Journal of Pharmaceutical & Biomedical Analysis
Vol. 6, No. 1, pp. 35–46, 1988
Printed in Great Britain

0731–7085/88 $3.00 + 0.00

Automated flow injection determination of salicylates using Trinder reaction for clinical analysis, assays and dissolution studies of formulations

M. A. KOUPPARIS* and P. I. ANAGNOSTOPOULOU

University of Athens, Department of Chemistry, Laboratory of Analytical Chemistry, 104 Solonos str., Athens 106 80, Greece

Abstract: An automated flow-injection technique is described for salicylate (25–250 or 80–800 mg l^{-1}), salicylamide (30–300 mg l^{-1}), methylsalicylate (100–1000 mg l^{-1}) and acetylsalicylic acid (after alkaline hydrolysis), based on their colour reaction with iron (III) in weak acid medium. The method was evaluated for the determination of salicylate in serum, the assay of the drugs in commercial formulations and automated dissolution studies of drug tablets. There were decreased interferences because of the short reaction time. The precision was good with RSD less than 1% in all cases. Recoveries of salicylate from spiked sera (100–1000 mg l^{-1}) varied from 96.4–102.5% (mean 99.3%), and from spiked sample solutions of acetylsalicylic acid, 97.8–103.0% (mean 99.6%). The results of the analysis of commercial drug formulations obtained with the proposed method agreed well with the current USP and BP procedures, with differences of 0.4–1.5% (mean 0.8%). High measurement rates of 180 or 95 per hour were achieved using manifolds without and with predilution respectively.

Keywords: *Salicylate; salicylamide; methyl salicylate; automated determination; flow-injection analysis; salicylate in serum; dissolution test.*

Introduction

Acetylsalicylic acid (aspirin), introduced in medicine in 1899, is still one of the most commonly used analgesic, antipyretic or anti-inflammatory drugs. After absorption from the gastrointestinal tract into the circulation, aspirin is completely converted by esterases to salicylate with a half-life of about 15 min [1]. The relationship between serum salicylate concentration and aspirin's therapeutic efficacy and toxicity is well established [2, 3]. From 150 to 300 mg l^{-1} salicylate in plasma represent therapeutic anti-inflammatory concentrations in the treatment of rheumatoid arthritis, while toxicity (e.g. tinnitus) is likely to occur above 300 mg l^{-1} [4]. Patients with various forms of arthritis generally require laboratory monitoring for serum salicylate to be maintained

*To whom correspondence should be addressed.

within the therapeutic range. In acute salicylate overdose, severity, prognosis and therapeutic intervention are largely based on serum salicylate concentrations [5].

These clinical needs and the widespread use of salicylates have prompted a large number of analytical procedures for serum salicylate. Currently, most are based on the formation of a purple complex when salicylate and ferric ions react in weakly acidic solution. The method was originally described by Brodie *et al.* [6] and adapted for clinical use by Trinder [7]. Mercuric chloride is used to precipitate proteins. Similar procedures, varying mainly in sample treatment, have also been proposed by others [8, 9]. Direct ultraviolet spectrometric [10] and fluorometric [11] methods have also been used. Recently, highly sensitive and specific GLC [12–16] and HPLC [17–19] methods have been developed but they do not seem to meet the requirements for easy and fast clinical methods.

Although the colorimetric method, based on iron(III) complex formation, is not selective [20], it is still useful for high anti-inflammatory salicylate doses and toxic drug level monitoring [21]. Besides the several commercial kits available for manual procedures, the method has been automated by adaptation on several analysers such as, Abbot ABA-100, American Monitor KDA, Du Pont ACA, microcentrifugal analyser, etc. [22–26]. The solubilization of proteins in these methods is achieved using Triton (polyoxyethylene ethers).

The determination of salicylic acid derivatives (i.e. sodium salicylate, acetylsalicylic acid, salicylamide and methyl salicylate) in a large number of single- or multi-component formulations, requires also an increased effort by the control laboratories of the pharmaceutical industry. The official methods in the current US Pharmacopoeia (USP) and British Pharmacopoeia (BP) are based on time consuming tedious titrimetric procedures so a fast automated method for routine analysis is highly desirable. Air-segmented continuous flow analysis has already been used for automation of the colorimetric method [27–28]. The dissolution test, adopted by the Pharmacopeias for capsules and tablets intensifies the demand.

