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Flow-through UV spectrophotometric sensor for determination of (acetyl)salicylic acid in pharmaceutical preparations

Antonio Ruiz-Medina, Maria L. Fernández-de Córdova, Pilar Ortega-Barrales, Antonio Molina-Díaz *

Department of Physical and Analytical Chemistry, Faculty of Experimental Sciences, University of Jaén, 23071 Jaén, Spain

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Abstract

The solid phase spectrophotometry technique, in which the absorbance of the species of interest sorbed on a solid support is measured directly, was applied to the determination of salicylic acid using flow injection-analysis. Salicylic acid was determined by monitoring of its intrinsic absorbance at 297 nm sorbed on Sephadex QAE A-25 resin placed in an appropriate flow-through cell. The method proposed improves the selectivity compared with the corresponding solution-phase method and the sensitivity is increased by a factor of 30 or more. The flow-through sensor proposed allows working with several calibration lines simply by varying the sample volume injected. Thus, linear dynamic ranges from 1 to 20 and from 2 to 40 μg ml^{-1} can be obtained by using 1000 and 300 μl, respectively, with detection limits being 0.064 and 0.135 μg ml^{-1}. Relative Standard Deviations (RSDs) of 0.52 and 0.38%, and sampling frequencies of 18 and 25 h^{-1}, respectively, were also achieved. The sensor also allows the indirect determination of acetylsalicylic acid previous hydrolysis on-line to salicylic acid. For acetylsalicylic acid, a linear dynamic range from 5 to 120 μg ml^{-1} and 25 h^{-1} of sampling frequency (300 μl of sample volume) were obtained. The proposed flow-through sensor has been successfully applied to the determination of both analytes in pharmaceutical preparations.

Keywords: Salicylic acid; Acetylsalicylic acid; Solid phase spectrophotometry; FIA; Pharmaceuticals

1. Introduction

In low concentrations, salicylic acid (SA) has keratoplastic activity, and in higher ones, it has keratolytic activity; SA has also a slight antiseptic action when it is applied topically to the skin. AcetylSA. (ASA) exhibits analgesic, anti-inflammatory, and antipyretic activity.

The large number of published methods for the determination of SA or ASA in biological fluids and pharmaceuticals, which make use of a large variety of analytical techniques, is not only indica-

* Corresponding author. Tel.: +34-953-012147; fax: +34-953-012141.

E-mail address: amolina@ujaen.es (A. Molina-Díaz).

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tive of the great interest in the determination of these compounds, but also of the problems encountered with their determination with regard to directness, specificity, simplicity, sensitivity, etc. The development of a simple, specific, sensitive and inexpensive method for the determination of SA or ASA, as an alternative to chromatographic techniques, would be highly advantageous.

A lot of batch and automated methods have been reported for the determination of SA such as: spectrophotometric (Glombitza and Schmidt, 1994; López Fernández et al., 1990; Saha and Baksi, 1985; Trinder, 1954), spectrofluorimetric (Graham and Rowland, 1972; Muñoz de la Peña et al., 1988; Villari et al., 1994), liquid membrane electrodes (Choi and Fung, 1982; Hassan and Hamada, 1988), immunoassays (Hendeles and Edwards, 1988) and amperometric (Neumayr et al., 1993) methods. Chromatographic methods have also been used for the simultaneous determination of SA and ASA. Liquid-chromatographic methods (Carlson and Thompson, 1987; Kees et al., 1996) have displaced gas–liquid chromatographic methods (Galante et al., 1981), where a previous chemical derivatization and a separation to avoid interferences of the additives with the chemical derivatization are required. With these chromatographic techniques, separation of the pharmaceuticals from tablet additives (excipients and antacids) is essential. ASA may be tested directly (Carlson and Thompson, 1987; Kees et al., 1996) or, more commonly, as SA (López Fernández et al., 1990; Villari et al., 1994).

Solid phase spectrophotometry (Ortega Barrales et al., 1998a) (SPS) is a technique that has been applied to the determination of both inorganic and organic analytes. The integration of SPS technique with flow injection analysis (FIA) (Ayora Cañada et al., 1999; Molina Díaz et al., 1999; Ortega Barrales et al., 1998b; Yoshimura, 1987) combines the advantages of this latter (automation, rapidity and low consumption of reagents) with those of SPS (high sensitivity and selectivity, as a consequence of the sorption of the analyte on the solid support). One of the most interesting cases of this integration occurs when the carrier itself elutes the species sorbed from the solid support, thus originating a transient retention of this and, consequently, a transient signal so increasing both the throughput and the solid support's lifetime (Molina Díaz et al., 1999; Ruiz Medina et al., 2000).

