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Three simultaneous dissolution profiles on a solid pharmaceutical formulation by a FIA manifold provided with a single spectrophotometric detector

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Abstract

This article deals with the simultaneous determination of three dissolution profiles in the same pharmaceutical formulation. The officially proposed procedure from the pharmacopoeias is adapted to the FIA methodology to obtain the officially recommended profile or "global profile", and two "individual" profiles, corresponding to dissolution rate of two different active principles present in the formulation; both drugs have overlapped UV–vis spectra. The simultaneous determination of several profiles is based on the derivative spectra and the zero crossing mathematical procedure for the "individual" profiles of an active principle; the "global" profile of the formulation is obtained from the order zero derivative. The empirical profiles were adjusted by regression analysis using the three-parameter (Higuchi equation) plot method which was selected as the most suitable. The analytical errors when the concentration of one drug is very small or very high are also checked.

Keywords: FIA; Pharmaceuticals; Dissolution profiles; In vitro availability tests; Bromhexine; Amoxicilline; Clavulanic acid

1. Introduction

The "in vitro" availability tests or dissolution tests are widely applied to different types of pharmaceutical formulations; the test has been established as compulsory by relevant international pharmacopoeias [1–3] with two main goals; namely, as a test of the availability of the formulation; and, as a way to check the reproducibility of the manufacture; recently it has extended to generic formulations. The results of this test are due to chemical contents but also to physical characteristics of the dosage form, the wettability of the dosage unit, the penetration ability of the dissolution medium, the swelling process, the disintegration and the deaggregation of the dosage form and a few factors that influence the dissolution characteristics of the drugs [4]. The former recommendations for oral solid dosage

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forms has been extended to other solid forms, even suspensions.

The officially recommended method resulted in a dissolution profile of the whole formulation. The recorded absorbance outputs are the sum of absorbances of all soluble compounds present in a formulation; insoluble products are on-line separated by a filter device. However, an interesting trend is to obtain dissolution profiles corresponding to one of the active principles present in a formulation. Of relevance is the automation of the whole process. Some interesting strategies have been studied [5,6]. The relevance of obtaining individual profiles relies on the information obtained from the "in-vitro" availability of each single active principle.

An automated option for dissolution profiles is the use of Flow Injection Analysis; it allows careful control of influential parameters, namely pH, temperature, stirring rate and ionic strength. A certain number of papers dealing on the FIA along with the resulting advantages can be found in analytical literature. The first reported basic advantage is to obtain the individual profile of one drug present in the mixture by implementing the official recommended assembly with a FIA manifold for developing the required chemical processes to obtain a single profile. The accuracy of the dissolution rates thus obtained is competitive with the "classical" non automatic method recommended in pharmacopoeias. Both give highly consistent results.

Koupparis and co-workers pioneered the application of FIA to dissolution assays by spectrophotometric measurements; paracetamol by oxidation with Fe (III) [7]; formation of Fe(III) complexes with salicylic acid, salicylamide or methyl salicylate [8]; and, indirect procedures based on the determination of (drug in chlorhydrate form) the chloride ion [9]. Other detection methods; namely, paracetamol fluorimetry, biamperometry and atomic absorption implementing an FIA manifold demonstrate that any detector is useful for the purpose of obtaining individual solution profiles with the aid of an FIA system [6–11]. As far as authors know, this article is the first attempt to obtain the simultaneous recording of three dissolution profiles; two corresponding to individual drugs and the "global" officially recommended in the same pharmaceutical formulation. This is performed with the aid of a FIA manifold provided with a single diode array UV–vis spectrophotometer which allowed simultaneous recording the absorbance of the solution at several wavelength values. For the present work were selected two different binary mixtures of pharmaceuticals: (a) amoxicilline and bromhexine; and, (b) amoxicilline and clavulanic acid. Both drugs of each pair presented overlapped spectra; to solve this was required the use of derivative spectra and the mathematical method of the zero crossing. Three order derivative spectra were obtained; zero, first and second. Then the first derivative spectrum was chosen for both mixtures.

The use, applications and advantages of derivative spectrophotometry to drug analysis have been reported elsewhere [12–19]. This paper follows former articles from this lab which dealt with the simultaneous determination of two individual profiles in a formulation with a FIA assembly provided with a single spectrophotometer [20,21].

Amoxicilline is active against a wide range of organisms. It has activity against penicillin-sensitive gram-positive bacteria as well as some gram-negative bacteria. Bromhexine is a mucolitic agent used in the treatment of respiratory disorders associated with viscid or excessive mucus. It has been shown that bromhexine enhanced the penetration of erythromycin into bronchial secretions. Bromhexine is often administrated with other antibiotics (for example with amoxicilline) as an adjuvent in the treatment of respiratory infections [22].

