Acetylsalicylic Acid Determination in Remedies Using a Gas-Diffusion Flow Injection Analysis - II[+]

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Abstract

In this work is proposed a very simple flow injection method for the analysis of acetylsalicylic acid (**asa**) in pharmaceutical preparations. The method is based on the **asa** alkaline hydrolysis forming a solution containing acetate and salicylate. This solution is mixed with a sulfuric acid solution. The acetic acid formed permeates through a PTFE membrane to a deionized water stream presenting less than 20 micro Siemens of conductance. The change in conductance was registered in a chart recorder. Six pharmaceutical preparations purchased in the local market were analyzed. The obtained results were compared with those obtained with the Pharmacopoeia titrimetric method. The statistical *t*-Student test was applied to compare the results obtained with the two independent methods. In all cases complete agreement was observed, in a confidence level of 95% (α=0.05). Considering six samples analyzed using the proposed method and three determinations for each sample, the observed mean RSD, is 1.1 %. The limit of detection is about 2×10^{-4} mol l^{-1} (3×SD) and the analytical frequency is 60 determinations per hour.

Keywords: flow injection analysis; acetylsalicylic acid; pharmaceutical preparations; conductivity; remedies

1. Introduction

The acetylsalicylic acid (**asa**) is a medicine with anti-inflammatory, analgesic and anti-pyretic properties. Beyond these properties, **asa** has been used to treat and to prevent circulatory and cardiac problems [1-3]. Despite the fact that it had been introduced in the clinic more than 100 years ago, it is still used mainly due to its efficiency and to the reduced collateral effects [4].

Since the years 1940 [5], several methods have been proposed for **asa** and salicylates quantitative analysis in pharmaceutical preparations and in biological samples [6]. Today there are several instrumental methods for this dosage. Among them, spectrophotometric [7-9], potenciometric [10-12], chromatographic [13-15], enzymatic [16-20], FIA spectrophotometric [21], reflectometric [22].

Despite this variety of methods it is clear that new ones can be envisaged, mainly those that use simplified and low cost procedures. Therefore, the method described in this work implies in a very simple and low cost procedure for the analysis of **asa** in pharmaceutical preparations, allied to the rapidity and functionality of the flow injection technique. The analyte is hydrolyzed with a sodium hydroxide solution. The acetate obtained in this reaction is transformed in acetic acid by the addition of a sulfuric acid solution. Then the acetic acid formed permeates through a PTFE membrane in a deionized water stream, being detected conductimetrically. The scheme of this very simple FIA system can be see in Fig. 1. The analytical results are similar to those obtained with the titrimetric Pharmacopoeia [23] method.

2. Experimental

2.1. Reagents

All reagents used in this work were of analytical grade. The water was distilled in a glass distiller and then deionized in a Milli Q Plus device. It was also boiled to eliminate carbon dioxide and kept in a closed glass bottle in order to avoid the absorption of CO_2.

The pharmaceutical preparations were purchased in the local market.

Sodium hydroxide 0.5 mol l^{-1} – This solution was daily prepared. 20 g of NaOH were dissolved in 1.0 litre of water recently boiled and carefully cooled.

Sulfuric acid solution 0.2 mol l^{-1} – 20 g (11 ml) of 98% sulfuric acid were dissolved in water to complete 1.0 litre.

Standard acetylsalicylic acid solutions – 0.0180 g, 0.0500 g, 0.1000 g, 0.2000 g, 0.3000 g, 0.4000 g and 0.5000 g of the acid were dissolved in 20.0 ml of the 0.5 mol l^{-1} sodium hydroxide and the volume was completed to 100.0 ml in order to obtain the respective concentrations 1.00×10^{-3} mol l^{-1}, 2.78×10^{-3} mol l^{-1}, 5.55×10^{-3} mol l^{-1}, 1.11×10^{-2} mol l^{-1}, 1.67×10^{-2} mol l^{-1}, 2.22×10^{-2} mol l^{-1} and 2.78×10^{-2} mol l^{-1}.

The deionized water of the acceptor stream, which conductance was always below 20 micro Siemens, was protected in a glass bottle from the CO_2 of the air. The respite of the bottle was constituted of a polyethylene tube (i.d. 1.5 mm) linked to a tube (i.d. 2 cm) containing NaOH (in order to retain the air carbon dioxide) sandwiched between two layers of $CaCl_2$, in both extremities, to protect the sodium hydroxide from the humidity.

[+] For paper I see ref. 7.