In this paper, we describe an automated colorimetric method for salicylate, salicylamide, methyl salicylate and acetylsalicylate (after hydrolysis), based on the ferric nitrate method, adapted for a flow injection analyser. Flow Injection Analysis (FIA) has several inherent advantages such as: short set-up and response time, high sample throughput, quick change of routine analyses, low sample and reagent consumption and automation of most analysis steps. The application of FIA in clinical [29, 30] and pharmaceutical [31–32] analysis is very promising and has been exploited with several examples in this laboratory [33–37]. The interface of the FIA analyser with conventional dissolution apparatus has produced a very useful system for automating the whole test [38]. A multipoint dissolution profile in the form of a series of absorbance peaks is obtained at the end of the procedure, allowing mathematical treatment of the dissolution rate.

Experimental

Apparatus

An automated homemade spectrometric FIA analyser [39] was used. An inexpensive microcomputer controls all the steps, i.e. sampling, injection, data collection and manipulation. The analytical manifolds used are shown in Fig. 1. In manifold (a), proposed for salicylate concentrations up to 250 mg l^{-1}, a sample volume of 200 μl is

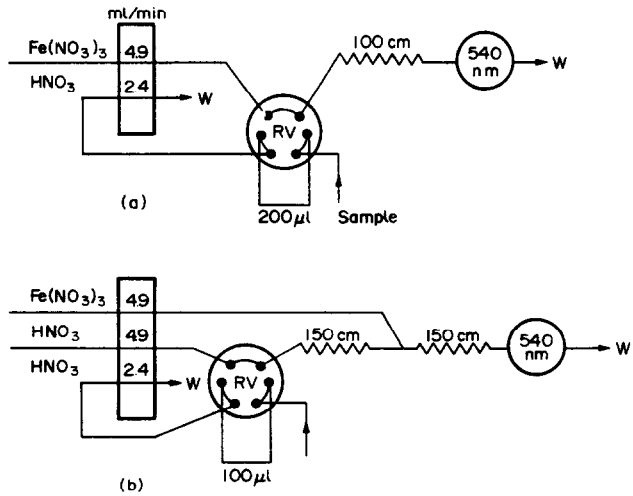

Figure 1
Schematic diagram of the FIA manifolds for salicylate determination, with limited dispersion (a) and large dispersion (b). W, waste; RV, rotating valve.

injected directly into the reagent stream and the complex formed in the reaction coil is monitored at 540 nm. In manifold (b), designed for high concentrations up to 800 mg l^{-1}, the injected sample of volume 100 µl is prediluted before merging with the reagent in a mixing-diluting coil.

For the dissolution studies of drug formulations, the FIA analyser was coupled with the USP rotating basket apparatus as previously described [38]. Figure 2 shows a diagram of this automated dissolution system. The dissolution medium is continuously circulated through the sample loop, and at preselected time intervals the loaded sample (volume 200 µl) is transferred by the carrier (H_2O), to be merged with the reagent for measurement.

When measuring sample solutions with undissolved material, either from other drugs or the excipients, the solution was centrifuged or a disposable filter (filter for pipette tips) was attached to the end of the sample probe to provide on-line filtration.

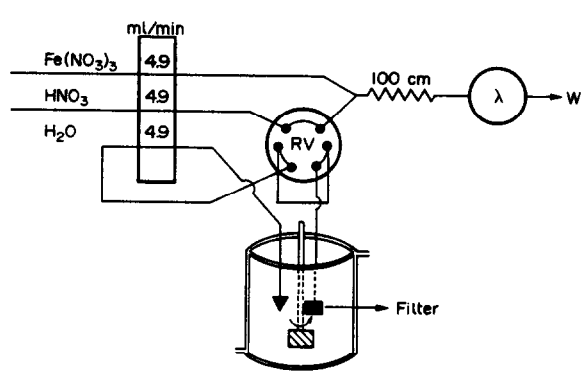

Figure 2
Schematic diagram of the automated FIA dissolution system.