On the other hand, the clavulanic acid is a white or almost white crystalline powder, is a naturally occurring non-competitive inhibitor of β-lactamase produced by gram-positive, and also many gram-negative bacteria. Although it has a β-lactam chemical structure, clavulanic acid has little antibacterial activity of its own. However, when clavulanic acid is administered concurrently with amoxicilline, it extends the activity of amoxicilline by preventing its destruction by bacterial enzymes. Also, the β-lactam structure of amoxicilline and clavulanate may stimulate some bacteria to produce more β-lactamase; it is easier for clavulanate

to protect amoxicilline against a small amount of enzyme than against a large amount [22,23].

2. Experimental

2.1. Reagents and apparatus

2.1.1. Reagents

Reagents used were all analytical grade unless stated: amoxicilline trihydrate and bromhexine hydrochloride (Guinama, Valencia, Spain), clavulanate potassium (GlaxoSmithKline, Madrid), hydrochloric acid and sodium chloride (Panreac, Barcelona), disodium hydrogenphosphate, ammonia and ammonium chloride (Probus, Barcelona, Spain), glycine and sodium hydroxide (Scharlau, Barcelona, Spain).

Aqueous solutions were prepared in pure deionised water (18 MΩ-cm) with the aid of the purifier system Sybron/Barnstead Nanopure II provided with a filter 0.2 μm pore size.

2.2. Flow-assembly

Two UV–vis photo-diode array spectrophotometers were used as FIA detector (both from Hewlett Packard, model HP8452 for preliminary studies and Agilent 8453 for the rest of the work) and provided with a flow-cell (from Hellma) of 1 cm light-path and inner volume 18 μl. 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 connectors of the "arrow tip" type. Sample aliquots from dissolution vessel were periodically inserted into the carrier stream, which forced the sample to the flow-cell of the detector. The finally proposed FIA manifolds are depicted in Fig. 1.

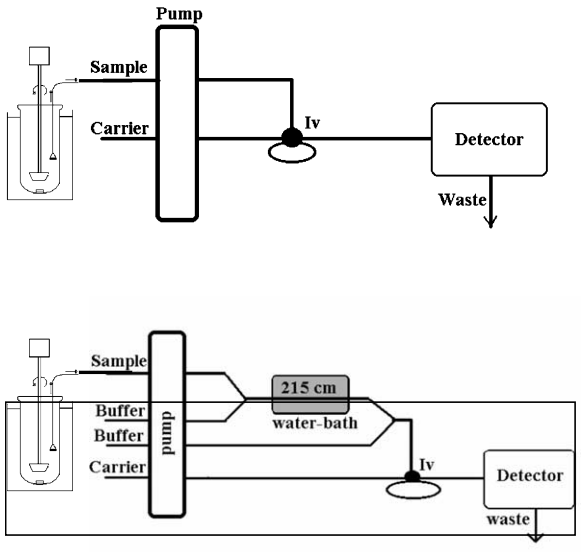

Fig. 1. (a) FIA assembly connected to dissolution standard vessel (USP Pharmacopoeia) to obtain dissolution profiles of amoxicilline and bromhexine. Sample solution in HCl mol l^{-1}, from the dissolution vessel; Carrier solution, HCl 0.10 mol l^{-1} flowing at 2.4 ml min^{-1}; Iv, injection valve for a sample volume of 467 μl; D, detector, spectrophotometer, integration time, 0.5 s. (b) FIA assembly for amoxicilline and clavulanic acid.

2.3. Commercially available samples studied and sample preparation

a) The studied commercially available formulations for the pair amoxicillin–bromhexine were: Ardine Bronquial, 500 mg capsules ("Antibioticos Farma, S.A.", Madrid, Spain); label claim: 500 mg of amoxicilline trihydrate and 8 mg of bromhexine hydrochloride, clamoxyl mucolitíco 500 mg capsules ("SmithKline Beecham, S.A.", Madrid, Spain); label claim: 500 mg of amoxicilline and 8 mg of bromhexine hydrochloride, Pulmo-Borbalan ® 500 ("Spyfarma", S.A., Sevilla, Spain); label claim: 500 mg of amoxicilline trihydrate and 6 mg of bromhexine.

b) Amoxicilline and clavulanic acid *Géminis* 500/125 mg EFG (a generic formulation); coated tablets EFG ("Merck" Biochemie GmbH, Kundl/Tyrol, Austria; entitled to "Merck Farma y Quimica, S.A.", Barcelona, Spain); label claim: 500 mg of amoxicilline trihydrate and 125 mg of clavulanic acid.

