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Analytical Techniques for Furosemide Determination

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Analytical Techniques for Furosemide Determination

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Abstract: Due to the clinical importance of furosemide, a large number of analytical procedures to detect the presence of this drug in pharmaceutical and physiological samples has been developed. In this manuscript, a review of the most frequent analytical techniques described to determine furosemide is presented. Special attention has been paid to spectrophotometric and chromatographic techniques, but also to relevant methods using capillary electrophoresis or flow-injection analysis, as well as the detection modes coupled to these techniques. The review also focuses on the different degradation pathways of the drug and cautions to prevent it, otherwise rarely or confusedly mentioned in the analytical reports. Some guidelines about purification of the raw product after synthesis are also addressed.

Keywords: Furosemide, purification, stability, separation procedures, pharmaceutical and biological samples

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INTRODUCTION

Furosemide or frusemide (4-chloro-*N*-furfuryl-5-sulfamoyl-anthranilic acid, see Figure 1) is formally a sulfonamide, an antibacterial agent. However, the intense and fast dieresis produced by this drug has extended its application as a powerful acidic ($pK_a = 3.8$ and 7.5) diuretic for diverse treatments in humans and veterinary medicine. Furosemide is often classified as a loop diuretic due to its predominant action in the nephron, where the drug interferes with the tubular re-absorption of sodium on Henle's loop (1). The renal excretion of ions is not limited to sodium and chloride, but it may also influence potassium, magnesium, calcium and, to a lesser extent, hydrogen carbonate ions (2). In the clinical practice, the effects of furosemide are applied in the treatment of oedema associated with pulmonary, cardiac, hepatic and renal disease, and of hypertension accompanied by fluid retention or impaired renal failure (3–7). A marked dieresis is also associated to a loss of weight, a side effect that has been incorrectly abused in the sport practice to achieve acute weight losses before competition, usually where weight categories are involved. Intense and fast dieresis may also mask the ingestion of other doping agents by reducing their concentration in urine (8). For this reason, the Medical Commission of the International Olympic Committee banned the use of furosemide among other diuretics in 1986 (9).

Furosemide is frequently administered as oral tablets, or through intravenous and intraduodenal injections. More recently, aerosolized furosemide has evidenced a new mechanism of action (10): inhaled furosemide provides a protective effect against respiratory infections by acting on the pulmonary system without causing dieresis.

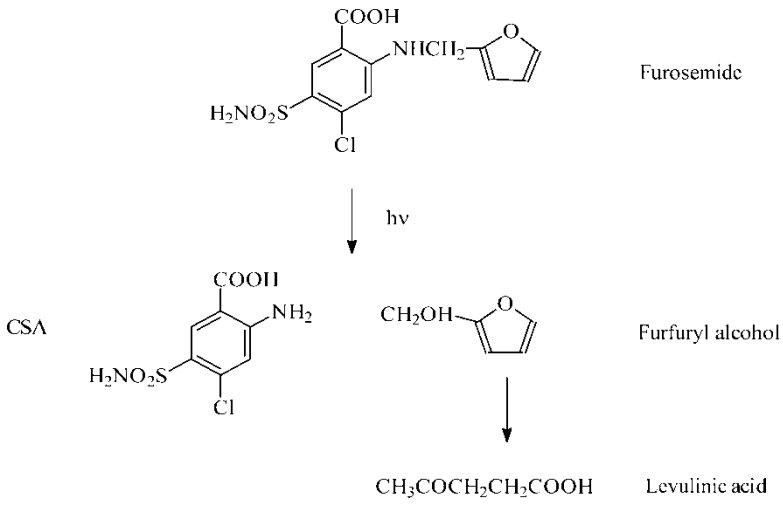

Figure 1. Photodegradation pathway of furosemide.

The pharmacokinetics and pharmacodynamics of furosemide studied in both healthy volunteers and patients with specific diseases are well documented and reviewed in the literature (11–16). Although the behavior is variable, the diuretic effect has been generally reported to appear approximately 30 min after oral dosing, or even before when intravenously administered, and to last for approximately 6–8 h. This means that the drug is readily absorbed with a fast diuretic action, which makes its administration very frequent. Furosemide is mainly excreted in urine as an unchanged drug and up to 14% conjugated with glucuronic acid (17). However, the bioavailability of the drug does not exceed 40–65%, which means that furosemide is incompletely absorbed. Unabsorbed furosemide has been reported to be excreted in human faeces and rabbit bile (18), which suggests alternative non-renal routes for the drug or its metabolites. No firm explanation has been given for the relatively low and variable bioavailability of furosemide, although various possible reasons such as catalyzed degradation in the stomach, or first-pass metabolism in the gut wall or in the liver have been considered as most likely (19, 20).