Reagents

All reagent solutions were prepared from analytical grade materials with deionised distilled water. Pure drugs (sodium salicylate, acetylsalicylic acid, salicylamide and methyl salicylate), were obtained from Merck and their purity tested using USP or BP procedures. The solutions for interference study were prepared from analytical or USP grade substances.

Iron(III) nitrate solution, 0.05 M. Dissolve 20.2 g of $Fe(NO_3)_3.9H_2O$ in 1 l of 0.02 M HNO_3 solution. For serum analysis add Triton X-100 to the reagent to concentration 1% (v/v).

Sodium salicylate standard solutions. Prepare a stock 2000 mg l^{-1} solution by dissolving 1.0 g of sodium salicylate in 500 ml of 0.02 M HNO_3. Prepare working standard solutions 25–250 mg l^{-1} (for serum analysis) and 100–800 mg l^{-1} (for formulation assays), by appropriate dilutions with 0.02 M HNO_3.

Salicylamide standard solutions. Prepare a stock 1000 mg l^{-1} solution by dissolving 0.5 g of salicylamide in 500 ml of 0.02 M HNO_3 solution. Prepare working standard solutions 30–300 mg l^{-1} by appropriate dilutions with 0.02 M HNO_3.

Standard methyl salicylate solutions. Prepare a stock 1000 mg l^{-1} solution by dissolving 0.5 g of the pure oily substance in 500 ml of 50 (v/v) aqueous alcohol. Prepare working standard solutions 100–1000 mg l^{-1} by appropriate dilutions with 50% aqueous alcohol.

Sodium hydroxide solution, 1 M. *Nitric acid solutions*, 0.5 M *and* 0.02 M.

Procedures

a. *Determination of salicylate in serum.* Dilute serum aliquots with 0.02 M HNO_3 solution so that the expected concentrations of salicylate are up to 250 mg l^{-1}. Measure the sample solutions together with at least three salicylate working standard solutions using the FIA analyser, with the manifold shown in Fig. 1a and the iron(III) reagent with Triton for protein solubilization. Use a 200 μl sample loop, 10 s for load-wash time and 5 s injection time. Calculate the salicylate concentration from the result obtained automatically, using the appropriate corrections for dilution.

b. *Determination of sodium salicylate and salicylamide in tablets.* Sample and treat tablets according to the current USP or BP. Dissolve an accurately weighed amount of the fine powder, equivalent to about 50–150 mg of sodium salicylate and 30 mg of salicylamide in 200 and 100 ml of 0.02 M HNO_3 respectively.

Measure the filtrates or supernatants of the sample solutions using the FIA analyser together with corresponding working standards. Use the manifold in Fig. 1b for sodium salicylate, with a 100 μl sample loop, 30 s load time and 3 s injection time. For salicylamide, use the manifold in Fig. 1a with the experimental parameters mentioned in procedure (a) without Triton.

c. *Determination of acetylsalicylate in tablets.* Dissolve an accurately weighed amount of the finely powdered tablets equivalent to 80–100 mg of the drug, in 50 ml tubes with 20 ml 1 M NaOH. Place the tubes in a boiling water bath for 10 min, cool and transfer

the solution to a 100 ml volumetric flask and dilute with 0.5 M HNO_3. Measure the sample solutions using the FIA analyser using the manifold in Fig. 1b together with sodium salicylate working standards. Calculate the drug content from the result obtained automatically using the appropriate corrections.

d. *Determination of methyl salicylate in lotions.* Dissolve and dilute 1.0 ml of the formulation in 50% aqueous alcohol, so that the concentration is in the range 100–1000 mg l^{-1}. Measure the sample solutions together with at least three working standards, using the manifold in Fig. 1b.

e. *Dissolution of salicylate and salicylamide tablets.* Load the "dissolution" program into the microcomputer's memory and provide the necessary information pertinent to the test. Obtain initially a calibration curve with the manifold in Fig. 2 using at least three standards of the drug examined, prepared in the dissolution medium used (water, 0.1 M HCl, phosphate buffer pH 7.2 or any other), and thermostatically maintained at 37°C. Then place a tablet into the 250 mesh screen basket, start the rotation (60 rpm), immerse the basket in the beaker of the dissolution apparatus containing the appropriate volume of dissolution medium at 37 ± 0.5°C, and run the program. At the end of the experiment the entire dissolution profile is presented on the chart recorder as a series of absorbance peaks versus time, and also on the computer's printer as a table, consisting of time, absorbance, drug concentration and percentage of dissolution.