This paper reports the determination of SA, and also that indirect by of ASA, in pharmaceuticals by using an integrated SPS–FIA system. SA shows an intense light absorption when it is sorbed on an ion-exchanger (Sephadex QAE-A25) placed into an appropriate flow-through cell. ASA could also be determined directly by use of its absorption fixed on this solid support, although its sensitivity is lower. It is, therefore, measured indirectly after on-line hydrolysis to SA and injection in the flow-through solid phase spectrophotometric sensing device.

2. Experimental

2.1. Apparatus

Spectra and UV measurements were made with an UV-Vis Lambda 2 (Perkin–Elmer) spectrophotometer. A 386 personal computer (PC) connected by means of a serial port and fitted with the PECSS V.5.0 software package (from Perkin–Elmer) was used for controlling the Lambda 2, storing the spectra, and performing the required calculations. HP DeskJet 690C printer was used for obtaining absorbance–wavelength graphs and spectra were recorded at a scanning speed of 240 nm/min. A four-channel Gilson Minipuls-3 peristaltic pump provided with a rate selector and three six-port rotatory Rheodyne 5041 injection valves (two of them connected as selection valves) were also used. The single-line manifold for the FI measurements was employed with 0.8 mm i.d. PTFE tubing. A Hellma 138-QS flow-through cell (1-mm optical path length, 50-μl inner volume), packed with the ion exchanger as the active microzone, was used (Fig. 1a). The cell was blocked with some glass wool to prevent outflow of the ion exchanger. A digital Crison model 2002 pH-meter fitted with a glass/saturated calomel electrode assembly and a temperature probe was used for all pH measurements.

2.2. Chemicals

Unless stated otherwise, all reagents used were of analytical grade. Doubly distilled water was used throughout.

2.2.1. SA and ASA standard solutions

A stock standard solution of 1000 μg ml^{-1} of the pure analytical generic form of SA or ASA, supplied by FLUKA, was prepared by directly dissolving the drug in water. These solutions were stored at 4°C. At this temperature, decomposition of ASA to SA was less than 2% within one month, and SA was stable for at least 2 months. Working standard solutions were prepared daily by appropriate dilutions of the stock standard solutions (with doubly distilled water).

Sodium chloride, tartrate, nitrate, sulphate and phosphate (from PANREAC) were used as 0.08 mol l^{-1} solutions at pH 10 (adjusting with NaOH). A 2 mol l^{-1} NaOH aqueous solution was also used to hydrolyze ASA. The carrier used in the experiments was NaOH (10^{-4} mol l^{-1})/NaCl (0.08 mol l^{-1}) solution. The eluting solution consisted of the same carrier solution. Sephadex QAE-A25 (ALDRICH) was used in the Cl^- form as the solid support without any pretreatment.

2.3. Sample preparation

Tablets or solutions of SA or ASA composite were directly dissolved in doubly distilled water with shaking for 10 min in an ultrasonic instrument. The solution was filtered through a 0.45 μm pore size Millipore membrane filter, and the filtrate plus washings were diluted to the mark in a 100 ml calibrated flask. Appropriate dilutions were made from this solution.

2.4. Procedure and manifold

Before using the flow system, it was purged with doubly distilled water and after this, with carrier solution. The system consisted of a single channel FIA configuration (Fig. 1b) in which the carrier solution NaOH (10^{-4} mol l^{-1})/NaCl (0.08 mol l^{-1}) was propelled. A sample solution containing SA or ASA was introduced into the system flowing at a constant flow rate of 1.20 ml min^{-1}. By actuating the selection valves SV_I and SV_{II} it is possible either to directly insert the sample stream into the carrier solution by means of the injection valve IV (direct determination of SA) or previously to merge with a 2 mol l^{-1} NaOH solution stream (to favor hydrolysis of ASA along the reactor (R), which was immersed in a thermostatic bath at 60°C; indirect determination of ASA). SA was retained on the anionic Sephadex QAE-A25 resin, its intrinsic absorbance (peak height) was measured continuously at 297 nm and it was eluted from the flow cell with the carrier solution itself after developing the analytical signal. When the recorder came back to the baseline, the next sample was then injected and determined as before. Calibration lines were con-