The solution profiles of the formulations were obtained as described in Pharmacopoeia [1], by placing a coated tablet into a basket which was in the tip of the rod stirrer (amoxicilline and bromhexine) or into the vessel solution (amoxicilline

and clavulanic acid); and introduced in a 0.1 mol l^{-1} HCl at 37 °C. The rotation speed was of 50 rpm or 75 rpm for the couple amoxicilline/bromhexine and amoxicilline/clavulanic acid respectively, and the time interval was 60 min for both. The tip of the PTFE tubing introduced in the solution vessel was provided with a filter to avoid the passage of insoluble excipients.

2.4. Procedures

Preliminary experiments in batch were performed to establish or to confirm the best medium for the simultaneous measurement of the two active principles. Once finished these assays the suitable assembly FIA was designed to "translate" the batch method to the continuous-flow and to perform the dissolution tests. Once selected the most appropriate assembly all chemical and hydrodynamic parameters were optimised. The residence time should be carefully tested to obtain measurements (including the whole spectra) only in the maximum of the transient FIA output. Finally the dissolution profiles were performed and the obtained results were adjusted by regression analysis by using the Statistica software.

3. Results and discussion

3.1. Preliminary results

Aqueous stock solutions were prepared for the three tested pharmaceuticals. A series of 30 mg l^{-1} solutions (for clavulanate solutions the weight is as clavulanic acid) were prepared by taking aliquots of the stock solution and adjusting the pH potentiometrically by drooping 0.100 mol l^{-1} HCl or 0.100 mol l^{-1} NaOH. The resulting solution was levelled to 100 ml. pH values were adjusted over the range 1–13 (1, 3, 5, 7, 9, 11 and 13 for amoxicilline and clavulanic acid). Due to low solubility of bromhexine in basic solutions the studied pH interval was from 1 to 5 (1, 3 and 5). Spectra were recorded from 190 to 350 nm; first and second order derivative spectra were obtained to find the zero crossings.

The selected pH and wavelength values as the most suitable for drug determination were 247 nm, and 259 nm, for amoxicilline (concentration 30 mg l^{-1}) and bromhexine (concentration 30 mg l^{-1}), respectively, both at the solution containing 0.100 mol l^{-1} HCl. For the study of the pair amoxicilline and clavulanic acid the pre-selected medium was the formed by an aqueous solution of NaOH, pH over the range 11–13. Obtained spectra at the pre-selected media (order derivative zero and first) are depicted in Fig. 2 for amoxicilline and bromhexine; and, in Fig. 3 for amoxicilline and clavulanic acid.

3.2. Amoxicilline–bromhexine

Then, spectrophotometric determinations of a series of solutions with different drug concentrations in 0.100 mol l^{-1} HCl, were performed at the selected wavelengths of the first derivative; namely, 247 and 259 nm, for amoxicilline and bromhexine, respectively. The linearity interval for

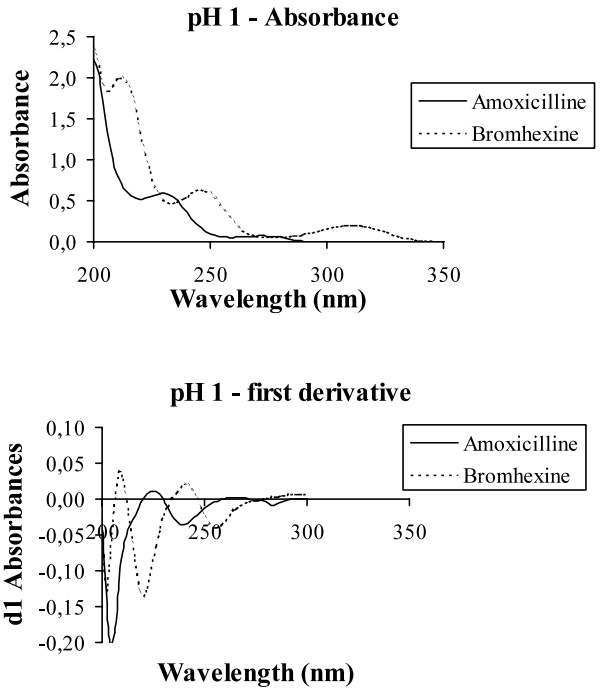

Fig. 2. Spectra of amoxicilline and bromhexine. Both at 30 mg l^{-1} in aqueous solution containing 0,100 mol l^{-1} HCl. Bottom: first derivative spectra of amoxicilline and bromhexine.

