Journal of Pharmaceutical and Biomedical Analysis 48 (2008) 519–532

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Review

Recent developments in analytical determination of furosemide

M. Espinosa Bosch [a], A.J. Ruiz Sánchez [b], F. Sánchez Rojas [c,*], C. Bosch Ojeda [c]

[a] Department of Pharmacy, General Hospital, University Hospital "Virgen del Rocío", Manuel Siurot s/n, 41013 Sevilla, Spain
[b] Department of Organic Chemistry, Faculty of Sciences, University of Málaga, Campus Teatinos s/n, 29071 Málaga, Spain
[c] Department of Analytical Chemistry, Faculty of Sciences, University of Málaga, Campus Teatinos s/n, 29071 Málaga, Spain

ARTICLE INFO

Article history:
Received 10 March 2008
Received in revised form 1 July 2008
Accepted 2 July 2008
Available online 11 July 2008

Keywords:
Furosemide
Review
Diuretics
Biological samples

ABSTRACT

Furosemide (FUR), a drug that promotes urine excretion, is used in the pharmacotherapy of various diseases and is considered as a doping agent in sports. FUR is a powerful diuretic (water pill). This medicine is used to treat excessive fluid accumulation and swelling (edema) of the body caused by heart failure, cirrhosis, chronic kidney failure, and nephrotic syndrome.

Owing to its extensive use as a powerful diuretic, FUR has long attracted the attention of many analysts. A variety of analytical methods have been proposed for the determination of FUR in biological fluids and pharmaceutical samples. The revision includes the most relevant analytical methodologies used in its determination from the nineties decade at present.

Contents

Abbreviations: AAS, atomic absorption spectroscopy; AGP, acidic glycoprotein; AM, amiloride hydrochloride; APCI, atmospheric pressure chemical ionization; BSA, bovine serum albumin; CE–LIF, capillary electrophoresis–laser induced fluorescence; CE–MS, capillary electrophoresis–mass spectrometry; CE–MS^2, capillary electrophoresis–negative electrospray ionization–ion trap tandem mass spectrometry; CL, chemiluminescence; CZE, capillary zone electrophoresis; DAD, diode-array detection; DDA, data dependent acquisition; DDPC, dodecylpyridinium chloride; DNA, deoxyribonucleic acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DPV, differential pulse voltammetry; EEM, excitation–emission matrix; EI, electron ionization; ESI, electrospray ionization; ESMS, electrospray ionization mass spectrometry; FD, fluorescence detection; FI, flow injection; FIA, flow-injection analysis; FI–CL, flow-injection–chemiluminescence; FUR, furosemide; GC/EI–MS, gas chromatography/electron impact–mass spectrometry; GC–MS, gas chromatography–mass spectrometry; HPLC, high performance liquid chromatography; HPLC–FD, high performance liquid chromatography–fluorescence detection; IDA, information dependent acquisitions; ID, internal diameter; IS, internal standard; ITP, isotachophoretic; LC, liquid chromatography; LC/MS, liquid chromatography/mass spectrometry; LC/MS/MS, liquid chromatography/mass spectrometry/mass spectrometry; MBTH, 3-methyl-2-benzothiazolinone hydrazone; MEKC, micellar electrokinetic chromatography; MLC, micellar liquid chromatography; MS, mass spectrometry; MS/MS, mass spectrometry/mass spectrometry; MUX, multiplexed; NED, *N*-(naphthyl)-ethylene-diamine; NQS, 1,2-naphthoquinone-4-sulfonate; PAD, pulsed amperometric detection; PIR, piretanide; PLS, partial least square; PVC, poly(vinyl-chloride); RAMs, restricted access materials; RP, reversed phase; RP-HPLC, reversed phase-high performance liquid chromatography; RP-LC, reversed phase-liquid chromatography; RuBPS, tris-(4,7-diphenyl-1,10-phenantrolinedisulfonic acid)ruthenium(II); SAL, saluamine; SDS, sodium dodecyl sulfate; SIA, sequential injection analysis; SIA-RAM, sequential injection analysis-restricted access material; SPE, solid-phase extraction; SPL, spironolactone; SPS, solid-phase spectroscopy; SRM, selected reaction monitoring; SWV, square wave voltammetry; TDPB, tridecylpyridinium bromide; TFA, trifluoroacetic acid; TLS, thermal lens spectrophotometry; TOPO, trioctylphosphine oxide; TRI, triamterene; UV, ultraviolet; UV–vis, ultraviolet–visible; VASS, variable-angle synchronous scanning.

