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# Simultaneous determination of amiloride and furosemide in pharmaceutical formulations by first digital derivative spectrophotometry

M. Inés Toral [a,*], Stefanie Pope [a], Silvia Quintanilla [a], Pablo Richter [b]

[a] *Faculty of Sciences, Department of Chemistry, University of Chile, P.O. Box 653, Santiago, Chile*

[b] *Faculty of Chemical and Pharmaceutical Sciences, Department of Inorganic and Analytical Chemistry, University of Chile, P.O. Box 233, Santiago, Chile*

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**Abstract**

This work presents a simple and fast method for the simultaneous determination of amiloride and furosemide by digital derivative spectrophotometry. HCl $1 \times 10^{-2}$ mol/l dissolved in ethanol was used as solvent and to extract drugs from formulations. Subsequently the samples were evaluated directly by first digital derivative spectrophotometry, using a smoothing factor of 8 and scale factor of $1 \times 10^{-4}$. The simultaneous determination of furosemide and amiloride can be carried out at 241.4 and 343.6 nm, respectively. In both cases, the zero crossing approach was used. When both compounds are present together in a sample, it is possible to quantify one in the presence of the other, without mutual interference. The determination range was found to be of $6.9 \times 10^{-8}$ to $16 \times 10^{-5}$ and $6.8 \times 10^{-8}$ to $8 \times 10^{-5}$ mol/l, for amiloride and furosemide, respectively. A good level of repeatability (RSD) of 0.9 and 0.6% was observed for amiloride and furosemide, respectively. The ingredients commonly found in commercial pharmaceutical formulations do not interfere. The proposed method was applied to the determination of these drugs in pharmaceutical formulations.



*Keywords:* Amiloride; Furosemide; Pharmaceutical formulation; Simultaneous determination and first digital derivative spectrophotometry

## 1. Introduction

Technological and scientific progress has led to the development of numerous synthetic drugs. It is therefore imperative to dispose of analytical methods to determine these drugs both in the quality control manufacturing phase of the pharmaceutical formulations and their determination in the human body. The drugs under survey in this study are amiloride as hydrated hydrochloric acid and furosemide, which are widely used in various types of diuretics, manufactured by national and international laboratories. These pharmaceutical formulations are administered for numerous therapeutical indications, such as arterial hyper-

* Corresponding author. Tel.: +56-2-6787386; fax: +56-2-2713888

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tension, cardiac insufficiency, and hepatic cirrhosis (Foye et al., 1995; Gringauz, 1997) since they increase the rate of urine formation, thus increasing the excretion of electrolytes, especially sodium, chloride and water. The main organ on which diuretics act is the kidney, where diuretic components interfere in the re-absorption of sodium and other ions (Foye et al., 1995).

Amiloride has the property of interfering with the process of cationic interchange in the distal tube (Foye et al., 1995). It blocks the absorption of sodium ions and the excretion of potassium ions. The degree of amiloride absorption when taken orally is around 50%, and its onset time lasts 10–12 h (Foye et al., 1995).

Furosemide forms part of the high-ceiling or loop diuretics which produce greater diuresis than the common diuretics (Foye et al., 1995). It acts by inhibiting the co-transporter of sodium, potassium and chloride, and further causes excretion of calcium, magnesium and bicarbonate ions (Foye et al., 1995). The diuretic effect appears after 30 min of administration and lasts up to approximately 6 h (Foye et al., 1995).

Since amiloride and furosemide make up various diuretics which are administered worldwide to humans, it is necessary to develop a simultaneous determination of these compounds in different matrixes.

Amiloride has been individually determined in biological fluids like urine and blood-plasma, utilizing isopotential fluorimetry (Murillo-Pulgarin et al., 1997), by capillary zone electrophoresis using fluorescence detection (Gonzalez et al., 1996) and by electrochemical techniques (Gunzel and Schlue, 1997).

