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Determination of furosemide in pharmaceutical formulations by diffuse reflectance spectroscopy

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Abstract

In this report an analytical method to determine furosemide by using diffuse reflectance spectroscopy is presented. This study shows that this technique can give quantitative results using spot test analysis, particularly in the case of pharmaceuticals containing furosemide. The color spot test could be obtained by reaction between furosemide with *p*-dimethylaminocinnamaldehyde, in acid medium. This reaction produced a stable complex on filter paper after heating to 80 °C for 5 min. All reflectance measurements were carried out at 585 nm and the linear range was from 7.56×10^{-3} to 6.05×10^{-2} mol l^{-1}, with a correlation coefficient of 0.999. The limit of detection was estimated to be 2.49×10^{-3} mol l^{-1} (R.S.D. = 1.7%) and the effect of common excipients on the reflectance measurements was evaluated. The method was applied to determine furosemide in commercial brands of pharmaceuticals. The results obtained by the proposed method were favorably compared with those of the official method, showing for the first time ever that quantitative spot test analysis by diffuse reflectance could be successfully used to determine furosemide in tablets.

Keywords: Diffuse reflectance spectroscopy; Furosemide; Spot test; *p*-Dimethylaminocinamaldehyde

1. Introduction

Diuretics are an important group of the drugs used in various clinical situations such as cardiac and renal insufficiency, nefrotic syndrome, edema, cirrhosis and hypertension [1]. Furosemide (4-chloro-*N*-furfuryl-5-sulphamoyl anthranilic acid, see Scheme 1) belongs to the class of loop diuretics and it is prescribed mainly for the control of the hypertension associated to renal and cardiac insufficiency [2].

In order to fulfill the requirements for quality control, several analytical methods have been described to determine furosemide in different pharmaceutical dosage forms. HPLC methods have been the main approach [3–6]. Other techniques reported are capillary electrophoresis [7,8], titrimetry [9,10], and spectrophotometry [10–13]. Some of these methods suffer interference from the tablet matrix, whereas, others are time consuming or require expensive equipment and consequently are not suitable for routine analysis. Titrimetric methods have suffered from a lack of selectivity and sensitivity, involve risks of environmental nature because it uses toxic solvent [10] and have not been applied to pharmaceutical formulations and not even to synthetic mixtures, thus precluding the assessment of their usefulness in real analysis. Spectrophotometric methods based on UV absorption present low selectivity, as all unsatured compounds display one or more bands in that region of the spectrum.

From the above consideration, the need for a fast, low-cost and selective method seems clearly apparent, especially for routine quality control analysis of pharmaceutical products containing furosemide. In the search for such a method, our attention was attracted to a quantitative spot test by diffuse reflectance spectroscopy, whose measurements are fast, easy to conduct and involve low consumption of reagents.

For many years, the use of reflectance spectroscopy as an analytical technique was limited to paints and pigments, paper, textile areas, ceramics, dye-stuffs and printing inks to evaluate properties such as color, whiteness, gloss, covering power, and so on [14]. Too little attention has been given to diffuse reflectance spectroscopy as a quantitative technique as it was not possible to attain highly precise

