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Flow-injection spectrophotometric determination of paracetamol in tablets and oral solutions

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Abstract

A flow injection method is proposed for the determination of paracetamol in pharmaceutical dosage forms. The method is based on the nitration of paracetamol with sodium nitrite, and the absorption of the reaction product is measured at 430 nm in alkaline medium. Unlike other colorimetric methods used for determination of paracetamol, this method does not require the use of heat. The influence of several operating parameters is studied. The method was applied to the determination of paracetamol in oral solutions and in tablets, alone or associated with caffeine. When the results were compared with those obtained by the official HPLC method (USP 24) the relative differences found were from 0.4 to 2.3%, with relative standard deviations below 1%.

Keywords: Paracetamol; Flow-injection spectrophotometry; Tablets and oral solution

1. Introduction

Paracetamol (4-acetamidophenol, acetaminophen, PAP) is an analgesic and antipyretic derived from phenacetin. It is widely used (alone or associated with other active substances such as caffeine) due to the lack of gastric upsets often associated with other analgesics such as acetyl salicylic acid [1].

When there are no significant spectral interferences, the determination of paracetamol in pharmaceutical products can be carried out by direct ultraviolet absorption spectrophotometry, such as in the Paracetamol Tablets monograph in the British Pharmacopoeia 1999 [2]. However, when formulated with other UV-absorbing substances such as excipients or active substances, where spectral overlap is possible, separative techniques such as high performance liquid chromatography (HPLC) are usually necessary, as prescribed, for instance, in several of the acetaminophen articles in the USP 24 [3]. A different approach is the use of colorimetric methods based upon chromogenic reactions that provide the required selectivity.

The determination of paracetamol has been reported employing flow-injection systems (FIA) [4–6] with colorimetric detection. Usually, these

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methods are based on the hydrolysis of paracetamol to 4-aminophenol, which is then transformed into a colored compound by a suitable reaction [7–9]. This strategy, however, has the disadvantage that the hydrolysis step requires high temperatures, which are usually attained by placing the flow reactor in a heating bath or inside a microwave oven. The hot flow coming out from the reactor should then be cooled to room temperature by means of an ice bath or similar cooling device, which adds up to the final complexity of the system. Thus, a reaction that could be carried out at room temperature would lead to a simpler system and should be preferred.

Paracetamol reacts with nitrous acid at room temperature under mild conditions (10% (m/v) $NaNO_2$, 50% (v/v) HCl) producing a derivative whose absorbance can be measured in alkaline solution at 430 nm. This reaction was investigated by Le Perdriel et al. [10] and by Chafetz et al. [11], and they concluded that in fact the reaction did not produce a nitroso-derivative but a nitro derivative, 2-nitro-4-acetamidophenol. In order to enhance the absorbance, pH is changed to alkaline by the addition of sodium hydroxide solution. Under this conditions a bathochromic and hyperchromic shift occurs, and measurements are carried out at about 430 nm.

The goal of this work was to develop a FIA method for the determination of paracetamol in pharmaceutical preparations based on this reaction.

2. Experimental

2.1. Flow system

The FIA system (Fig. 1) was composed of a multichannel peristaltic pump (Gilson Minipuls 2 (Villiers le Bel, France)) and an auxiliary peristaltic pump (Ismatec (Zürich, Switzerland) MS-CA2/820), both fitted with Tygon® and Viton® tubing, an electronically controlled six-port injection valve (Valco (Houston, USA) Cheminert fitted with 50-μl loop). The detector was a Shimadzu (Kyoto, Japan) UV-240 UV–visible recording spectrophotometer fitted with a Hellma (Müllheim, Germany)