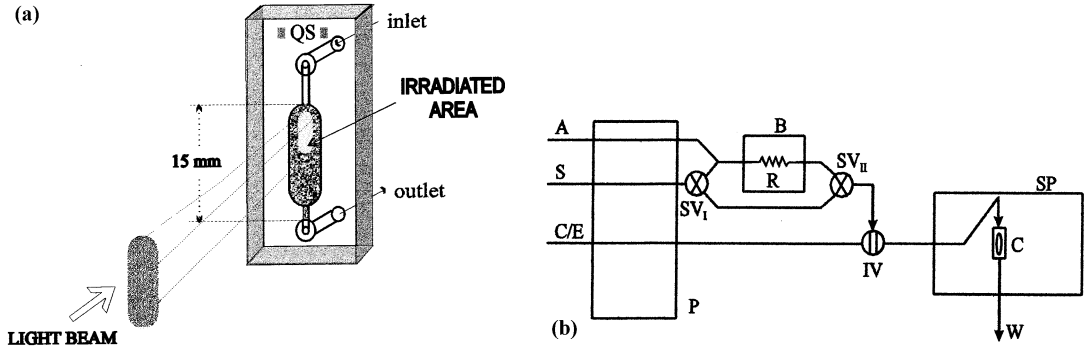

Fig. 1. (a) Flow-through cell. (b) Schematic diagram of the FI system for determination of SA or ASA. C/E: carrier/eluting solution; P: peristaltic pump; IV: injection valve; SV_I and SV_{II}: selection valves; S: sample; SP: spectrophotometric detector; C: flow cell; W: waste; R: reactor; B: thermostatic bath; A: 2 mol l^{-1} NaOH solution. Flow-rate: 1.20 ml min^{-1}.

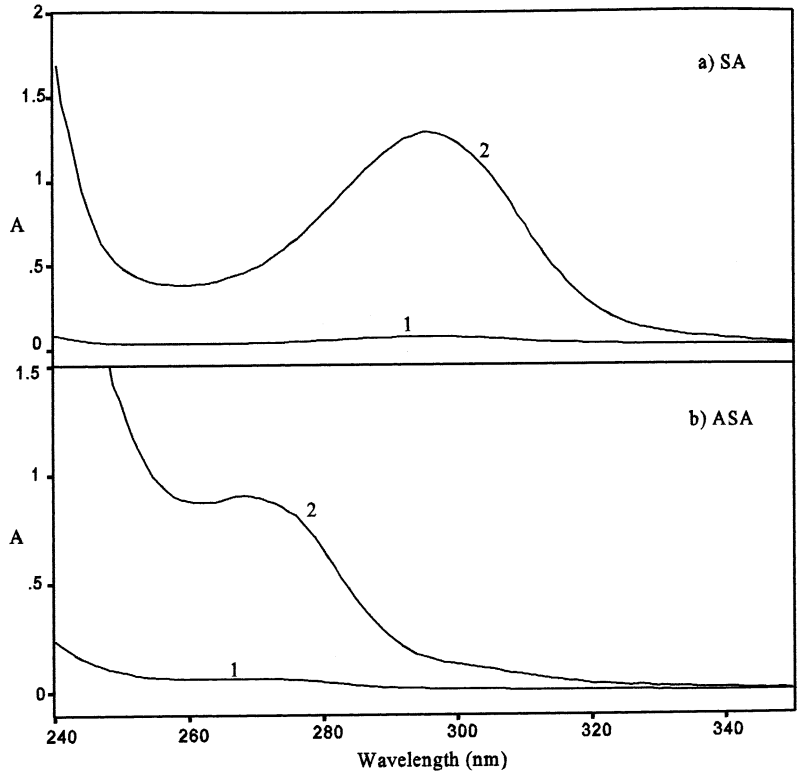

Fig. 2. Absorption spectra of SA (a) and ASA (b) in solution flow (1) and sorbed on Sephadex QAE A-25 (2). 25 mg l^{-1} SA; 200 mg l^{-1} ASA; 1000 μl of injected sample; 1-mm optical path length.

structed by injecting standard solutions of SA and ASA as above indicated.

