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Automated flow-injection spectrophotometric determination of catecholamines (epinephrine and isoproterenol) in pharmaceutical formulations based on ferrous complex formation

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Abstract

A novel automated flow-injection spectrophotometric method for the determination of catecholamines (epinephrine and isoproterenol) has been developed based on the formation of their coloured complexes with Fe(II) in aminoacetic-carbonate buffer pH 8.3 and measuring of the absorbance peaks at the λ_{max} of 530 nm. A fully automated FIA system controlled by home-made software (FIA-MOD) was used for optimising the chemical and manifold parameters and running of routine measurements. The calibration graph was linear in the range of 5–200 mg l^{-1} for epinephrine with an RSD of 0.24% ($n = 5$; $c = 150$ mg l^{-1}) and 10–300 mg l^{-1} for isoproterenol with an RSD of 0.13% ($n = 5$; $c = 200$ mg l^{-1}). Measurement throughput was 120 h^{-1} ensuring a sample throughput of 40 h^{-1} analysed in triplicate. Common excipients for tablets and injections were found not interfering. The proposed method was applied for the assay of various commercial pharmaceutical formulations containing epinephrine and isoproterenol and for the content uniformity test for the isoproterenol tablets. The assay results with RSD 2–4% ($n = 3$) were comparable with those obtained with the official USP XXIII methods (mean difference 1.9%).

Keywords: Flow injection; Spectrophotometry; Isoproterenol; Isoprenaline; Epinephrine; Adrenaline; Pharmaceutical analysis; Content uniformity; Ferrous complex

1. Introduction

Epinephrine or adrenaline (**I**)-[(*R*)-1-(3,4-dihydroxyphenyl)-2-(methylamino)ethanol]-and isoproterenol or isoprenaline (**II**)-[bis[(*RS*)-1-(3,4-dihydroxyphenyl)-2-isopropylaminoethanol]-are catecholamine drugs that are widely used in

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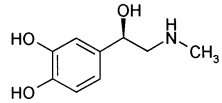
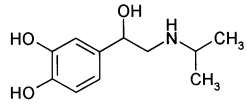
the treatment of allergic emergencies, status asthmaticus, bronchial asthma, venticular bradycardia, cardiac arrest, glaucoma and as styptic [1].



(I)



(II)

A great number of various methods have been developed for the determination of these drugs. The determination of catecholamines in biological fluids, where they are found in relatively low concentrations, usually requires the use of selective and of high detectability techniques like HPLC with fluorimetric [2] or electrochemical [3,4] detection. Pharmaceutical formulations containing relatively large amounts of the drugs can be analysed using batch spectrophotometric methods, most of which are based on the oxidation of catecholamines prior to the absorbance measurement. Several oxidants such as metavanadate, [5] periodate, [6] bromate [7] and phenanthroline-iron(III) complex [8] have been proposed. A spectrofluorimetric method using oxidation by 2-cyanoacetamide [9] has also been described.

Due to the great importance of these drugs, an automated fast method for routine analysis and quality control of commercial formulations is very desirable. Flow injection (FI) techniques, characterised by high measurement throughput, low reagent consumption and high precision, have gained widespread recognition in the automation of methods of pharmaceutical analysis [10–14]. Several FI methods have been described for the determination of **I** based on: its oxidation with a solid-phase reactor of manganese dioxide incorporated in polyester resin beds and fluorescence measurement [15], photochemical inhibition on the photo-reduction of phloxin by EDTA which is monitored fluorimetrically [16], the use of an immobilised tyrosinase enzyme reactor and L-ascorbic acid with potentiometric detection [17], chemiluminometric monitoring after the oxidation with permanganate [18] or Fenton reagent [19] or using an aerobic oxidation catalysed by micelles and manganese(II) [20]. A chemiluminometric determination based on the inhibition of the chemiluminescence reaction between luminol and hypochlorite by both catecholamines has also been reported [21].

Only two FI methods with spectrophotometric detection for the determination of catecholamines have been described in the literature. The first method is based on the online oxidation of **I** by a manganese dioxide solid reactor and measuring the absorbance of the product at 300 nm [22]. The second is based on the oxidation of **I** and **II** with periodate and monitoring the absorbance of the aminochrome produced at 491 nm [23]. Neither of these methods used a fully automated FI procedure.