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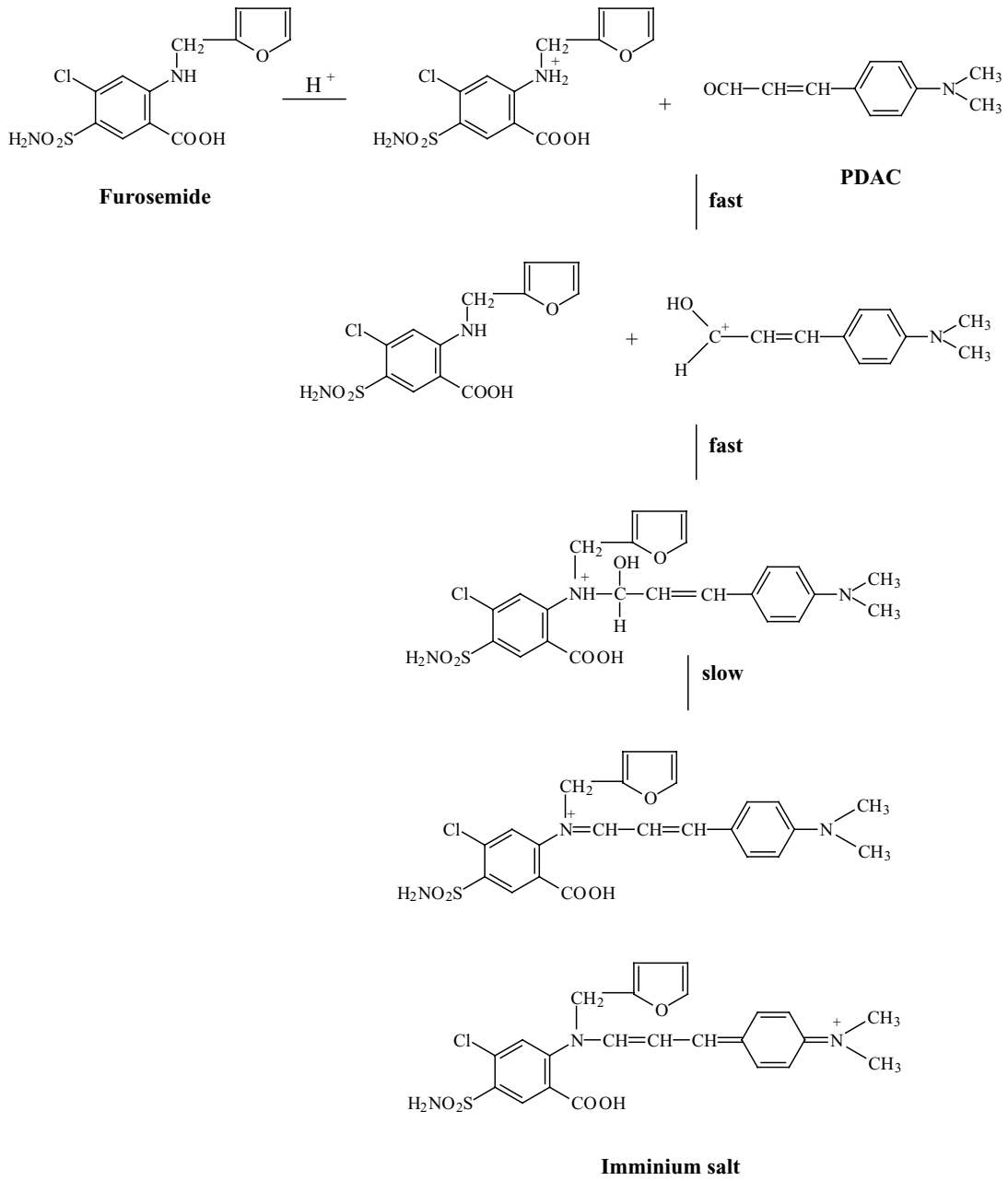

Scheme 1.

measurements from conventional spot tests [15]. However, with the development of optical devices including optical fibers and reflectance spheres, this situation has changed and the quantification from spot tests by diffuse reflectance, on inert support, yield good precision and selectivity [16].

Few methods intended for quantitative analysis spot testing using diffuse reflectance have been reported in literature [17–24]. Many of these showed that the appropriate use of diffuse reflectance spectroscopy could yield reliable results evidencing the potential of this technique for quantitative analysis of ions [17–20,24]. Therefore, there has not yet been any application of spot test by diffuse reflectance for quantitative analysis in the area of pharmaceutical quality control.

The aim of this study was to develop an analytical method employing diffuse reflectance spectroscopy for the routine analysis of furosemide in pharmaceuticals. The proposed method is based on the reflectance measurements in the visible region of the spectrum of the violet compound produced from the spot test reaction between furosemide and *p*-dimethylaminocinnamaldehyde (PDAC) using a filter paper as solid support, in acid medium, after heating to 80 °C for 5 min. The reaction of secondary aromatic amines with PDAC in the presence of hydrochloric acid has been used for spectrophotometric determination of some sec-

ondary aromatic amines [25–28]. The proposed mechanism for the reaction between secondary aromatic amines and PDAC involve the condensation of protonated secondary amino group with carbonyl group of the reagent to produce imminium salt [28] as shown in Scheme 1.