3. Results and discussion

3.1. Flow-through cell and solid support

Two main aims must be pursued in the design of a suitable cell for a flow-through sensor: (1) the concentration of the monitored product on the support in an area of the cell as small as possible of that; (2) the incident light beam must be focused on this area without loss of light to the surrounding zone. Typical variables of the flow-through sensor, such as the type of cell and the type of support in the flow cell were optimized. In previous works (Molina Díaz et al., 1999; Ortega Barrales et al., 1998a, b) we found a Hellma 138-QS cell of 1-mm optical path to be the most appropriate cell. The level of solid support in the cell was as necessary to fill it up to a sufficient height, permitting the light beam to pass completely through the solid layer (15 mm) (Fig. 1a). Several ion exchange and gel filtration resins were tested: Sephadex QAE-A25, DEAE-25, Dowex 1 × 8, G-15 and G-75. Of all them, Sephadex QAE-A25 gave the best results, and fixation was quicker.

In order to obtain high sensitivity in on-line detection, the analyte to be determined should be retained on the resin and have a high molar absorptivity. The sensitivity improvement achieved by using the proposed method was clear from the early steps of method development. To check for similarities between the spectra of the dissolved and retained products, the normal FIA manifold (solution-only method) required analyte concentrations 25 times higher to obtain recordings comparable to those provided by the proposed flow-through sensor: Fig. 2 shows the spectra of SA and ASA in both

solution flow alone (flow cell without packing) and sorbed on the solid phase. This drastic improvement in sensitivity is a remarkable feature of the sensor and it is a result of the transient concentration of the analyte on the resin beads in the detection area of the spectrophotometer. No appreciable change was found in the absorption maxima of the spectra (297 nm for SA and 270 nm for ASA) of the analytes in homogeneous solution and sorbed on the anion exchanger Sephadex QAE-A25.

The absorbance (really attenuation), at the working wavelength on the solid support, A, consists of several components:

$$A = A_A + A_R + A_S$$

where A_A is the absorbance of the analyte sorbed on the resin, A_R is the absorbance of the background ($A_R \approx 1.3$), and A_S, that of the interstitial solution between the resin beads (which can be neglected as compared with the other terms). The packing of the resin beads in the flow cell affects the values of A_A and A_R, but when the system is flowing for a few seconds, the packing keeps constant, so the baseline shows a constant value equal to $A_R + A_S \approx A_R$. Therefore, the analytical peak, A_A, corresponds to the difference between A and $A_R + A_S$. In this way, in the flow-through solid phase spectrophotometric system, the analytical signal can be obtained directly by measuring at one only wavelength and successive measurements are performed on the same resin packing. However, in SPS batch mode each measurement is performed on a different resin batch and in each case, the packing is also different, so rendering no reproducible measurements when they are performed at one only wavelength (Ortega Barrales et al., 1998a). For this reason, measurements at two different wavelengths are needed in SPS in batch mode (one at the absorption maximum of the species of interest, and another at the wavelength where only the resin absorbs light).

The net intrinsic absorbance of the analyte sorbed, A_A, is given by $A_A = \varepsilon_R l_R C_R$, where ε_R is the apparent molar absorptivity of the analyte in the ion exchanger phase as observed in the flow-through system (kg mole^{-1} cm^{-1}), l_R the mean light path length through the resin layer which can be supposed equal to 0.1 cm (although it usually will not be exactly this value) and, C_R, the analyte concentration in the solid phase. When V (ml) of a sample at concentration C_o (mol/l) of analyte is injected in the system, supposing a high value of the distribution ratio (as it is usual) the concentration on the solid phase (C_R, mol/kg) will be:

$$C_R = C_o \times 10^{-3}\ V/m_r$$

where m_r is the mass of resin (kg) in which the analyte is retained. Therefore:

$$A_A = \varepsilon_R l_R V C_o/m_r \quad (1)$$

where m_r is expressed in g, so keeping V constant. As can be seen, there is a linear relationship between the analytical signal and the initial concentration, C_o, of the analyte in the injected solution; $\varepsilon_R l_R V/m_r$ is the slope of the calibration line.