The clinical interest and wide use of furosemide has promoted the development of accurate and specific analytical techniques that permit the routine quality control of pharmaceutical products containing furosemide, and the measurement of the drug in biological samples at different therapeutic or doping levels. In this review, the relevant characteristics concerning the most frequent analytical techniques involved in the identification and determination of furosemide in pharmaceutical and biological samples are discussed.

Furosemide is also known to suffer from photodegradation, but curiously, information on the stability of furosemide solutions, although fundamental to achieve reliable analytical results, is somewhat limited in the analytical reports. Concise information regarding prevention of photosensitivity and photodegradation products of furosemide is here provided. Purification guidelines of the raw product are also addressed. The patent of furosemide expired in the early 1980s, and the product is currently manufactured by several international pharmaceutical laboratories. A complete description of the synthesis procedure employed to obtain the raw material is not, however, available in the literature.

PURIFICATION OF THE RAW PRODUCT

The preparation of pharmaceutical products of adequate quality for human or veterinary consumption requires sample purification after synthesis. Furosemide is obtained as an impure yellow powder. A purification method is described for furosemide in both the British Pharmacopoeia (21) and USP (22). After synthesis, a simple recrystallization of the drug in ethanolic aqueous solution is recommended to obtain a white or slightly yellow product with high purity. This method can be also applied with a pre-treatment of the raw product with activated carbon. García-Valdés et al. (23) proposed

recently an alternative procedure based on the formation of the sodium salt of furosemide, followed by treatment with activated carbon and restitution of the base by hydrolysis. This procedure eliminates the recrystallization step in the ethanolic solution, which implies a large consumption of ethanol, and can be easily applied in routine work.

Purification of furosemide is not a frequent practice among analysts. It should be remembered that furosemide is commercialized as a standard reagent for laboratory use with analytical-grade purity $\geq 99\%$, which makes the purification stage in the laboratory unnecessary.

DRUG STABILITY

Therapeutic and doping control of a drug requires knowledge of its main metabolic pathways, and the percentages of the dose eliminated unchanged using different routes of administration, and doses. Moreover, the identity of the metabolic products is essential in order to consider potential interferences in the analytical procedures. The existence of alternative degradation pathways, such as photochemical degradation or any kind of adverse photosensitivity responses, is another factor to take into account. For furosemide, both degradation routes have been described, but degradation problems in the analysis of biological samples are rarely reported. On the other hand, potential interference from its major degradation products has not been considered for the majority of analytical methods. This may limit their usefulness not only in the therapeutic and doping monitoring, but also in pharmacokinetic bioequivalence studies of the drug.

Metabolic Pathway

Data concerning the biotransformation of furosemide are conflicting. The major metabolite of furosemide was believed to be 2-amino-4-chloro-5-sulfamoylanthranilic acid (CSA), the hydrolyzed product of furosemide. In early studies, Hadjù and Häussler (24), and Häussler and Wicha (25), reported that CSA was the only metabolite in man, dog and rat, according to the results obtained by paper chromatography and spectrofluorimetry of urine samples from subjects given furosemide. Pérez et al. (26) reported that CSA and a product resulting from conjugation of furosemide with glucuronic acid were found by gas chromatography (GC), in urine samples from patients with acute pulmonary edema. Andreasen et al. (27) found that between 1.0 and 4.4% of furosemide was hydrolysed to CSA after 1 h in gastric juices, but no CSA trace was found in duodenal juices. On the contrary, Beerman et al. (28) found no evidence of CSA in human samples of plasma, gastrointestinal aspirates, urine or faeces, when they were analyzed by measuring the radioactivity levels of ^{35}S-furosemide.

The use of assay methods that in some cases lacked specificity may be the reason for these initial controversies. Since the 1980s (13, 29, 30), the glucuronide is generally considered as the main metabolite of furosemide. Hydrolysis with beta-glucuronidase of urine samples containing furosemide, followed by chromatographic elution, seems to have provided enough evidence of the presence and absence of the glucuronide and CSA, respectively, in physiological samples. Other metabolites were identified in bile samples of rabbits by Sekikawa et al. (18), and Rachmel et al. (31), as the acyl migration isomers of the glucuronide metabolite.