Furosemide, on the other hand, has been individually determined in pharmaceutical formulations by extractive-spectrophotometry (Sevillano-Cabeza et al., 1997). Also, in biological fluids it has been determined by HPLC (Okuda et al., 1996) and HPLC-mass spectrometric analysis (Abdel-Hamid and Mohammed, 2000).

Different methods have been presented for the determination of amiloride in presence of other drugs in pharmaceutical formulations (Zivanovic et al., 1996) and in biological fluids (Wood et al., 2000).

In the case of determination of furosemide together with other drugs, their determination has been reported in tablets and urine by HPLC-EC (Barroso et al., 1996a), by micellar electrokinetic chromatography (Lalljie et al., 1997) and by HPLC with amperometric detection (Barroso et al., 1996b). The simultaneous determination of amiloride and furosemide together with other drugs has been reported in urine by screening of diuretics using isocratic reversed phase LC with micellar organic mobile phase (Carda-Broch et al., 2000) and by HPLC using a micellar mobile phase of sodium dodecyl sulfate (Rosado-Maria et al., 2000).

Few methods have been presented for the simultaneous determination of amiloride and furosemide. A HPTLC method has been described for determination of both drugs in pharmaceuticals (Argekar et al., 1995).

Although at present it is easy to find commercial pharmaceutical formulations containing both drugs, the analytical simultaneous determination has not been reported yet in the actual pharmacopoeia (Anon, 2000).

In this study an inexpensive method is proposed for simultaneous determination of both drugs by first derivative spectrophotometry. Also the optimization of the kind of solvent and the spectral variables are included, in order to obtain precise procedures and accurate results in the application of the proposed method for the determination of these drugs. The proposed method was applied in a commercial pharmaceutical formulation that contains both drugs and that is commonly prescribed.

## 2. Material and methods

### 2.1. Instruments

A Shimadzu UV-1603 spectrophotometer with 10-mm quartz cell was used for measurement of the absorbance and derivative absorption spectra. For all the tested solutions, the first derivative spectra was recorded over the range 450.0–190.0 nm against solvent. The spectral data are pro-

cessed by software Shimadzu kit version 3.7 (P/N 206-60570-04).

### 2.2. Materials and reagents

All reagents were of analytical reagent grade. Amiloride and furosemide were provided by Laboratorio Chile, Santiago, Chile.

Stock solutions $1.0 \times 10^{-3}$ mol/l of amiloride and furosemide were prepared by dissolving $3.0 \pm 0.01$ and $3.3 \pm 0.01$ mg of each compound to 10 ml using HCl $1 \times 10^{-2}$ mol/l in ethanol as solvent. Other ranges of concentrations were prepared by appropriate dilution using the same solvent. Different lots of tablets, Tensuren (Laboratorio Chile) and Furdiuren (Chemopharma, Chile), containing these drugs, were also dissolved with the same solvent. Furthermore, in order to carry out a study of the solvent effect on the spectral behavior, stock solutions $1.0 \times 10^{-3}$ mol/l of amiloride and furosemide were prepared by dissolving the same amount of each drug in different solvents and other ranges of concentrations were prepared by appropriate dilution, using the respective solvent.

The drugs were dried at 105 °C before use and kept in hermetic flasks. The exposure to yellow light of the standard solutions was avoided by storing in dark bottles (Anon, 2000). Therefore all of the experiments were done using drugs and standard solutions that were maintained in the above conditions.

### 2.3. Calibration procedure for determination of amiloride and furosemide in mixture

Aliquots of stock solution of amiloride and furosemide were simultaneously diluted in HCl $1 \times 10^{-2}$ mol/l in ethanol to obtain the concentration range $4.0 \times 10^{-5}$–$16.0 \times 10^{-5}$ mol/l. The calibration graphs were carried out for each compound in presence of $6.0 \times 10^{-5}$ mol/l of the other. In all cases, the corresponding absolute values of the first derivative spectra at 343.6 and 241.4 nm for amiloride and furosemide respectively were obtained, and the values were plotted against the corresponding concentration.