* Corresponding author.
E-mail address: fsanchezr@uma.es (F. Sánchez Rojas).

0731-7085/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2008.07.003

1. Introduction

The group of diuretics includes compounds with wide differences in molecular structures and physico-chemical properties: Basic (potassium sparing diuretics, such as amiloride and triamterene), neutral (aldosterone antagonists, such as canrenone and spironolactone), weakly acidic (carbonic anhydrase inhibitors, such as acetazolamide and diclofenamide; thiazides and related agents, such as chlorthalidone) and strongly acidic compounds (loop diuretics, such as FUR, bumetanide, piretanide and etacrynic acid).

Furosemide or frusemide is a loop diuretic, which is an anthranilic acid derivative (5-(aminosulfonyl)-4-chloro-2-[(2-furanylmethyl)amino]benzoic acid) as can be see in Fig. 1, used in the treatment of congestive heart failure and edema. His medication is also used to treat high blood pressure (hypertension). FUR works by blocking the absorption of salt and fluid in the kidney tubules, causing a profound increase in urine output (diuresis). The diuretic effect of FUR can cause body water and electrolyte depletion. Therefore, careful medical supervision is necessary during treatment.

On the other hand, the use of diuretics has been forbidden by the Medical Commission of the International Olympic Committee because it was shown that they were misused in sports for two main reasons: to achieve acute weight losses before competition, in sports where weight categories are involved, and to mask the ingestion of other doping agents by reducing their concentration in urine. This effect may be accomplished either by increasing the urine volume, or by increasing the urinary pH (carbonic anhydrase inhibitors) and, thus, reducing the excretion in urine of basic doping agents.

Evidently, a sensitive and reliable analytical method to determine FUR in plasma as well as urine is a prerequisite to correlate diuretic activity with drug kinetics under various conditions. A review of the analytical methodology described to detect diuretics in urine keeping in mind the requirements of doping control is presented by Ventura and Segura [1] in 1996. More recently, Zendelovska and Stafilov describe reversed phase-high performance liquid chromatography (RP-HPLC) methods for determination of diuretics in different human body fluids [2], and also, Ruiz-Angel et al. [3] present a revision of the most frequent analytical techniques to determine FUR.

This review consists of papers mainly reported from the nineties decade until today about the analytical methodology for FUR determination.

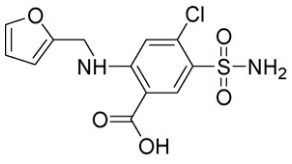

Fig. 1. Chemical structure of furosemide.

2. Titrimetric methods

For the determination of the concentration of the major compounds, titrimetric methods are more convenient that other instrumental methods. In this way, FUR contains acidic hydrogen atoms and can be determined by alkalimetric titration. However, FUR is water insoluble; therefore, its determination is performed in a dimethylformamide solution with the detection of the titration end point using Bromothymol Blue [4].

Because of the extremely low solubility of FUR in water, the titration curve of a saturated solution of FUR has no inflection points and its determination is impossible. However, titration curves of FUR in micellar solutions of surfactants exhibit clearly defined inflection points. It was found that the maximum solubility of FUR is observed in aqueous–micellar solutions of tridecylpyridinium bromide (TDPB) and dodecylpyridinium chloride (DDPC) because of the formation and solubilisation of ion pairs of the anionic form of FUR with surfactant cations. For this reason, a procedure was proposed for the titrimetric determination of FUR in solutions of cationic surfactants with the detection of the titration end point either pH-metrically or using Bromothymol Blue as the indicator [5].

