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Automated flow-injection colorimetric determination of acetaminophen for assays and dissolution studies of multicomponent dosage forms

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Summary

An automated flow-injection determination of acetaminophen, based on its oxidation with iron(III) and subsequent chelation of the produced iron(II) with 2,4,6-tripyridyl-S-triazine (TPTZ), is described. Acetaminophen in the range 50–500 μg/ml can be rapidly determined with a precision of 0.4% with a rate of 120 measurements per hour. Salicylates and phenothiazines interfere seriously. The method was evaluated by performing recovery studies and analyzing commercial formulations. The method was also used to monitor the dissolution of acetaminophen formulations. Full automation of the dissolution studies was achieved. A complete dissolution profile in tabulated form and graphical presentation were provided at the end of the study. Results obtained by the proposed method were found to agree well with those derived from established techniques.

Introduction

Acetaminophen (paracetamol, N-Acetyl-4-aminophenol) occupies a prominent position amongst the extensively employed antipyretic analgesics. The availability of acetaminophen in a great number of formulations in combination with a variety of drugs, demands an analytical method that is rapid, sufficiently specific and able to be automated for routine analyses and dissolution studies.

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Acetaminophen has been assayed by titrating its phenolic group with a base in dimethylformamide (Walash et al., 1972; Agarwal and Walash, 1974) or its aromatic amino group (after hydrolysis) with nitrite (Inamdar et al., 1973). However, these methods are time consuming, they cannot be automated and they lack specificity.

Various colour reactions involving hydrolysis to 4-aminophenol and formation of a Schiff base with a substituted benzaldehyde (D'Souza and Shenoy, 1968; Kalatzis and Zarbi, 1976) or diazotization and coupling (Inamdar et al., 1974) have been utilized for the estimation of acetaminophen. Other spectrophotometric methods are based on indophenol formation (Goncalves, 1974; Ellcock and Fogg, 1975; Davis et al., 1974), nitrosation and subsequent chelation (Belal et al., 1979), ultraviolet absorption (British Pharmacopoeia, 1973; U.S.Pharmacopoeia, 1980) and its change with pH (Elsayed et al., 1979). Recently, the reaction of acetaminophen with 2-iodylbenzoate (Krishna et al., 1984) has been proposed as the basis for a new spectrophotometric method. Most of these methods require lengthy treatments and lack the simplicity needed for routine analysis.

Several automated methods have been developed, using commercial analyzers. The reaction of acetaminophen with nitrous acid under mild conditions, to form 2-nitro-4-acetaminophenol which can be measured by its color in alkaline solution (Le Perdriel et al., 1968) has been adapted to the Beckman AMA 40 analyzer (Chafetz et al., 1971) and to the air-segmented continuous flow analyzer (Daly et al., 1972).

In recent years Flow Injection Analysis (FIA), a new rapidly developing analytical technique, has found a wide application in various fields of routine analysis (Ruzicka and Hansen, 1981; Rocks and Riley, 1982; Ruzicka, 1983). The FIA technique is very flexible in performing a variety of sample pretreatment functions on-line and in using various analytical detectors for measuring. This technique has been successfully interfaced with dissolution apparatus in order to automate dissolution studies of drugs (Koupparis et al., 1984). This combination provides exploitation of all the FIA benefits (small sample consumption, rapid analysis, full automation, and flexibility in analytical procedures) in dissolution studies.

In this paper, the development of an automated flow-injection determination of acetaminophen and its application for routine assays and dissolution studies is described. The reaction of acetaminophen with iron(III) in an acetate buffer of pH 3.6 to produce iron(II) which subsequently reacts with 2,4,6-tris(2-pyridyl)-S-triazine (TPTZ) to form the highly coloured complex $Fe(TPTZ)_2^{2+}$ absorbing at 593 nm ($\epsilon = 22{,}600$) (Collins et al., 1959), was chosen for adaptation. This principle has been used by Liu and Oka (1980) to measure acetaminophen in serum by a manual procedure involving extraction of drug with ethyl acetate followed by the proposed reaction in the organic layer.

Materials and Methods

Reagents

All solutions were prepared in de-ionized water from analytical reagent grade

materials. Acetaminophen and its formulations were obtained from commercial sources [1-8]. The purity of acetaminophen powder was tested using a USP Acetaminophen RS.