### 2.4. Procedure for determination of amiloride and furosemide in pharmaceutic formulation

A lot containing a total of 10 tablets of each pharmaceutical formulation, were weighed and powdered. A quantity of powder around 57 mg of each tablet containing both drugs was accurately weighed and transferred into 100 ml calibrated flask and dissolved in HCl $1 \times 10^{-2}$ mol/l in ethanol to the mark. The content of the flasks, was shaken for 10 min. Later, the suspension was centrifuged and the supernatant solution was evaluated by the first derivative spectrophometry.

The same procedure as mentioned before was carried out for different lots of Tensuren and of Furdiuren.

## 3. Results and discussion

Taking into account that the pharmaceutical formulations contain amiloride and furosemide in a 8/1 ratio, it was necessary to carry out a detailed study of different variables.

### 3.1. Solvent effect on the spectra

The structures of amiloride dihydrochlorohydrate and furosemide are shown in Fig. 1(a) and (b). As can be seen the structures of these drugs are quite different, consequently the spectral behavior of these compounds in various solvents could also be expected to be different.

The spectral behavior of amiloride and furosemide in a concentration of $1 \times 10^{-3}$ mol/l was studied in the following solvents: methanol, ethanol, dioxane, acetonitrile, dimethyl sulfoxide, and dimethylformamide. Further, a study was conducted on the alkaline and acid effect on methanol and ethanol using HCl $1 \times 10^{-2}$ mol/l and NaOH $1 \times 10^{-2}$ mol/l (Table 1). In all cases the spectral behavior of the drugs in the mentioned solvents showed no alteration during 48 h.

To select the appropriate solvent, chemical characteristics and economic factors pertaining to each of the eight solvents were taken into consideration. Dimethyl sulfoxide was discarded since no optimum wavelength was found to determine

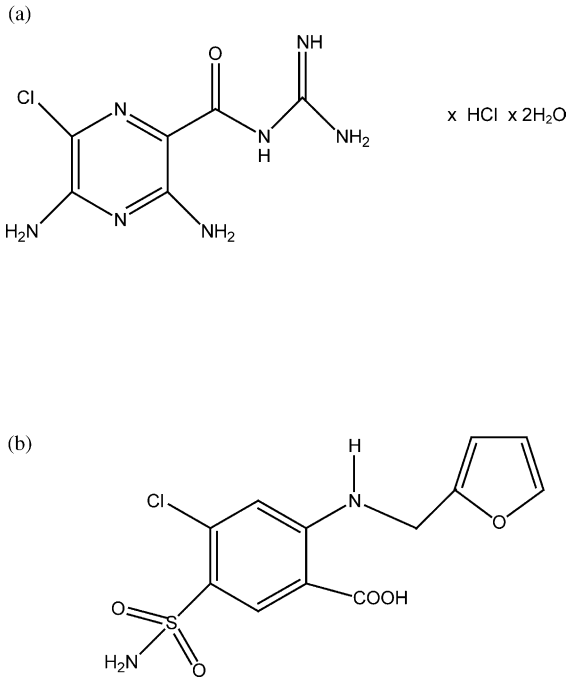


Fig. 1. Structures of amiloride dihydrochlorohydrate and furosemide. (a) Amiloride dihydrochlorohydrate; (b) furosemide.

furosemide, and because the spectra present a zone of high overlapping of the bands at wavelengths near to 300.0 nm. Dimethylformamide was also discarded because the spectra present small analytical signals at 301.0 nm.

The remaining solvents met the necessary requirements for simultaneous determination. Ethanol–NaOH was not considered since it does not present good solubility and could become a future source of error. The chosen solvent was ethanol–HCl, since it is less volatile and the cost is lower.