An important feature of these flow-through sensing devices is derived from Eq. (1): keeping C_o constant, A_A increases as V increases. So a linear relationship between A_A and the injected sample volume could be expected, that is, sensitivity is proportional to the sample volume used for analysis and it can easily be increased just by increasing the sample volume.

3.2. Nature of the carrier

Small signals were obtained when only water was used as carrier. The pH of the carrier solution was studied in the range 2–12 by adjusting it with HCl or NaOH (Fig. 3). A basic medium might favor the retention and detection steps because of the deprotonation of carboxylic and hydroxyl groups; so, suitable conditions were obtained between pH 8 and 11. It was decided to work at pH 10. The efficiency of elution of the analyte from the resin increased with increasing concentration in an electrolyte in the carrier. Several electrolytes (sodium salts of chloride, nitrate, tartrate, phosphate and sulphate) were tested, and the best results were obtained by using sodium chloride. Its concentration was tested between 0.02–0.15 mol l^{-1} (inset Fig. 3). A 0.08 mol l^{-1} NaCl

solution was chosen as a compromise solution between obtaining a decrease in the response of the sensor or smaller peak width time than that with high concentrations. Thus, a NaOH (10^{-4} mol l^{-1})/ NaCl (0.08 mol l^{-1}) solution was used as carrier/eluting solution. The sample pH value did no influence the analytical signal when its value was maintained in the 3–11 range, and hence, there is no need to adjust the sample pH.

3.3. Hydrolysis of ASA

ASA solutions undergo hydrolysis, and this process is accelerated in an alkaline medium. The hydrolysis was performed by merging the sample stream (ASA) with a 2 mol l^{-1} NaOH solution stream, the hydrolysis taking place along the reactor coil (R). The length of this latter must be as short as possible in order to minimize the dispersion of the sample. Lengths from 0.5 to 5 m were tested and a 3.5 m reactor was found to be the most appropriate. This reactor was immersed into a thermostatic bath (60°C) to complete the hydrolysis. The analytical signal decreased below 60°C probably because of hydrolysis was only partial.

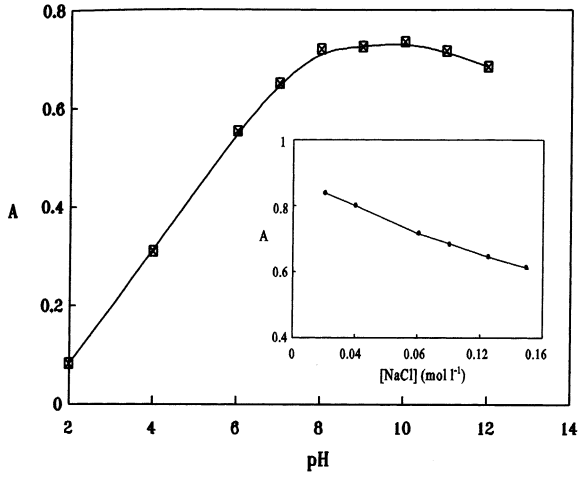

Fig. 3. Influence of the pH of the carrier solution. Inset: Influence of the NaCl concentration in the carrier solution (pH of the carrier solution: 10). SA 20 μg ml^{-1}; flow rate 1.20 ml min^{-1}; 600 μl sample volume.

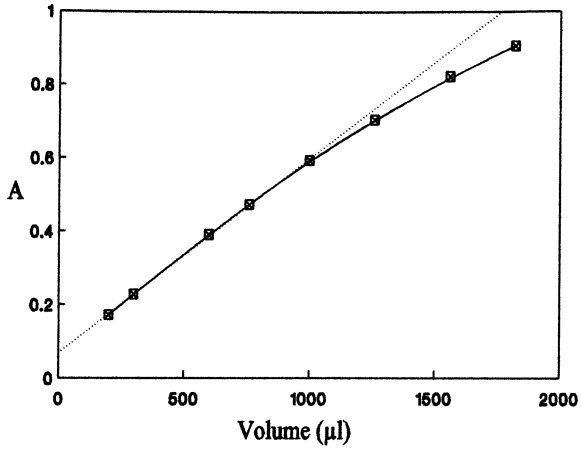

Fig. 4. Influence of the sample volume in increasing order: 200, 300, 600, 800, 1000, 1300, 1600, and 1900 μl. SA 10 μg ml^{-1}; flow rate 1.20 ml min^{-1}.