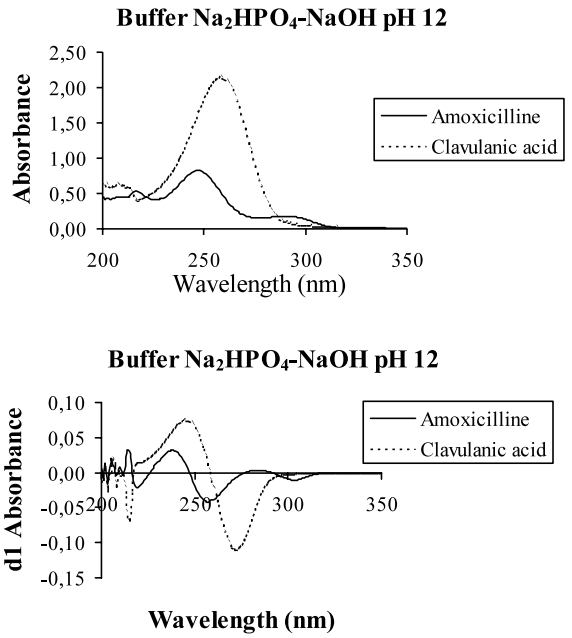

Fig. 3. Spectra of amoxicilline and clavulanic acid at pH 12.00. Both at 30 mg l^{-1} in aqueous solution containing buffer Na_2HPO_4–NaOH. Bottom: first derivative spectra of amoxicilline and clavulanic acid.

amoxicilline from 1 to 100 mg l^{-1} fitted the equation $y = -0.00049x - 0.000021$ with a correlation coefficient of 0.9996; for bromhexine and the interval 1–40 mg l^{-1} the linear equation was $y = -0.00088x - 0.00020$, with correlation coefficient 0.9985; where y, means the first derivative absorbance and x, the drug concentration in mg l^{-1}.

3.2.1. FIA assembly and optimisation

A new spectrophotometer was used and the spectra screening revealed the zero crossings at 258 and 247 nm.

Then it was decided that batch assays did not merit further research and the required FIA assembly was designed on the basis of two points: (a) the official recommendations in which aliquots of the drug solution in 0.1 mol l^{-1} HCl were inserted in a carrier stream; and (b) on the basis a few basically characteristics: solubility parameters of two drugs, especially bromhexine which is very slightly soluble in water, the physiological conditions, and the absence of the official recommendations for dissolution testing for capsules containing the combination amoxicilline–bromhexine.

The optimisation of the FIA assembly included the influence of the parameters sample volume and the carrier flow-rate. The optimisation was performed with the aid of the univariate method and by performing two series of experiments, the second was a re-optimisation of the former results by testing a smaller range in the vicinity of the best results. The re-optimisation was not performed with a single concentration of amoxicilline or bromhexine; a series of solutions of four different drug concentrations (calibration graph) for each tested value. Then and by using the optimised hydrodynamic parameters the HCl concentration was re-optimised to test the chemical robustness of the method; and finally, the re-optimisation included the integration time. At last, new series of calibration graphs were performed with the final optimised flow assembly to test the robustness of the system.

The studied ranges for the optimisation of the FIA parameters were: carrier flow-rate, 0.4–3.1 ml min^{-1}; and, sample volume, 115–1120 μl. From this experiments the selected values for further work were: carrier flow-rate: 2.5 ml min^{-1}; and sample volume: 366 μl. A further re-optimisation was performed by adjusting the intervals to the best observed results and adding the integration time. Tested ranges were as follows: carrier flow-rate, 2.3–2.6 ml min^{-1}; sample volume, 316–467 μl; HCl concentration, 0.09–0.11 mol l^{-1}; and, integration time, 0.1–1.0 s. From the series of experiments the finally selected values were: carrier flow-rate, 2.4 ml min^{-1}; sample volume, 467 μl; and, integration time, 0.5 s.

The accuracy of these conditions for obtaining solution profiles and, if required, analytical determinations, was formerly tested by calculating the analytical errors observed when testing dissolution containing low or high concentrations of the drug; corresponding with the first and final stages of the dissolution test. The studied solutions contained different concentrations of the mixture of both drugs; amoxicilline and bromhexine. The calibration equations were: bromhexine at 258 nm (1–10 mg l^{-1}): $y = -0.00097x - 0.00006$, r^2 0.99996;

amoxicilline at 245 nm (1–150 mg l^{-1}): $y = -0.00066x - 0.0012$, r^2 0.9985

The analytical determination of both drugs in laboratory prepared mixtures was performed to test the relative errors by comparing the concentration of the drug empirically obtained with the added amount. Different ratio concentrations were prepared. These relative errors were minor than 4% for bromhexidine. However, relative errors for amoxicilline determinations were usually over 10% for low concentrations of it, under 10 mg l^{-1}.

