Flow Injection Spectrophotometric Determination of Furosemide in Pharmaceuticals by the Bleaching of a Permanganate Carrier Solution

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Abstract

A flow injection spectrophotometric method based on furosemide reaction with potassium permanganate is described. A flow system using as carrier a 3.0 x 10^{-4} mol/l potassium permanganate solution was developed. This system used a 50 cm sample loop and a 100 cm reactor, which was kept at 50°C. The permanganate flow rate was 2.2 ml min^{-1}. The analytical signal was the bleaching of the carrier solution, caused by the reaction between furosemide and potassium permanganate. The detection at 550 nm presented a linear range from 1.0 to 6.0 x 10^{-4} mol/l, with a calibration equation equals to $y = 587.5x + 0.09$ (in which $r = 0.995$, $n = 6$), the limit of detection (3σ/slope) was 1.1 x 10^{-5} mol/l. The proposed method was applied to commercial samples from three different brands, as tablets and ampoules. It presented an adequate analytical frequency (40 measurements per hour), with results according to the labeled values.

Keywords Antihypertensive drug analysis; furosemide determination; flow injection spectrophotometry

1. Introduction

Furosemide (4-chloro-N-furfuril-sulphamoylantranilic acid)[1] represents a powerful loop diuretic, widely used in the treatment of hypertension and edema, its structure is presented in Fig. 1. The reference drug is Lasix, from Aventis®, commercialized as tablets and bulks. Nowadays, with the advent of generic drugs, the number of pharmaceutical formulations increased, demanding for quality control.

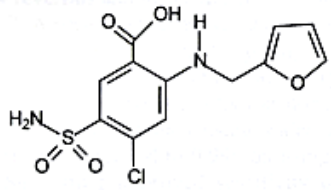

Fig. 1 Structural formula of furosemide.

For furosemide determinations in aqueous solutions, analysts must consider the adequate solubility in alkaline solutions [2,3], or in the presence of surfactants [4,5].

Some authors determined furosemide by static or flow injection procedures based on complexation with different compounds and spectrophotometric detection [6]. Direct simultaneous determinations in samples containing furosemide and amiloride were developed through derivative spectrophotometry [7]. Titrimetric procedures using acid-base reactions were developed by Kulichencko and co-workers [4,5].

Still aiming furosemide determination, differential pulse and square wave voltammetry have been used [8]. Dias *et al.* [9] developed ion-selective electrodes for furosemide intending its potentiometric determination, these sensors used tricaprilmetilamonium as ionic par. Published reports have also demonstrated the applicability of lanthanides in the determination of derivatives of the anthranilic acid, like furosemide, using its reaction and complexation, and exploration of their luminescence properties, obtaining high sensibility. Among lanthanides they report cerium [10], europium [11], terbium [12,13], among others. The chemiluminescence properties of ruthenium complex reagents have also been applied as described in Xi *et al.* [14].

Chromatographic procedures have been used in the determination of the analyte in tissues, serum and urine; using UV, fluorimetric or amperometric detection [15-18].

Reactions with potassium permanganate have been studied to determine some drugs as methotrexate [19] and norfloxacin [20], applying flow injection fluorimetry and kinetic spectrophotometry, respectively. Korenaga *et al.*[21] developed a system applying cerium (IV) sulphate as oxidizing agent in order to determine chemical oxygen demand (COD), in this case the analytical signals were the decrease of absorbance due to the oxidation processes.

The present work proposes a simple and fast method for furosemide determination by flow injection with spectrophotometry based on its reaction with a permanganate carrier solution.

2. Experimental

2.1. Reagents and Standard Solutions

Furosemide was obtained from Natural Pharma (Brazil) and it was characterized as standard by nuclear magnetic resonance (^{1}H-NMR), Fourier transformed-infrared spectrometry (FT-IR), elemental analysis (EA), differential scanning calorimetry (DSC), and high-performance liquid chromatography equipped with a diode array detector (HPLC-DAD). The water was obtained from a Milli-Q Waters system (USA). All reagents were of analytical grade from Mallinckrodt (USA).

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The carrier consisted of a 3.0 x 10^{-4} mol/l $KMnO_4$ in 2.0 mol/l H_2SO_4 solution. A 1.0 x 10^{-2} mol/l furosemide stock solution was prepared by dissolving the reagent in 0.02 mol/l NaOH. This solution was used for the preparation of the working solutions by diluting the desired volumes with 0.02 mol/l NaOH.

2.2. Flow System

The applied flow system consisted of a Genesys 20 Spectronics (USA) spectrophotometer, set up at 550 nm, and a peristaltic pump Ismatec IPC 8 (Switzerland). Tygon pumping tubes of different internal diameters, from Cole Parmer (USA)were used. The sample loops and other pathways were made of polyethylene tubes (0.8 mm i.d.), were from Protubos (Brazil).

During the optimization step the temperature of the reactor was controlled by a thermostatic bath from Marconi (Brazil). The system is demonstrated in Fig. 2.