3.4. Flow injection variables

The FI variables studied were the sample volume, which influenced both the sensitivity and the sampling rate of the method, and the flow-rate, which determines the rate at which the analyte can be eluted, and hence, also the sampling frequency.

The influence of flow-rate on the measurement of a 20 μg ml^{-1} SA solution (300 μl of sample) was studied. A change in this variable from 0.57 to 1.93 ml min^{-1} decreased the sensitivity by 27%. Nevertheless, very low flow-rates were incompatible with short residence times and rapid baseline restoration; thus, in all subsequent experiments, the total flow-rate was maintained at 1.20 ml min^{-1}.

Fig. 4 shows that A (absorbance) increases linearly with an increase in the sample volume from 200 to 1000 μl (at the same concentration of SA, 10 μg ml^{-1}); A is expressed as a empirical function of the sample volume V (μl): $A = 0.065 + 5.29 \times 10^{-4}\ V$ (correlation coefficient = 0.9998). This was theoretically shown in Section 3.1 The rate of increase is not linear for a sample volume greater than 1000 μl. This is usual because the central zone pH value of the sample plug does not allow all the analyte to be completely retained on the resin. As large sample volumes resulted in

decreasing sampling frequency but increased sensitivity, injection volumes of 300, 600 and 1000 μl were selected for SA.

3.5. Features of the proposed method

Individual calibration curves were run from standards of each analyte (SA or ASA). The equations of the calibration lines obtained for SA (300, 600 and 1000 μl), the linear concentration ranges and regression coefficients, precision (expressed as RSD% for ten separately prepared samples), and sampling frequencies are listed in Table 1. It should be emphasized that the proposed method shows a very good sensitivity in spite of its UV nature.

Under the optimum working conditions, ASA standards of concentrations between 5 and 120 μg ml^{-1} were injected (300 μl) into the proposed manifold. The calibration graph was linear over this range. The equation of the calibration line obtained was $A = 0.023 + 8{\cdot}1 \times 10^{-3}$ [ASA] (μg ml^{-1}), with a regression coefficient larger than 0.999. It should be noticed that the slope of the calibration line is lower than that obtained for SA calibration. This could be attributed to the dilution of the sample by mixing it with the NaOH solution stream, in addition to the influence of the ionic strength supplied by the latter. The reproducibility found for ten independent samples of ASA, with a concentration of 60 μg ml^{-1} injected in triplicate, was 0.41% and the detection limit was 0.321 μg ml^{-1}. The reproducibility is also very high taking into account that the measurements are made in a solid phase. The sampling frequency was 25 h^{-1}.

Table 1
Analytical features for the determination of SA

Parameter	Volume of sample loop (μl)		
	300	600	1000
Linear dynamic range (μg ml^{-1})	2–40	1.5–28	1–20
Calibration graph			
Intercept	0.021	0.029	0.037
Slope (ml μg^{-1})	0.022	0.035	0.051
Correlation coefficient	0.999	0.999	0.999
Detection limit (μg ml^{-1})	0.135	0.083	0.064
RSD (%) ($n = 10$)	0.38[a]	0.47[b]	0.52[c]
Sampling frequency (h^{-1})	25	22	18

[a] [SA] = 30 μg ml^{-1}.
[b] [SA] = 20 μg ml^{-1}.
[c] [SA] = 10 μg ml^{-1}.

Table 2
Study of interferences (determination of 10 μg ml^{-1} of SA)

Foreign species	Interference/analyte Ratio (w/w) tolerated
Glucose, saccharose, boric acid, codeine, caffeine, nicotinamide, undecylenic acid, Zn(II)	>100
Lactic acid	50
Camphor	10
Betamethasone	5
Paracetamol, ascorbic acid, saccharin, Cu (II)	1
Benzocaine	0.5

3.6. Influence of foreign species

Table 2 shows the effect of foreign species usually accompanying SA. A relative error equal or less than 3% was considered to be within the range of experimental error for such a low sample concentration. Maximum ratio interference/analyte tested was 100 (w/w). The determination of SA and ASA by conventional UV spectrophotometry is not possible in the presence of species such as codeine, caffeine and nicotinamide, commonly found along with them in pharmaceuticals, due to the extensive spectral overlap. However, the tolerance to these species in the proposed method is drastically increased by the selective fixation of the analytes on the solid support in the cell. The most serious interference is due to ascorbic acid, saccharin, paracetamol, Cu (II) and benzocaine, but it is necessary to point out that the tolerance to these species is higher than the amounts of these usually accompanying SA and ASA in pharmaceuticals.

