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Furosemide assay in pharmaceuticals by Micellar liquid chromatography: study of the stability of the drug

S. Carda-Broch [a], J. Esteve-Romero [b], M.C. García-Alvarez-Coque [a,*]

[a] *Departament de Química Analítica, Facultat de Química, Universitat de València, Dr. Moliner 50, 46100 Burjassot (València), Spain*
[b] *Area de Química Analítica, Universitat Jaume I, Cra. Borriol s/n, 12080 Castelló, Spain*

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Abstract

A simplified high-performance liquid chromatographic procedure is described for the determination of furosemide (4-chloro-*N*-furfuryl-5-sulphamoylanthranillic acid), which makes use of UV detection, a C_{18} reversed-phase column, and micellar mobile phases of sodium dodecyl sulphate (SDS) and 1-propanol at pH 3 buffered with phosphate system. The most adequate experimental conditions to handle furosemide solutions in the analytical laboratory are studied. The mixture of furosemide and its degradation products which are formed upon light exposition was resolved with a mobile phase of 0.04 M SDS-2% propanol. Separation of furosemide from its common impurities and the hydrolytic product, 4-chloro-5-sulphamoylanthranillic acid, was also possible. A mobile phase of larger elution strength, such as 0.06 M SDS-8% propanol was preferred to assay furosemide in several dosage forms (tablets, capsules, injectables and drops). The validity of the procedure was checked by analysing 27 pharmaceuticals commercialised in several countries. The label claim percentages and coefficients of variation were in the 95–102% and 0.05–1.3% ranges, respectively. The results showed that the procedure is suitable for routine analysis of the diuretic.

Keywords: Furosemide; Degradation products; Impurities; Stability conditions; Pharmaceuticals; Micellar liquid chromatography

1. Introduction

Furosemide (4-chloro-*N*-furfuryl-5-sulphamoylanthranillic acid) is a potent diuretic of rapid action, used in the treatment of edema associated with hypertension, congestive heart failure [1,2], pulmonary and renal diseases [3], cirrhosis of the liver [4] in humans, and epistaxis [5] in race horses, and normally administered as tablets or intravenous and intramuscular injectables. Furosemide has a secondary amine group and is therefore susceptible to acid catalysed hydrolysis. At high temperature, it hydrolyses to 4-chloro-5-sulphamoylanthranillic acid (CSA) and furfuryl alcohol [6–8], which is quickly converted to levulinic acid [9,10] (Table 1).

* Corresponding author. Tel.: +34-96-3983003; fax: +34-96-3864436.
E-mail address: celia.garcia@uv.es (M.C. García-Alvarez-Coque).

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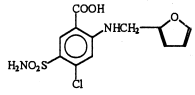
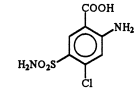
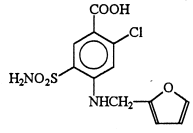
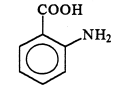
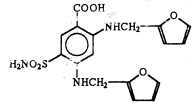
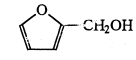
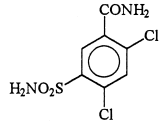
Photochemical degradation of furosemide has been extensively reported. In acidic solution, rapid photolytic degradation takes place. Several authors have investigated the nature of the degradation products in aqueous and methanolic solution under the influence of UV light. Furosemide seems to undergo photooxidation, photohydrolysis and photodechlorination. Rowbotham et al. [11] found that UV irradiation of furosemide during 48 h, in alkaline solution, produced 4-chloro-5-sulphoantranillic acid by oxidation of the sulphamoyl group to sulphonic acid with hydrolysis of the furfuryl group, which is also formed by hydrogen peroxide oxidation of the hydrolysis product, CSA. Moore et al. [12,13] first reported that both furosemide and CSA suffer photodechlorination to *N*-furfuryl-5-sulphamoylanthranillic acid (FSA) and 5-sulphamoylanthranillic acid, respectively, in oxygen-free solutions, and used reversed-phase liquid chromatography and gas chromatography both coupled to mass spectrometry (RPLC-MS and GC-MS) to investigate the nature of the compounds. The authors separated isocratically the drug from its photodecomposition products using a mobile phase of water:methanol 75:25 (v/v) with an analysis time of 38 min. The lost of chlorine decreased the retention of furosemide and CSA. According to Bundgaard et al. [8], furosemide loses the chlorine atom to give FSA, but its substitution with a hydroxyl group derived from an aqueous solvent may also be imaginated. Recently, Vargas et al. [14] analysed by ^{1}H- and ^{13}C-NMR spectroscopy, IR and GC-MS, the products formed under irradiation in aerobic and anaerobic conditions, in methanolic and buffered (pH 7.4) aqueous medium. Three main products were found where furosemide suffered photodechlorination to obtain FSA, or decarboxylation with hydrogen or hydroxyl abstraction.

Table 1
Retention times and maximum wavelengths for the peaks obtained in the solution of several compounds protected from light

Compound	Structure	t_R (min)	λ (nm)	Compound	Structure	t_R (min)	λ (nm)
Furosemide		12.8	236, 274, 344	CSA		2.3	230, 268, 330
CFS		17.1	226, 286, 322 (shoulder)	Anthranillic acid		33.3	220, 330
BFS		22.3	258, 290, 328	Furfuryl alcohol		4.2	226, 278
DSB		4.0	212, 232 (shoulder)	Levulinic acid	$CH_3COCH_2CH_2COOH$	1.7	266

Numerous analytical methods are available for the assay of furosemide in pharmaceutical preparations that include titrimetric [15], spectrophotometric [16,17], flow injection-spectrophotometric [18], fluorimetric [19], coulometric [20], voltammetric [21], ^{1}H-NMR [22], and HPLC methods [23–30]. There are also several reports on the chromatographic separation of furosemide and its degradation products [8,10,13,31–33], or impurities [27,34].

The comments found in the literature about the adequate medium to prepare furosemide solutions are contradictory. In this work, we study the experimental conditions needed to prevent the degradation of this diuretic in the analytical laboratory. A simple chromatographic procedure with micellar mobile phases of sodium dodecyl sulphate (SDS) is developed, which is applied to the control of numerous pharmaceuticals in several dosage forms. The procedure resolves also furosemide from its photodegradation products or usual impurities, which is useful to check the purity of the solutions.