Results and Discussion

Salicylate, salicylamide and methyl salicylate react with iron(III) through the possession of a free phenolic group adjacent to the −COR group (R = $-OH$, $-NH_2$ or $-OCH_3$ respectively). Acetylsalicylate cannot react without hydrolysis of the phenolic group.

Optimisation of the method

The reaction is rapid, so the time and the experimental parameters controlling it in a FIA system (length of coil and flow rate) have no critical effect on the response. The effect of the concentrations of iron(III) and nitric acid were studied using the manifold in Fig. 1a. The results are shown in Figs 3 and 4 for a salicylate standard of 100 mg l^{-1}. It will be seen that the peak height increases as iron(III) concentration increases up to 0.05 M, and this concentration was selected as the optimum. The effect of nitric acid concentration is opposite and more dramatic. The final concentration chosen, 0.02 M, is a compromise between increased response and the avoidance of iron precipitation.

To apply the method to clinical analysis, where more sensitivity is required, and to routine analysis of formulations, where high concentrations are obtained, two manifolds were designed as shown in Fig. 1. With manifold (a) the calibration curve is linear up to 250 mg l^{-1} and the dispersion D of the sample zone (measured experimentally using a dye) is 2.8. Manifold (b) allows measurement of salicylate up to 800 mg l^{-1} with sample dispersion of 9.8. To avoid errors from extreme variations in the pH of the sample solutions, samples and standards are made in 0.02 M HNO_3. Measurement of salicylate solutions with pH in the range 2–12 gave exactly the same absorbance peaks as the corresponding working standard made in 0.02 M HNO_3. Thus, the acid concentration in

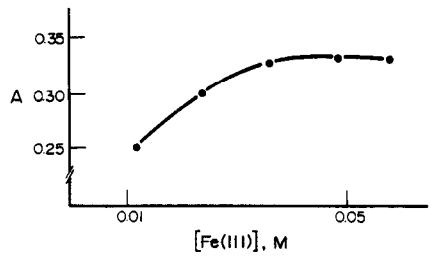

Figure 3
Effect of iron(III) concentration on the FIA response of 100 mg l^{-1} salicylate using manifold (a).

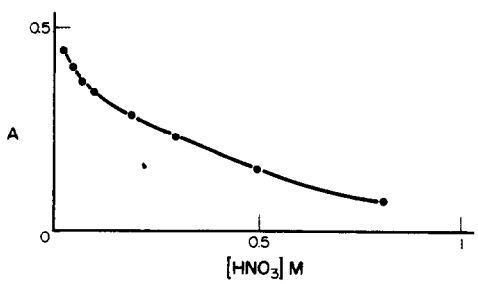

Figure 4
Effect of nitric acid concentration on the FIA response of 100 mg l^{-1} salicylate using manifold (a).

the reagent flow, in conjunction with sample dispersion, ensure the necessary pH adjustment of the reacting mixtures.

The effect of various acids on the response was examined so that they might be used instead of HNO_3 for sample treatment or as dissolution media. Solutions of salicylate in 0.1 M HNO_3, HCL, $HClO_4$, CH_3COOH and CCl_3COOH gave the same response, while for 0.1 M H_3PO_4 and H_2SO_4 the response was drastically decreased to 8.2 and 28.6% of its value respectively. This decrease is caused by the formation of iron(III) complexes with the acid anions.

Validation of the method

Data relevant to the calibration curves for the drugs determined with the proposed method are summarised in Table 1. The precision is good with the RSD in all cases less than 1% (N = 10). The linearity of the calibration curves is also good, the correlation coefficient (r) varied from 0.9996 to 0.99994. The measurement rates are 180 and 95 samples per hour, for manifold (a) and (b) respectively.

