5, 6, 8 y 10 mg l^{-1} $Y = -0.0006$ x-1E-04, 0.9950.

A further test was to analyse the contents of each of both drugs in synthetic and mixtures prepared in the lab or commercially (Mutabase from Schering-Plough) as reported for the previous studied sulphametoxazole/trimethoprim mixture; also different concentrations (from low to high in the linear range) and different amitriptyline/perphenazine ratios were tested. The analyses were performed by dissolving the samples in mixture of buffer solution containing citric acid/$NaHPO_4$ at pH 5 and 20% ethanol. The calculated errors were under the normal level for analysis, lower than 3% as the relative error,

excepting for low concentrations of amitriptyline, under 7 mg l^{-1} which were in the vicinity of 7%. Another test was to prepare samples (from 1 to 10 mg l^{-1}) of only one drug and compare the outputs for amitriptyline and perphenazine at the same concentration in the range studied (mimicking the solution profile process, a solution with continuously increasing concentrations). The ratio of signals was very similar from 3.285 to 3.813.

The FIA manifold prepared should allow the formulation being dissolved in 0. 1 M hydrochloride acid medium to be monitored in the medium of the buffer solution containing citric acid/$NaHPO_4$ at pH 5 and 20% ethanol. In addition, the dispersion of the solution aliquots must kept the dispersion as low as possible bearing in mind the concentration of both drugs in the formulation is not high. Taking those requirements into account the proposed manifold has the configuration depicted in Fig. 3. The solution aliquots merged with the buffer and ethanol solution combining both flow-rates to provide a low sample dispersion; then follows an inert reactor, a PTFE tubing, 4.6 cm long and 0.5 cm internal diameter filled with 0.5 mm diameter glass beads. The resulting mixture was inserted trough a 6-

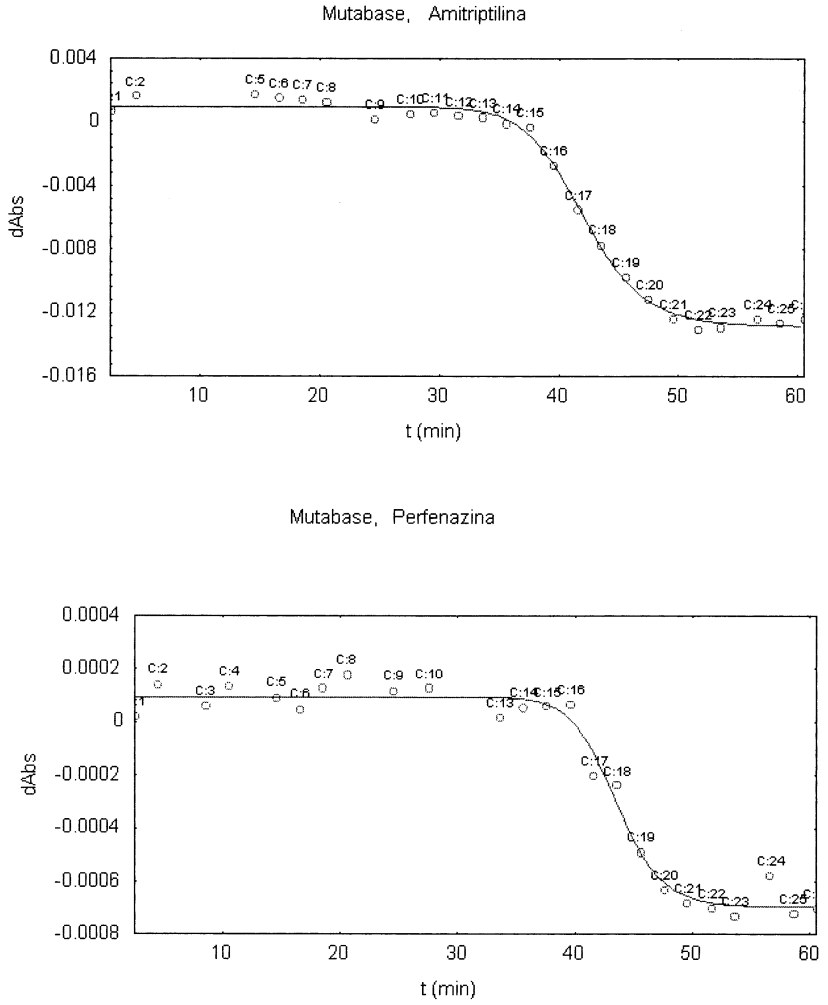


Fig. 5. Dissolution tests for the pharmaceutical formulation Mutabase, amitriptyline (top) and perphenazine.