2. Experimental

2.1. Reagents

Furosemide standards were either kindly donated by Hoechst (Frankfurt-am-Main, Germany), or obtained from Sigma (St. Louis, MO, USA). Furfuryl alcohol was from Sigma and levulinic acid from Aldrich (Milwaukee, WI, USA). The impurities 4-chloro-5-sulphamoylanthranillic acid, 2,4-dichloro-5-sulphamoylbenzamide (DSB), 2-chloro-4-(2-furfurylamino)-5-sulphamoylbenzoic acid (CFS), and 2,4-bis(2-furfurylamino)-5-sulphamoylbenzoic acid (BFS) were supplied by Hoechst. The pharmaceuticals Furosemide 1%, Frusemide Injection BP, and Diflux were donated by Inibsa, Evans Medical and Merck Química, respectively. Information about the pharmaceuticals Salidur and Nuriban were kindly given by Grupo Farmacéutico Almirall and Roux–Ocefa, respectively.

Other reagents were sodium dodecyl sulphate (Merck, Darmstadt, Germany), sodium dihydrogenphosphate, disodium hydrogenphosphate, sulphamic acid (Panreac, Barcelona, Spain), HCl, NaOH (Probus, Badalona, Spain), acetonitrile, methanol, 1-propanol (Scharlau, Barcelona), ethanol (Prolabo, Paris, France), *N*-(1-naphthyl)ethylenediamine chlorhydrate (NED, Merck), and sodium nitrite (Fluka, Buchs, Switzerland). Nanopure water (Barnstead, Sybron, Boston, MA, USA) was used to prepare the aqueous solutions.

2.2. Apparatus

Hydrolysis of furosemide was performed in a thermostatic bath (model Precis-Term, Selecta, Barcelona). The HPLC system consisted of a Hewlett-Packard chromatograph (model HP 1050, Palo Alto, CA, USA), provided with an isocratic pump, an autosampler (series 1100 model G1313A), and a UV/VIS diode-array detector (series 1100 model G1315A). The signal was acquired with a PC computer connected to the chromatograph through an HP Chemstation. The software MICHROM was used for the treatment of the chromatographic data and to perform the optimisation studies [35].

The analytical separation was accomplished using an ODS-2 C_{18} reversed-phase column (5 μm particle size, 125 × 4.6 mm i.d.), connected to a 30-mm guard column of similar characteristics (Scharlau). The chromatographic system was washed weekly with 60 ml of water to eliminate the surfactant, and afterwards, with 60 ml of methanol.

2.3. Standard solutions

Stock standard solutions containing 100 μg/ml of furosemide, its impurities, furfuryl alcohol or levulinic acid were prepared. Furosemide was dissolved in 10 ml of ethanol with the aid of an ultrasonic bath (model 617, Selecta), and was made up to the mark in a 100 ml volumetric flask with water or 0.1 M SDS, usually in the pH range 3–7 buffered with phosphate system. Similar solutions were prepared for the impurities, furfuryl alcohol and levulinic acid in 0.1 M SDS at pH 3. For the stability studies, furosemide solutions

were diluted to 10 μg/ml with the same SDS solution. For the analysis of the pharmaceuticals, several standard solutions were prepared in the range 4–20 μg/ml. All solutions were protected from light (except some used in the stability studies) with aluminium foil, and kept in the dark at 4°C overnight.

2.4. Procedure

The pharmaceuticals analysed were tablets, capsules, intravenous and intramuscular injectables and drops. The average weight per tablet was calculated from 10 units. The tablets were ground and reduced to a homogeneous fine powder in a mortar. Several portions of this powder and capsule contents equivalent to ca. 5 mg of furosemide were accurately weighed and sonicated in the presence of a small amount of ethanol in an ultrasonic bath. A 0.1 M SDS solution at pH 3 was added to favour the extraction of the analyte, using again the ultrasonic bath. A dilution was then made with the same micellar solution to a final concentration of ca. 10 μg/ml.

The injectables and drops were contained in amber glass vials and non-transparent plastic containers, respectively. Aliquots of 25 μl of the injectables were taken with a micropipette of 20–200 μl from Eppendorf (Hamburg, Germany) and diluted with the 0.1 M SDS solution up to 25 ml. The mixture was vigorously shaken. A similar dilution was made with the drops, taking 15 μl with a micropipette Transferpette of 10–50 μl from Brand (Wertheim, Germany).

The excipients in the tablets and capsules were not soluble in the micellar medium, hence the sample solutions should be filtered before their injection into the chromatograph. The standard solutions of furosemide were also filtered. However, the filtration was performed directly into the autosampler vials through 0.45 μm nylon membranes of 13 mm diameter (Micron Separations, Westboro, MA, USA). The transparent glass vials in the autosampler were protected from light with aluminium foil to avoid photochemical degradation of the drug.

2.5. Chromatographic conditions

The micellar mobile phase used to resolve the mixture of furosemide and its degradation products, in the stability studies, was 0.04 M SDS-2% propanol-0.01 M NaH_2PO_4 at pH 3. A mobile phase of larger elution strength, 0.06 M SDS-8% propanol-0.01 M NaH_2PO_4 at the same pH, was used to analyse the pharmaceuticals. The pH was adjusted before the addition of propanol to the micellar solution. The mobile phases were filtered through 0.45 μm Nylon membranes of 47 mm diameter (Micron Separations). The flow-rate was 1 ml/min and the injection volume, 20 μl. The chromatographic runs were carried out at the laboratory temperature. The detection was performed at several wavelengths: 220, 240, 274, and 340 nm in the optimisation study of the resolution of the mixture of furosemide and its photodegradation products, and at 274 nm to assay furosemide in the pharmaceuticals.