### 3.2. Spectral behavior

Amiloride dissolved in HCl $1 \times 10^{-2}$ mol/l in ethanol, was evaluated directly against the solvent. As can be seen in Fig. 2A, the spectrum of this compound shows three absorption maxima (Table 1), in similar conditions furosemide spectrum (Fig. 2B) also presents three absorption bands (Table 1) and these bands are strongly overlapped. Numerical methods based on the mathematical resolution of multivariate signals, like the ones obtained from UV-visible spectroscopic data, is an alternative to simultaneously determine these analytes in spite of the fact that their bands are strongly overlapped. The partial least-squares regression with a single dependent variable (PLS-1) is a type of numerical method (Ferraro et al., 2001). However, the derivative spectrophotometry technique was adopted in order to determine binary mixture of constituents, because it is a reliable technique which does not require complex processing of mathematical data. Therefore, it is much easier to apply than the numerical type method (Toral et al., 2001, 2002, 2000).

Table 1
Wavelengths of the spectral classic bands for amiloride and furosemide in different solvents

| Solvent | Wavelengths/nm | |
|---|---|---|
| | Amiloride | Furosemide |
| Methanol | 215.6 | 234.0 |
| | 286.0 | 273.0 |
| | 361.2 | 341.0 |
| Methanol–HCl | 211.6 | 234.0 |
| | 285.8 | 272.8 |
| | 361.4 | 343.2 |
| Methanol–NaOH | 261.8 | 228.0 |
| | 283.0 | 274.0 |
| | 364.6 | 330.0 |
| Ethanol | 215.0 | 234.0 |
| | 286.0 | 273.0 |
| | 362.0 | 337.0 |
| Ethanol–HCl | 215.0 | 234.0. |
| | 286.0 | 272.0 |
| | 362.0 | 342.0 |
| Ethanol–NaOH | 219.0 | 227.0 |
| | 283.0 | 270.0 |
| | 364.6 | 332.0 |
| DMF | 286.8 | 276.0. |
| | 364.6 | 332.0 |
| DMS | 288.4 | 275.4 |
| | 364.4 | 340.2 |
| Dioxane | – | – |

Despite the fact that amiloride and furosemide present overlapped bands, the derivative method proposed by the Savitzky and Golay (1964) was

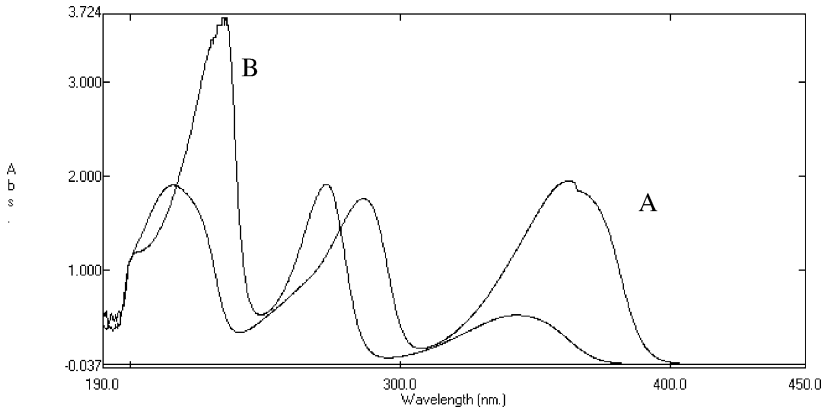


Fig. 2. Absorption spectra of amiloride and furosemide in HCl $1 \times 10^{-2}$ mol/l in ethanol against solvent. (A) amiloride, $1.0 \times 10^{-4}$ mol/l and (B) furosemide, $1.0 \times 10^{-4}$ mol/l.

adopted in this study, because with this mode it was possible to resolve the spectral bands and to obtain a control of the noise of the baseline and the analytical signal. Under this condition the higher signal/noise ratio was found.