Conditions for alkalimetric determination of hydrophobic acids and FUR with the use of emulsions stabilized by cationic surfactant have been suggested by Kulichenko and Shevchenko [6]. However, for highly hydrophobic compounds it is practically impossible to achieve the threshold concentration to achieve their precise determination. This obstacle can be overcome through substitution of aqueous micellar systems with “oil-in-water” emulsions stabilized by surface-active compounds. These emulsions become convenient for performing titration of hydrophobic organic acids. The same authors investigate protolytic characteristics of fat row carbonic acids in the emulsion media stabilized by Triton X-100. The procedure for the determination of FUR main substance content in pharmaceuticals using Triton X-100-stabilized emulsion was recommended to dissolve the sample of FUR (0.05–0.10 g) in the emulsion of 0.25 g of oil in 25 mL 2.5% Triton X-100 solution. The titration is performed with 0.025 M KOH aqueous solution and the equivalent point was determined from the differential titration curve or by addition of bromothymol blue solution until to the emulsion colour changed from yellow to blue [7]. Recently, an acidified solution of FUR is titrated directly with bromate–bromide mixture using methyl orange as indicator and applicable over 2–20 mg range [8].

At the present time, liquid chromatography (LC) is the most widely used technique for the separation and determination of drugs and acetonitrile is the most widely used organic modifier in LC. For this reason, the study of the acid–base behaviour of analytes in the widely used acetonitrile–water media therefore could be very important for predicting the influence of pH on retention and selectivity in LC. With this purpose, Barbosa et al. [9,10] determine the dissociation constants of series of compounds, including FUR in several acetonitrile–water mixtures and the results were subjected to factor analysis. The p*K* values of the studied substances, including

FUR, were determined from titrations of appropriate solutions of acid species in acetonitrile–water mixtures up to 70% (w/w), using potassium hydroxide solutions in the same mixture as titrant and approximately 7×10^{-3} mol L^{-1} KCl for the correct response of the electrode system.

3. Optical methods

3.1. Spectrophotometric methods

FUR determination was carried out in many ways and matrices applying spectrophotometric procedures, even in the presence of other drugs, exploiting the chemical reaction possibilities allowed by FUR structure were used. Some systems were developed using UV and visible absorption spectrophotometry, with or without previous reactions, as well as static and flow procedures as can be see in Table 1.

Although complexation reactions are simple and sensitive, no related method for the determination of FUR has been reported until now. Based in this reaction type, Gölkü present a method for the determination of FUR by complexation reaction with Cu(II) that is simple, sensitive, and accurate [19].

Other interesting area is the flow-injection analysis (FI), which is characterized by its simplicity, speed and the use of inexpensive equipment; its results are accurate and precise and there are clear advantages because of the short time required for each assay. The usefulness of FI methods for routine analysis has been shown in a large number of determinations developed for clinical, pharmaceutical, food and environmental analyses. In this way, García et al. development a FI method that is simple, inexpensive and rapid for routine determination of FUR in pharmaceuticals [21]. The proposed procedures are based on the visible absorption of the complex formed between FUR and Pd(II). The FI methods proposed are useful for the quality control of FUR in pharmaceutical dosage forms since there is no interference from the common additives and excipients that might be found in commercial preparations.

3.1.1. Mixtures resolution

Although UV–vis spectrophotometry is a rapid, sensitive and inexpensive analytical tool, however, the lack of specificity of the UV–vis absorption usually hinders the application of this technique in case of mixtures of absorbing species, due to spectral overlap. In this way, in recent years, the literature has been showing an increase in the methods based on the derivative spectrophotometry. It allows enhancement of the resolution of overlapping spectra and selective discrimination of sharp bands over broad bands, thereby offering effective approaches to analysis of drug association and impurities. Dias et al. describes the first derivative spectrophotometry method for determination of FUR in pharmaceutical commercial formulations, in the presence of its degradation product [25].

Finally, numerical methods based on the mathematical resolution of multivariate signals, such as UV–vis spectroscopic data, have been shown to allow the resolution of complex mixtures with high speed and acceptable accuracy and precision. Among them, the partial least squares regression with a single dependent variable (PLS-1) has found important applications in pharmaceutical analysis, providing an interesting alternative to the more demanding chromatographic techniques. Ferraro et al. describe a rapid, precise and accurate procedure for the simultaneous determination of FUR and amiloride hydrochloride in synthetic samples and commercial combined tablet preparations, based on the joint use of the PLS-1 algorithm and UV–vis spectrophotometric data [26].