Interference studies

The reaction of salicylate with iron(III) is not selective and its use in clinical analysis, for the determination of therapeutic and toxic levels, relies on the high concentrations of the drug relatively to those of the interferants. The coexisting drugs and excipients in the formulations of the studied drugs do not interfere in the technique, so it can be used reliably for drug analysis. The speed of the FIA measurement minimises any interference caused by slow reactions with iron(III) which are apparent in the manual colorimetric procedures.

A large number of possible interferants were studied, these are compounds with similar structure present in serum and in drug formulations. Solutions containing 100 mg l^{-1} of the compounds studied were measured, and the apparent "salicylate" (salicylate

Table I
Calibration curves for the automated FIA determinations

Drug	Manifold used	Concentration range (mg l^{-1})	Equation of linear regression	r
Sodium salicylate	(a)	25–250	$A = (-0.0067 \pm 0.0080) + (0.00465 \pm 0.00006).\ C$	0.9997
	(b)	80–800	$A = (-0.0299 \pm 0.0154) + (0.00131 \pm 0.00003).\ C$	0.9998
Salicylamide	(a)	30–300	$A = (-0.0047 \pm 0.0058) + (0.00403 \pm 0.00003).\ C$	0.99994
Sodium methyl salicylate	(b)	100–1000	$A = (-0.0216 \pm 0.0146) + (0.00119 \pm 0.00002).\ C$	0.9996

concentration giving equal absorbance peak) and the % comparability to salicylate (ratio of absorbance peaks given by equal concentrations) were calculated. Those interferants showing a comparability to salicylate more than 5% are shown in Table 2. It will be seen that serious interference is caused by phenothiazines because they produce colour free radicals with iron(III), by substances with a phenolic group activated by substitutions in the *o*-position, and by theobromine, tetracyclines and antipyrine.

The following substances showed a comparability of less than 5%: histidine, tyrosine, tryptophan, valine, methionine, cysteine, phenylalanine, glycine, ephedrine, chloramphenicol, mandelic acid, creatinine, hippuric acid, benzoic acid, citric acid, phenylacetic acid, lidocaine, amphetamine, phenylbutazone, indole, 3-chlorobenzoic acid, maleic acid, ascorbic acid, 4-chloro-3-nitrobenzoic acid, 2,4-dinitrophenol, 4-aminobenzene-sulfonic acid, oxalic acid, barbital sodium, 2,6-dichlorophenol, amitryptiline, riboflavine, cholic acid, imipramine, cholesterol, propranolol, heparin, veronal, nembutal, nitrazepam, theophyline, streptomycine, thiamine, 2-aminobutyric acid and phenacetin. The interferences found with the proposed rapid FIA method are considerably less than those found with the manual Trinder procedure for some compounds studied in ref. [20]. The common excipients used in tablet formulations showed no interference.

Table 2
Interferences with the FIA determination of salicylate

Interferant (100 mg l^{-1})	Absorbance peak	Apparent "salicylate"	% comparability to salicylate
Hemoglobin	0.026	9.2	6.2
Phenol	0.020	8.8	5.7
Tannic acid	0.173	41.9	40.8
Methyldopa	0.022	8.3	5.2
Levodopa	0.024	8.7	5.7
4-aminosalicylate	0.242	57.3	57.1
4-aminophenol	0.039	12.2	9.2
5-sulfosalicylate	0.359	83.4	84.7
4-chlorophenol	0.031	10.4	7.3
3,5-dinitrosalicylate	0.114	28.9	26.9
o-aminophenol	0.373	86.6	88.0
Chlorpromazine	0.986	223.1	232.5
Oxytetracycline	0.122	30.6	28.8
Theobromine	0.226	53.7	53.3
Antipyrine	0.138	34.2	32.6

Applications

The automated FIA method was tested by analyzing quality control serum (Wellcome Reagents Ltd., Basingstoke, UK), spiked with salicylate. The spiked samples, containing 100–1000 mg l^{-1}, were treated according to procedure (a), 0.5 ml of serum being diluted with 2.0 ml of 0.02 M HNO_3. The results in Table 3 show a mean recovery of 99.3% (96.4–102.5%). Within-run precision varied from 0.2–0.9% RSD (3 measurements on a single diluted sample). Spiked serum samples containing 50, 200 and 500 mg l^{-1} and diluted 1 : 1 with 0.02 M HNO_3 gave within-run precision of 1.2, 0.8 and 0.6% RSD respectively ($N = 5$). The method can be used for the determination of therapeutic and toxic levels of salicylate without removal of proteins.