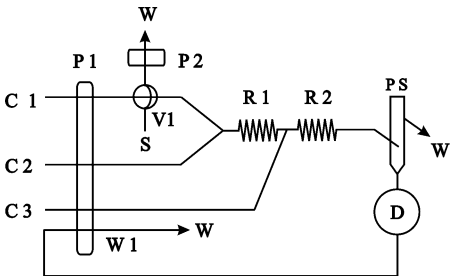

Fig. 1. Flow injection system for the determination of paracetamol. P1, main peristaltic pump. P2, auxilliary peristaltic pump. V1, 6-port injection valve (fitted with 50 μl loop). R1: 100 cm, 0.8 mm ID. R2, 200 cm, 0.8 mm ID. PS, phase separator. C1, 50% (v/v) HCl, 1.3 ml/min; C2, 1% $NaNO_2$, 1.1 ml/min; C3, 10% (v/v) NaOH, 4.8 ml/min. W1: 5.1 ml/min; S, sample. D: spectrophotometer; W, waste.

178.010 quartz flow cell (internal volume 80 μl) and operating at 430 nm in the time-recording mode. Reactors and connections were made from 0.8-mm (ID) FEP tubing and flangeless Valco Cheminert fittings. In order to save sample, the auxiliary pump was switched on only when loading the valve loop.

The spectrophotometer was operated at 430 nm. Graphical recording of the absorbance signal and peak-height measurements was obtained directly from the instrument.

In order to eliminate the gas bubbles generated by the reactions, a lab-made glass phase-separator (PS in Fig. 1) was inserted before the flow cell. Differential pumping was used to drive the liquid from the flow cell to waste.

2.2. Hardware and software

Timing and control of the system was made from a 486-notebook computer (Canon Innova Book 10). The Valco injector was controlled via the RS-232 serial port, while the auxiliary pump was switched on and off as required by means of a lab-made optoisolated triac switch controlled from the computer via the LPT parallel port.

The system was operated by a program compiled in QuickBASIC 4.0 and running under MS-DOS 6.0. In this way the operation of the system was automated, the operator being required only for sample changing.

2.3. Reagents and samples

Paracetamol (Fluka (Buchs, Switzerland) 99.8%) was used as received. The rest of the reagents were of analytical reagent grade. Distilled water obtained from an all-glass still was used throughout.

Aqueous solutions of 1% (m/v) sodium nitrite, 50% (v/v) hydrochloric acid and 10% (m/v) sodium hydroxide were prepared according to usual laboratory practices.

Standard solutions of paracetamol were prepared by dissolving the substance in water and diluting with the same solvent as necessary.

Commercial samples of Pirantil® (Szabó, Uruguay), and Dolex® (Roemmers, Uruguay) oral solutions, and of Zolben® (Novartis, Uruguay) and Saridon® (Roche, Mexico) tablets (the latter containing also caffeine) were obtained from local drugstores.

2.4. System operation

When valve V1 is in the ''load'' position, the sample or standard solution is pumped by the auxiliary pump P2 through the sampling loop of the valve for 15 s. After that time P2 is turned off and V1 switched to the ''inject'' position, The sample bolus is carried by the HCl stream (C1) towards the point where it is mixed with the $NaNO_2$ stream (in reactor R1) and then with NaOH solution (C3) in reactor R2. Then the phase separator or debubbler PS eliminates the gas bubbles formed in the reaction and the stream is carried through the flow cell in the spectrophotometer (D) and pumped to waste by means of the W1 pump tube. The flow rate of W1 is lower than the sum of the flow rates C1+C2+C3, thus ensuring the differential pumping and a constant flow through the flow cell.

2.5. Analysis of oral solutions and tablets

Oral solutions were diluted with water to a final concentration of paracetamol around 240 mg/l.

Tablets were ground in a mortar. An amount of powder (accurately weighed) equivalent to 240 mg of paracetamol was extracted with 75 ml of water in an ultrasonic bath for 15 min and the volume was made up to 100 ml with water. The extract was filtered and further diluted with water to obtain a final concentration of about 240 mg/l.

Individual tablets were sonicated for 15 min with 75 ml of water in a 100-ml volumetric flask. The volume was then made up to the mark and the contents filtered and diluted to the final concentration of about 250 mg/l.