Photochemical Degradation

Several diuretic drugs give rise to adverse photosensitivity responses in vivo, and many have been the subject of photodegradation and other photochemical studies in vitro (32, 33). The most important photoreactive diuretics are characterized by an absorption spectrum in the sunlight region above 280 nm; among these, chlorothiazide (318 nm) and furosemide (330 nm) are the most studied. Furosemide is known to initiate adverse light-induced biological effects, and has been found to produce photosensitivity, phototoxicity, oxygen-dependent photohemolysis and lipid photoperoxidation (34), but its degradation photoproducts do not seem to be phototoxic in vitro (35).

In aqueous and organic solution, furosemide suffers photochemical degradation under the influence of UV radiation: it hydrolyses to CSA and furfuryl alcohol (36–38), which would be quickly converted into levulinic acid (39, 40). A scheme of this degradation is shown in Figure 1. The process is faster in acidic solution (34, 36–38), and in the presence of sulfate ions (39), as in normal urine. The preparation of furosemide solutions in alkaline media protected from light is then mandatory to avoid degradation problems (40, 41). In fact, Rowbotham et al. (42) found that unprotected alkaline solutions of furosemide still produced CSA by oxidation of the sulfamoyl group to sulfonic acid with hydrolysis of the furfuryl group, after UV irradiation during 48 h. It should be noted that CSA may be an impurity in the pharmaceutical preparation of furosemide.

The nature of the photodecomposition process is still, however, subjected to controversy. Moore et al. (43, 44) reported that both furosemide and CSA suffer photodechlorination to *N*-furfuryl-5-sulfamoylanthranilic acid (FSA) and 5-sulfamoylanthranilic acid, respectively, in oxygen-free solutions. This was confirmed by reversed-phase liquid chromatography (RPLC) and GC, both coupled to mass spectrometry (MS). According to Bungaard et al. (36), furosemide losses the chlorine atom to give FSA, but its substitution with a hydroxyl group may also be imaginated. Vargas et al. (34) analyzed by ^{1}H- and ^{13}C-NMR spectroscopy, IR and GC-MS, the products formed under aerobic and anaerobic conditions in methanolic and buffered (pH 7.4) aqueous medium. Three main products were found after photodechlorination

or decarboxylation, with hydrogen or hydroxyl abstraction. The analysis of furosemide in pharmaceutical dosage forms using ^{1}H-NMR spectroscopy revealed only the formation of CSA (45).

More recent reports have extended the stability studies of furosemide to different media. Carda-Broch et al. (46) found that furosemide solutions prepared in micellar media of sodium dodecyl sulfate (SDS) were stable at pH 3–5, when protected from light. Exposed to light, the degradation gave rise to several products. The degradation rate was higher under sunlight exposure than under artificial laboratory light with half-lives of 8 h and 9 days, respectively, being also faster in aqueous-organic solutions. This point was also confirmed by Guzmán et al. (47). In all cases, apparent first-order kinetics was found. Furfuryl alcohol and CSA were identified among other degradation products. CSA was observed to suffer also an extensive and rapid decomposition. Furosemide photodecomposition was also investigated in spiked and excreted urine samples (48). In this matrix, furosemide was stable at pH < 4. A plot of the degradation rate of furosemide in urine micellar solutions under different light sources is shown in Figure 2.