### 3.3. Selection of spectral variables

#### 3.3.1. Derivative order

Derivative spectra of different orders were obtained from the zero-order spectra using digital differentiation (Fig. 3). Fig. 3a and b shows that the first and second derivative could be used for simultaneous determination of amiloride and furosemide because in all cases the derivatives present characteristic zones for each compound. When the derivative order increases, the sensitivity decreases. When the first derivative is used, the simultaneous determination can be achieved easily, since both the spectra present well-defined zones for determination of each analyte and the sensitivities are greater. Thus, the first derivative was selected. The second, third and fourth derivatives were discarded, because they do not present analytical advantages.

#### 3.3.2. Selection of the smoothing factor

By using the first derivative, the smoothing factor was varied and the following values were used: 2, 4, 8 and 16. These values are defined by default, which are in relation to the range of wavelength in which the spectra were scanned.

The $\Delta\lambda$ value of differentiation is constant and corresponds to 260.0 nm, when the smoothing factor is increased the heights of the derivative signal decrease but the noise decreases faster, increasing in this way the signal/noise ratio. A value of 8, was selected because it presents maximum sensitivity without affecting the signal/noise ratio.

#### 3.3.3. Selection of the scale factor

The scale factor must be studied in order to observe whether the system presents a distortion effect of the spectra. Further, the selection of this parameter permits improvement of the reading of the analytical signal. Considering this, a scale factor of $10^4$ was selected.

#### 3.3.4. Selection of analytical wavelength

The selection of the analytical wavelengths was carried out using the spectral parameters previously selected. This selection is based on sensitivity of the signals in the first derivative spectra.

The first derivative spectra of amiloride dissolved in HCl $1 \times 10^{-2}$ mol/l in ethanol, was evaluated directly against solvent, presenting three principal zero crossing at 215.0, 241.4 and 285.0 nm (Fig. 4). These points could be used for the determination of furosemide. The wavelength of 215.0 nm was discarded because it was very near to solvent absorption and the analytical signal offers low sensitivity. On the other hand, the zero crossing point at 285.0 nm was discarded, because this