Table 3
Regression analysis using the 4-parameters equation for Amitriptyline (top) and Perphenazine in Mutabase by application of the computerised program STATISTICA

	a	b	c	d	R
Amitriptyline					
Assay 1	−0.0152	43.488	7.148	0.0008	0.985
Assay 2	−0.0172	46.082	12.408	0.0011	0.997
Assay 3	−0.0141	50.516	14.530	0.0010	0.992
Ensayo 4	−0.0128	42.082	16.720	0.0010	0.971
SRD (%)	12.38	8.14	32.27	15.93	1.21
Perphenazine					
Assay 1	−0.0006	41.127	10.900	6.2207	0.931
Assay 2	−0.0009	47.078	12.941	0.0002	0.966
Assay 3	−0.0012	53.244	8.287	0.0002	0.973
Assay 4	−0.0007	43.496	22.523	9.4664	0.970
SRD (%)	31.18	11.40	45.42	120.30	2.04

ports injection valve into a carrier stream of the buffer and ethanol mixture.

To conduct the dissolution tests on commercially available tablets, some preliminary assays were performed with the aid of the FIA assembly to optimise the procedure. The optimisation studies included the chemical and the hydrodynamic parameters and fixing the residence time for recording the spectral data.

After several preliminary and optimisation assays the definitive conditions were adopted; (a) discussion similar to that presented for the mixture sulfadiazine/trimethoprim do not merit further description. Carrier the mixture buffer–ethanol, flowing at 1.0 ml min^{-1}; (b) sample solution 0.1 mol l^{-1} HCl at flow-rate 0.6 ml min^{-1}; (c) auxiliary solution for sample dilution (channel B), the same mixture as the carrier and flow-rate 0.3 ml min^{-1}; injected sample volume 452 μl; and, distance injection valve-detector flow-cell, 19 cm PTFE tubing of 0.8 mm internal diameter.

With these conditions the residence time was established at 33 s; the reported profiles using the 3-parameter equation were suitable for amitriptyline but a no well fitting curve-plots were observed for perphenazine. It should be noted Mutabase is not in a tablet form, it is presented as sugar coated pills in which the pharmaceutical mixture is covered by a lactose layer. During the first step of the dissolution the layer is dissolving and no signal appeared; then when the cover is completely dissolved the drug solution is causing a sudden variation with time.

As the dissolution profiles for this mixture are different to the observed with the mixture sulphametoxazole-trimethoprim and the 3-parameters equation did not provide the required accuracy, a new equation was tested and proposed to include the intercept. This is the so called 4-parameters equation

$$V_2 = \frac{a - d}{1 + (b/V_1)^c} + d$$

where a means the output when the formulation solution is completed; b, is the time for obtaining an output half of the maximum; c, exponent related with the slope of increasing interval; and, d, is the intercept.

Best fittings (see Table 3) can be observed for amitriptyline; the not so poorer fitting for perphenazine could be due to the minor concentrations (higher analytical errors) and to using the second order derivative which means less analytical sensitivity compared with the zero or first orders.

4. Conclusions

For the first time, a method to obtain simultaneously two dissolution profiles of active principles present in the same pharmaceutical formulation and whose spectra are overlapping is described.

The whole process is carried out by using a FIA assembly connected to only one detector. The empirical conditions for the dissolution are these officially recommended by USP, British and Spanish pharmacopoeias.

The method has been applied to commercially available formulations and it resulted in a simple and quick procedure, after optimum conditions had been established.

Absorbance figures were recorded and through the derivative spectra independent data were obtained for each drug. No chemical derivatisation of the analyte was required.

A discussion on the suitable regression fitting is included; the 3-parameter equation offers a suitable approach to describe the dissolution test curve of tablets containing the mixture sulfadiazine-trimethoprim and the 4-parameter equation for the sugar coated pills containing amitriptyline/perphenazine. Each parameter is related to one step of the global dissolution procedure and has a meaning in the 'in vitro' availability test.

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