For the assay of pure substances the United States Pharmacopoeia (USP) XXIII [24] recommends a non-aqueous acidimetric titration for **I** and an HPLC method with UV detection for **II**. The content of pharmaceutical formulations is determined either by an HPLC method with UV detection or spectrophotometrically after a colour-formation reaction with ferro-citrate reagent. For pure substances of both catecholamines British Pharmacopoeia [25] recommends only non-aqueous titration, while for pharmaceutical formulations utilises all three methods mentioned. European Pharmacopoeia [26] describes also only non-aqueous acidimetric titrations for pure substances. The HPLC methods, albeit selective, are rather time-consuming and expensive, so an automation of the colorimetric method for routine analysis is very desirable.

By employing the colour derivative reaction with ferrous-citrate for the determination of catecholamine drugs in formulations, any interference from excipients causing problems to the direct UV measurements is avoided.

The purpose of this work was to develop a simple, rapid and fully automated method for the routine quality control (assay and content uniformity) of formulations of **I** and **II**, using the FI technique and based on the complex-formation of these drugs with Fe(II). After optimising the manifold and chemical parameters of the system, the method was evaluated by assaying commercial formulations and comparing of the results with those obtained by the USP XXIII official method.

2. Experimental

2.1. Apparatus

The automated FI system consisted of: a four-channel peristaltic pump (Gilson Minipulse 3, Worthington, OH, USA) fitted with Tygon pump tubings for the propulsion of fluids, a Rheodyne (Model 5020, Cotati, CA, USA) four-way injection valve controlled with an electrical actuator (Watrex, Prague, Czech Republic), a spectrophotometer (Spekol Model 10, Zeiss Jena, Germany) with a flow cell of 10 mm optical path and 18 μl dead volume, an IBM compatible personal computer (486-DX66, 8 MB RAM) to which the analogue output of the spectrophotometer was fed through a home-made interface circuit utilising a 10-bit analogue-to-digital converter (ADC-1005CCJ, National Semiconductors). The pump and the injection valve were controlled by the computer through the parallel digital I/O section of the interface circuit and a home-made computer programme (FIA-MOD 2.1).

2.2. Reagents

All reagents used were of analytical-reagent grade and distilled water was used throughout.

Isoproterenol and epinephrine were obtained from Sigma (St Louis, MO, USA) and their exact purity was determined using the official European Pharmacopoeia titrimetric methods [26]. Stock solutions of **I** and **II** (500 mg l^{-1}) were prepared by dissolving the appropriate amount of the drug in 0.010 mol l^{-1} sodium hydrogen sulphite using sonication and stored in amber bottles. The solutions remained stable for at least 2 weeks if kept refrigerated. More dilute working solutions were prepared daily by the appropriate dilution of stock solutions with the same solvent.

The optimal carrier solution was a 0.010 mol l^{-1} sodium hydrogen sulphite aqueous solution, prepared by dissolving of 1.04 g of $NaHSO_3$ in 1 l of distilled water.

The aminoacetic (1.0 mol l^{-1}) -carbonate (2.0 mol l^{-1}) buffer solution of pH 8.3 was prepared by dissolving 42.0 g of sodium hydrogen carbonate and 50.0 g of potassium hydrogen carbonate in 200 ml of water, mixed with a solution containing 37.5 g of aminoacetic acid and 50 ml of diluted ammonia (6 mol l^{-1}) in 150 ml of water and adjusted to volume of 500 ml.

The Fe(II) (0.050 mol l^{-1}) -citrate stock solution was prepared daily by dissolving of 1.39 g of $FeSO_4(7H_2O$ in 75 ml of water, adding of 0.5 g of sodium hydrogen sulphite, 5.0 ml of hydrochloric acid (0.10 mol l^{-1}) and 5.0 g of sodium citrate. The final volume was adjusted to 100 ml with distilled water. The Fe(II)-citrate working solution was prepared freshly by mixing of Fe(II)-citrate stock solution and aminoacetic-carbonate buffer of pH 8.3 at the optimal ratio 1:4.