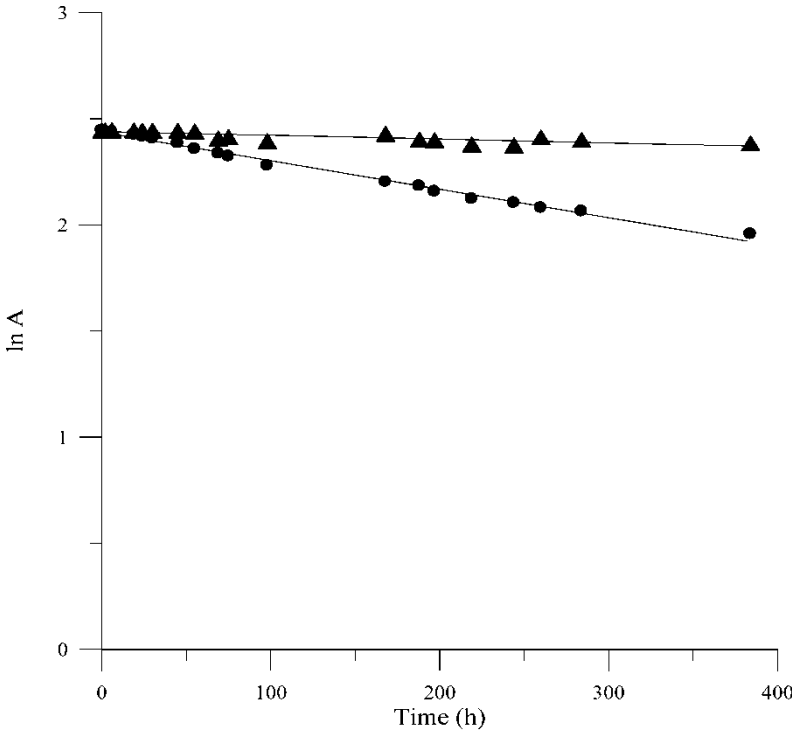

Figure 2. Degradation rate of furosemide in a urine matrix buffered at pH 3 in micellar media: protected (▲) and exposed (●) to laboratory artificial light at room temperature. The concentration of furosemide was 10 μg ml^{-1}.

More recently, Fiori et al. (49) found that other drugs, such as triamterene, had a photoprotective effect over furosemide degradation. In acetonitrile-phosphate buffer (0.02 M; pH 7.4) 20:80 (v/v) under UV radiation, furosemide half-life was 24 h versus 44 h in the absence and presence of triamterene, respectively.

It should be pointed out that the glucuronic metabolite of furosemide was also found to be unstable in physiological samples, and in both neutral and alkaline buffer solutions (18, 50). The metabolite degrades rapidly to form acyl migration isomers by hydroxyl ion catalysis and is still light sensitive.

ANALYTICAL TECHNIQUES

Spectroscopic Methods

Ultraviolet absorbance spectrophotometry is a rapid, sensitive and inexpensive analytical tool, highly appropriate for pharmaceutical dosage control. The official procedures for furosemide in pharmaceutical preparations propose the use of spectrophotometry (21, 22), based on alkaline or acidic hydrolysis of the diuretic to form the primary amine as a preliminary step, and followed by reaction of the amine with an appropriate reagent. The oxidation of furosemide by an isopolyanion of molybdenum(VI) and further analysis by spectrophotometry was also proposed (51). Iron(III) chloride (52), and 3-methyl-2-benzothiazolinone hydrazone in conjunction of iron(III) chloride (53), were also found to be useful for the spectrophotometric determination of furosemide. Sevillano-Cabeza et al. (54) developed an extractive-spectrophotometric study based on the reaction of furosemide with 1,2-naphthoquinone-4-sulfonate to determine the drug in pharmaceutical samples.

Nevertheless, the use of UV-visible absorption suffers from lack of specificity, which usually hinders the application of the technique in the presence of other absorbing species, due to spectral overlapping. On the other hand, the use of spectrofluorimetry to determine fluorescent drugs, such as furosemide, although interesting on account of its high selectivity and sensitivity, presents the same problem of simultaneous drug determination when the analytes exhibit broad spectral bands that tend to overlap. This entails the use of prior separation procedures or specific methods, both of which result in increased analysis time and cost. Since furosemide and other active principles are frequently administered jointly to obtain synergic pharmacological effects, the need to improve the existing methods to expedite the analysis is still persistent.

This challenge has been met by using different techniques. García-Sánchez et al. (55) first used variable-angle synchronous scanning fluorescence spectroscopy for the simultaneous determination of furosemide, triamterene and piretanide. This approach results in more simple and

narrow spectra than those obtained with conventional spectrofluorimetry, and permits to increase the selectivity avoiding previous sample treatment. However, the application of this technique to real samples is not always possible, since strong spectral overlaps are sometimes encountered.

Computers in the analytical laboratory have enabled the development of multicomponent methodologies for complex samples that process the vast amount of information provided by currently available instruments. Among these methods, partial least-squares regression, which does not require previous knowledge of individual spectra for each analyte and interference, has been successfully applied coupled to spectrofluorimetry in pharmaceutical analysis of multicomponent drug products containing furosemide (56, 57).