Acetaminophen standard solutions. A 1000 μg/ml stock solution is prepared by dissolving 500.0 mg of pure substance in 500 ml of water. This solution, if kept in refrigerator, is stable for a long time. Working standard solutions in the range of 50–500 μg/ml are prepared by appropriate dilutions of the stock solution with water.

Acetate buffer, 0.30 M, pH 3.6. It is prepared by dissolving 3.1 g of sodium acetate trihydrate in 800 ml of H_2O, adding 16 ml of glacial acetic acid, adjusting the pH at 3.6 and diluting to 1.0 litre.

Mixed Fe(III) 0.050 M-acetate buffer solution. 20.2 g of $Fe(NO_3)_3 \cdot 9H_2O$ are dissolved in 1 litre of acetate buffer solution.

2,4,6-Tris(2-pyridyl)-S-triazine (TPTZ), 1.0 mg/ml (3.2×10^{-3} M) solution. It is prepared by dissolving 0.50 g of the reagent [9] in 500 ml of 3.0×10^{-2} M HCl solution and stored in an amber bottle. It is refrigerated when not in use.

Dissolution medium-phosphate buffer 0.01M, pH 5.8. It is prepared as recommended by USP XX for acetaminophen.

Instrumentation

The home-made automated photometric flow-injection analyzer (Koupparis and Anagnostopoulou, 1984) was used for the determination of acetaminophen. This analyzer provides automatic aliquoting of samples from the turntable, or manually extracted solutions, injection of an accurate adjustable sample volume in the reagents flow stream, measuring the absorbance peaks of the reacting mixtures and manipulating the analytical data. The analyzer is controlled by a versatile inexpensive microcomputer.

The analytical manifold designed for the acetaminophen determination, along with the flow rates of the reagents is shown in Fig. 1.

For the automated dissolution studies of acetaminophen solid dosage forms, the flow-injection analyzer was coupled with a rotating basket apparatus as described elsewhere (Koupparis et al., 1984).

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[1] Depon tablets (500 mg of acetaminophen), Lot 4D036 ER Squibb and sons, New York-Athens.

[2] Depon elixir (120 mg of acetaminophen/5 ml), Lot 4E061, Squibb A.E.B.E. Athens, Greece.

[3] Distalgesic tablets (325 mg acetaminophen, 32.5 of dextroproxyphene hydrochloride), Lot 62916 A.E, Dista Products, Liverpool, U.K.

[4] Dolal elixir (125 mg acetaminophen/5 ml), Lot 8404 36 Famar S.A., Athens, Greece.

[5] Lonarid tablets (400 mg of acetaminophen, 20 mg of dimethyl-N-octyl-(β-benzilic acid-ethylester)-ammonium bromide, 10 mg of codeine phosphate, 30 mg of amobarbital, 50 mg of caffeine), Lot 46063, Boehringer Ingelheim, Athens, Greece.

[6] Norgesic tablets (450 mg acetaminophen, 35 mg of orphenadrine), Lot 3783, Cana S.A. Athens, Greece.

[7] Panadol tablets (500 mg of acetaminophen), Lot 4C38 Sterling Drug Hellas S.A.

[8] Panasorb elixir (120 mg of acetaminophen/5 ml), Lot X029YE, Sterling Drug Hellas S.A.

[9] Aldrich.

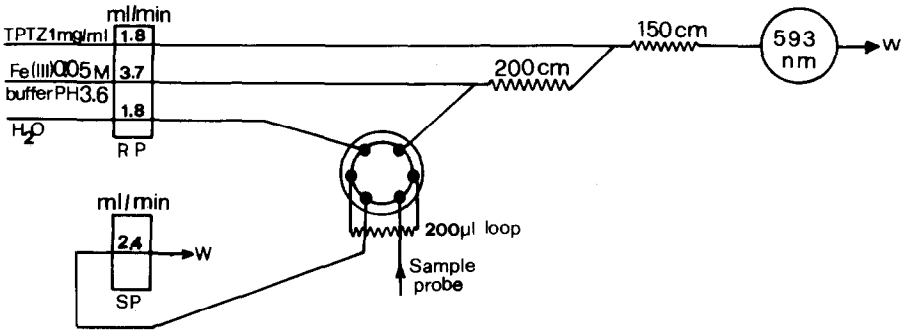

Fig. 1. Analytical manifold for the FIA determination of acetaminophen. Key: RP, reagent pump; SP, sample pump; TPTZ, 2,4,6-tripyridyl-S-triazine; W, waste.