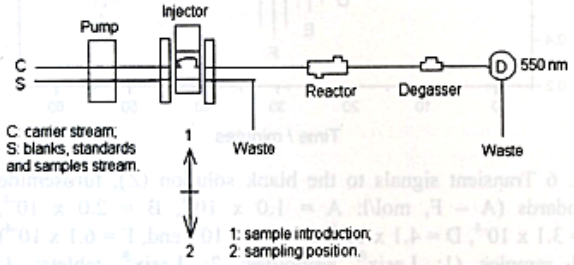


Fig. 2 Flow system design.

2.3. Sample Preparation

The pharmaceutical formulations analyzed in this work were presented as tablets and ampoules, from three different brands commercialized in the brazilian market (Lasix, from Aventis®; Neosemid, from Neoquimica®; and Furosemida, from Biosintetica®).

In order to perform the determinations, tablets were weighed and triturated in a mortar until a fine and homogeneous powder is obtained. Suitable amounts of these powders were weighed accurately (±0.1 mg) and solubilized in 15 ml 0.02 mol/l NaOH, the systems were sonicated during 10 minutes and the solutions were then filtered. The volumes were completed with the same NaOH solution to 25 ml in volumetric flasks.

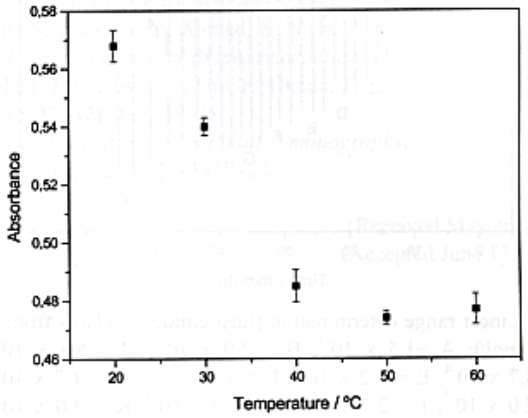
The ampoule samples were prepared by direct dilution of adequate aliquots in 0.02 mol/l NaOH in 25 ml volumetric flasks.

3. Results and Discussions

3.1. Optimizations On The Flow System

Intending to optimize the flow injection set up, three parameters were initially studied: flow rate, sample loop, and reactor extension. In these studies, the temperature was initially fixed at 22°C. The parameters were optimized according to a factorial design (2^n): sample loop varied from 10 and 50 cm, reactor extension varied from 30 and 100 cm, and the flow rate varied from 0.8 ml min^{-1} and 2.2 ml min^{-1}. In these tests some tendencies were observed. When the sample loop and reactor extension were increased, the analytical signals tend to higher values; on the other hand, the increase in the variable flow rate generated a decrease in the signal since the residence time is not enough for the reaction completed. Under such conditions, a low analytical frequency resulted. In order to compensate the necessity of higher flow rate the effect of temperature was also evaluated.

In temperature optimization, as expected, the reaction showed to be strongly dependent on. This variable were studied from 20 to 60°C (Fig. 3). The best temperature found was 50°C,



which demonstrated best analytical signal and repeatability.

The optimized parameters allowed 40 measurements per hour, with no memory effect.

Fig. 3 Temperature optimization.

3.2. Linear Range

In order to determine the linear region for furosemide determination, under these conditions eleven standard solutions with different concentrations (from A to K, Fig. 4) between 1.0 x 10^{-4} and 3.0 x 10^{-3} mol/l were introduced into the optimized flow system.

The curve showed an exponential behavior, as demonstrated by the Fig. 5.

The sensibility decreases with the increase of furosemide concentrations, probably due to the limited reaction with MnO_4^- under these conditions. A linear range from 1.0 to 6.0 x 10^{-4} mol/l was observed.

3.3. Analytical Applications

In the described method the analytical signal was the bleanching of the carrier solution, it is necessary to define how to determine the analytical signal. As the blank generates dilution signal, it must be subtracted from the analyte signal; in order to solve this situation an equation was proposed:

Bleanching = [(baseline signal – furosemide solution signal) – (baseline signal – blank signal)]; in this equation the second term is the dilution effect.

The analytical curves were obtained by using standard solutions from 1.0 to 6 x 10^{-4} mol/l (n = 6), as presented in Fig. 6. The linear interval was applied in order to calibrate the system, and is represented by the following equation: $y = 587.5x + 0.09$ ($r = 0.995$, $n = 6$), in which y means bleanching defined above and x represents furosemide concentration.

The system was used to determine furosemide in some commercial samples of ampoules and tablets, as cited before. The labeled values and found results are presented in Table 1.