In Table 2 are summarized the more important methods proposed for the simultaneous determination of FUR and other compounds based in UV/vis spectroscopy.

3.2. Spectrofluorimetric methods

Spectrofluorimetry has been widely used to determine trace elements in different fields such as clinical, biomedical and environmental analysis on account of its high selectivity, sensitivity and relatively low cost. However, the analytes usually exhibit broad spectral bands that tend to overlap when the sample contains several components; this entails the use of prior separation procedures or high specific methods, both of which result in increased analysis times and costs. There is thus a persistent need to improve existing methods, not only to improve their selectivity and detection limits, but also to expedite analyses. In recent years, advances in analytical instrumental and the general use of computers have promoted the application of mathematical algorithms for treating the large amount of data that modem instrumentation can provide, increasing the selectivity of analytical methods.

This challenge has been met by using variable-angle fluorescence spectroscopy, luminescence spectroscopy, the whole fluorescence excitation–emission spectrum (known as the "excitation–emission matrix", EEM) or, very frequently, derivative fluorescence spectra of both the conventional and the synchronous type.

A novel approach of conventional synchronous scanning that offers considerable flexibility is variable-angle synchronous scanning (VASS), where the wavelength separation between the two monochromators is varied. There are three different instrumental configurations for performing VASS. First, the speed of the monochromators can be manipulated by two different motors scanned at different rates. A second approach consists of acquiring the excitation–emission matrix and storing the data on the interfaced microcomputer, and the desired angle (linear or nonlinear) is determined by using the appropriate software. Recently, the research group of Garcia-Sanchez has modified a commercial digital instrument to generate the VASS directly from the spectrofluorimeter output and their applicability has been demonstrated for the resolution of mixtures of three diuretics (furosemide, triamterene, piretamide) with closely overlapping fluorescence profiles. This approach has permitted the simultaneous determination of the three compounds at the μg to ng mL^{-1} level, with a relative standard deviation ≤5% [34].

Luis et al. addressed the simultaneous determination of FUR and triamterene (TRI) with strongly overlapped spectra and very different intensity of fluorescence. The analytical signals obtained by conventional and synchronous fluorescence spectroscopy were processed by using a multivariate calibration method (PLS) and carefully selecting the wavelength range and number of factors to be used. One of the most salient advantages of them is that the vast amount of information provided by the whole spectrum of the sample is not required, this makes analyses simple and fast [35].

On the other hand, multicommutation is the use of three-way solenoid valves controlled automatically by use of appropriate software. The method has several advantages compared with conventional FIA, for example better reproducibility, lower sample and reagent consumption, easy sample handling, and minimal waste generation. The solenoid valves are individually switched on and off by means of an electric pulse, so enabling a great variety of configurations in the system merely by changing the configuration of the tubing. Combinations of solid-phase spectroscopy (SPS) with FIA, so-called "flow-through optosensors", have advantages such as speed, selectivity, and sensitivity. The combination of multicommutation and flow-through optosens-