Procedures

(A) Acetaminophen assay in dosage forms

Sample preparation. (a) Tablets. Not less than 20 acetaminophen tablets are weighed and finely powdered. An accurately weighed portion, equivalent to 50–500 mg of acetaminophen, is transferred to a 100 ml volumetric flask and diluted with water to volume. Using an ultrasonic bath or mechanical shaker the powder is completely disintegrated and the solution is allowed to clear or filtered. 10.00 ml of the solution is diluted with water in 100 ml volumetric flask. The samples along with at least three standards are loaded on the turntable of the analyzer or extracted manually to the sample probe. *(b) Capsules.* The contents of not less than 20 capsules are weighed and mixed and an accurately weighed portion, equivalent to 50–500 mg of acetaminophen, is treated as described above for tablets. *(c) Elixirs.* An accurately obtained volume is diluted with water in a volumetric flask so that the acetaminophen concentration is in the range of 0.05–0.5 mg/ml.

Procedure. The photometer of the FIA analyzer is set at 593 nm, the reagents are pumped and the 100% transmittance is set. The number of standards, samples, runs per standard and sample, the injection and wash time (15 and 20 s, respectively) are provided to the microcomputer's routine program. The determination then proceeds automatically by measuring the standards, constructing the calibration curve by linear regression analysis, measuring the samples, calculating and printing their concentration.

(B) Dissolution studies

The "dissolution" program is loaded in the microcomputer's memory and a calibration curve is obtained using at least three acetaminophen standards (50–500 µg/ml) thermostated at 37°C. Then a dosage form is placed into a 250 mesh screen basket and the whole is immersed and rotated at 60 rpm in a double-wall beaker containing 900 ml of dissolution medium, thermostated at 37 ± 0.5°C. All other conditions concerning the automatic aliquoting of 200 µl samples from the dissolution medium and the manipulation of the analytical data were as described previously (Koupparis et al., 1984).

Results and Discussion

Optimization of the method

The designed analytical manifold shown in Fig. 1, is a compromise of short analysis time, low reagent consumption and good linearity up to 560 μg/ml (the maximum concentration obtained in dissolution studies of 500 mg acetaminophen dosage forms in 900 ml of dissolution medium). The sensitivity of the analytical method can be considerably increased by using longer reaction coils, i.e by increasing the reaction time.

The effect of iron(III) and TPTZ concentrations was studied. The increase of iron(III) concentration showed negligible effect on absorbance peak heights, but the background absorbance of the reagents flow increased. In contrast, the increase of TPTZ concentration enhanced peak height. The final TPTZ concentration chosen, is a compromise of maximum solubility of TPTZ, the need for low reagent consumption in prolonged continuous operation and the wide analytical range required.

The sample volume injected affects seriously the peak height. Using 100 μl, the analytical range can be expanded to 1000 μg/ml.

A reaction medium of a buffer of pH 3.6 has been chosen as a compromise of keeping Fe(III) in solution by preventing the formation and precipitation of iron hydroxide and achieving quantitative $Fe(TPTZ)_2^{2+}$ complex formation since the complex is stable in the pH range 3.4–5.8 (Collins et al., 1959).

Using the aforementioned experimental conditions, the reaction of acetaminophen with Fe(III) is allowed to proceed only for 30 s, i.e. it is completed only to a few percent. Thus, the method can be considered as a kinetic fixed time analytical procedure showing the inherent advantage of FIA technique for utilization of uncompleted slow reactions.

Validation of the method

Typical FIA recorded peaks used for the calibration curve are shown in Fig. 2 in increasing and decreasing concentration order to show the absence of any carryover effect. Results for a calibration curve along with the statistical treatment are shown in Table 1. As is shown, the precision and linearity of the developed method are excellent and the sample throughput is high (120 measurements per hour, with 15 s injection time and 20 s wash time).

To study the interference of some common additives used in tablet preparation, synthetic sample solutions containing 100 μg/ml acetaminophen and various excess amounts of each additive were analyzed. The undissolved material was filtered before measurement. The recovery results are shown in Table 2. As is shown, only sodium lauryl sulfate in large excess (10-fold) and carbopol (carboxypolymethylene) interfere. The latter causes an enhancement of the peak height generating non-reproducible highly coloured aggregates. All other common excipients showed an average recovery of 99.8%. Therefore, the assay, as well as measurements for dissolution studies, can be obtained with the developed method without any error from this source.