2.3. Preparation of sample solutions

The common pharmacopoeia sampling and treatment procedures were used for solid formulations. The **II** tablets were finely powdered and an accurately weighed portion equivalent to about 15 mg of IS was dissolved in 100 ml of 0.010 mol l^{-1} sodium hydrogen sulphite solution. By using an ultrasonic bath, the powder was completely disintegrated and the suspension was filtered through a Whatman cellulose nitrate membrane, pore size 0.45 μm. Liquid formulations of **I** and **II** were appropriately diluted with 0.010 mol l^{-1} sodium hydrogen sulphite. No other treatment of the sample was required. For the content uniformity test each tablet was dissolved in 200 ml of 0.010 mol l^{-1} sodium hydrogen sulphite.

2.4. Manifold and procedure

The two-channel FI manifold developed and optimised for the determination is shown in Fig. 1. All connecting tubings were of PTFE (i.d. 0.5 mm).

Carrier ($NaHSO_3$) and reagent (Fe(II)-citrate) were pumped through Tygon tubes. The spectrophotometer was set at 530 nm (λ_{max} of the resulting complexes). After the stabilisation of the baseline absorbance, 210 μl sample volumes of **I** or **II** were injected into the carrier stream by

means of the Rheodyne injection valve. The sample plug was merged with the reagent stream and left to react in the 0.5-m PTFE reaction coil.

The absorbance peaks of the coloured complexes produced were displayed on the computer screen and printed. The peak height was measured and linearly related to the concentration of **I** or **II**. Five replicate injections per sample or standard solutions were performed.

2.5. Computer program

All the operations of the FI system were under computer control. The computer programme FIA-MOD 2.1 was written in Turbo-Pascal 6.0 and enables the control of the multichannel peristaltic pump (motor on/off, speed and direction of the flow), injection valve status (loading and sampling position with the associated timing) and signal processing from the detector.

The FI-grams obtained are displayed on the screen in real-time and the calculation of the peak-height or peak-area is performed automatically. The algorithm used for the calculation of the peak height is based on a quadratic fit of the upper most 11 signal-time points to minimise the effect of noise spikes. More details about the programme FIA-MOD 2.1 have been presented earlier [27].

3. Results and discussion

Most of the poly-hydroxy-phenyl compounds react with Fe(II) to form coloured complexes. This widely known reaction was described a long time ago [28] and it is still employed in many methods described in pharmacopoeias [24–26]. The colour (λ_{max} and intensity) of the formed complexes varies with the pH of the reacting mixture. Upon alkalising a slightly acidic solution containing epinephrine or isoproterenol and ferrous salt, a blue colour appears at a pH about 6.5, which gradually changes to the characteristic red-blue colour. The maximum intensity is obtained at the pH range of 8.0–8.5. Fig. 2 shows the dependence of absorbance (at $\lambda = 530$ nm) on pH under flow conditions for both catecholamines. The stoichiometry of the reaction is always 2 moles of catecholamines to 1 mole of Fe(II), therefore the chosen flow rate and concentration of the Fe(II) reagent ensure an adequate excess of iron to provide maximum colour development. The complex is formed rapidly and it is stable for several hours. The experimental molecular absorptivity, ε, of the Fe(II)-complex in the optimised flow and chemical conditions (Table 1) was found to be 910 and 810 mol^{-1} cm^{-1}, for **I** and **II**, respectively. From these values, and assuming reliable absorbance peak measurements at the

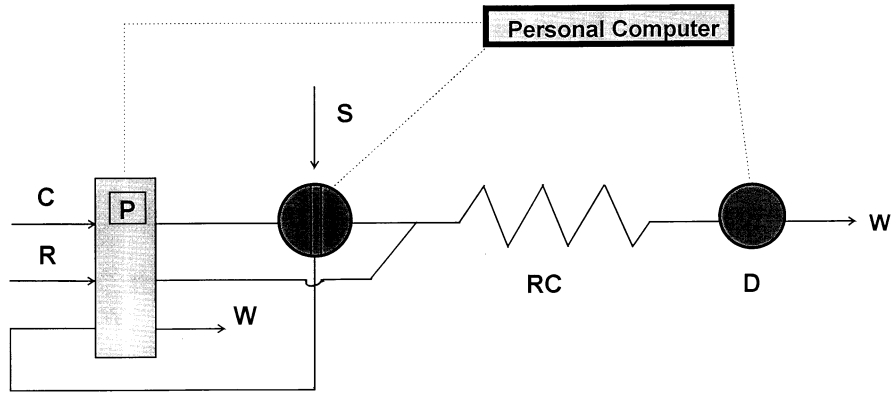


Fig. 1. Schematic FI manifold for the determination of epinephrine and isoproterenol. C: carrier solution, 0.010 mol l^{-1} sodium hydrogen sulphite, 1.3 ml min^{-1}; R, reagent (mixture of Fe(II)-citrate with aminoacetic-carbonate buffer pH 8.3), 1.3 ml min^{-1}; S, sample (210 μl); P, peristaltic pump; W, waste; RC, reaction coil (0.5 m); D, flow spectrophotometric detector ($\lambda = 530$ nm).