The solutions were injected and the peak height measured. The calibration was carried out by means of a five-point calibration curve in the range 0–400 mg/l.

3. Results and discussion

3.1. Concentration of nitrite

The presence of nitrite causes a number of problems in a FIA method, because its decomposition produces gas bubbles, which may disturb the flow pattern and cause extraneous signals. In the original batch method [11], excess nitrite is eliminated by means of ammonium sulfamate, a reaction that also generates a large amount of bubbles.

In order to avoid these problems, the concentration of $NaNO_2$ was reduced from the original value of 10% used in the batch method according to Chafetz et al. Under these conditions, elimination of excess $NaNO_2$ would not be necessary and there would be no need to use an ammonium sulfamate stream. Several concentrations of $NaNO_2$ were tested. With a 1% concentration, the reaction proceeds satisfactorily and only a small amount of bubbles is generated, which are efficiently eliminated by the phase separator. Thus a concentration of 1% (m/v) was chosen for sodium nitrite.

3.2. Influence of operating conditions

3.2.1. Reactor length

Several experiments were carried out to determine the behavior of the system with different styles and lengths of reactors R_1 and R_2. For both reactors, two different styles of coiling (i.e. helical

and serpentine (shaped in "8")), and three lengths (50, 100 and 200 cm) were tested and compared in terms of peak height and precision. A solution of paracetamol (240 mg/l) was injected five times in each instance.

The nitration reaction is carried out in reactor R_1. Results for this reactor (Fig. 2) suggest that the optimum reactor is that shaped in "8" and 100 cm in length.

For reactor R_2, again the 8-shaped reactor gives higher signals. 200 cm was chosen as the length as it allows the best precision to be obtained. These results (Fig. 3) suggest that mixing with the alkaline solution was not complete with the shorter reactors.

3.2.2. Flow rate

The system was operated at four total flow rates corresponding to 40, 60, 80 and 100% of the maximum pump speed. The lower flow-rates produce slightly higher signals but precision is poorer (Fig. 4). Thus 80% was chosen as operating speed, which corresponds to the flow rates indicated in Fig. 1. Higher flow rates give no significant advantage and increase tubing wear.

3.2.3. Interferences

The possible interference of caffeine was studied by analyzing an aqueous solution of caffeine (22.2 mg/l), and a solution containing 259 mg/l paracetamol plus 22.2 mg/l caffeine. The peak heights of the absorbance signals were compared with those of a standard solution of paracetamol (259 mg/l).

The signal from the caffeine solution was undistinguishable from that of a water blank, and that from paracetamol plus caffeine was equivalent to the signal produced by the paracetamol standard alone. A solution containing 259 mg/l of paracetamol plus 222 mg/l of caffeine (i.e. an amount of caffeine ten times higher) gave similar results. According to these findings it was concluded that under the proposed conditions caffeine produced no measurable interference enabling the method to be tested in commercial samples containing paracetamol and caffeine.

3.2.4. Linearity

Calibration curves (seven concentration levels plus zero) in the range 0–480 mg/l paracetamol, were fitted by means of the least-squares method.