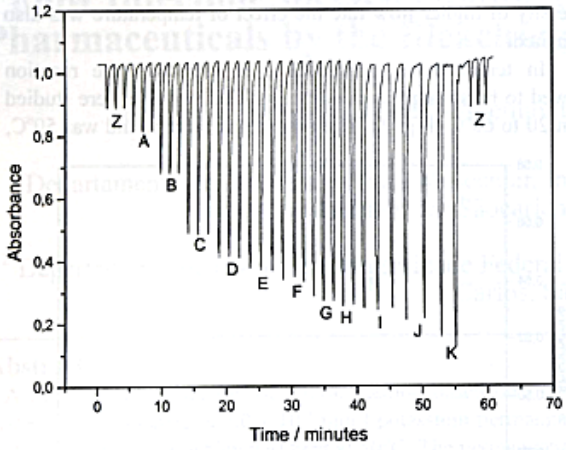


Fig. 4 Linear range determination (furosemide standards from A to K, mol/l: A =1.5 x 10^{-4}, B = 2.9 x 10^{-4}, C = 5.8 x 10^{-4}, D = 8,7 x 10^{-4}, E = 1.2 x 10^{-3}, F = 1.5 x 10^{-3}, G = 1.7 x 10^{-3}, H = 2.0 x 10^{-3}, I = 2.3 x 10^{-3}, J = 2.9 x 10^{-3}, K = 3.0 x 10^{-3}, Z means blank solution).

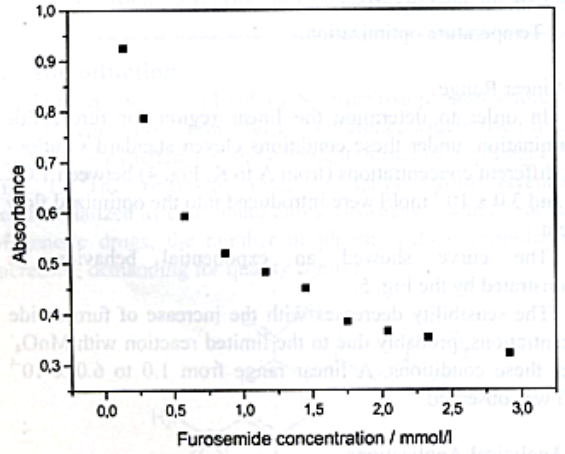


Fig. 5 Exponential behavior of the analytical signal.

3.4. Comparison Method

In order to evaluate comparatively the developed procedure the same samples were submitted to a direct Uv-vis spectrophotometric method, proposed by the United States Pharmacopoeia [22], in which furosemide was extracted from the matrices using NaOH. In this case, the static detection was carried out in 271 nm; in this case a single point calibration is suggested.

Comparing the obtained results by Student *t*-test, no statistical difference was observed (95 and 99% confidence levels, $n = 3$). These results are presented in Table 1.

4. Final Comments

The developed method showed to be fast, and easy applying simple instrumentation and low accessories. The analytical frequency showed to be greater than others reported in literature (close to 20 measurements per hour). Although the linear range was short, it was used to analyze some pharmaceutical formulations as ampoules and tablets, with adequate results.

Although permanganate is a strong oxidizing agent that can react with several organic substances, it seems that the excipients present in the analyzed samples did not interfered in the proposed method. This makes the proposed procedure suitable for the determination of furosemide in anti-hypertensive formulations.

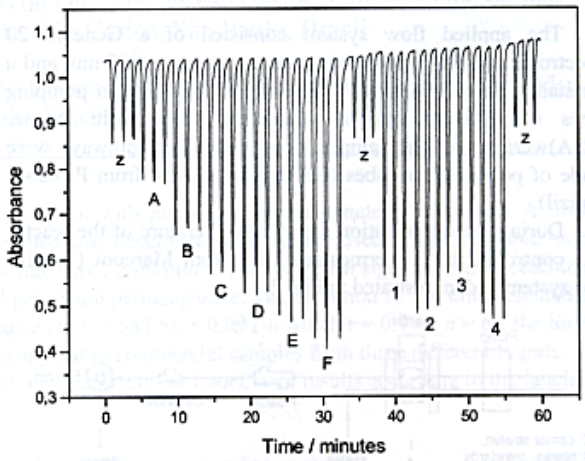


Fig. 6 Transient signals to the blank solution (Z), furosemide standards (A – F, mol/l: A = 1.0 x 10^{-4}, B = 2.0 x 10^{-4}, C = 3.1 x 10^{-4}, D = 4.1 x 10^{-4}, E = 5.1 x 10^{-4} and, F = 6.1 x 10^{-4}) and samples (1: Lasix® ampoules; 2: Lasix® tablets; 3: Neosemid® tablets, and 4: Biosintetica® tablets).

Table 1 Found results and t-test values.

Sample	Label values	Proposed Method[c]	Comparision Method[c]
Lasix® ampoule	10[a]	10.6 ± 0.1	9.6 ± 0.3
Lasix® tablet	40[b]	42.5 ± 0.1	40.5 ± 1.5
Neosemid® tablet	40[b]	42.7 ± 0.1	41.8 ± 0.1
Biosintetica® tablet	40[b]	40.3 ± 0.1	40.6 ± 0.9

[a] mg furosemide per ml;
[b] mg furosemide per tablet;
[c] mean values and standard deviations.

5. Acknowledgements

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