3. Results and discussion

3.1. Influence of pH on the retention of furosemide

Furosemide has two protonable groups in non-micellar aqueous medium, with protonation constants $\log K_1 = 7.5$ and $\log K_2 = 3.8$. The cationic species dominates at $pH < 3.8$ and the anionic species at $pH > 7.5$. The presence of micelles of the anionic surfactant SDS increases the stability of the cationic drug, and consequently, the value of $\log K_2$. We have also observed that the acid-catalised degradation is slower in micellar solution with respect to aqueous medium [8,33,36]. When the retention factor of furosemide was measured at increasing pH and different concentrations of surfactant and propanol in the mobile phase, it was found that this constant was slightly modified (Fig. 1A, B). The retention decreased at increasing pH, due to repulsion of the anionic fusosemide by the negatively charged heads of the monomers of surfactant adsorbed on the stationary phase, and free silanol groups. At $pH > 6$, furosemide eluted at the dead time. The efficien-

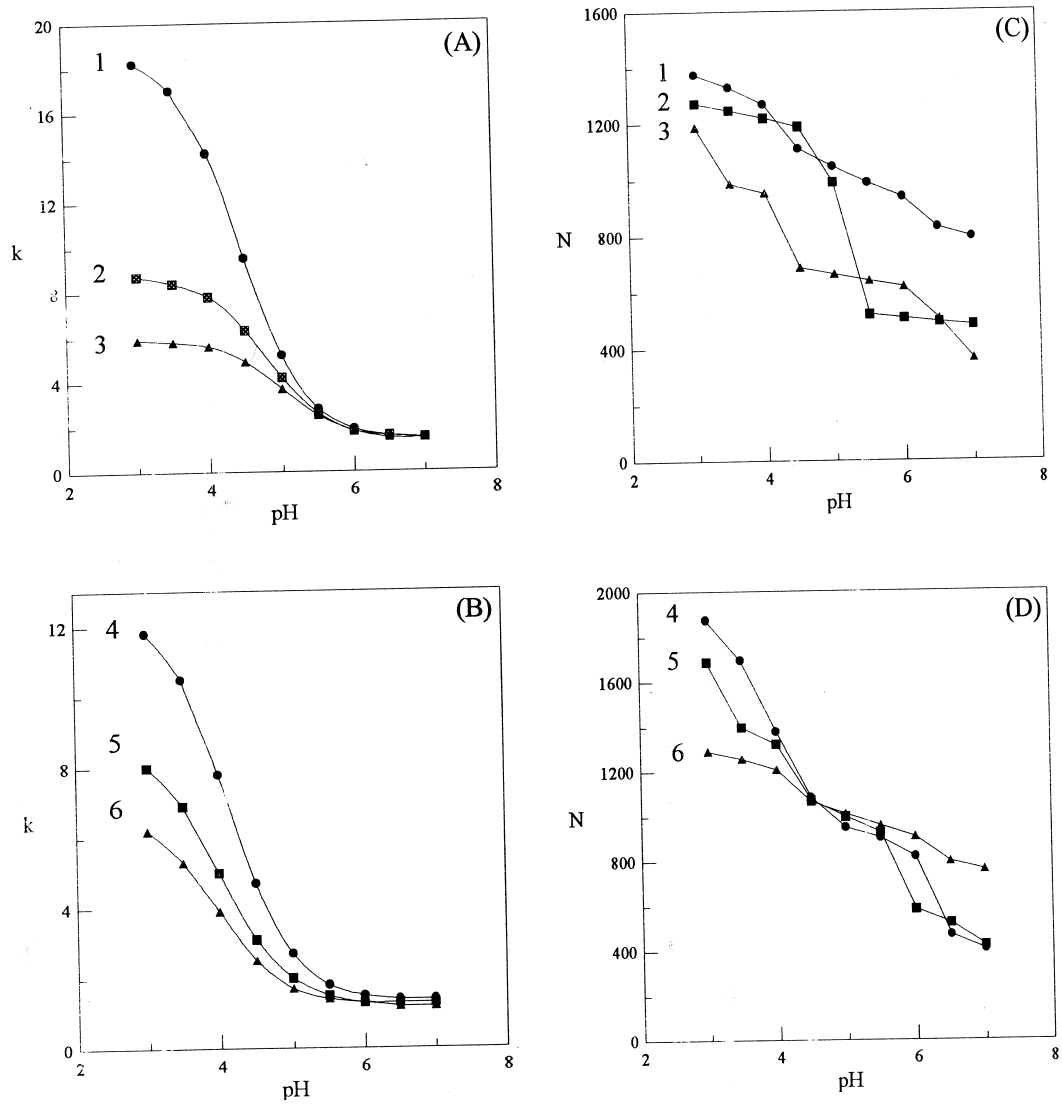


Fig. 1. Influence of the mobile phase pH on the retention factor (k) and efficiency (N) of neutral solutions of furosemide. Mobile phase composition: (A, C) absence of modifier and varying concentrations of SDS: 0.04 M (1), 0.09 M (2), and 0.14 M (3); (B, D) 0.04 M SDS and varying concentrations of propanol: 2% (4), 5% (5), and 8% (6).

cies also deteriorated at increasing pH of the mobile phase (Fig. 1C, D).

In the experiments shown above, non-buffered solutions of furosemide in micellar medium were injected. When the solutions were buffered at pH 3, the behaviour was different: the retention times in mobile phases at pH 3 and 5 were coincident, whereas at pH 7 the peak appeared at the dead time, as expected. In these conditions, the pH range where the cationic species dominates was increased, which suggests an impediment in the establishment of the acid–base equilibria inside the chromatographic column.

The analysis of furosemide samples should be carried out with mobile phases buffered at pH 3–5, since the retention times and plate counts are

excessively low at higher pH. The following studies were made with a mobile phase at pH 3. Neil et al. [31] reported that the HPLC procedures for furosemide, utilising an acidic mobile phase, suffer from the disadvantage of the breakdown of the diuretic during chromatography on account of its acid lability, and suggested the use of a neutral or alkaline mobile phase, such as 1-propanol-0.02 M KH_2PO4 (25:75 v/v) containing 0.25% cetrimide at pH 7. However, other authors [28,29,37–42] used an acidic aqueous-organic mobile phase without making any observation about the degradation.