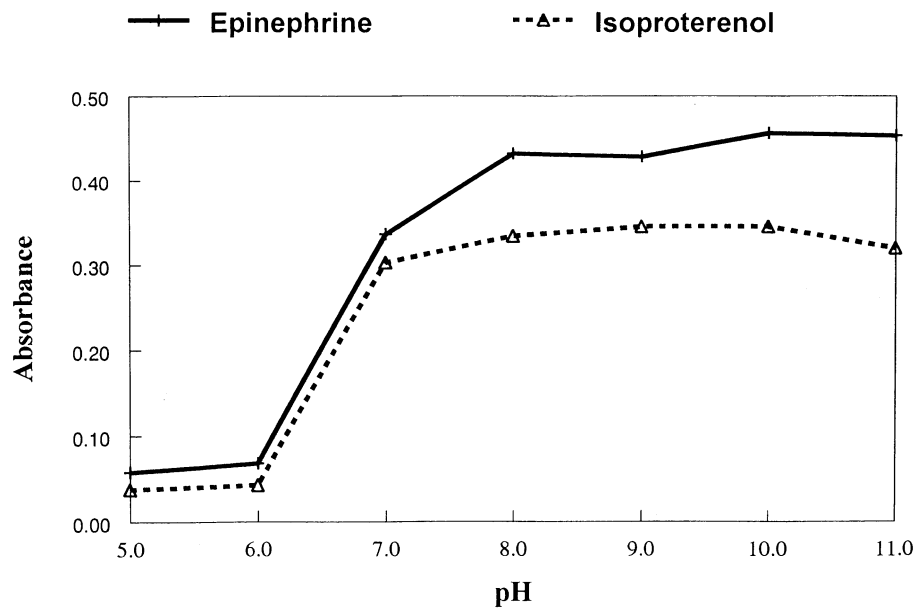


Fig. 2. Effect of pH on the absorbance of the complex. Concentrations of **I** and **II**: 50.0 mg l^{-1}.

level of 0.01 units, quantification limits of the level of about 3 mg l^{-1} are expected. Although the detectability and sensitivity of the reaction are not high, the large amounts of catecholamines present in pharmaceutical formulations allow the use of this reaction for the assay of the two drugs.

Catecholamine solutions undergo oxidation in the presence of oxygen, especially in neutral or alkaline solutions. Therefore a 0.01 mol l^{-1} sodium hydrogen sulphite solution was thoroughly used as the carrier reagent and as a solvent for samples and standards to prevent oxidation and stabilise the colour of the complex developed.

Several preliminary tests indicated that this colour reaction is rather selective for compounds possessing at least two phenolic groups at adjacent carbon atoms. No colour was produced with phenol, resorcinol, hydroquinone, phloroglucinol and neo-synephrine (*m*-methylaminoethanolphenol).

3.1. Optimisation of chemical and instrumental variables

Optimisation of chemical variables (concentration of the Fe(II) reagent, and composition, concentration and pH of the buffer) and instrumental variables (volume of injected sample, length of the reaction coil, flow rate, wavelength) was performed using the univariate optimisation procedure (changing one variable in every turn and keeping the others at their optimum values). All experiments were done under constant laboratory temperature.