The results obtained from this present study showed the good performance of this technique, suggesting its use as a reliable and advantageous alternative to most other previously reported method in the routine control of furosemide in pharmaceutical formulations.

2. Experimental

2.1. Materials, chemicals and solutions

Whatman 41 filter paper was used as solid support. All reagents utilized were of analytical reagent grade. The excipients used in the interference study were of pharmaceutical grade. Solvents used were acetone (p.a. grade) and methanol (HPLC grade) from Mallinckrodt, Xalostoc, Mexico. PDAC (Riedel-de haën, Germany) was used to prepare a 0.4% (w/v) solution in methanol and it was kept refrigerated for no more than 1 week. Hydrochloric acid (Mallinckrodt, Xalostoc, Mexico) at 6.3% (w/v) in methanol was prepared by adequate dilution of the concentrated acid (37%). Furosemide standard was purchased from Purifarma, Brazil (purity >99.99%). A 15.2×10^{-2} mol l^{-1} stock standard solution of furosemide in acetone was freshly prepared. Working standard solutions were prepared by appropriate dilution of the stock solution with acetone in order to construct an analytical curve from 7.56×10^{-3} to 6.05×10^{-2} mol l^{-1}.

2.2. Pharmaceutical formulations

The analyzed products were purchased locally or directly from the manufacturers and all were tested prior to the listed expiration date. Six pharmaceutical formulations containing furosemide and other components were analyzed. All the commercial brands of pharmaceuticals studied were package labeled to contain 40 mg furosemide per tablet.

2.3. Apparatus

The detection was performed by coupling a Labsphere RSA-HP-8453 reflectance sphere integrator (76 mm, 5 W halogen source) to a Hewlett–Packard HP 8453A diode array spectrophotometer.

2.4. Procedure

For the spot test reaction, the solutions were spotted onto Whatman 41 filter paper. To carry out measurements, first 10 μl of the analyte solution was spotted, then 20 μl of the acid solution and finally, 20 μl of the reagent solution. The solutions were spotted onto the center of the filter paper using a micropipette fixed in a holder according to procedure described by Tubino et al. [24]. In the sequence, the filter paper was heated to 80 °C for 5 min in an oven with temperature control and then the reflectance measurements were carried out at 585 nm. The blank with 10 μl of the acetone, 20 μl of the acid solution and 20 μl of the reagent solution was spotted using the same procedure.

2.5. Study of interferences

Since the aim of this study was to determine furosemide in pharmaceuticals, the effects of the most commonly used excipients were carefully examined. The excipients studied were starch, talc, magnesium stearate, lactose, ethylcelulose, silicon dioxide and sodium croscarmelose. For this study, solutions containing furosemide and each of the excipients taken separately in concentrations equal or 10 times greater than that of furosemide were shaken with acetone in a magnetic mixer for 10 min, diluted and analyzed under the same conditions described in Section 2.4.

2.6. Sample preparation

Twenty tablets of each commercial brand pharmaceutical to be studied were weighed and finely powdered. A portion of this powder, equivalent to approximately 100 mg of furosemide was accurately weighed. The sample was shaken with acetone in a magnetic mixer for 10 min and filtered in Whatman 42 filter paper. This solution was then diluted with acetone in a calibrated 10 ml flask and an aliquot of this solution was taken for the spot test reflectance analysis.

3. Results and discussion

The PDAC has already been used for spectrophotometric analysis of furosemide [25]. In our study an intense violet color appeared on the surface of the filter paper indicating the formation of chromogen. Fig. 1 shows the reflectance spectrum with maximum value of A_R (optical density for reflectance measurement) at 585 nm.

According to Wendlant and Hecht [14] the color of the spot test should be uniform over the entire surface in order to ensure reproducible reflectance measurements. In a sense, we have taken into consideration important details as previously described [19,24] to perform the spot test reaction, such as order and rate of reagent addition, quality of filter paper and volume of solution added. All these details are important for the uniformity of the color spot test. An interesting fact in our study was that the color of the spot test obtained from analyte, reagent, and acid solution, in this order of addition, was more intense on the borders than in the center of the spot. But we observed that when the reagent solution was added last, the color of the spot was more intense in the center and much more uniform.