3.2. Optimisation of the separation of furosemide and its degradation products

A micellar solution of 10 μg/ml of furosemide at pH 3, stored unprotected from daylight, was used to study the separation of the parent drug from its main degradation products. After 2 h of exposition to sunlight (beside a laboratory window), the vial containing the furosemide solution was kept in the dark covered with aluminium foil. This solution remained practically unchanged during the measurements (about 1 week). Furosemide and its degradation products were eluted with mobile phases of SDS at pH 3 containing a small amount of propanol, not greater than 8% (v/v). The concentration of SDS was in the range 0.04–0.14 M. As can be observed in Fig. 1A, B, the effect of the surfactant on the retention of furosemide (the compound which showed the larger retention) is similar to that of propanol, in the studied factor space. The retention time of the diuretic was 3.4 min for 0.14 M SDS-8% propanol and 20.2 min for 0.04 M SDS. The retention time in these mobile phases for the least retained degradation product was 2.4 and 7.9 min, respectively.

The optimisation of the separation of furosemide from its degradation products was performed using a procedure based on the prediction of retention times with a model equation [35]. The chromatographic data from five mobile phases were used: 0.04 M SDS-1% propanol, 0.14 M SDS-1% propanol, 0.09 M SDS-4% propanol, 0.04 M SDS-8% propanol, and 0.14 M SDS-8% propanol. Some changes in the elution order of the decomposition products were produced when a small amount of propanol (1%) was added to pure micellar phases (without modifier), which increased the errors in the prediction of retention times. For this reason, the experimental design did not include mobile phases without propanol.

Modelling of the retention of some degradation products was troublesome due to the lack of standards. We did not know their identity either. However, the diverse compounds had different absorption spectra, and monitoring at several wavelengths (220, 240, 274, and 340 nm) permitted the knowledge of the changes in elution order of each peak, or the retention times of overlapped peaks. In this study, we took the chromatograms measured at 274 nm as reference (Fig. 2A). It was observed that at 220 nm, the peak height of the degradation product labelled as III increased with respect to peak I, and peak II was smaller than peaks I and III. At 240 nm, peaks II, IV and V increased, whereas peaks I and III were much smaller. Finally, at 340 nm, peaks II, III and V decreased, and peaks I and IV were not observed. All these peaks were also found in solutions exposed to artificial light, except peak IV which only appeared under sunlight.

The global error in the prediction of the retention was 0.9%. Maximum resolution was achieved in a wide range of concentrations of SDS (0.04–0.06 M SDS) and the whole range of concentrations of propanol. The elution order was the same in this region ($I < II < III < IV < V$) (see Fig. 2A). However, at increasing concentrations of SDS (above 0.075 M and low concentrations of propanol), or at decreasing concentrations of propanol (below 7% and large concentrations of SDS), compound I retarded its retention and reversed its elution order with respect to compound II. For 0.14 M SDS-1% propanol the elution order was $II < I \approx III < IV < V$. A mobile phase of low elution strength was finally selected: 0.04 M SDS-2% propanol at pH 3, which permitted the observation of further degradation of the drug with appearance of new peaks. The retention time of furosemide with this mobile phase was 12.7 min.

3.3. Photolytic degradation of the drug

A study was performed on the stability of furosemide solutions at several pH values. Since furosemide is only sparingly soluble in water, it was dissolved in a small amount of ethanol, and further diluted with water or 0.1 M SDS solution. The final ethanol contents was 10% (v/v); this amount of alcohol has been reported to increase the photostability of furosemide solutions [10,36]. Several solutions were buffered at pH 3, 5, 7 and 10, and protected from light or exposed to standard laboratory lighting (fluorescent light Osram 40 W, Germany, at 1.5 m), at room temperature. No precautions were taken to prevent contact of the samples with air, and no efforts were made to expel oxygen from the solutions, which were analysed to know their degradation degree.

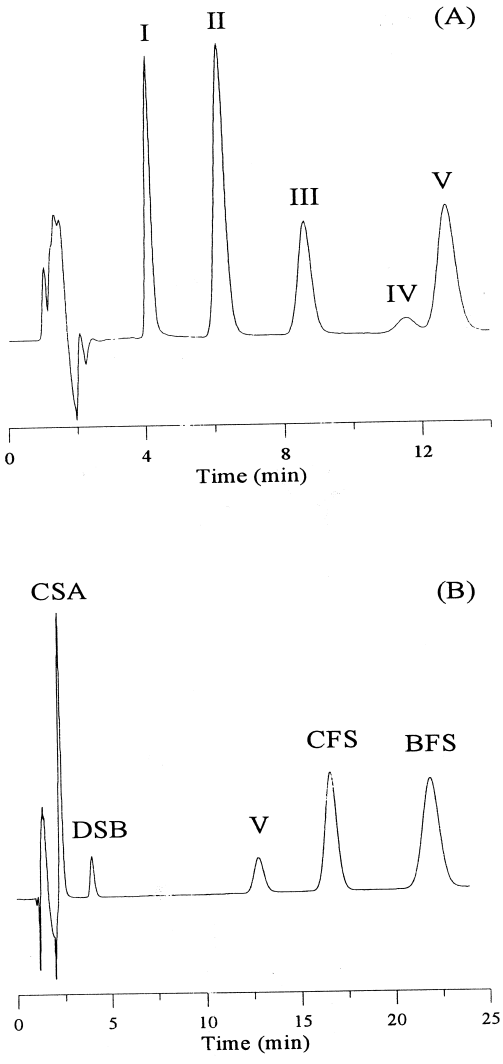


Fig. 2. Chromatograms of: (A) furosemide solution prepared at pH 3 and exposed to sunlight, and (B) mixture of furosemide and some usual impurities. Compounds: furfuryl alcohol (I), furosemide (V), 4-chloro-5-sulphamoylanthranillic acid (CSA), 2,4-dichloro-5-sulphamoylbenzamide (DSB), 2-chloro-4-(2-furfurylamino)-5-sulphamoylbenzoic acid (CFS), and 2,4-bis(2-furfurylamino)-5-sulphamoylbenzoic acid (BFS). Mobile phase: 0.04 M SDS-2% propanol at pH 3.