3.2.2. Dissolution tests from commercially available tablets

Connecting the officially recommended solution assembly to the proposed FIA manifold were performed dissolution tests. Samples were injected at 1 minute intervals. The wavelength for the corresponding measurements was 245, 258 and 258 for amoxicilline, bromhexine (first derivative) and global profile (zero derivative), respectively. Eight different and independent dissolution profiles were performed for each tested formulation. Then regression analysis of the obtained plots was performed.

In a former paper [21] the suitability of the Higuchi equation over polynomial regression analysis was demonstrated. Three were the possible reasons to explain these 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.

This equation [25] established for the mathematical fitting of the hyperbolic type plots is

$$y = a/(1 + (b/x)^c)$$

The 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 corresponding to the slope of the climbing interval of the profile. The Higuchi equation was tested in the two version of three-parameters and the four-parameters; the first fitted best for both mixtures amoxicilline–bromhexine and amoxicilline–clavulanic acid. Computerised calculations were performed with the aid of the program "Statistica" working in windows (Copyright Statsoft. Inc 1993). See obtained equations in Table 1.

3.3. Amoxicilline–clavulanic acid

The empirical strategy for these two drugs was similar to reported for amoxicilline and bromhexine. Aqueous stock solutions of 30 mg l^{-1} were prepared by placing aliquots of the stock solution in a beaker and adjusting the pH potentiometrically by drooping 0.100 mol l^{-1} HCl or 0.100 mol l^{-1} NaOH. pH values were adjusted over the range 1–13. Finally, spectra were recorded from 190–350 nm; first and second order derivative spectra were obtained to find the zero crossings.

The pre-selected medium was the formed by an aqueous solution of NaOH, pH clearly basic, over the interval 11–13. A recently published paper for simultaneous batch determination of amoxicillin and clavulanic acid proposed a basic medium and after boiling. [24]. Afters some preliminary assays we studied the basic media to arrange a flow manifold able to change the medium of the aliquots from the dissolution vessel in the way to detector flow-cell. Due to that, a study for testing the best buffer solution was performed. Tested buffer solution and pH intervals were: NH_4OH–NH_4Cl (from 9.0 to 9.8); glycine–NaOH (from 9.0 to 11.1); and, NaOH–Na_2HPO_4 (from 11.0 to 12.0).

The study of different basic media reported above resulted in selecting for the couple amoxicilline–clavulanic acid, pH 12 with the buffer NaOH–Na_2PO_4 and working with the first derivative at 257 nm for amoxicilline and at 271 nm for clavulanic acid. Obtained spectra at the selected media (order derivative zero and first) can be seen in Fig. 3.

3.3.1. FIA assembly and optimisation

Preliminary flow studies resulted in zero absorbances for clavulanic acid in basic media and at room temperature; due to rate of hydrolysis of clavulanate flow measurements were too quick avoiding the hydrolysis of the drug. Previously to obtain the suitable pH and medium, an study of the influence of the temperature on the hydrolysis rate was performed by preparing a vessel im-

Table 1
Three-parameters (Higuchi equation) for the obtained dissolution profiles in amoxicilline–bromhexine formulations