Investigations were carried out to establish the most favorable conditions for the spot test reaction on the filter paper in order to achieve maximum color development at

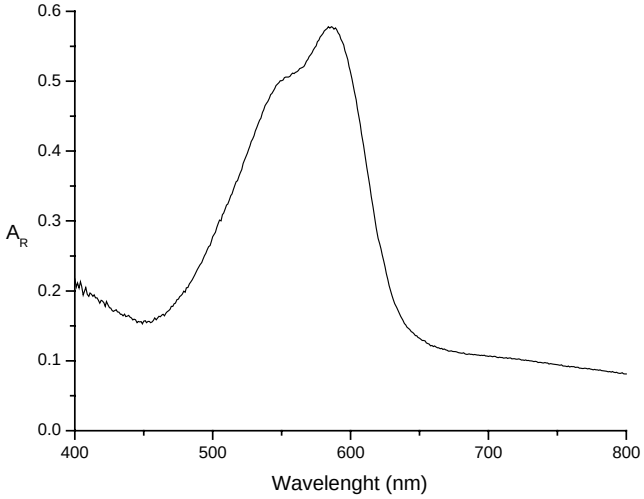

Fig. 1. Reflectance spectrum of the spot test reaction on filter paper from furosemide, PDAC, in HCl medium. A_R values were taken after heating to 80 °C for 5 min. The maximum value of A_R was at 585 nm. Furosemide concentration = 3.02×10^{-2} mol l^{-1}.

585 nm. The effect of PDAC and HCl volumes, and heating time and temperature on the color intensity and uniformity of the spot test were evaluated using evolutionary operation (EVOP). So, it was possible to vary two parameters simultaneously: PDAC and acid volume and/or heating time and temperature. The reflectance response for fixed volume of furosemide, heating time and temperature can be optimized as a function of PDAC and acid volume. Similarly, for fixed PDAC and acid volume the reflectance response can be optimized as a function of heating time and temperature. The conditions tested are summarized in Table 1.

The reflectance values obtained from the spot tests were statistically analyzed. Comparisons between averages were performed using ANOVA and the statistical significance of the differences between averages was estimated using the Tukey test ($P \leq 0.05$). The results demonstrated that the highest reflectance measurements without loss of uniformity of the spot test were reached with 20 μl of PDAC and 20 μl of HCl, differing significantly ($P > 0.05$) from the other conditions studied. From these results, different heating times and temperatures were then evaluated and the reflectance measurements obtained at 80 °C for 5 min were significantly higher ($P > 0.05$) that those obtained with the other heating times and temperatures. It should be mentioned that spot tests obtained with heating at 90 °C presented traces of carbonization in the paper and it could not be read. For this reason the EVOP was discontinued. Fig. 2 shows the three-dimensional graphs obtained from the experimental data and fitted to a response surface model. Analyzing the fitted surface, it is possible to see the points referring to the conditions chosen, which showed the highest reflectance values.

Table 1
Variables studied during EVOP

Volume (μl)		Heating	
HCl[a]	PDAC[b]	Time (min)	Temperature (°C)
20	10	5	60
20	20	5	80
15	15	3	70
10	10	2	60
10	20	2	80

[a] HCl 6.3% (w/v) in methanol.
[b] PDAC 0.4% (w/v) in methanol.

The calibration curve was constructed from 7.56×10^{-3} up to 6.05×10^{-2} mol l^{-1} furosemide standard solutions. A linear relationship ($r = 0.999$) was obtained by plotting A_R versus log concentration of furosemide (mol l^{-1}). A_R values for the concentration range were fitted by the equation: $A_R = -0.17768 + 0.41338C$, where C = log (10^3 [Furosemide]/mol l^{-1}). The factor 10^3 was used to adjust the calibration graph to log values higher than zero. The limit of detection was estimated to be 2.49×10^{-3} mol l^{-1} (R.S.D. = 1.7%), according to the analytical curve data and using the criteria of the mathematical model given by Miller and Miller [29].