When freshly prepared micellar solutions of furosemide buffered at pH 3–5 and protected from light were chromatographed, only the peak at 12.7 min was observed. However, at pH > 5.5, a small peak appeared at 8 min, whose area was increased with the pH of the injected solutions, whereas the peak of furosemide was shifted to lower retention times: from 12.5 min at pH 5.5 to 10.9 min at pH 10. At increasing pH, the peak of furosemide was also wider and shorter. Otherwise, another peak at 12.9 min appeared at pH > 6.5, apparently due to changes in the refraction index, which difficulted the integration of the peak of the diuretic. Several authors have recommended the use of alkaline medium at pH 7–10 to prevent acid-catalysed degradation [11,31,32,34]. Also, furosemide injections are usually commercialised in alkaline medium in the pH range 8–9.3 [27]. However, as shown, the integration of the peak of furosemide injected in neutral and alkaline medium is troublesome and the solutions suffer a slight degradation, even when protected against light.

Similar chromatograms were achieved for furosemide solutions prepared in non-micellar medium (ethanol:water 10:90, v/v) at varying pH. The preparation of furosemide solutions in pure methanol has also been suggested [13,26,37]. We checked however that the injection of methanolic solutions into SDS micellar and conventional acetonitrile-water mobile phases produces shorter peaks with a more pronounced heading.

The plot of the retention factor versus pH of furosemide solution was similar to that shown in

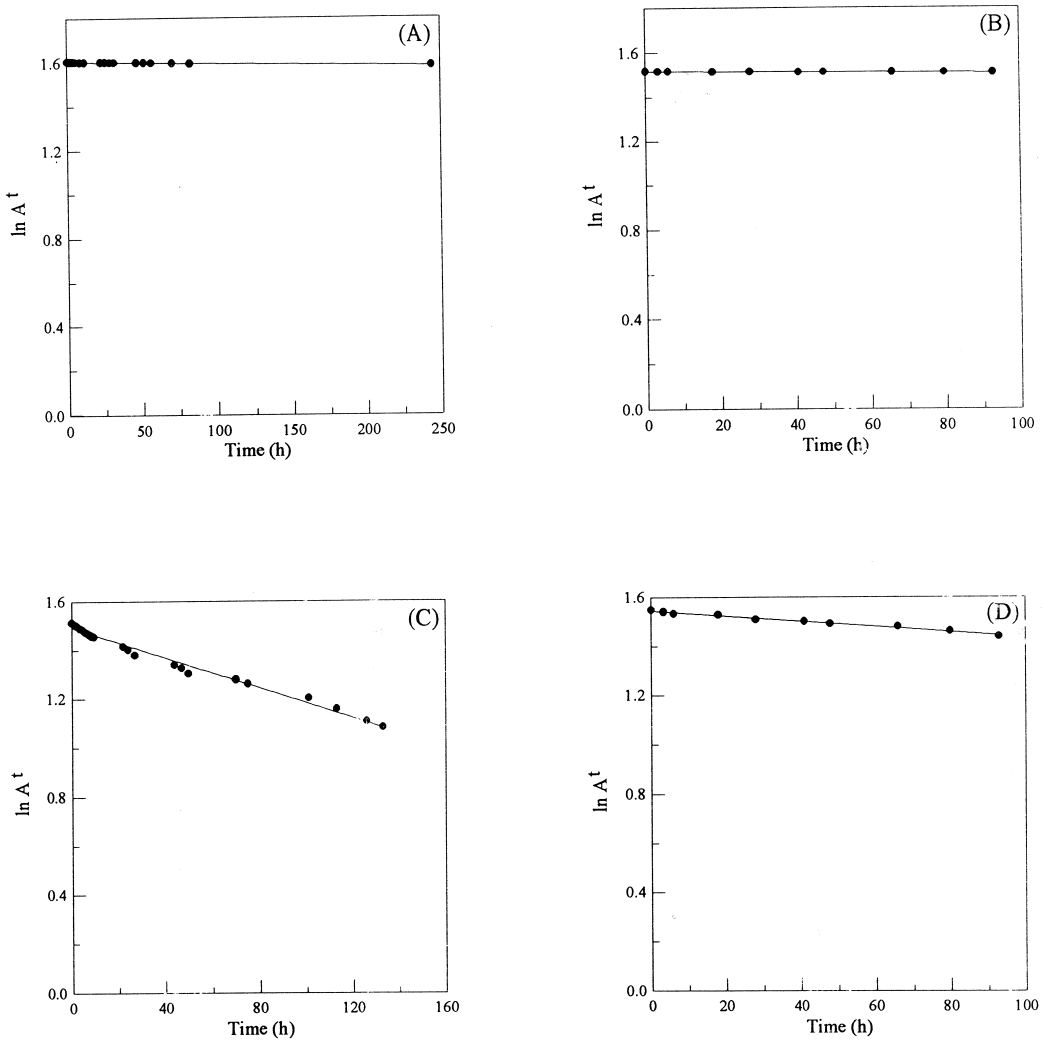


Fig. 3. First-order plots for the photodegradation of furosemide: (A, B) solutions protected from light, and (C, D) solutions exposed to artificial laboratory light at room temperature. The pH of the solutions was: 3 (A, C), and 5 (B, D).

(Fig. 1A, B) although shifted to larger pH values. Also, the reduction in the retention factors was smaller. The apparent displacement of the acid–base equilibrium is similar to that observed when acidic solutions of the drug were injected into mobile phases at increasing pH. Apparently, the equilibrium is not re-established inside the column at the pH of the mobile phase (pH 3). The mobile phase was buffered with phosphate system and the injection volume was 20 μl, therefore, the pH of the mobile phase was not changed.