Amoxicilline			Bromhexine			Global profile		
a	b	c	a	b	c	a	b	c
Ardine bronquial								
−0.1544	4.2149	7.3096	−0.0136	6.9252	3.1354	1.1496	6.7714	2.8015
−0.1570	3.9628	7.2317	−0.0138	6.7859	2.7927	1.1275	6.4851	2.7980
−0.1582	3.7606	7.6380	−0.0144	6.9557	2.6574	1.1615	6.1523	2.4513
−0.1502	3.7905	6.2004	−0.0133	6.9687	2.3731	1.1500	6.4940	2.3779
−0.1455	3.8324	7.0465	−0.0143	7.6844	2.0351	1.1569	6.6992	2.2204
−0.1279	5.0515	7.5604	−0.0120	8.5157	3.2675	1.0188	8.4602	2.6489
−0.1243	4.5108	6.6597	−0.0125	7.5586	2.9223	1.0317	7.4548	2.6051
−0.1303	4.0970	8.4979	−0.0128	7.1690	2.7638	1.0766	6.6877	2.6366
Average (dsr, %)			Average (dsr, %)			Average (dsr, %)		
−0.1435 (−9.7)	4.1526 (10.6)	7.2680 (9.4)	−0.0133 (6.5)	7.3204 (7.9)	2.7434 (14.5)	1.1091 (5.3)	6.9006 (10.6)	2.5675 (7.9)
Clamoxyl mucolítico								
−0.1459	3.9378	9.2029	−0.0131	4.9322	5.1315	1.0359	4.8496	4.7691
−0.1402	3.5272	6.5115	−0.0135	4.9208	3.7163	1.0719	4.7942	3.4911
−0.1701	3.8808	9.5052	−0.0150	5.5464	3.9851	1.1229	5.3086	3.9474
−0.1682	3.1057	7.7199	−0.0151	4.5984	4.0499	1.0815	4.4268	4.0991
−0.1646	4.0460	11.3892	−0.0146	5.6711	4.3156	1.0882	5.4905	4.2867
−0.1350	4.5959	11.1727	−0.0131	5.8638	5.2673	1.0179	5.6439	5.3350
−0.1324	4.2910	8.0038	−0.0126	5.6851	4.5017	1.0221	5.7991	4.0543
−0.1275	4.7095	12.6295	−0.0122	6.0657	5.1051	0.9663	6.0524	4.3016
Average (dsr, %)			Average (dsr, %)			Average (dsr, %)		
−0.1480 (−11.6)	4.0117 (13.3)	9.5168 (22.0)	−0.0136 (−8.1)	5.4104 (9.7)	4.5091 (13.2)	1.0508 (4.7)	5.2956 (10.6)	4.2855 (13.0)
Pulmo-Borbalan								
−0.1583	4.7571	5.8829	−0.0103	6.9492	3.1281	1.1223	8.0060	2.7186
−0.1594	4.8838	6.2545	−0.0109	7.0574	3.1804	1.1557	7.6396	2.5455
−0.1591	4.0011	7.9475	−0.0122	6.3037	3.2735	1.1626	6.7675	2.4983
−0.1853	3.0975	5.9937	−0.0121	4.9796	2.7450	1.2758	6.3548	1.8376
−0.1732	2.9504	7.4597	−0.0136	4.9947	2.8359	1.2126	5.4213	2.4695
−0.1505	3.5406	5.1643	−0.0105	5.5516	2.8075	1.1091	5.8768	2.1916
−0.1429	5.1510	6.2348	−0.0102	7.3388	3.2772	0.9997	7.9895	2.5215
−0.1261	4.6083	4.9730	−0.0102	7.3388	3.2772	1.0130	7.3177	2.6641
Average (dsr, %)			Average (dsr, %)			Average (dsr, %)		
−0.1569 (−11.5)	4.1237 (20.6)	6.2388 (16.4)	−0.0113 (−11.2)	6.3142 (16.0)	3.0656 (7.5)	1.1313 (8.2)	6.9217 (14.1)	2.4308 (11.8)

mersed in a water bath and containing the buffer solution NaOH–Na_2HPO_4 at pH 12; when clavulanate solution was added the solution was pumped to the spectrophotometer flow-cell in a close circuit. Fig. 4 depicts the variation of the absorbance vs time for different temperatures. Selected temperature for further work was 37 °C.

Different flow assemblies were tested. Finally the proposed FIA manifold was the depicted in Fig. 1b. Calibration graphs of both drugs were obtained with this assembly at this pH and different zero crossings wavelengths (277 and 281 nm for clavulanic and 258 nm for amoxicilline) to check the suitability; 281 nm was the selected wavelength for clavulanic acid. A series of assays were performed to re-optimise the flow assembly. Tested ranges (at 60 mg l^{-1} for both drugs) were: carrier flow-rate, 1.3–3.4 ml min^{-1}; sample volume, 115–915 μl; and, sample flow-rate, 0.3–1.0 ml min^{-1}; also the same values for the medium to have a resulting solution from mixing 50%–50%. A second re-optimisation was performed around

Table 2
Three-parameters (Higuchi equation) for the obtained dissolution profiles in amoxicilline–clavulanic formulations