Although chemometric methods are gaining in popularity, other methodologies, such as derivative spectroscopy, are being still investigated for the analysis of co-formulated products. First- or ratio-spectra derivative spectroscopies (58, 59) were successfully applied for the determination of furosemide and amiloride, and furosemide and spironolactone, respectively, in commercial formulations. These techniques are rapid, easy to apply and utilize inexpensive instrumentation.

More recently, diffuse reflectance spectroscopy has been introduced in pharmaceutical quality control. For years, this analytical technique was limited to paints, pigments or textile areas to evaluate properties such as color and whiteness (60). It was not possible to attain highly precise measurements from conventional spot tests (61). The development of optical fibers and reflectance spheres has changed this situation. Gotardo et al. (62) employed this methodology to quantify furosemide in commercial samples with good precision.

Reversed-Phase Liquid Chromatography

A great number of RPLC procedures have been published in the literature for the analysis of furosemide. Official procedures for the dosage of pharmaceutical forms included RPLC in the early 1990s (63). Liquid chromatographic procedures were first developed as an alternative to improve the specificity of spectroscopic methods, and were applied either to pharmaceutical or physiological samples. In early reports, some problems associated to the fast elution of some furosemide decomposition products, which limited the performance of stability studies in dosage forms, have been frequently addressed (41, 64–66). In many of these studies, the presence of CSA as an impurity in pharmaceutical samples or other compounds identified as furosemide metabolites could be the result of a photodecomposition process, since cautions to prevent furosemide decomposition under laboratory light seemed not to be adopted.

RPLC is frequently employed to perform stability, pharmacokinetic or bioequivalence studies of furosemide (16, 49, 67, 68). Several RPLC

procedures have been also described to screen diuretics including furosemide (69–73). The elution of furosemide is usually achieved using octadecylsilane (C_{18}) columns with 10 μm particle size. The employment of 5 μm particles allowed the reduction of column length to obtain the same chromatographic efficiency, and a substantial reduction in the analysis time. Phenyl (16), cyano (67), and recently, deactivated columns (49) have been also employed. Acetonitrile is the preferred solvent for the mobile phases, which are buffered in the pH range 3–4.5. Occasionally, mobile phases containing the anionic surfactant SDS have been used to analyze furosemide or to screen diuretics (46, 48). In general, RPLC methods described for furosemide utilize isocratic elution, but gradient elution has been also employed (49, 74).

UV detection between 235 and 274 nm is used in nearly all RPLC applications reported in the literature for furosemide. In some cases, diode array detection (DAD) was employed to facilitate the identification of the detected peaks (46, 48). Fluorescence detectors have been also used allowing more sensitive detection (16, 75). More recently, liquid chromatography coupled to mass spectrometry (LC–MS) has been employed. This method provides better efficiency in the detection and quantitation processes, especially when working with biological samples or analytes not completely resolved, or contaminated by endogenous constituents. LC–MS has been successfully applied in pharmacokinetic and photostability studies of furosemide (67, 76).

An RPLC method with amperometric detection at a glassy carbon electrode using a detection potential of +1.20 V versus Ag/AgCl reference electrode was reported to determine furosemide in pharmaceuticals and urine (77, 78). An improved electrochemical detection of furosemide and other diuretics, based on post-column online photolysis and applying a detection potential of +0.20 V versus Pd, was also described (79). Guzmán et al. (48) developed an RPLC determination of furosemide using pulsed amperometric detection in a cylindrical carbon fiber microelectrodes. The use of microelectrodes allowed an improvement in the signal-to-noise ratio with respect to that obtained with conventional-size electrodes.

Other Chromatographic Techniques

In the late seventies, a number of thin layer chromatography (TLC) procedures appeared in the literature, describing the assay of furosemide or its degradation product, CSA, in biological fluids (80–82). The components separated by TLC were quantitated with UV or fluorescence detection. TLC cannot, however, compete with RPLC, which provides the simplicity and specificity necessary to carry out the analysis of furosemide samples.

Gas chromatography (GC) has also been used for the control of furosemide and other diuretics (83–89). GC methods are more sensitive, but the polar nature of most diuretics makes the direct analysis difficult.