Table 3
Recovery of salicylate from spiked serum samples

Salicylate ($mg\ l^{-1}$) Added	Found (±SD)*	Recovery %
100.0	101.0 ± 0.9	101.0
200.0	200.0 ± 1.6	100.0
300.0	292.5 ± 1.8	97.5
400.0	387.0 ± 2.0	96.8
500.0	512.5 ± 2.7	102.5
600.0	583.5 ± 2.3	97.3
700.0	710.5 ± 2.1	101.5
800.0	771.0 ± 3.1	96.4
900.0	911.0 ± 1.8	101.2
1000.0	986.5 ± 3.9	98.7
	mean	99.3

*Three measurements on a single sample.

Table 4
Results of pure acetylsalicylic acid determination

Acetylsalicylic acid (mg) Taken	Found	Error (%)
33.3	34.4	3.3
66.7	65.8	−1.3
133.3	128.6	−3.5
200.0	203.6	1.8
266.7	266.0	−0.3
333.3	323.1	−3.1
	mean	2.2

The accuracy of the method for the determination of acetylsalicylic acid using procedure (c) was tested by measuring samples of pure aspirin. The results in Table 4 show a mean error of 2.2% (0.3–3.5%). The method was then evaluated by analyzing commercial formulations and comparing the results with those obtained by the USP, BP or other established procedures. The results in Table 5 show a mean percentage difference of 0.8 (0.1–1.5%). The precision was in all cases better than 1.5% RSD. It must be emphasised that some of the formulations analyzed contain other drugs. Recovery experiments of acetylsalicylic acid, performed with solutions of commercial formulations, gave a mean of 99.6% (97.8–103.0%) (Table 6).

Figure 5 shows typical FIA results for the dissolution profile of a salicylamide tablet in 0.1 M HCl and 0.01 M phosphate buffer pH 7.2, together with the calibration curve used for the test. Similar automated dissolution tests were performed for sodium salicylate tablets. Dissolution experiments performed on three tablets of the same lot gave an average standard deviation of 2.1 (% of dissolution). Considering the inherent tablet to tablet variability the precision of the proposed technique is good. A semiautomated dissolution test of aspirin tablets can also be performed by using the FIA system for

Table 5
Comparison results of commercial formulation assays

Formulation	Drug content Stated	FIA (±SD)[1]	Official	Difference[2] %
A. Acetylsalicylic acid		mg/tablet		
Aspirin for children	100	100.4 ± 0.9	101.1	−0.7
Aspirin (Bayer)	500	494 ± 6	489	1.0
Aspirin-C[3]	400	401 ± 6	405	−1.0
Neospir P[4]	80	80.6 ± 1.0	81.8	−1.5
Upsalgin[5]	330	326 ± 2	328	−0.6
Codaphen[6]	250	247.8 ± 0.4	248	−0.1
Corisidin-D[7]	388.8	383.4 ± 0.9	382	0.4
Dolviran[8]	200	200 ± 3	197	1.5
Kalmaline AC[9]	500	501 ± 5	508	−1.4
B. Salicylamide				
Ilvico-Neo[10]	150	155.0 ± 0.5	156	−0.6
Methyl salicylate		mg ml^{-1}		
Sloan's lotion[11]	—	149.4 ± 0.6	150	−0.4
			mean	0.8

(1) Mean of 4 measurements; (2) FIA — official; (3) + ascorbic acid 240 mg; (4) + glycine 89 mg; (5) + ascorbic acid 200 mg; (6) + phenacetin 250 mg + codeine phosphate 10 mg; (7) + chlorpheniramine maleate 2 mg + phenylephrine 10 mg + caffeine 32.4 mg; (8) + phenacetin 200 mg + codeine phosphate 10 mg + caffeine 50 mg + phenobarbital 25 mg; (9) + caffeine 30 mg; (10) + brompheniramine maleate 3 mg + ascorbic acid 30 mg + propylphenazone 75 mg + caffeine 10 mg; (11) + oleoresin capsicum + turpentine + menthol + eucalyptus + camphor.