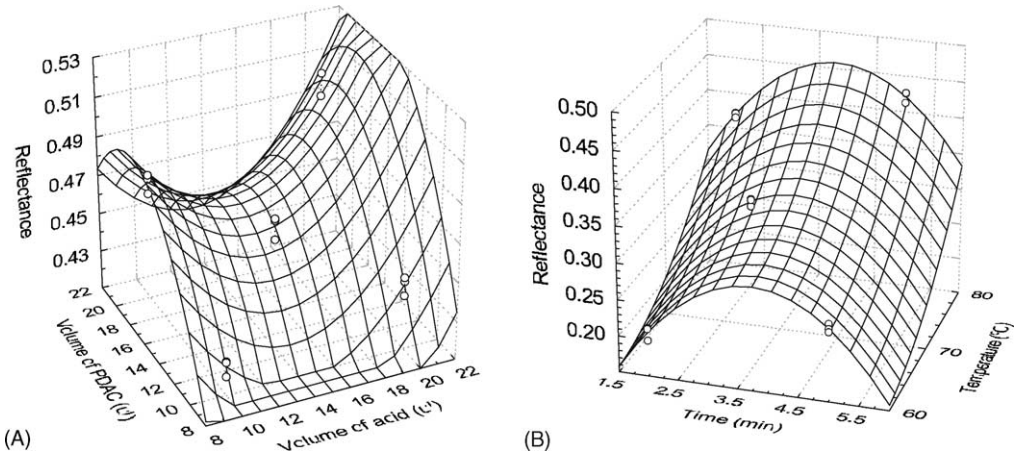

Fig. 2. Surface response obtained to establish optimum conditions of volumes of PDAC and acid (A) and of heating time and temperature (B).

Table 2
Furosemide determination in commercial pharmaceuticals

Sample	Proposed method			Official method [10]
Furosemide tablets[a]	Found[b]	t value (4.30)[c]	F value (19.00)[c]	Found[b]
A	39.4 ± 0.5	1.72	2.04	38.6 ± 0.7
B	39.6 ± 1.1	0.45	2.98	40.2 ± 2.0
C	40.8 ± 1.5	1.23	1.79	40.9 ± 1.1
D	39.3 ± 1.5	1.17	1.70	41.0 ± 2.0
E	39.2 ± 1.0	0.97	4.08	39.4 ± 0.5
F	39.3 ± 0.8	0.72	9.21	39.2 ± 0.5

[a] Package labeled to contain 40 mg furosemide/tablet.
[b] Average ± S.D. (mg per tablet), $n = 3$.
[c] Theoretical values of t and F at 95% confidence level.

3.1. Applications

The proposed method was applied to determine furosemide in commercial pharmaceuticals. The results of the comparison of the proposed method with the official method (spectrophotometric method) [10] are shown in Table 2. For all formulations assayed, the results obtained by the official and proposed methods were compared by applying the F test and t test at 95% confidence level. In all cases, the calculated F and t values did not exceed the theoretical values, indicating that there is no significant difference between either method regarding precision and accuracy in determining furosemide in pharmaceuticals. The average recoveries obtained by the proposed method ranged from 98.0 to 102.1% for the commercial preparations.

3.2. Study of interferences

The effect of each excipient was considered to be an interference when the signal showed an error more than or equal to 5% in the determination of the drug. The percentage of furosemide found in the spiked solutions was in the range of 96 to 104, with R.S.D. values less than 5% for three replicates. So, no interference was observed from these excipients under the studied conditions.

4. Conclusion

This study showed the feasibility of diffuse reflectance spectroscopy to determine furosemide in pharmaceutical formulations by using a spot test on a filter-paper surface. The order of addition and the volumes of PDAC and acid solution significantly affected the spot test reaction. The best conditions for the color spot test reaction were obtained from 10 μl of furosemide, 20 μl of hydrochloric acid solution and 20 μl of PDAC solution in this addition order, heated to 80 °C for 5 min. This method was successfully applied to the analysis of furosemide in different commercial brands of tablets. The diffuse reflectance spectroscopy using spot test offers advantages over other techniques used at present for determine furosemide, such as simplicity, rapidness and extremely low consumption of reagents. So, the further development of diffuse reflectance spectroscopy methods for analysis of pharmaceutical samples is encouraged in this study.

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