Table 1
Spectrophotometric methods

Reagent	Experimental conditions	Results	Applications	Ref.
Ferric chloride	pH 5.2–6.2; red water-soluble complex; at 513 nm	Linear up to 8 mmol L^{-1}; detection limit 0.03 mg mL^{-1}	Tablets and ampoules	[11]
Pd(II) chloride	With KCl and pH 10 Britton–Robinson buffer; at 527 nm	Linear range of 0.25–3.5 mmol L^{-1}; $\varepsilon = 0.86 \times 10^2$	Bulk drug, tablets and ampoules	[12]
2,2-Diphenyl-1-picrylhydrazyl (DPPH)	Reduction of violet colour of DPPH with formation of yellow coloured 2,2-diphenyl-1-picrylhydrazine; at 520 nm on methanolic solutions of the reagent and drugs	Linear range of 2–15 μg mL^{-1}	Pure form and pharmaceutical preparations	[13]
Solution of CH_3ONa in benzene	Spectrophotometric titration in non-aqueous differentiating solvents of mixture clonidine hydrochloride-furosemide	Best results in dimethylformamide and isopropyl alcohol	Directly both acidic components of mixtures, without previous separation	[14]
Sevron blue 5G	Chloroform extract of the ion-association complex at pH 7.0; at 655 nm	–	Pharmaceutical formulations	[15]
1,2-Naphthoquinone-4-sulfonate (NQS)	NaH_2PO_4–Na_2HPO_4 pH 7.5, NQS 7.7×10^{-3} M, heating 30 min at 70 °C and isoamyl alcohol as extractant	–	Pharmaceutical samples	[16]
Based upon simple diazotization reaction	Method optimized for acidity, amount of reagents required and heating time; at 480 nm	Linear range of 0.4–10 μg mL^{-1}; $\varepsilon = 1.0 \times 10^4$ L mol^{-1} cm^{-1}; detection limit 0.16 ppm	Pharmaceutical preparations	[17]
Bromate–bromide mixture and methyl orange	Fixed and known amount of bromate–bromide mixture, acidified solution of drug, determination of residual bromine by reacting with fixed amount of methyl orange; at 520 nm	Linear range of 0.25–3.50 μg mL^{-1}; $\varepsilon = 6.11 \times 10^4$ L mol^{-1} cm^{-1}; detection limit 0.07 μg mL^{-1}	Bulk drug and formulations	[8]
Fe(III) and ferricyanide(III)	Based on a redox reaction; resulting Prussian blue measured at 760 nm	Linear range of 0.4–4.0 μg mL^{-1}; $\varepsilon = 4.03 \times 10^4$ L mol^{-1} cm^{-1}; detection limit 0.09 μg mL^{-1}	Bulk drug and formulations	[18]
Cu(II)	At pH 3.2 using Mclivaine buffer solution to produce a green adduct; at 790 nm	Linear range of 5–30 μg mL^{-1}; detection limit 0.23 μg mL^{-1}	Tablets	[19]
N-(naphthyl)-ethylene-diamine (NED)	In a sodium dodecyl sulfate micellar medium of pH 1 by conventional and thermal lens spectrophotometry (TLS, 100 mW of pump power at 514.5 mn)	Detection limit 1.9×10^{-7} M for conventional and 4×10^{-9} M for TLS	–	[20]
$PdCl_2$	72 μL of furosemide injected into inert carrier stream, which then joined the reagent stream of 3×10^{-3} M $PdC1_2$ at pH 5.0; peak height measured at 410 nm	Linear range of 2×10^{-5}–4×10^{-4} M	Pharmaceutical preparations	[21]
4-Chloro-5,7-dinitro-benzofurazan and 7-chloro-4,6-dinitro-benzofuroxane	FI performed in reverse single-channel mode using a setup equipped with a D1 plunger pump and a flow photometric unit including a 6 μL cell with optical pathlength of 0.5 cm; Acetonitrile–aqueous buffer, pH 6.86 (15:85)	Linear range of 1.65–13.22 μg mL^{-1}; detection limit 0.33 μg mL^{-1}	Medicinal forms and technological reaction media	[22]
Potassium permanganate	FI system using a 50 cm sample loop and a 100 cm reactor, at 50 °C; at 550 nm	Linear range of 1×10^{-4}–6×10^{-4} mol L^{-1}; detection limit 1.1×10^{-5} mol L^{-1}	Tablets and ampoules	[23]
Fe(III) ions in ethanol media	As carrier flow single line system configuration used an ethanolic 10^{-2} mol L^{-1} Fe(III) at 1.0 mL min^{-1}, 50 cm sample loop (250 μL total sample injection), and 50 cm long reactor coil; at 513 nm	Linear range of 10^{-4}–10^{-2} mol L^{-1}; detection limit 3×10^{-5} mol L^{-1}	Commercial samples from different suppliers, as tablets and ampoules, and a synthetic urine sample spiked with analyte	[24]

ing is the next stage of improving the features of flow analysis. The advantages of each method are maintained, so providing further scope for applications using this new approach. Llorent-Martinez report a multi-commuted flow-through optosensor for simultaneous determination of the diuretics FUR and TRI by measurement of their native fluorescence when retained on a cationic resin, Sephadex SPC-25. A mini-column placed before the flow cell and filled with the same resin was used to separate the drugs.