Table 6
Recovery of acetylsalicylic acid from formulation sample solutions

Formulation	Acetylsalicylic acid, mg l^{-1} Found	Added	Recovered	Recovery %
Aspirin for child.	47.2	40.0	39.1	97.8
Aspirin	51.5	40.0	39.2	98.0
Aspirin-C	40.1	40.0	39.4	98.5
Neospir-P	47.6	40.0	41.2	103.0
Upsalgin	41.7	40.0	39.2	98.0
Codaphen	45.4	40.0	39.8	99.5
Coricidin-D	52.3	40.0	41.2	103.0
Dolviran	46.2	40.0	40.2	100.5
Kalmaline AC	45.8	40.0	39.4	98.5
			mean	99.6

automated sampling of the dissolution medium at preselected time intervals, collecting the sample in tubes and then measuring the drug content according to the proposed procedure.

Conclusions

The proposed automated FIA method is rapid and flexible for a range of applications (clinical analysis, assays of drugs in formulations, automated dissolution tests on tablets

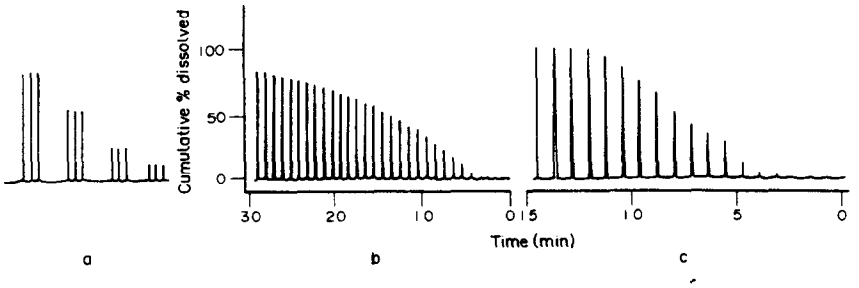

Figure 5
Typical FIA dissolution test for a 150 mg salicylamide tablet. (a) calibration curve with standards: 50, 100, 200 and 300 mg l^{-1}; (b) dissolution in 0.1 M HCl; (c) dissolution in 0.01 M phosphate buffer pH 7.2. The volume of dissolution medium was 400 ml.

containing salicylate and salicylamide). There is promising usefulness for the automated FIA technique in pharmaceutical analysis. Interference is decreased because of the short reaction time.