The stock solution Fe(II) reagent was prepared in an almost neutral citrate buffer in the presence of hydrogen sulphite to avoid precipitation and

Table 1
Optimisation of experimental variables

Variable	Unit	Range studied	Optimum found
Flow rate	ml min^{-1}	0.3–3.6	2.6
Mixing coil length	M	0.2–4.0	0.5
Sample volume	μl	50–400	210
Wavelength	Nm	400–650	530
Fe(II)-citrate	mol l^{-1}	0.001–0.1	0.01
Carrier ($NaHSO_3$)	mol l^{-1}	0.001–0.5	0.01
pH		5.0–11.0	8.3

Table 2
Linear regression equations and precision for the determination of catecholamines (**I** and **II**)

Drug	c (mg l^{-1})	n	Equation	r	LOD[a] (mg l^{-1})	LOQ[b] (mg l^{-1})	% RSD[c] ($n=5$)
I	5–200	7	$A_p = 0.018(\pm 0.0003) + 4.66(\pm 0.05)\times 10^{-3}c$	0.9997	1.0	3.3	0.24
II	10–300	9	$A_p = -0.004(\pm 0.0001) + 3.23(\pm 0.08)\times 10^{-3}c$	0.9993	1.2	4.0	0.13

[a] LOD detection limit (signal to noise ratio = 3).
[b] LOQ quantitation limit (signal to noise ratio = 10).
[c] 150 mg l^{-1} (**I**) and 200 mg l^{-1} (**II**).

oxidation. The working Fe(II) solution was prepared freshly by mixing with the selected optimised alkaline buffer. Different types of buffers of various concentrations were tested (aminoacetic, phosphate, tetraborate, Clark-Lubs). The aminoacetic-carbonate buffer was found as the best as far as concern the baseline stability and the reaction sensitivity. The optimised composition of the Fe(II) reagent is similar to that of the Pharmacopoeia Bohemoslovaca [29]. It is simple in preparation and sufficiently stable. pH 8.3 was found to be optimum for the complex formation (Fig. 2).

The ranges of the various experimental variables tested and their optimum values finally chosen are summarised in Table 1.

3.2. Calibration graphs and statistical data

Under optimised conditions, the calibration graphs were linear for epinephrine over the range 5–200 mg l^{-1} with a relative standard deviation (RSD) of 0.24% ($n=5$; $c=150$ mg l^{-1}) and for isoproterenol over the range 10–300 mg l^{-1} with an RSD of 0.13% ($n=5$; $c=200$ mg l^{-1}). Deviation from linearity was observed at higher concentrations. The detection limits ($3\times$ SD of the most dilute standard) were 0.8 mg l^{-1} for **I** and 1.1 mg l^{-1} for **II**, while the quantitation limits ($10\times$ SD) were 2.6 mg l^{-1} for **I** and 3.6 mg l^{-1} for **II**. The linear regression equations of calibration curves along with precision data for the determination of both catecholamines are summarised in Table 2.

3.3. Interference studies

The influence of several compounds (possessing phenolic, carboxylic, and hydroxylic group(s) and common excipients) was studied in synthetic mixtures (filtered if necessary prior analysis). The results shown in Table 3 reveal that none of the common excipients (sodium chloride, EDTA, magnesium stearate, lactose, talc and starch) cause any serious interference on the proposed method at the 1:1 concentration ratio. EDTA at higher concentrations causes a negative error due to the complexation of the Fe(II) reagent. Magnesium stearate at high concentrations forms micelles, which effect the absorbance measurements. It is noticeable that compounds possessing phenolic group(s) (phenol, *m*-dihydroxybenzene (resorcinol), *p*-dihydroxybenzene (hydroquinone), and 1,3,5-trihydroxybenzene (phloroglucinol)) show no interference revealing the selectivity of the reaction towards the *ortho*-dihydroxy group (epinephrine, isoproterenol). Carboxylic acids of various types (*ortho*-dicarboxylic benzene (phthalic acid), *ortho*-hydroxy-carboxylic benzene (salicylic acid) and 2-hydroxypropanoic acid (lactic acid) have also no interference.

3.4. Assay of pharmaceutical preparations

The proposed method was applied for the assay of the two drugs in two commercially available epinephrine formulations (Adrenalin injections, Léčiva, Czech Republic; Solution Adrenalinii Hydrogentartarici, Léčiva, Czech Republic) and in

two isoproterenol formulations (Isuprel injections, Sterling-Winthrop, France; Saventrine tablets, Pharmax, UK). The results shown in Table 4 were found in a good agreement (t-test) with those obtained using the USP Pharmacopoeia UV-spectrophotometric method (differences: 1.3–2.4%, mean: 1.9%). The relative standard deviations ranged from 1.7 to 3.7%. Assuming three measurements per sample, about 40 sample solutions can be analysed in 1 h.