In view of these results, we studied the stability of furosemide solutions prepared at pH 3 and 5, and exposed or protected from light. Decomposition of furosemide obeys first-order kinetics [8,33,36]. The logarithms of the difference between the area at any time, A_t, and the final area of the chromatographic peak, $A_\infty(=0)$, were plotted against time in Fig. 3 for diverse conditions. The apparent first-order rate constants were obtained from the slopes of the linear segment according to Eq. (1)

$$\ln(A_t - A_\infty) = \ln(A_0 - A_\infty) - k_1 t \quad (1)$$

where A_0 is the area at time zero, and k_1 is the apparent first-order constant. As can be seen, the solutions protected from light (Fig. 3A, B) were stable during long periods, whereas those unprotected (Fig. 3C, D) suffered photodegradation with half-lives of 9 and 27 days, at pH 3 and 5, respectively. Simultaneously, the formation of the degradation products I, II and III was followed. These peaks were also observed in the solutions protected from light, with very small areas (at pH 3, 10 days after the preparation of the solutions, the degradation was $< 0.35\%$ against 11.5% for the exposed solutions, measured as the ratio between the areas of the largest peak among the degradation products and the peak of furosemide). The plot of the areas of peaks I-III versus. time in the exposed solutions was also linear.

When the solutions were directly exposed to sunlight, the diuretic was completely decomposed after 8 h at pH 3. Also, a brown-yellow colour appeared in solutions containing 100 μg/ml of furosemide at pH 3 and 5 after 2 months, and at pH 7 after 3 months, which probably corresponds to the formation of furfuryl alcohol in large amounts. Ionic strength does not affect the photodegradation of furosemide [7,36].

3.4. Chromatographic-spectral study of the degradation products of furosemide and usual impurities

In the literature, the identity of the photochemical degradation products of furosemide has been discussed extensively, being subject to controversy. We checked that the retention time and absorption spectrum of peak I (at 4.2 min), in (Fig. 2A) agrees with furfuryl alcohol, but none of the remaining peaks corresponds to CSA. However, after keeping the solutions of furosemide exposed to standard laboratory lighting during more than 24 h, a new peak at 2.3 min appeared which was assigned to CSA (Fig. 4). The peak of furfuryl alcohol in the injected solutions indicates that CSA should suffer an extensive and rapid transformation, in weak acidic medium, which gives rise to peaks at short retention times. The presence of a free amine group in

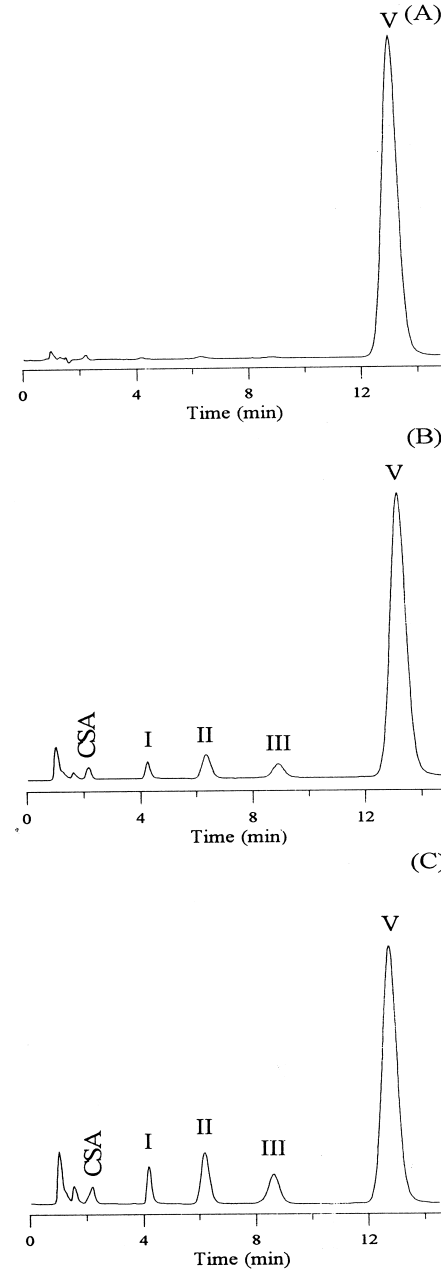


Fig. 4. Chromatograms of a furosemide solution at pH 3 exposed to artificial light at different times after its preparation: (A) 1 min, (B) 24 h, and (C) 89 h. Mobile phase: 0.04 M SDS-2% propanol at pH 3.

Table 2
Retention times and maximum wavelengths for the peaks obtained in the solutions of several compounds exposed to artificial light[a]

Compound	t_R (min)	λ (nm)
Furosemide	1.7	222, 266, 328
	2.3	230, 268, 332
	4.2	226, 278
	6.2	236, 274, 326
	8.9	222, 274, 342
	11.4[b]	238, 276, 328
	12.9	236, 274, 342
CSA	1.9	226, 266, 326
	2.3	230, 268, 330
CFS	2.2	210, 282
	4.2	230, 278
	11.2	226, 286
	14.4	210, 288
	17.1	226, 286, 322
BFS	4.2	230, 278
	6.8	252, 276, 328
	22.3	258, 290, 328

[a] 24 h of light exposition.
[b] Direct sunlight exposition.

the solutions was revealed by the formation of a NED azodye, which was not observed in the solutions protected from light [43]. It should be reminded however that furosemide can also be transformed in other products without breaking the alkyl-amine bond.

In a parallel study, a solution of furosemide in a strong acidic medium was heated at 100°C in the dark to avoid photodegradation. The surfactant was not added, since it is decomposed at high temperature. Aliquots of this solution were taken at different times and cooled rapidly in a light-protected water bath to stop the reaction. The pH of the solution was increased by dilution with 0.1 M SDS at pH 3, previously to injection. Under these conditions, a peak was observed at the retention time of CSA, together with other unidentified peaks which did not agree with the peaks obtained in the photodegradation study (included the peak of furfuryl alcohol), but the formation of an azodye was observed with NED. Otherwise, the peak of furosemide dropped to zero after 2 min of heating. The hydrolysis of furosemide in acidic media also allows a first-order kinetics [7,8].

We attempted to assign the peaks of the degradation products in the solutions of furosemide exposed to light, with the aid of the information found in the literature about their nature [11–14]. We investigated also several usual impurities of the drug (DSB, CFS and BFS) using the mobile phase of 0.04 M SDS-2% propanol (Fig. 2B). Identification of the degradation products by direct coupling of MLC with MS is problematic, owing to the high concentration of surfactant in the mobile phase. A similar problem is found in other surfactant-mediated separation techniques. Only recently, a possible solution has been provided for micellar electrokinetic chromatography, filling only a part of the capillary with an electrolyte solution containing micelles, which allows a separation without the surfactant entering the mass spectrometer [44,45].