Amoxicilline			Clavulanic			Global profile		
a	b	c	a	b	c	a	b	c
Geminis EFG								
−0.0747	13.5403	2.8278	−0.0261	13.2976	2.8975	2.5829	14.1744	3.0771
−0.0580	12.3445	2.5011	−0.0244	14.1740	2.4273	2.4215	13.3920	2.1959
−0.0543	8.9735	2.9380	−0.0232	10.3738	2.8215	2.2957	9.6613	2.5826
−0.0503	6.7191	2.7075	−0.0255	10.6100	1.9227	2.3781	8.4989	1.9764
−0.0456	8.0855	2.9439	−0.0233	11.1817	2.4891	2.1822	9.7947	2.3521
−0.0724	12.4378	3.1948	−0.0278	13.6871	3.0341	2.7478	13.0342	3.0089
−0.0725	12.8337	2.3741	−0.0295	15.5523	2.0520	2.9268	14.5384	2.0938
−0.0786	16.0003	2.4039	−0.0318	19.6798	2.1030	3.0190	17.3418	2.2397
Average (dsr, %)			Average (dsr, %)			Average (dsr, %)		
−0.0633 (−20.0)	11.3668 (27.5)	2.7364 (10.7)	−0.0264 (−11.6)	13.5695 (22.7)	2.4684 (17.0)	2.5693 (11.8)	12.5545 (23.9)	2.4408 (16.9)
EFG-Merck								
−0.0682	10.9281	2.5741	−0.0229	13.0945	2.3526	2.5440	11.9597	2.3932
−0.0675	12.2897	2.5915	−0.0204	14.4827	2.3339	2.5189	13.5509	2.3811
−0.0606	9.1238	2.7103	−0.0147	10.5162	2.3402	2.1033	9.8274	2.4714
−0.0865	17.8591	1.7967	−0.0244	26.9374*	1.4921*	3.1113	20.7212*	1.6567
−0.0701	11.8630	2.3957	−0.0157	13.5632	2.1578	2.3893	12.7996	2.2504
−0.0711	11.0695	2.0589	−0.0190	12.7071	1.9845	2.4714	11.7890	1.9974
−0.0795	14.3178	2.0753	−0.0222	16.1879	2.2097	2.7587	14.9787	2.0740
−0.0678	12.3311	2.8725	−0.0200	14.0082	2.5426	2.3947	12.9613	2.7117
Average (dsr, %)			Average (dsr, %)			Average (dsr, %)		
−0.0714 (−11.2)	12.4728 (21.1)	2.3844 (15.6)	−0.0199 (−17.1)	13.5085 (12.9)	2.2745 (7.8)	2.5364 (11.7)	12.5524 (12.8)	2.2420 (14.6)

* Rejected values (from the applied statistical Q assay).

the best values (flow-rate; sample, 0.5 ml min^{-1}; carrier, 2.3 ml min^{-1}; sample volume, 517 μl) using the following ranges: sample flow-rate, 0.2–0.5 ml min^{-1}, carrier (buffer) flow-rate 2.1–2.4 ml min^{-1}; sample volume, 417–567 μl. Selected values were: 0.5 ml min^{-1}; 2.3 ml min^{-1}; and 417 μl, respectively.

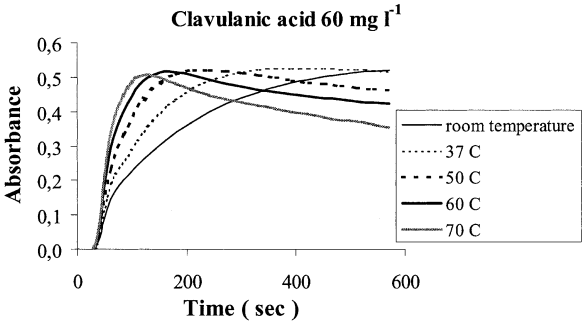

Fig. 4. Variation of the absorbance vs time for different temperatures. Clavulanic acid in 60 mg l^{-1}; pH 12.0 with the buffer solution Na_2PO_4–NaOH.

To avoid the detector signal saturation due to high concentration of amoxicilline an additional channel was added immediately after the water bath (for the hydrolysis of clavulanic finished). The observed linear intervals and fitting equations at the selected values were: (a) amoxicilline (258 nm): $y = -0.00011x - 0.0016$, correlation coefficient 0.9984, over 5–900 mg l^{-1}; and, (b) clavulanic acid, $y = -0.00013x - 0.000016$, correlation coefficient 0.9999, over the range 5–200 mg l^{-1}. The observed errors for mixtures amoxicilline–clavulanic acid were minor than 12% for amoxicilline under 100 mg l^{-1}; and, for clavulanic acid under 3% for concentrations minor than 5 mg l^{-1}.

3.3.2. Dissolution tests from commercially available tablets

Next work consisted in obtaining dissolution profiles with the commercial formulations *Géminis* 500/125 mg EFG (a generic formulation); and, the coated tablets EFG-Merck to test the reproducibility. Some results can be seen in Fig. 5.