References

[1] M. Rowland and S. Riegelman, *J. Pharm. Sci.* **51**, 1313–1319 (1968).
[2] G. Levy, *Br. J. Clin. Pharmac.* **10**, 2855–2905 (1980); *ibid, Drug Metab. Rev.* **9**, 3–19 (1979).
[3] M. Mandelli and G. Tognoni, *Clin. Pharmacokinetics* **5**, 424–440 (1980).
[4] E. Mongan, E. Kelly, P. Niesh, W. Porter and H. Paulus, *J. Am. Med. Assoc.* **226**, 142–145 (1973).
[5] A. K. Done, *Pediatrics* **26**, 800–807 (1960).
[6] B. B. Brodie, S. Udenfriend and A. F. Coburn, *J. Pharmac. Exp. Ther.* **80**, 114–117 (1944).
[7] P. Trinder, *Biochem. J.* **57**, 301–303 (1954).
[8] G. Levy and J. A. Procknal, *J. Pharm. Sci.* **57**, 1330–1335 (1968).
[9] N. A. Farid, Q. S. Born, W. V. Kessler, S. M. Shaw and W. E. Lange, *Clin. Chem.* **21**, 1167–1168 (1975).
[10] J. I. Routh, N. A. Shane, E. G. Arredondo and W. D. Paul, *Clin. Chem.* **13**, 734–743 (1967).
[11] P. A. Harris and S. Riegelman, *J. Pharm. Sci.* **56**, 713–716 (1967).
[12] L. J. Walter, D. F. Biggs and R. T. Coutts, *J. Pharm. Sci.* **63**, 1754–1758 (1974).
[13] J. Tischio, *J. Pharm. Sci.* **65**, 1530–1533 (1976).
[14] M. Rowland and S. Riegelman, *J. Pharm. Sci.* **56**, 717–720 (1967).
[15] M. J. Rance, B. J. Jordan and J. D. Nichols, *J. Pharm. Pharmac.* **27**, 425–429 (1975).
[16] B. H. Thomas, G. Solomraj and B. B. Coldwell, *J. Pharm. Pharmac.* **25**, 201–204 (1973).
[17] R. L. Stevenson and C. A. Burtis, *J. Chromatogr.* **61**, 253–261 (1971).
[18] P. P. Ascione and G. P. Chrekian, *J. Pharm. Sci.* **64**, 1029–1035 (1975).
[19] G. W. Peng, M. A. F. Gadalla, V. Smith, A. Peng and W. L. Chiou, *J. Pharm. Sci.* **67**, 710–712 (1978).
[20] E. S. Kang, T. A. Todd, M. T. Capaci, K. Schwenzer and J. J. Jabbour, *Clin. Chem.* **29**, 1012–1014 (1983).
[21] W. Sadee and G. C. M. Beelen, *Drug Level Monitoring*. John Wiley and Sons, New York, 1980.
[22] S. Levy, C. Dioso and B. Nerie, *Clin. Chem.* **25**, 1089 (1979).
[23] T. Higgins, *Clin. Biochem.* **13**, 139–140 (1980).
[24] S. Natelson, in *Micro-Techniques of Clinical Chemistry for the Routine Laboratory*, pp. 73–80. Masson Publishing, USA, NY, 1979.
[25] J. H. Eckfeldt and K. M. Nelson, *Clin. Chem.* **29**, 839–841 (1983).
[26] W. E. Juhl and R. D. Kirshhoefer, *J. Pharm. Sci.* **69**, 544–548 (1980); *ibid* **69**, 548–550 (1980).
[27] K. B. Wrightman, R. E. Barber and R. F. McCadden, in *Automation in Analytical Chemistry*, pp. 12–21. Technicon Symposia 1961, Mediad Inc.
[28] M. S. Kling and J. J. Jennings, Jr., *Drug Autoanalysis Manual*. FDA, 1978, method No. 86.
[29] B. Rocks and C. Riley, *Clin. Chem.* **28**, 409–421 (1982).
[30] C. Riley, *Talanta* **31**, 879–888 (1984).
[31] J. B. Landis, *Pharmaceutical Analysis*. (J. W. Munson, Ed.). Marcel Dekker, New York, 1984.
[32] A. Rios, M. D. De Castro and M. Valcarcel, *J. Pharm. Biomed. Anal.* **3**, 105–121 (1985).
[33] M. A. Koupparis, P. I. Anagnostopoulou and H. V. Malmstadt, *Talanta* **32**, 411–417 (1985).
[34] P. I. Anagnostopoulou and M. A. Koupparis, *J. Pharm. Sci.* **74**, 886–888 (1985).

[35] M. Koupparis, P. Macheras and C. Tsaprounis, *Int. J. Pharm.* **27**, 349–359 (1985).
[36] P. Anagnostopoulou and M. Koupparis, *Anal. Chem.* **58**, 322–326 (1986).
[37] M. A. Koupparis and A. Barcuchova, *Analyst* **111**, 313–318 (1986).
[38] M. Koupparis, P. Macheras and C. Reppas, *Int. J. Pharm.* **19**, 339–344 (1984).
[39] M. Koupparis and P. Anagnostopoulou, *J. Auto. Chem.* **6**, 186–191 (1984).

[*Received for review 28 February 1986; revised manuscript received 8 July 1986*]