The retention times and maximum wavelengths of several standards chromatographed with 0.04 M SDS-2% propanol at pH 3 are shown in Table 1. For these analyses, the solutions were kept from light. The data obtained for some of these compounds exposed to light are also given (Table 2). As commented, the photodegradation gives rise to breaking of the alkyl–amine bond and lost or substitution of chlorine or the carboxylic group. It should be noted that anthranillic acid, which lacks the sulphamide group, has a much higher retention than furosemide. Therefore, all the degradation products showing the benzene ring must keep this group. The amine group should be also probably present in all the compounds.

The peak at 1.6–1.9 min that appears after exposing furosemide solutions to light during at least 24 h may be due to CSA dechlorination. This peak was also obtained with irradiated CSA solutions. Other authors [31,33] have also reported that unprotected CSA gives a peak at shorter retention times. The peak of furfuryl alcohol at 4.2 min was also observed in the chromatograms of the solutions of CFS and BFS (see Table 1) exposed to light. As expected, this peak is not obtained in the solutions of DSB.

CFS and BFS seem to decompose at similar rate since the ratio of the areas of the peak of furfuryl alcohol in both chromatograms is 1:2, for the same molar concentration of both compounds. On the other hand, the peak at 6.2 min may belong to a compound formed from CSA where the carboxylic group is released. This should increase significantly the retention, due to its lower polarity. The peaks at greater retention times (8.9 and 11.4 min) may correspond to furosemide dechlorination. The peak at 11.4 min only appears upon direct sunlight irradiation and can be assigned to a compound formed by lost of the carboxylic group of the diuretic. The peak at 8.9 min may correspond to FSA.

CFS, an impurity similar to furosemide with the substituents at the positions 2 and 4 switched in the benzene ring (Table 1), has longer retention (17.1 min) than the diuretic. The chromatogram of its degradation products (Table 2) is simpler, and shows a peak at 2.2 min that may correspond to a compound similar to CSA, which seems to be more stable than this compound. The retention of BFS is even larger (22.3 min), owing to the two furfuryl alcohol molecules. Likely, the double peak at 6.8 min for the solutions of this compound may be due to the release of both furfuryl groups, yielding diaminesulphamoylbenzoic acid and diaminesulphamoylbenzene.

3.5. Analysis of pharmaceutical formulations

The solutions of furosemide protected from light are highly stable, but exposed to light decompose at greater rate at pH 3 than at pH 5. However, above pH 5, the peak of furosemide is shifted progressively to shorter retention times, and its quantification is problematic. For this reason, we decided to select pH 3 to prepare the solutions of furosemide for the control of the diuretic in the pharmaceuticals. For these analysis, we preferred a mobile phase of 0.06 M SDS-8% propanol which produced a shorter retention time (5.4 min) for furosemide than the mobile phase used in the stability studies. The resolution obtained with this mobile phase also allowed to control the formation of the photodegradation products along the analytical process.

Twenty-seven pharmaceuticals from several countries (Spain, France, Belgium, England, Canada, Argentina, Paraguay, Costa Rica, Nicaragua and El Salvador) were analysed (Table 3). No degradation was observed when the tablets or furosemide solid standard reagent were exposed to daylight during 8 h. However, the analyses were performed avoiding any exposure to light. Thus, after grinding and homogenising the tablets, the fine powder was kept in a container protected from light with aluminium foil. The refractometric peak that appears close to the peak of furosemide for solutions buffered at $pH > 6.5$ can be problematic for the analysis of the intravenous and intramuscular solutions, which are commercialised in the pH range 8.4–8.9, except Nuriban with pH 6.6. These solutions were diluted before analysis to decrease their pH.

Calibration curves were constructed measuring the areas of the chromatographic peaks of duplicate injections of furosemide solutions, at five increasing concentrations in the range 4–20 μg/ml. Standard solutions were freshly prepared periodically and kept protected from light. The analyses were carried out in different days along 4 months, consecuently, seven different calibration curves were used. The parameters of the fitted straight-lines are given in Table 4. Coefficients of regression were always $r > 0.999$, the intercepts were almost null and the slopes similar. The accuracy and precision of the analyses were evaluated in assays performed during the same day and along consecutive days. The values shown in Table 5 correspond to within-day and day-to-day analyses of an aqueous solution containing 7 μg/ml of furosemide. The low variability and high precision of the results obtained in different days are evident. The limit of detection (3 s criterion) for furosemide was 7 ng/ml.

For each pharmaceutical, five samples were analysed with duplicate injections. Table 3 gives the values declared by the manufacturers and the values found, together with the label claim percentages and precisions. The label claim percent-

Table 3
Analysis of pharmaceuticals containing furosemide with micellar reversed-phase liquid chromatography