3.7. Application of the method

In order to check the applicability of the proposed method for SA and ASA, it was used to analyze various pharmaceutical preparations. The results obtained are summarized in Tables 3 and 4, respectively. The samples were injected in triplicate into the FI system after being dissolved as described under sample preparation. The determination of both compounds was made by using a 300 μl sample volume injection. As can be seen, in all the cases the data were in good agreement with the labeled amounts.

The accuracy of the proposed method was checked with a recovery study of various amounts of SA and ASA added to the respective pharmaceuticals. The results are shown in Tables 3 and 4, respectively.

4. Conclusions

For both SA and ASA, the FI-spectrophotometric determination in the solid phase offers a number of advantages as compared with the techniques used at present. This SPS–FIA integrated method is a significant step forward owing to the urgent need for simple, rapid, automated and more sensitive methods for these analytes. The proposed method has a lot of advantages over other methods such as: (a) high selectivity and sensitivity (as a consequence of the sorption of the analyte on the resin), (b) neither a previous separation step nor a chemical derivatization procedure are required, so increasing the sampling frequency and making it extraordinarily simple and inexpensive; and (c) it is a more precise, more rapid and less expensive alternative to HPLC methods for the determination of SA or ASA in the presence of a not very complex matrix.

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Table 3
Determination of SA in pharmaceutical preparations

Sample[a]	SA amount labelled (mg)	SA amount added (mg)	Recovery mean ± RSD[b] (%)
Callix-D (Pérez Giménez, Ltd.)	150[d]	–[d]	98 ± 2
		20[d]	99.2 ± 0.8
		40[d]	98.5 ± 0.9
Isdín Antiverrugas[c] (Isdín, Ltd.)	167[e]	–[e]	102 ± 2
		20[e]	99 ± 1
		40[e]	99.2 ± 0.9
Acnosán (Bescansa, Ltd.)	1200[e]	–[e]	101 ± 1
		100[e]	101.1 ± 0.6
		200[e]	100.3 ± 0.5
Diprosalic (S. Plough, Ltd.)	30[e]	–[e]	100.1 ± 0.6
		3[e]	100.1 ± 0.7
		5[e]	99 ± 1

[a] Other species accompanying: Callix-D: Lactic acid 100 mg, Benzocaine 33.3 mg, Collodion flexible q.s.; Isdín Antiverrugas: Lactic acid 167 mg, Collodion flexible q.s.; Acnosán: Undecylenic acid 8.75 g, Zn sulphate 0.13 g, Boric acid 6.45 g, Resorcinol 1.94 g, Cu sulphate 0.13 g; Diprosalic: Betamethasone 0.5 mg.

[b] Mean of three determinations.

[c] Standard addition calibration graph method: 5, 10, 20 and 30 μg ml^{-1} were added for a 10 μg ml^{-1} sample solution.

[d] Per g.

[e] Per l.

Table 4
Determination of ASA in pharmaceutical preparations

Sample[a]	ASA amount labelled (mg)[b]	ASA amount added (mg)[b]	Recovery mean ± RSD[c] (%)
Dolvirán (Bayer, Ltd.)	400	–	100 ± 1
		40	100 ± 1
		60	101 ± 1
Aspirina (Bayer, Ltd.)	500	–	99.2 ± 0.9
		50	100 ± 1
		80	101 ± 1
Veganín (Parke-Davis, Ltd.)	250	–	100.1 ± 0.9
		25	100.2 ± 0.9
		50	100.9 ± 0.9
Dolmen (J. Uriach, Lab.)	500	–	98 ± 1
		50	99.5 ± 0.5
		80	99.8 ± 0.6

[a] Other species accompanying: Dolvirán: Codeine 9.6 mg, Caffeine 50 mg; Veganin: Codeine 10 mg, Paracetamol 250 mg; Dolmen: Codeine 10 mg, Ascorbic acid 250 mg, Saccharin 15 mg.
[b] Per tablet.
[c] Mean of three determinations.

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