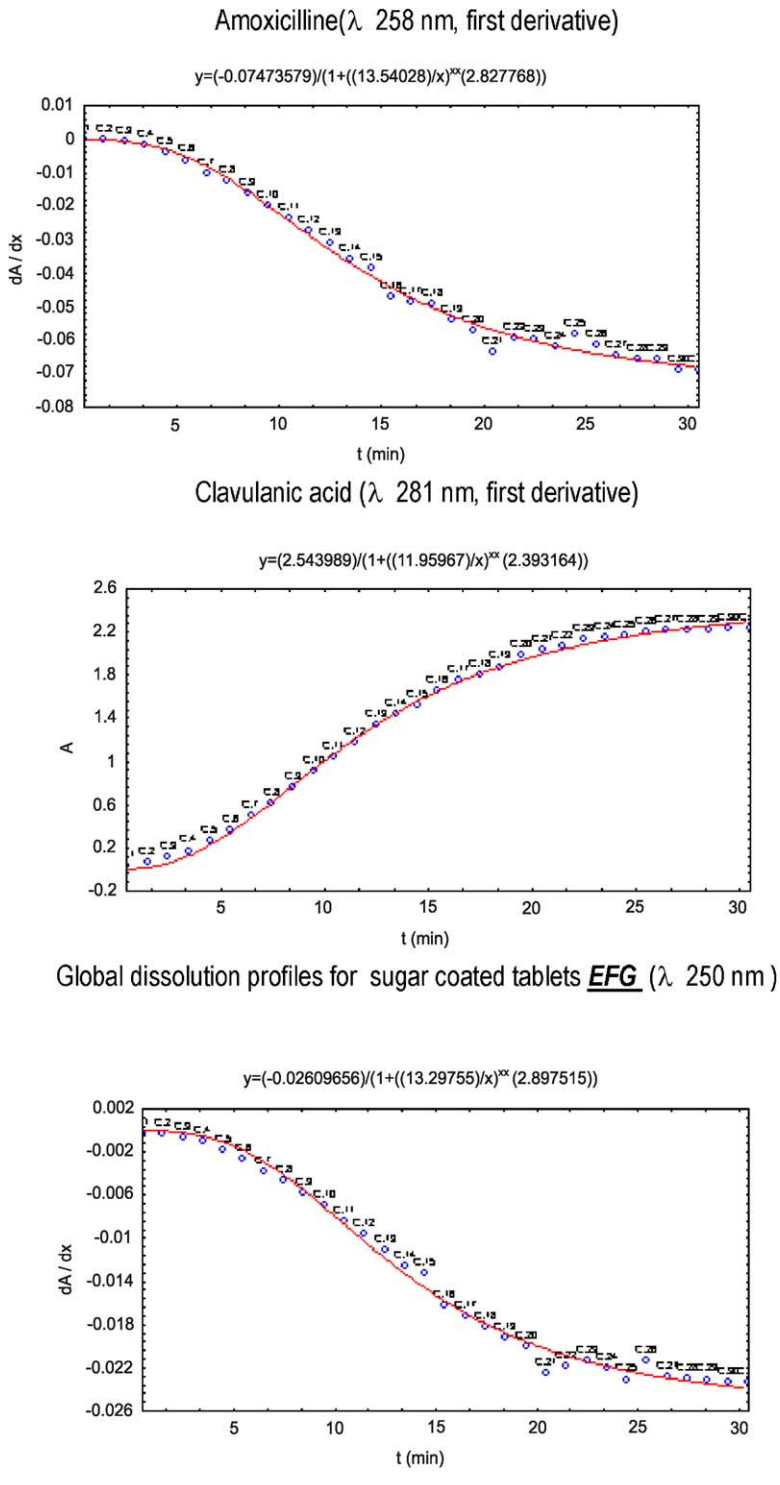

Fig. 5. Dissolution test curves for commercially available pharmaceutical formulations of oral administration.

The regression analysis of the obtained plots was also performed as reported for the amoxicilline–bromhexine pair and the results can be seen in Table 2.

4. Conclusions

It is described, for the first time, how to obtain the officially recommended from the pharmacopoeias dissolution profile of a pharmaceutical formulation of oral administration simultaneously with the individual dissolution rate of the two active principles present in the same pharmaceutical formulation and whose spectra are overlapped.

The whole process is automated by using a FIA assembly provided with a single spectrophotometer.

The method is tested with two different pharmaceutical pairs in several pharmaceutical formulations.

A discussion on the suitable regression fitting is included; the three-parameters equation offers a suitable approach to describe the dissolution test curve. 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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