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2.2. Apparatus

The FIA system shown in Fig.1, is constituted by the following parts.- *Gas diffusion cell* (**DC**): Similar to the cell that has been described by van der Linden [24]. *Sampling valve* (**V**): Has been previously described [25-26]. *Sampling inlet* (**S**): 20 cm of a polyethylene tube with internal diameter equal to 1 mm. T-*form mixer* (**T**) made in PTFE. *Reaction coil* (**B**): 50 cm of a polyethylene tube with internal diameter equal to 1 mm. *Peristaltic pump* (**P**): Ismatec mp13 GJ4. *Conductimeter*: Micronal model B-331. *Conductimetric flow cell*: Has been previously described [27]. *Chart recorder* (**CR**): Barnstead-Thermoline, model LR92425.*Pumping tubes*: Ismatec two-stop tubes, blue-blue, Tygon®, internal diameter 1.65 mm. *Conducting tubes*: Polyethylene tubes, internal diameter 1 mm. *Membrane*: Commercial polytetrafluoroethylene, PTFE, tape. Stream of deionized water ($\mathbf{A_1}$). Stream of the aqueous 0.2 mol l^{-1} sulfuric acid solution ($\mathbf{A_2}$). Stream of deionized water, carrier of the sample ($\mathbf{A_3}$). Waste (**W**).

2.3. Calibration curves

Calibration curves were performed using acetylsalicylic acid dissolved in deionized water. These solutions, in the range from 2.78×10^{-3} mol l^{-1} to 2.78×10^{-2} mol l^{-1}, are above described as standard acetylsalicylic acid solutions.

2.4. Preparation of the samples

Sample treatment – the tablets were triturated and dissolved in about 20 ml of the 0.5 mol l^{-1} NaOH solution at room temperature. The volume was completed to 50.0 or 100.0 ml volumetric flasks according to the desired final concentration considering the range of the calibration curve.

2.5. Measurements

The conductimetric measurements were performed in the 200 micro Siemens scale (1.0 cm = 6.4 micro Siemens) and registered on the chart recorder. Peak heights in centimetres, measured to ± 0.05, were treated statistically.

2.6. The proposed method

The aliquot containing the analyte were placed in a small beaker. Some milliliters (20 ml) of a 0.5 mol l^{-1} NaOH solution were added and the whole transfered to a volumetric flask. The volume was completed with water. Based on the nominal **asa** content of medicine, the final concentration was estimated to be in the working range of the calibration curve. A small volume of this solution was introduced in the flow system through the valve **V**, as shown in Fig. 1. There is a merging zone in **T** with a sulfuric acid solution, forming acetic acid. This acid permeates through the membrane in the cell diffusion, **CD**, to the deionized water flow, $\mathbf{A_1}$, which passes through the conductimetric cell, **C**, where the conductance of the solution is measured and registered in the chart recorder, **CR**.

2.7. The Pharmacopoeia method

The results obtained with this method were compared with those obtained with the Pharmacopoeia [23] procedure in which 1.5 g of the medicine is treated with 50.0 ml of a 0.5 mol l^{-1} NaOH solution, heated to the boiling temperature where it must remain during 10 minutes. The solution is then cooled and titrated, with a 0.25 mol l^{-1} H_2SO_4 solution.

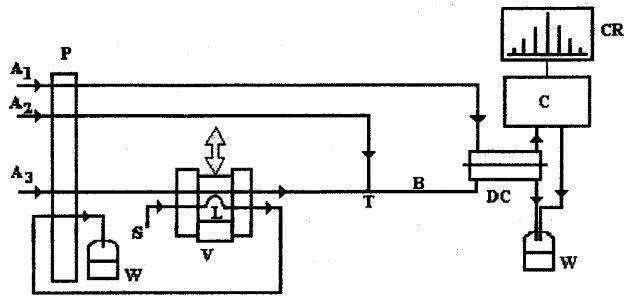

Fig.1 Flow injection system. $\mathbf{A_1}$- deionized water; $\mathbf{A_2}$- 0.2 mol l^{-1} sulfuric acid solution; $\mathbf{A_3}$- deionized water; **CR**- chart recorder; **C**- conductimetric cell and conductimeter; **B**- (reaction coil) distance from the **T**-form mixer to the diffusion cell; **DC**- diffusion cell; **L**- sampling loop; **P**- peristaltic pump; **S**- sampling inlet; **V**- sample valve system; **W**- waste. Total flow rate = 1.8 ml min^{-1} with $\mathbf{A_1} = \mathbf{A_2} = \mathbf{A_3}$.

3. Results and discussion

In the proposed method, **asa** determination was based on the conductimetric detection of the acetic acid that permeates the PTFE membrane in the diffusion cell. This acetic acid is formed by the action of the sulfuric acid on the acetate generated in the alkaline **asa** hydrolysis.

In order to optimize the method, the influence of the sampling volume, of the total flow rate, of the sulfuric acid concentration and of length of the reaction coil, **B**, where the sulfuric acid solution is merged with the sample solution, were studied.