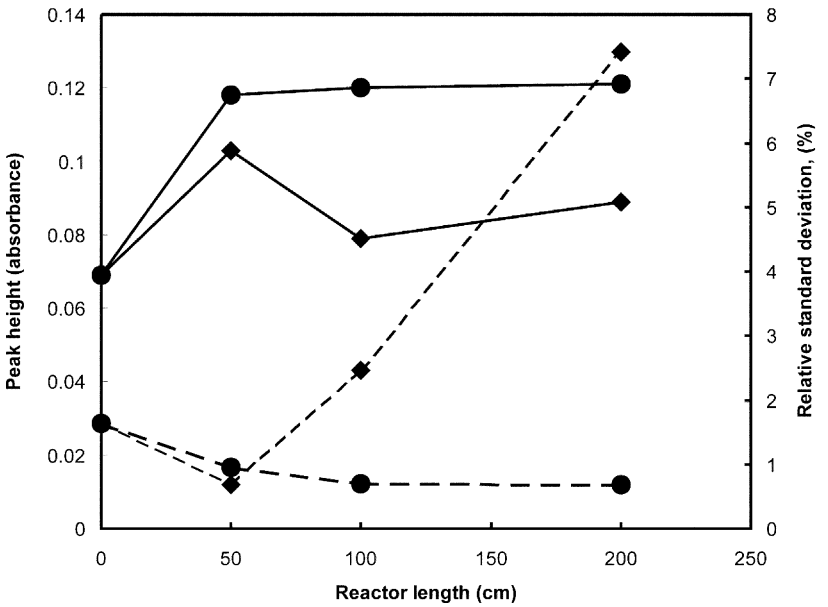

Fig. 2. Variation in signal height (—) and precision (----) with length of reactor R_1, for coiled (◆) and serpentine (●) reactors. Sample, paracetamol 240 mg/l, 50 μl.

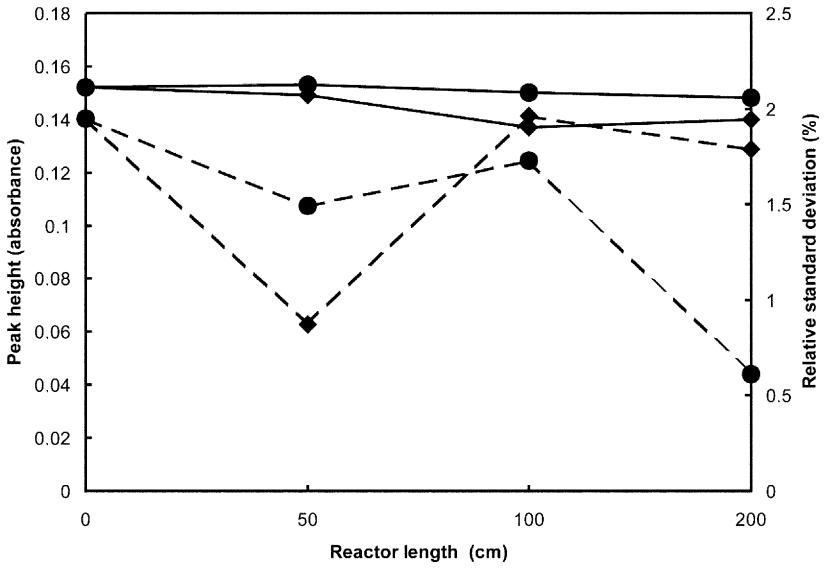

Fig. 3. Variation in signal height (—) and precision (----) with length of reactor R_2, for coiled (◆) and serpentine (●) reactors. Sample, paracetamol 240 mg/l, 50 μl.

In this range, a slight curvature was found. A second-degree polynomial was the best-fitting regression model, with $R^2 = 0.9996$ ($h = -7.0 \times 10^{-7}C^2 + 0.001C + 0.0174$, being h the peak height in absorbance and C the concentration of paracetamol in mg/l).