The effect of various drugs incorporated with acetaminophen in various formula-

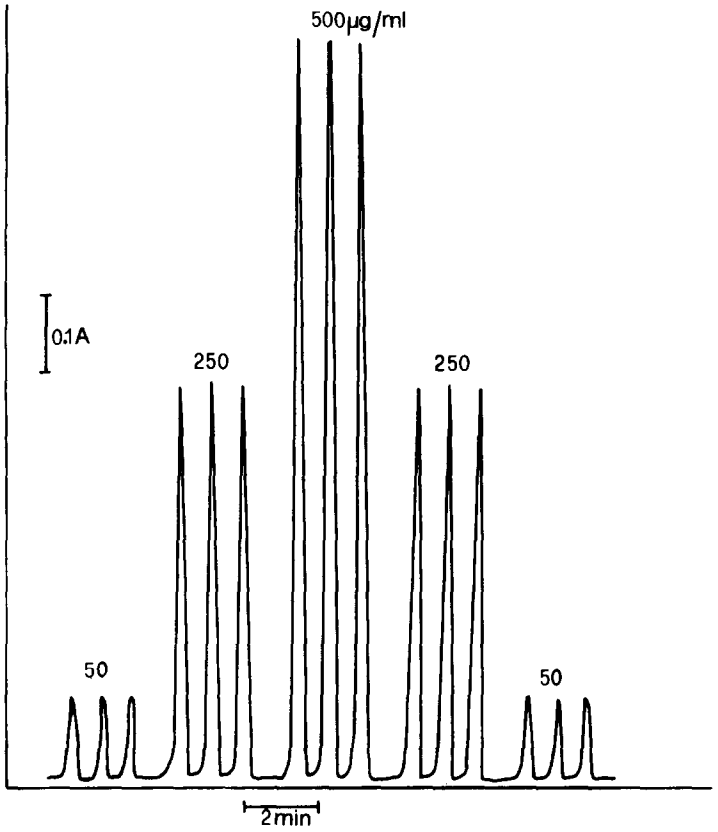

Fig. 2. Typical recorded absorbance peaks for calibration curve for FIA acetaminophen determination. Injection time 15 s, wash time 40 s.

tions was tested by analyzing pure solutions of each drug and performing analytical recovery experiments in synthetic mixture solutions with acetaminophen. Table 3 shows those drugs which interfere with the proposed method. These drugs react with iron(III) to form complexes or free radicals absorbing at the same wavelength.

TABLE 1
RESULTS FOR FLOW INJECTION ANALYSIS DETERMINATION OF ACETAMINOPHEN

Acetaminophen (μg/ml)	Average peak (absorbance)	% RSD (n = 5)
50.0	0.1107	0.4
100	0.2137	0.3
250	0.5158	0.2
350	0.7114	0.3
500	1.028	0.2

Calibration curve: peak height = $0.0086 + 0.002029 \times C$ (μg/ml); $r = 0.99992$, $S_{yx} = 0.005$.

TABLE 2
ANALYTICAL RECOVERY OF ACETAMINOPHEN (100 μg/ml) FROM VARIOUS TABLET ADDITIVES

Additive	Concentration ratio (additive/acetaminophen)	Recovery % (n = 3)
Starch	10	100.0
Galactose	10	100.3
Glucose	10	99.8
Lactose	10	100.3
Garbowax [a]	10	100.0
Sodium lauryl sulfate	10	124.8
Sodium lauryl sulfate	5	99.2
CAHP [b]	10	100.9
Carbopol [c]	10	199.2
Carbopol [c]	5	181.1
Carbopol [c]	1	134.8
Carbopol [c]	0.5	130.6
Magnesium stearate	10	98.7
Gelatin	5	98.3

[a] Polyethylene glycol 4000.
[b] Cellulose acetate hydroxyphthalate.
[c] Carboxypolymethylene.

p-Aminophenol reduces iron(III) more rapidly than acetaminophen. The following drugs or other substances formulated with acetaminophen had no effect on the determination at the concentration in μg/ml specified in parentheses: acetylsalicylic acid (1000), alcohol (10% v/v), aluminum ions (250), amobarbital (30), barbital (200), bromide ions (100), caffeine (1000), calcium gluconate (500), citrate ions (200), codeine phosphate (100), dimethyl-N-octyl-(β-benzilic acid-ethylester)-ammonium bromide (20), homatropine hydrobromide (16), magnesium ions (250), meperidine hydrobromide (500), orphenadrine (50), phenacetin (200), phosphate ions (200) and propoxyphene hydrochloride (300).