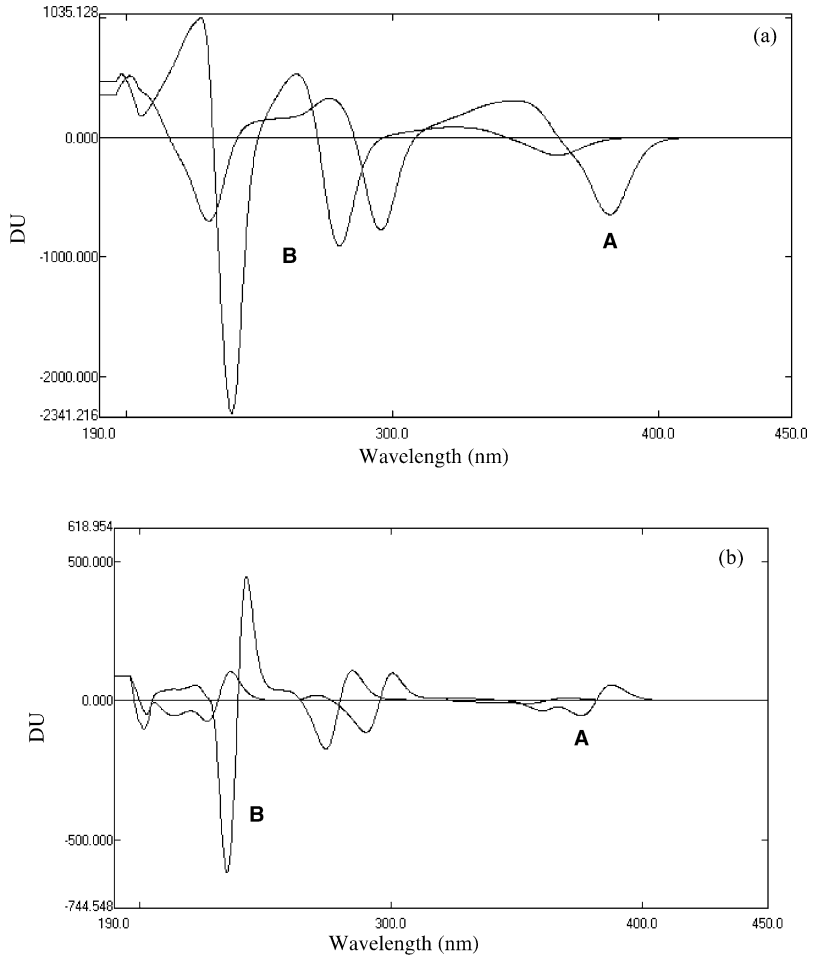


Fig. 3. Derivative spectra of amiloride and furosemide dissolved in HCl $1 \times 10^{-2}$ mol/l in ethanol against solvent. (A) amiloride, (B) furosemide. (a) First derivative spectra; (b) second derivative spectra. DU: derivative unit.

point is too close to the first derivative spectral band of amiloride. The selected wavelength for furosemide determination was 241.4 nm because the analytical signal is highly sensitive and it is located in the center of the peak of the first derivative spectra.

In similar conditions, the first derivative spectra of furosemide present five zero crossing at 232.0, 249.0, 271.0, 296.0 and 343.6 nm (Fig. 4). Besides, furosemide spectrum presents a wavelength range between 390.0 and 400.0 nm where it does not absorb, but amiloride presents absorption. Therefore, many wavelengths could be selected for amiloride determination. At 232.0, 249.0, 271.0 and 296.0 nm the results could be more uncertain, because an overlapping of the derivative spectra could occur when furosemide concentration increases. The graphic method was not selected because the sensitivity is low, due to the fact that the measurement must be carried out near the end of the first derivative spectral of amiloride.

Taking into account the behavior of the first derivative spectra, the best analytical wavelength to determine amiloride is of 343.6 nm, since at this

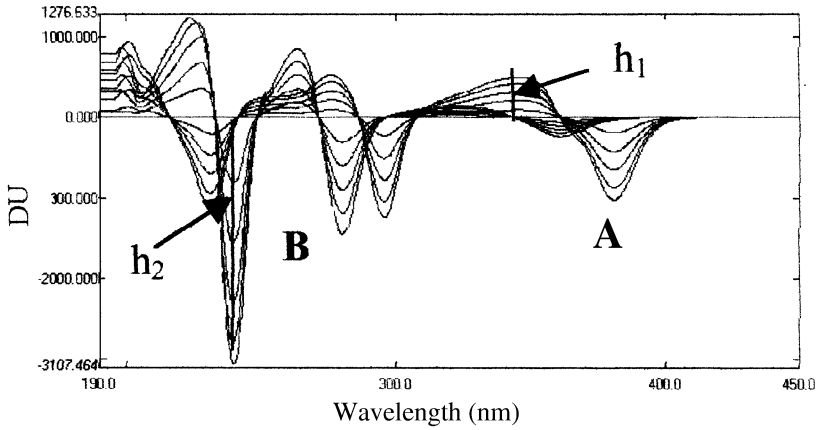


Fig. 4. (a) Concentration effects of furosemide ($6 \times 10^{-5}$ mol/l) over amiloride ($2 \times 10^{-5}$, $4 \times 10^{-5}$, $6 \times 10^{-5}$, $8 \times 10^{-5}$, $10 \times 10^{-5}$, $12 \times 10^{-5}$, $14 \times 10^{-5}$ and $16 \times 10^{-5}$ mol/l) at 343.6 nm. (b) Concentration effects of amiloride ($6 \times 10^{-5}$ mol/l) over furosemide ($2 \times 10^{-5}$, $4 \times 10^{-5}$, $6 \times 10^{-5}$, $8 \times 10^{-5}$ mol/l) at 241.4 nm.

point there is no possible overlapping, the analytical signals are well defined, and it is possible to expect more accurate results.

According to the above analysis, the simultaneous determination of furosemide and amiloride can be carried out at 241.4 and 343.6 nm, respectively.