A laborious extraction procedure and derivatization of the drug to make it volatile is thus needed prior to analysis. In doping control, silylation is the derivatization procedure of choice (83–85), but it is not useful for diuretics due to the instability of the trimethylsilyl derivatives of sulfonamide functions (86). Therefore, methylation is the procedure usually selected to derivatize these drugs. Extractive methylation has been described for the determination of furosemide (87, 88), which has been also applied in screening procedures for diuretics including furosemide, in physiological samples (89). This procedure involves the extraction of the organic acid as an ion pair into an organic solvent where the methylation reaction occurs. A quaternary ammonium salt is used as phase–transfer reagent to extract the organic acid from the alkaline aqueous–phase into an aprotic reagent with low solvation power, for anions containing the methylation reagent (methyl iodide).

Other Methods

Capillary electrophoresis (CE) has gained popularity in the analysis of drugs, vitamins and excipients, due to its low cost and short analysis time. In the past few years, CE of furosemide and other diuretics in absence and presence of micelles was investigated in several laboratories (90). A capillary zone electrophoresis (CZE) method was reported by Jumppanen et al. (91), who used a 3-(cyclohexylamino)-1-propanesulfonic acid buffer and UV detection. Sádecká and Polonský (92) described a capillary isotacophoresis method with conductimetric detection. Lalljie et al. (93) developed a micellar electrokinetic capillary chromatography (MEKC) assay employing pH 9 borate buffer with SDS micelles and diode array detection. These methods were applied to the analysis of urinary extracts containing furosemide. Two methods that used MEKC and CZE were also tested in the resolution of the mixture formed by furosemide, triamterene and chlorothiazide, and performed in the analysis of pharmaceutical formulations (94).

Recently, CE with laser-induced fluorescence detection (95, 96) and coupled to atmospheric pressure electrospray ionization mass spectrometry (97, 98) showed to permit direct analysis of drugs in body fluids, thereby avoiding time consuming sample preparation. These techniques were applied to the analysis of furosemide in patient samples (99).

In a lesser extent, flow-injection analysis with UV (100), chemiluminiscent (101) and pulsed amperometric detection (47), and sequential-injection analysis (SIA) procedures, were proposed for furosemide determination (102). The development of SIA based on access restricted material, a support that combines size exclusion of proteins and other macromolecular matrix components to allow the retention of low molecular mass analytes, achieved the pre-concentration, screening and direct determination of furosemide in human serum in short times. Although this method permits a

complete automation of the process, it suffered from lack of sensitivity, probably caused by sample diffusion of the zones in a column of high diameter and particle size.

ANALYSIS OF SAMPLES

Pharmaceutical Formulations

Furosemide is usually co-administered with other drugs. The most frequent co-drugs are the diuretics amiloride, chlorothiazide, hydrochlorothiazide, piretanide, spironolactone, and triamterene (49, 55, 56, 58, 59, 68, 94), the β-blocker propranolol (68), and the sulfonamide sulfathiazole (100). The analysis of furosemide in pharmaceutical preparations does not require a complex sample treatment. Several tablets or capsules are usually weighed and powered; a portion is accurately weighed and diluted with an aqueous-organic mixture or the same mobile phase if an RPLC analysis is being undertaken. After sonication in an ultrasonic bath or centrifugation, an aliquot is taken to be analyzed. If furosemide is determined from injections, an aliquot is taken and conveniently diluted before the analysis. Some examples of procedures are shown in Table 1, where furosemide is determined in pharmaceuticals employing different techniques. No significant differences between spectroscopic and chromatographic procedures are found for drug recovery (91–100%).

Biological Samples

The analysis of furosemide in biological samples requires previous treatment of the sample. Urine and plasma are the most frequent samples, but control of furosemide has been also reported in milk. The drug can be found in residue concentrations in milk for human consumption, due to administration to dairy cattle for the treatment of mammary gland edema (103). Liquid–liquid extraction with ethyl acetate is most widely used to isolate the unmodified diuretic. The sample is agitated on a vortex and centrifuged, the organic layer is then evaporated to dryness, usually under nitrogen gas stream, and the residue is reconstituted with methanol, acetonitrile, or the mobile phase for RPLC analysis.

Solid-phase extraction was also evaluated for furosemide assay, using a Bond-Elut C_2 column, a mixture of water and methanol to wash the column, and acetonitrile as eluent (16). A similar treatment was carried out for the screening of diuretics (8).