TABLE 3
INTERFERENCE OF DRUGS FORMULATED WITH ACETAMINOPHEN ON FLOW INJECTION ANALYSIS DETERMINATION

Drug	Concentration ratio (interferant/acetaminophen [a])	Recovery (%)
Promethazine	0.1	157
Salicylamide	10	356
Salicylic acid	2	172
p-Aminophenol [b]	0.1	200

[a] Acetaminophen used 100 μg/ml.
[b] *p*-Aminophenol is the hydrolysis product of acetaminophen.

TABLE 4
DETERMINATION OF ACETAMINOPHEN IN COMMERCIAL FORMULATIONS WITH THE FLOW INJECTION ANALYSIS METHOD AND COMPARISON WITH A REFERENCE METHOD [a]

Formulation	Acetaminophen			% Difference (FIA–Ref.)
	Claimed	Found		
		FIA [b]	Ref. [c]	
(A) Tablets [d] (mg)				
(1) Depon	500	508 ±2	494 ±5	+2.8
(2) Panatol	500	504 ±4	495 ±4	+1.8
(3) Lonarid	400	403 ±2	394 ±5	+2.3
(4) Norgesic	450	397 ±4	407 ±4	−2.4
(5) Distalgesic	325	326 ±2	317 ±3	+2.8
(B) Elixirs [d] (mg/ml)				
(6) Depon	24	24.3±0.2	23.2±0.4	+4.7
(7) Panasorb	24	23.4±0.3	22.5±0.3	+4.0
(8) Dolal	25	24.6±0.1	23.9±0.4	+2.9
				mean ± 3.0%

[a] Belal et al., 1979.
[b] Average of 3 samples, measured 3 times, ± S.D.
[c] Average of 3 samples, measured once.
[d] See footnotes 1–8.

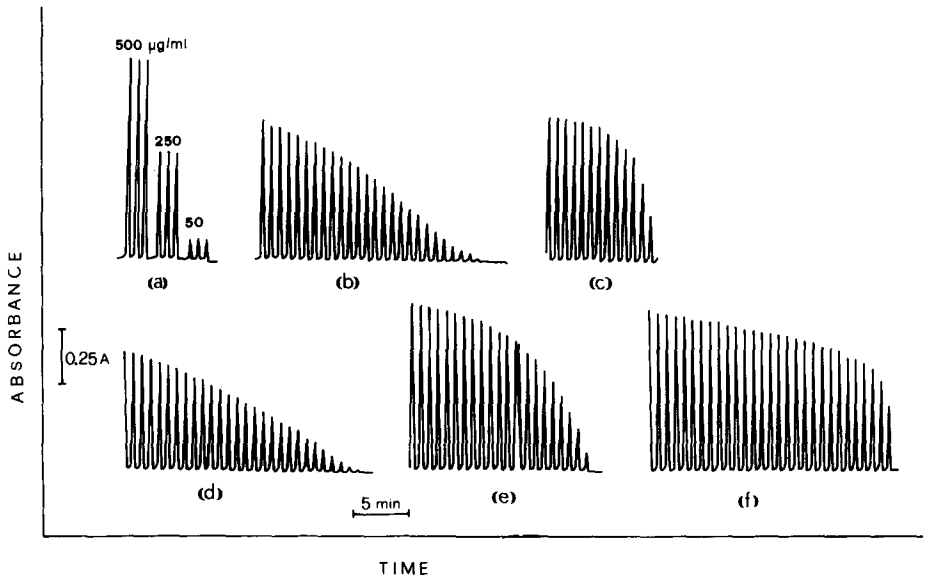

Fig. 3. Dissolution profiles of commercial acetaminophen tablets. Key: (a) calibration curve; (b) Distalgesic; (c) Lonarid; (d) Norgesic; (e) Panatol; (f) Depon. The drugs contained in each tablet can be seen in the footnotes appearing in Methods. The absorbance axis can be converted to % dissolution using the calibration curve slope and the theoretical maximum concentration of acetaminophen in the dissolution medium.