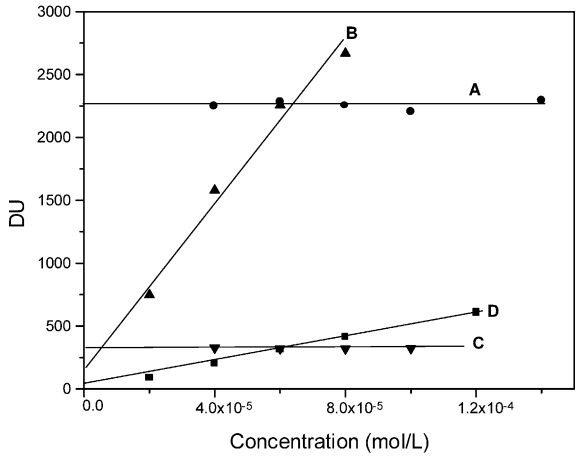


Fig. 5. Calibration curves of amiloride in presence of furosemide and viceversa and the effect of the concentration of one analyte over the other: (A) Effect of the furosemide concentration on analytical signal of amiloride at 241.4 nm; (B) calibration curve for furosemide at 241.4 nm in presence of amiloride; (C) effect of the amiloride concentration on analytical signal of furosemide at 343.6 nm; (D) calibration curve of amiloride at 343.6 nm in presence of furosemide.

### 3.4. Analytical features

By using a smoothing factor of 8, a scale factor of $10^4$ and a $\Delta\lambda$ of 260.0 nm, calibration graphs (Fig. 5) were obtained by plotting the first-derivative value, $h_1$ for amiloride ($\lambda = 343.6$ nm) and $h_2$ for furosemide ($\lambda = 241.4$ nm), versus the respective analyte concentration. The linear regression equations and the correlation coefficients calculated for mixtures of both analytes were:

$$\text{Amiloride } h_1 = 5.1 \times 10^6 \text{C (M)} + 0.97$$
$$r = 0.999;$$

$$\text{Furosemide } h_2 = 3.2 \times 10^7 \text{C (M)} + 203.8$$
$$r = 0.989;$$

where, $h$ is in derivative units and C(M) corresponds to the analyte concentration in mol/l.

Furthermore, as can be seen in Fig. 5 a good linearity is obtained and the analytical signals do not present mutual interference among the determination of these drugs. All analytical features are shown in Table 2.

In order to establish the ratios at which one analyte can be accurately measured in presence of the other, the recoveries of samples containing standard solutions of mixtures of amiloride and furosemide in different concentration ratios were carried out. The results are shown in Table 3. The

Table 2
Analytical features

| Analytical parameters | Amiloride | Furosemide |
|---|---|---|
| Detection limit[a] (mol/l) | $2.1\vert \times 10^{-8}$ | $2.0 \times 10^{-8}$ |
| Determination limit[b] (mol/l) | $6.9 \times 10^{-8}$ | $6.8 \times 10^{-8}$ |
| Determination range (mol/l) | $6.9 \times 10^{-8}$–$16 \times 10^{-5}$ | $6.8 \times 10^{-8}$–$8 \times 10^{-5}$ |
| Repeatability[c] (RSD/%) | 1.26 | 1.29 |

[a] $3\sigma$ criterion.
[b] $10\sigma$ criterion.
[c] Relative standard deviation ($n = 8$).

content of each compound can be determined, if the concentration ratio is between 1:8 and 8:1 for amiloride:furosemide. According to the results it is possible to conclude that this method has a wide range of application and besides it permits the simultaneous determination of both drugs in real pharmaceutical formulations.

### 3.5. Application

The accuracy of the method was determined by analysis of synthetic formulation samples containing 5 mg of amiloride and 40 mg of furosemide mixed with 195 mg of excipients (approximately magnesium stearate + gelatin 3–5% and lactose–starch 95–97%). The recoveries were found to be $99.2 \pm 0.9$ and $98.8 \pm 0.6$%, for amiloride and furosemide, respectively. According to the result, it is possible to establish that common excipients normally found in tablets do not interfere in the proposed method.