The sensor was used for determination of both analytes in pharmaceuticals [36]. Sequential injection analysis (SIA) is based on forward and reverse movement of a piston of a syringe pump, which together with a multi-position selection valve enables precise sampling of chemicals into the system and propelling of the sequenced zones to the reactors and detector. Automation, velocity of the analysis and low consumption of sample and reagents are the most important features that favour the SIA technique for application in many fields of analysis; in recent years, special solid-phase extrac-

Table 2
Simultaneous determinations of furosemide with other compounds by spectrophotometric methods

Compounds	Procedure	Applications	Ref.
Spironolactone (SPL)	SPL at 238 nm; FUR at 276 nm	Combination preparations	[27]
SPL	Multiwavelength spectroscopy (at 242 nm for SPL and 228 and 274 nm for FUR) and 1st derivative	Two component tablet formulations	[28]
SPL	Using Vierordt's method and 1st and 2nd derivative applying the zero-crossing technique	Synthetic mixtures and dosage forms	[29]
SPL	1st derivative for routine analysis	Combination formulations	[30]
SPL	By use of ratio spectra derivative spectrophotometry	Capsule formulation	[31]
In presence of its degradation product, saluamine (SAL)	Stability-indicating method based on the use of derivative spectrophotometry; 1st derivative at 254 nm for SAL and 262 nm for FUR; 2nd derivative at 265 nm for SAL and 272 nm for FUR	Dosage forms either as tablets or injections	[32]
SAL	By 1st derivative at 262 nm; photolitic degradation carried out by putting samples in stove at 45 °C under artificial light during 28 days; after 7, 14, and 28 days, samples collected and analyzed	Tablet and liquid pharmaceutical preparations	[25]
Amiloride hydrochloride (AM)	Stock solution of AM and FUR simultaneously diluted in HCl 10^{-2} M in ethanol to obtain the concentration range 4×10^{-5} to 16×10^{-5} mol L^{-1}; calibration graphs carried out for each compound in presence of 6×10^{-5} mol L^{-1} of the other; 1st derivative spectra at 343.6 nm for AM and 241.4 nm for FUR	Commercial pharmaceutical formulation	[33]
AM	PLS-1: A four-level full factorial design training set of 16 samples for calibration, by convenient dilution of the stock solutions of FUR and AM in MeOH–H_2O (1:1) to final concentrations in the range of 8.0–13.0 mg L^{-1} for FUR and 1.0–1.6 mg L^{-1} for AM	Synthetic mixtures and commercial formulations	[26]

tion (SPE) supports possessing restricted access properties have been developed to allow the direct injection of untreated biological samples into on-line SPE liquid chromatography (LC) systems. These sorbents called restricted access materials (RAMs), combine size exclusion of proteins (without destructive accumulation) and other macromolecular matrix components with the simultaneous enrichment of low-molecular analytes, which can be retained and extracted selectively. In this way, a method for direct determination of FUR in serum comprising on-line sample preparation based on SIA–RAM hybrid technique has been proposed by Huclová et al. [37]. The integration of RAM material into SIA enabled creation of a comprehensive on-line sample cleanup technique combined with fluorescence quantitation of analyte (emission filter 385 nm). The developed methodology has the potential to fill the gap between the traditional HPLC (high performance liquid chromatography) and manually performed sorbent extraction. The proposed method involving sample preparation can be simply automated and shows the possibility of restriction of manual sample handling. The main disadvantage of the proposed SIA–RAM method is low sensitivity.