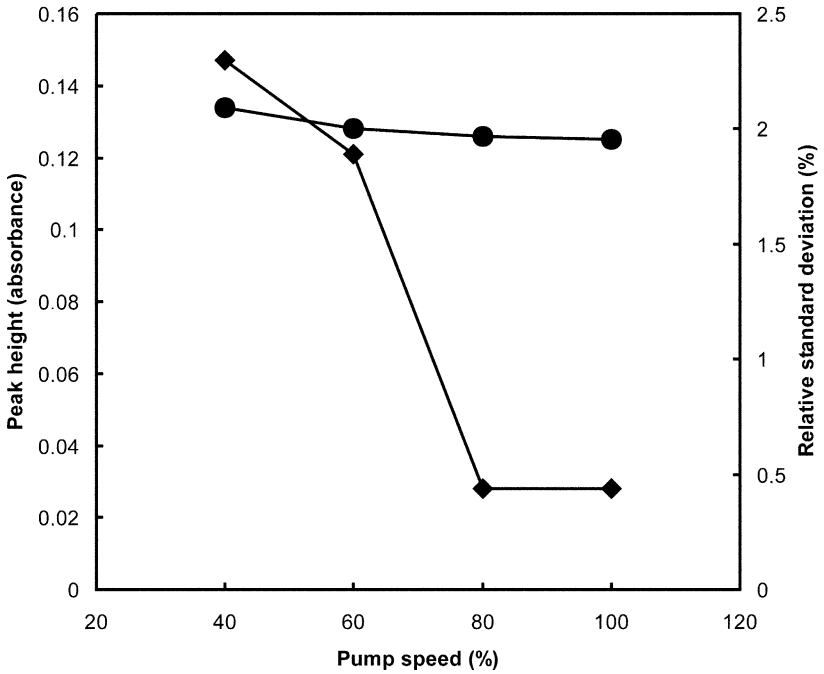

Fig. 4. Variation in signal height (●) and precision (◆) with pump speed for serpentine reactors. Sample: paracetamol 240 mg/l, 50 μl.

Table 1
Contents found (average of five injections) and R.S.D. (s_r (%)) obtained for commercial samples analyzed by the FIA and USP 24 (HPLC) methods

	Nominal contents	Contents found and R.S.D., FIA	Contents found and R.S.D., HPLC	Relative difference of contents (%)
Solutions		g/dl	g/dl	
Pirantil	2.4 g/dl	2.45 (0.46%)	2.51 (0.70%)	−2.3
Dolex	4.8 g/dl	4.87 (0.41%)	4.91 (0.69%)	−0.8
Tablets		mg/tablet	mg/tablet	
Zolben	500 mg	509 (0.41%)	503 (0.80%)	1.2
Saridon	500 mg + 50 mg caffeine	498 (0.45%)	496 (0.69%)	0.4

In the reduced concentration range of 180–300 mg/l, a straight line ($h = 0.00061C + 0.06867$, $R^2 = 0.99993$) was the best fitting line, determined with a five point calibration curve.

3.2.5. Sampling frequency

With the operating parameters chosen (load time 15 s, injection time 15 s) a sampling frequency of 120 per h could be attained.

3.2.6. Stability

In order to assess the operating long-term stability of the system, a 259-mg/l standard solution of paracetamol was injected during a 60-min period. No significant drift or sensitivity changes were noticed during this period. It was also verified that the gas separator efficiently handled the small amounts of bubbles generated by the chemical reaction and thus no alteration of the signal was noticed.

3.3. Analysis of commercial samples

Two commercial samples of paracetamol oral solution (label claim 2.4 and 4.8 g/dl), one of paracetamol tablets (500 mg) and one of paracetamol and caffeine tablets (500 and 50 mg, respectively) were analyzed by the FIA method and by the corresponding official HPLC method (USP 24).

The results were compared in terms of precision and average value. Table 1 shows the result (average of five injections) and relative standard deviation (R.S.D.) (%).

Analytical repeatability was evaluated by repeating five times the complete analytical procedure, and R.S.D. of the five analytical results was calculated. The results were 0.74% for tablets and 1.3% for oral solutions.

4. Conclusions

A flow-injection spectrophotometric method for the determination of paracetamol without the need of heating was developed. The method was found to be robust and provided extremely precise results, appropriate even for the evaluation of contents uniformity of tablets. When applied to commercial samples it produced results that do not differ significantly from the official method (USP 24).

The lack of interference from caffeine allows the determination of paracetamol in tablets of paracetamol and caffeine.

The overall performance of the method is considered to be satisfactory for quality control purposes.

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