However, in order to insure, that in absence of light, the ingredients and its degradation products in pharmaceutical formulations do not interfere through time in the simultaneous determination of amiloride and furosemide one portion of each tablet was diluted individually in HCl $1 \times 10^{-2}$ mol/l in ethanol, so that the solution contains $1 \times 10^{-5}$ mol/l of amiloride and $8 \times 10^{-5}$ mol/l of furosemide. Aliquots of these solutions were taken every hour for a period of 12 h. These aliquots were scanned in the spectrophotometer and the first derivative was registered. In all cases, the classical spectra and the first derivative did not present changes in its form, this means, that the height and the maximum wavelength of the bands were maintained. Furthermore, the values of $h_1$ and $h_2$ did not change, which indicates that the components of the tablets do not interfere in the simultaneous determination of amiloride and furosemide, under the experimental conditions proposed in this method.

On the other hand, the content of amiloride and furosemide in Tensuren (Laboratorio Chile) and in Furdiuren (Chemopharma) was analyzed. Both pharmaceutical formulations contain nominally 5 mg of amiloride and 40 mg of furosemide. The average weight of one lot of Tensuren tablets and of one lot of Furdiuren tablets is 240 and 150 mg, respectively. Following the application of the method, amiloride presents an average of $4.95 \pm 0.09$ mg and furosemide one of $38.99 \pm 0.038$ mg in one lot of Tensuren tablets. Similar results, with

Table 3
Amiloride and furosemide recuperation using standard solution in different ratio (mg/100 ml)

| Ratio amiloride/furosemide | Amiloride added (mg) | Recovery found[a]/% (RSD/%) | Furosemide added (mg) | Recovery found[a]/% (RSD/%) |
|---|---|---|---|---|
| 1:1 | 0.029 | 99.1 (1.0) | 0.034 | 99.1 (0.6) |
| 1:2 | 0.029 | 99.0 (1.0) | 0.067 | 101.1 (0.7) |
| 1:4 | 0.029 | 97.3 (0.8) | 0.135 | 98.7 (0.6) |
| 1:8 | 1.16 | 98.8 (0.9) | 9.28 | 97.9 (0.5) |
| 2:1 | 0.058 | 101.4 (1.0) | 0.034 | 99.1 (0.7) |
| 4:1 | 0.116 | 97.4 (0.8) | 0.034 | 100.1 (0.7) |
| 8:1 | 0.233 | 98.7 (0.9) | 0.034 | 98.7 (0.6) |

[a] Average of five determination.

the same standard deviation, were obtained for one lot of Furdiuren tablets, even though they contain different quantities of excipients. The results obtained from the analysis of different lots of Tensuren and of Furdiuren, are also similar to the ones presented previously.

## 4. Conclusion

The simultaneous determination of drugs by the technique of digital derivative spectrophotometry in liquid phase is generally easier and more useful when the spectral classic bands are overlapped.

In order to carry out the simultaneous determination of amiloride and furosemide by digital derivative spectrophotometry in liquid phase an exhaustive analysis of solvent effect was done due to the fact that the molecular forms of the drugs are different. Their derivative spectrum tend to overlap in all of the solvents that were under study, including in ethanol–HCl, the solvent chosen to carry out the simultaneous determination. However, in this solvent it was possible to distinguish two wavelengths where the simultaneous determination of the analytes by first digital derivative spectrophotometry could be carried out, because at these points there is no overlapping of the derivative spectral bands and the analytical signals are highly sensitive and well defined.

The validation of the method to simultaneously determine furosemide and amiloride by digital derivative spectrophotometry in liquid phase, is an important contribution, because it has not been reported by the actual pharmacopoeia. Further, the proposed method requires few instruments, is reliable, easy and quick to apply, besides being inexpensive.

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