FI technique is particularly suitable for performing content uniformity tests of pharmaceutical

Table 3
Recovery of epinephrine and isoproterenol from synthethic mixed solutions containing various common compounds and excipients

Interferent	Concentration ratio[a]	Recovery (%)	
		Epinephrine[b]	Isoproterenol[c]
Phenol	10	100.1	102.4
Resorcinol	10	100.6	98.3
Hydroquinone	10	98.3	97.6
Phloroglucinol	10	98.8	100.0
Salicylic acid	5	100.3	105.1
Phthalic acid	5	100.2	99.9
Lactic acid	10	100.0	99.7
Sodium chloride	50	100.8	99.0
Sodium hydrogen sulphite	50	99.3	99.3
EDTA	10	93.7	92.9
EDTA	1	98.3	98.3
Magnesium stearate	1	106.9	95.6
Lactose	50	102.1	98.7
Talc	50	101.3	98.5
Starch	10	99.1	97.7

[a] Interferent/drug (w/w).
[b] [Epinephrine] = 100 mg l^{-1}.
[c] [Isoproterenol] = 150 mg l^{-1}.

Table 4
Determination of catecholamines (**I**, epinephrine; **II**, isoproterenol) in pharmaceutical formulations by using flow injection (FI) and reference method

Drug	Formulation (manufacturer)	Claimed	Drug content (mg) ± standard deviation ($n = 3$)[a]		
			Found		
			FI method	Reference[b]	t-test[c]
I	Adrenalin injection (Léčiva, Czech Republic)	1.0 mg ml^{-1}	1.07 ± 0.03	1.05 ± 0.03	0.826
I	Solution Adrenalinii Hydrogentartarici solution (Léčiva, Czech Republic)	1.0 mg ml^{-1}	1.08 ± 0.04	1.06 ± 0.03	0.700
II	Isuprel injection (Sterling-Winthrop, France)	0.2 mg ml^{-1}	0.214 ± 0.006	0.209 ± 0.010	0.743
II	Saventrine tablets (Pharmax Ltd, UK)	30 mg	29.4 ± 0.50	29.8 ± 0.73	0.784

[a] Average of three samples measured in triplicate.
[b] UV-method
[c] t_{teor} (95%, $n = 3+3$) = 2776.

Table 5
Results of content uniformity test of Saventrine[a] tablets of 30 mg of isoproterenol (dissolved in 200 ml of 0.010 mol l^{-1} sodium hydrogen sulphite)

Tablet	Concentration (mg ml^{-1})	Percentage of average content[b]	RSD (%)
1	0.1497	97.3	1.2
2	0.1550	100.7	0.8
3	0.1609	104.5	0.7
4	0.1583	102.8	1.1
5	0.1426	92.6	1.3
6	0.1648	107.2	0.8
7	0.1568	101.9	1.4
8	0.1365	88.7	0.9
9	0.1580	102.7	0.6
10	0.1568	101.9	1.4

[a] Pharmax, UK.
[b] Grand mean: 30.78 mg per tablet, RSD = 5.6%, range = 5.66 mg; each sample was measured in triplicate.

formulations. This test was performed for the Saventrine tablets and the results for ten individual tablets are shown in Table 5.

The tablets were found to meet the general Pharmacopoeia requirements (all ten tablets examined fell within the limits of 85–115% of the average content). The time required for the 30 measurements of the test was only 15 min.

4. Conclusions

The proposed automated flow-injection method for the spectrophotometric determination of epinephrine and isoproterenol in pharmaceutical formulations was found to provide results comparable with those of the official UV spectrophotometric method. It is simple, fast (results are obtained in a few min), accurate, with the advantages of simple operation with a friendly computer programme and the automated control of timing, which provide results of high precision (RSD < 0.3%). In addition, it features a high measurement throughput (120 h^{-1}), adequate sensitivity and detectability (quantitation limits (4 mg l^{-1}) and low reagent consumption. The method can be used as a routine method for quality control of catecholamine formulations (assay, content uniformity and dissolution studies) [12]. The interface of an automated FIA method with a dissolution apparatus results in the full automation of the very useful dissolution test for tablets and capsules. A complete multipoint profile of dissolution can be obtained using the FI-dissolution technique.

The use of an automated flow-injection system with a suitable software enables the achievement of a high productivity in routine pharmaceutical analysis with less consumption of reagents and accuracy comparable with that of official pharmacopoeia methods.

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