The influence of the concentration of the sodium hydroxide solution, used to hydrolyze the sample, was studied from 0.1 mol l^{-1} to 1.5 mol l^{-1}. The signal height is not very much affected by the hydroxide concentration in the studied range. However that correspondent to the 0.5 mol l^{-1} NaOH solution is the highest among all and 38% higher than the lower one that corresponds to the 0.1 mol l^{-1} sodium hydroxide concentration.

The influence of the sulfuric acid concentration was studied from 0 mol l^{-1} to 2.0 mol l^{-1}. The signal height rapidly increases until the acid concentration *ca.* 0.2 mol l^{-1}. Beyond this concentration the signal increasing is smaller. Considering that at 0.2 mol l^{-1} the signal presents a good intensity and that above this concentration there is not a great signal increase, this concentration was selected.

The influence of the sample volume was studied from 100 micro litters to 350 micro litters. The signal height increases almost linearly in this range. Considering that the intensity of the signal correspondent to 150 micro litters is quite satisfactory and that smaller volumes imply also in higher analytical frequencies, this volume was selected to perform this work.

The length of the reaction bobbin, **B** in Fig. 1, was varied from 15 cm to 100 cm. The intensity of the signal varied little with the length increase and the 50 cm bobbin was selected due to practical convenience.

The rates of the flows $\mathbf{A_1}$, $\mathbf{A_2}$ and $\mathbf{A_3}$ were maintained equal among them. The effect of the total flow rate, on the acetylsalicylic acid determination, was examined in the range from 1.2 ml min^{-1} to 13.2 ml min^{-1}. The signal height decreases rapidly until the flow *ca.* 2 ml min^{-1} remaining almost constant beyond this. The analytical signal correspondent to the total flow of 5.4 ml min^{-1} had been considered quite satisfactory and was used in this work.

Obeying the above established conditions, *i.e.*, 0.5 mol l^{-1} sodium hydroxide solution, total flow rate of 5.4 ml min^{-1}, sulfuric acid concentration of 0.2 mol l^{-1} and sample volume of 150 micro litters, the calibration curve was obtained in the range from 0 mol l^{-1} to 0.028 mol l^{-1}. This curve is described by the equation, $\mathbf{h} = -0.34 + 11.86 \times 10^2\ \mathbf{C} - 1.51 \times 10^4\ \mathbf{C}^2$ (R=0.99996), where **h** is the signal height in centimetres (1.0 cm = 6.4 micro Siemens) and **C** is the acetylsalicylic acid concentration in the aliquot introduced in the flow system, in mol l^{-1}. This curve was applied to the analysis of the acetylsalicylic acid contained in six different solid remedies.

Table 1 summarizes the analytical results for the remedies using the proposed method. These results are compared, through *t*-test [28], with the concentrations found using the Pharmacopoeia titrimetric procedure [23]. It can be observed a complete agreement between the two methods under the degree of freedom $\nu=4$ ($n_1 = n_2 = 3$; $\nu = n_1 + n_2 - 2 = 4$) and at the confidence coefficient $(1 - \alpha) = 0.95$ (95% confidence level). Therefore it can be concluded that the results obtained with the two analytical methods are statistically identical.

The simplicity of the proposed method becomes clear when it is compared with the recommended by Pharmacopoeia that uses alkaline hydrolysis of the acetylsalicylic acid during 10 minutes under heating, followed by titration of the excess of the added hydroxide with an H_2SO_4 standard solution, after cooling of the hydrolyzed solution to room temperature.

Table 1. Comparison using the statistical Student's *t*-test between the results obtained by the proposed conductimetric FIA method and by the titrimetric analysis recommended by Pharmacopoeia [23]. Tabled $t = 2.78$ for the degree of freedom $\nu = 4$ ($n_1 = n_2 = 3$; $\nu=n_1 + n_2 - 2 = 4$) and at confidence coefficient $(1 - \alpha) = 0.95$ (95% confidence level).

Sample	Nominal content / (mg)	Proposed method / (mg) ± SD	Pharmacopoeia method / (mg) ± SD	Calculated *Student's* *t* values
1	100[a]	108.3 ± 1.8	107.7 ± 0.5	0.45
2	200[b]	209.1 ± 1.8	209.2 ± 0.5	0.08
3	500	567.0 ± 2.1	565.3 ± 1.0	1.03
4	500	541.3 ± 4.3	539.2 ± 1.0	0.67
5	500[c]	567 ± 11	564.5 ± 1.3	0.32
6	500	568.4 ± 4.7	568.2 ± 1.3	0.06

Also contain: [a] yellow dye and flavour; [b] paracetamol 150 mg and caffeine 50 mg; [c] caffeine 30 mg.

4. Conclusions

Considering the operational simplicity of the proposed method, added to the sampling frequency of 60 samples per hour, the detection limit (2×10^{-4} mol l^{-1} (3×SD)), the mean relative standard deviation (1.1%) and the low cost, it can be recommended for the routine analysis of acetylsalicylic acid in pharmaceutical preparations.

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