Pharmaceutical (laboratory)	Composition\mg	Found/mg	Label claim (%)	CV (%) ($n = 5$)
Seguril (Hoechst Pharma, S. Feliu de Llobregat, Barcelona, Spain)	Per tablet: Furosemide (40), excipients	40.0	100.0	0.05
Seguril Inyectable (Hoechst Pharma)	Per 2 ml: Furosemide (20), excipients (NaOH, NaCl), water for injections (2 ml)	19.9	99.6	0.45
Lasilix (Laboratoires Hoechst, Paris, France)	Per tablet: Furosemide (40), excipients	39.9	99.8	0.05
Lasix 20 (Hoechst Belgium, Brussel, Belgium)	Per 2 ml: Furosemide (20), excipients	20.2	101.2	0.94
Lasix Furosemida (Hoechst Marion Roussel, Capital Federal, Paraguay)	Per 2 ml: Furosemide (20), excipients	20.2	100.9	0.30
Lasilacton 100 (Hoechst, San Isidro, Buenos Aires, Argentina)	Per capsule Furosemide (20), Pironolactone (100)	20.1	100.7	0.20
Logirene(Laboratoires Upjohn, Paris)	Per tablet: Furosemide (40), excipients amiloride (5 mg), excipients	39.7	99.2	0.45
Furosémide-ratiopharm (Laboratoire Lafon-ratiopharm, Maisons Alfort, France)	Per tablet: Furosemide (40), excipients	40.0	99.9	0.05
Furosémide RPG (Laboratoires Biogalénique, Paris)	Per tablet: Furosemide (40), excipients	39.9	99.8	0.05
Furosemida (Mckesson, San Salvador, El Salvador)	Per tablet: Furosemide (40), excipients	40.1	100.4	0.20
Diuremide (Laboratorios Rarpe, Managua, Nicaragua)	Per 2 ml: Furosemide (20)	19.0	95.2	0.58
Furosemida (Laboratorios Solka, Masaya, Nicaragua)	Per tablet: Furosemide (40), excipients	38.9	97.3	0.36
Frusemide Injection BP (Evans Medical, Leatherhead, England)	Per 2 ml: Furosemide (20), sodium chloride BP (15), sodium hydroxide BP, water for injections BP	20.3	101.3	0.59
Aldalix (Monsanto France, Division Searle, Paris)	Per tablet: Furosemide (20), spironolactone (50), excipient	19.9	99.4	0.20
Salidur (Grupo Farmacéutico Almirall, Barcelona)	Per tablet: Furosemide-xanthinol (77.6), triamterene (25), excipients	39.8	99.6	1.3
APO-Furosemide (Apotex, Toronto, Canada)	Per tablet: Furosemide (40), excipients	40.0	100.0	0.05
Furosemida (Stein, Cartago, Costa Rica)	Per tablet: Furosemide (40), excipients	38.9	97.2	0.36
Nuriban Furosemida (Roux-Ocefa, Buenos Aires)	Per tablet: Furosemide-diethylaminoethanol (50), excipients	37.6	101.8	0.35
Nuriban Inyectable (Roux-Ocefa)	Per 5 ml: Furosemide-diethylaminoethanol (50), excipients	37.1	100.5	0.22
Nuriban Gotas (Roux-Ocefa)	Per 15 ml: Furosemide-diethylaminoethanol (300), excipients	222.9	100.6	0.22
Furosemida (Lavimar, Córdoba, Argentina)	Per 2 ml: Furosemide (20)	19.6	98.0	0.97
Furosemida Vannier (Vannier, Buenos Aires)	Per tablet: Furosemide (40), excipients	39.8	99.5	0.10
Furodur (Boss Pharma, Brussel)	Per tablet: Furosemide (40), excipients	39.8	99.5	1.3

Table 3 (*Continued*)

Pharmaceutical (laboratory)	Composition\mg	Found/mg	Label claim (%)	CV (%) ($n = 5$)
Furosemide EG (Eurogenerics, Brussel)	Per tablet: Furosemide (40), excipients	40.0	99.9	0.10
Diflux (Volpino Laboratorios, Buenos Aires)	Per tablet: Furosemide (40), amiloride (20), excipients	40.0	100.1	0.12
Furosemida 1% (Inibsa, Lliçà de Vall, Barcelona)	Per 2 ml: Furosemide (20), excipients (NaOH, NaCl), water for injections (2 ml)	20.0	99.8	0.65
Furtenk (Biotenk, Buenos Aires)	Per tablet: Furosemide (40), excipients	39.7	99.3	0.10

age values were in the 95–102% range and the coefficients of variation in the range 0.05–1.3%. The excipients were eluted with the dead time or did not absorb at the measuring wavelength. The compounds administered in combination with furosemide (except diethylaminoethanol) yielded chromatographic peaks at retention times longer than furosemide (5.4 min), and did not interfere the analyses: xanthinol (12.5 min), amiloride (13.2 min), triamterene (20 min), and spironolactone (27.5 min). Diethylaminoethanol probably eluted at the dead time. Xanthinol in Salidur and diethylaminoethanol in Nuriban are added as counterions of furosemide in a 1:1 molar ratio. It should be indicated that the impurities (CSA, DSB, CFS and BFS) were not observed in the furosemide standards (even by injection of 100 μg/ml), or in the solutions of the pharmaceuticals analysed in this work.

4. Conclusions

The studies performed in this work have shown that furosemide solutions, protected from light, are stable at pH 3–5. Exposed to light, the degradation is complex, giving rise to several products. Furosemide can be completely resolved from its degradation products and usual impurities using a mobile phase of 0.04 M SDS-2% propanol at pH 3. The chromatographic procedure can be applied to control the decomposition degree of the solutions of standards and pharmaceuticals during analysis, or the quality of the pharmaceuticals. It

Table 4
Calibration parameters obtained in the control of furosemide in pharmaceutical preparations, using 0.06 M SDS-8% propanol as mobile phase[a]

Intercept	Slope	r
0.01 ± 0.04	0.437 ± 0.003	0.99993
-0.04 ± 0.03	0.439 ± 0.003	0.99994
-0.14 ± 0.04	0.437 ± 0.003	0.99993
0.01 ± 0.07	0.446 ± 0.006	0.99974
-0.05 ± 0.04	0.457 ± 0.003	0.99992
-0.03 ± 0.04	0.457 ± 0.003	0.99994
	Mean	CV (%)
	0.447 ± 0.010	2.2

[a] Duplicate injections of five solutions were made in the concentration range 4–20 μg/ml.

Table 5
Inter-and intra-day accuracy and precision for micellar aqueous solutions of furosemide

Added concentration (μg/ml)	Measured concentration (μg/ml)[a]
7.28	7.250 ± 0.006
	7.242 ± 0.006
	7.2372 ± 0.0019
	7.231 ± 0.005
	7.223 ± 0.012
Mean	7.237 ± 0.010
Accuracy (%)	−0.59
Precision (%)	0.14

[a] Inter-day values correspond to five-fold injections.

shows good accuracy, repeatability and selectivity, and is simpler than most procedures reported for this compound. Good results were obtained in the determination of furosemide in diverse dosage forms. Since no interference from common additives, excipients or drugs that might be found in commercial preparations is noticed, a previous extraction of the drug or the use of an internal standard are unnecessary. The analyses of 27 pharmaceuticals indicate the validity of the procedure.

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