Direct injection of biological samples can be achieved using the surfactant SDS in micellar liquid chromatography (MLC) (48). The proteins rather than precipitating on the column are solubilized and swept harmlessly

Table 1. Analytical procedures for the analysis of furosemide associated with other compounds in pharmaceuticals

Compounds	Technique/detection mode	LOD	Recovery (%)	Ref.
Furosemide	Micellar liquid chromatography/UV	7 ng/mL	95–102	Carda-Broch (46)
Furosemide triamterene	RPLC/UV	60 ng/mL	98–100	Fiori et al. (49)
Furosemide triamterene piretanide	Variable-angle scanning fluorimetry	—	97–110	García-Sánchez et al. (55)
Furosemide amiloride	UV Spectrophotometry (PLS); RPLC/UV	—	93–99	Ferraro et al. (56)
Furosemide amiloride	First digital derivative spectrophotometry	0.02 μmol/L	98–101	Toral et al. (58)
Furosemide spironolactone	Ratio spectra derivative spectrophotometry	—	100–104	Millership et al. (59)
Derivatised furosemide	Diffuse reflectance spectroscopy	2.5×10^{-3} mol/L	98–102	Gotardo et al. (62)
Furosemide propranolol	RPLC/UV	40 ng/mL	100	El-Saharty (68)
Chlorothiazide hydrochlorothiazide furosemide	MEKC/UV	1.2 μg/mL	100	Luis et al. (94)
Furosemide sulfathiazole	FIA/UV	5.5 μmol/L	99–100	García et al. (100)
Furosemide	FIA/chemiluminiscence	0.22 μmol/L	95	Rao et al. (101)

Table 2. Analytical procedures for the analysis of furosemide in biological samples

Compounds	Sample	Technique/Detection mode	LOD	Recovery (%)	Ref.
Furosemide	Milk	RPLC/Pulsed amperometric detection	0.55 μg/mL	95	Guzmán et al. (47)
Furosemide	Urine	Micellar liquid chromatography/DAD	0.15 μg/mL	99–101	Carda-Broch et al. (48)
Furosemide	Plasma	RPLC/MS	0.1 ng/mL	89–97	Abdel-Hamid (67)
Furosemide propranolol	Plasma	RPLC/UV	0.1 μg/mL	97–103	El-Saharty (68)
Furosemide	Urine	Capillary electrophoresis/UV, laser induced fluorescence, MS	<1 μg/mL	—	Thormann (99)
Furosemide	Serum	Sequential injection extraction/fluorescence, UV	3 μg/mL	101–103	Huclová et al. (102)

away, eluting with or shortly after the solvent front. This allows repetitive serial injections with no increase in system pressure, no noticeable clogging of the injection valve or analytical column, no change in retention factors, or system contamination. However, the analytical column should be protected with a guard pre-column to saturate the micellar mobile phase with silica. Carda-Broch et al. (48) made a study of the most commonly co-administered drugs with furosemide to avoid possible interferences.

Recently, capillary electrophoresis with laser-induced fluorescence and mass spectrometry detection allowed the analysis of urine samples as received or after dilution with water (99). This makes the assays very attractive compared to CZE and MEKC with UV detection. For biological samples, recoveries in the range 90–100%, and detection limits below 0.5 μg/mL were usually found (Table 2).

CONCLUSION

The intense and fast diuresis caused by furosemide makes its administration very frequent. Consequently, the development of procedures to carry out pharmacokinetic or bioequivalence studies, as well as control of the drug in pharmacological or biological samples is of great interest in the clinical field. Spectrophotometry and RPLC are the techniques most widely chosen to develop furosemide analysis. RPLC offers simplicity, and the sensitivity may be conveniently adjusted by coupling different detectors. Spectrophotometric methods are attractive due to their low cost and rapidity. The development of chemometric tools and new technologies (e.g., diffuse reflectance spectroscopy) in the analytical laboratories has enabled to overcome important drawbacks from the past (e.g., selectivity) associated to spectrophotometry.

Achievement of reliable results in furosemide analysis relies on proper manipulation of drug solutions in the laboratory, and knowledge of its metabolic pathway in the organism. Under UV light exposure, furosemide half-life varies between 8 and 24 h depending on light source, nature of the media and pH. Cautions to prevent photochemical degradation of the drug in standard and biological samples are thus needed to avoid drug under estimation. Furosemide has been proved to be stable in aqueous-organic alkaline media or acidic micellar media protected from light.

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