TABLE 5
COMPARISON STUDY FOR THE DISSOLUTION OF AN ACETAMINOPHEN FORMULATION [a] WITH THE MANUAL OFFICIAL (M) AND AUTOMATED FLOW INJECTION ANALYSIS (A-FIA) PROCEDURES

Time (min)	3.75	6.25	8.75	11.25	13.75	16.25	22.5
% Dissolved M [b]	35.7	62.1	67.7	84.2	90.0	92.4	95.7
acetaminophen A-FIA [c]	34.2	58.5	72.1	80.4	85.2	88.6	93.0
Difference [d]	−1.5	−3.6	+4.4	−3.8	−4.8	−3.8	−2.7
Mean difference			+3.5				

[a] Norgesic tablet.
[b] Mean of 3 runs.
[c] Mean of 2 runs.
[d] Automated-manual value.

TABLE 6
PRECISION OF AUTOMATED FLOW-INJECTION ANALYSIS DISSOLUTION STUDY OF A COMMERCIAL TABLET [a]

Injection number [b]	Time (min)	Average % dissolution of acetaminophen	Standard deviation (% dissolution) (n = 3)
6	5.50	7.9	2.5
7	6.42	12.6	2.8
8	7.33	16.9	2.7
9	8.25	21.2	2.8
10	9.17	25.2	2.6
11	10.08	28.9	2.7
12	11.00	33.5	2.6
13	11.92	36.6	2.5
14	12.83	41.3	2.2
15	13.75	44.4	2.2
16	14.67	47.8	1.8
17	15.58	51.7	2.1
18	16.50	54.6	2.0
19	17.42	58.1	1.9
20	18.33	61.0	1.5
21	19.25	64.6	1.7
22	20.17	67.2	0.5
23	21.08	71.0	0.6
24	22.00	74.0	0.5
25	22.92	76.4	1.0
26	23.83	79.0	0.8
27	24.75	81.8	0.5
28	25.67	84.1	0.3
29	26.58	85.5	1.4
30	27.50	87.3	1.1
			range: 0.3–2.8
			mean: 1.7

[a] Norgesic tablet.
[b] Injection every 55 s.

The proposed method was also evaluated by analyzing acetaminophen commercial formulations and comparing the obtained results with the well-established nitrosation method (Belal et al., 1979) (Table 4). The mean difference found was $\pm$ 3.0%.

To evaluate if the proposed technique is capable of automatic monitoring of acetaminophen formulations dissolution, a number of dissolution experiments were carried out. Acetaminophen concentrations were followed by the FIA method and the USP XX procedure. The dissolution profiles obtained were identical. Fig. 3 shows typical FIA dissolution profiles of commercial tablets. Table 5 shows some of the comparison results. The mean differences of the two methods, expressed as percentage of dissolved drug, were less than 5% for all time points through the period of study. This difference can be considered reasonable since it includes also the variation in content uniformity and the run-to-run variability.

The results presented in Tables 4 and 5 demonstrate that the method, when compared to established techniques, proved to be reliable. It must be emphasized that some of these formulations contain other drugs and excipients which interfere with the official methods. Thus, time-consuming separation with column chromatography is required and recommended by USP XX.

The precision of the analytical procedures was tested thoroughly during the measurements of the standard and sample solutions as well as during the analysis of the commercial formulations. The within-run precision was always better than 0.4% (% RSD, n = 5) and the between-run precision (different sample solutions prepared from the same homogenized formulation sample) was always better than 1.5% (Tables 1 and 4).

The precision of the automated dissolution procedure (in which the formulation variability is included) was tested by performing dissolution experiments for 3 tablets of the same acetaminophen formulation. Table 6 shows the results. As it is shown, an average standard deviation of 1.7% dissolution (range 0.3–2.8) was obtained. Considering the variability of the tablets, the precision of the proposed technique is excellent.

Conclusions

Flow-injection analysis proved to be a versatile new analytical technique with promising successful applications in pharmaceutical analysis. The proposed automated method for acetaminophen can be used for routine assays and dissolution studies ensuring rapid, accurate and precise results. The set up time is very short, sample throughput is high and reagent consumption is low. Interferences from excipients and other drugs are limited.

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