As can be see above, previous papers had showed the wide application of fluorescence spectroscopy in the detection and quantification of FUR, not only due to the improvement of sensitivity, but also to maximize the selectivity of chromatographic procedures. Very recent, the determination of FUR based on two simple flow-injection systems with fluorimetric detection is described by Semaan et al [38]. The first configuration used a phosphate buffer solution pH 3, 0.2 ionic strength solution flowing at 3.0 mL min^{-1} as carrier, a 80 cm sample loop (400 μL total sample injection), and a 40 cm long reactor coil, which was kept at room temperature; the second has a unique difference: the introduction of a new channel of surfactant solution with reduction of flow-rate. The excitation and emission were carried out at 270 and 410 nm, respectively. The surfactant micelles remarkably improved the sensitivity. This work shows an alternative procedure to determine FUR in some matrices as tablets, ampoules, and synthetic urine. The limits of detection found with phosphate buffer showed to be compatible or lower than those described before in literature with the advantages of higher analytical frequency and lower reagent consumption.

Also, recently, the interaction of FUR with bovine serum albumin (BSA) has been investigated, at physiological acidity (pH 7.40), by fluorescent technique. Displacement experiment with site markers and synchronous fluorescence clearly reveal that there are non-specific binding sites of FUR with BSA [39].

3.3. Luminescence methods

The methods based on the measurement of the native fluorescence of FUR and similar compounds are not always very sensitive and indirect methods involving complexation of the drug with lanthanide ions have been investigated. The lanthanide ions in solutions of simple salts and complex compounds possess the luminescence properties. The excitation of lanthanide ion in complex solutions occurs at the expense of the intramolecular energy transfer from excited organic molecule to lanthanide ion. The sensitization of luminescence of lanthanide ions in complexes with organic ligands has been extensively used during the past 30 years in various applications, including the investigation of biological systems, immunoassays and DNA hybridization assays, quantification of organic compounds and chromatographic detection. Beltyukova et al. [40] studied the possibility of application of luminescence sensitization of lanthanide ions in complexes with organic ligands relating to the drugs with the purpose of determination the latter. The Tb(III) and Eu(III) ions possessing the highest luminescence intensity in solution of complex compounds were chosen. It was shown that a result of intramolecular energy transfer from the ligand molecule to the lanthanide ion increases the luminescence intensity of the latter by 10^8–10^{10} times. The highly sensitive method for luminescence determination of FUR was developed, with a detection limit of 0.05 μg mL^{-1}.

Also, in the same time, Ioannou et al. report the results of a study on the intrinsic fluorescent properties of the three anthranilic acid derivatives, including FUR, and on their ability to sensitize terbium ion fluorescence in both aqueous and non-aqueous solutions and report a sensitive and simple method for the determination of furosemide and mefenamic and tolfenamic acids in serum based on terbium-sensitized fluorescence in methanolic solutions [41].

Previously reported data for the determination of anthranilic acid derivatives have been obtained in organic solvents. The purpose of the work, realized by Arnaud and Georges [42], was to investigate the luminescent properties of these drugs when complexed with terbium in aqueous solutions. The influence of pH, surfactant and trioctylphosphine oxide (TOPO), as co-ligand, on the properties of the complexes is studied and the luminescence lifetimes are determined under optimum conditions. Finally, the performance of these complexes for the determination of the drugs in water is considered. The method based on the formation of a ternary luminescent complex with terbium and TOPO in aqueous solutions of Triton X-100 is one to two orders of magnitude, more sensitive than previously reported methods based on native fluorescence or terbium-sensitized luminescence in methanol.

3.4. Chemiluminescence methods

Analytical procedures applying chemiluminescence (CL) methods combine the advantages of simplicity of equipment and sensitivity of detection, and have been applied frequently in the determination of pharmaceutically important compounds, including diuretics. The CL properties of fluorophore-sensitized Ce(IV) reactions with sulfur-containing drugs in acidic medium have been widely studied. FI–CL methods were developed previously for the determination of some compounds in different pharmaceutical formulations. FUR which contains an aminosulfonyl group is a weak reducing drug and may be oxidised by Ce(IV) to produce CL emission like the other sulfur-containing pharmaceutical compounds mentioned above. The primary aim of the study carried out by Rao et al. was to explore the possibility of developing a CL-based FI method, applying the CL reaction of cerium(IV) with FUR in sulfuric acid, sensitized by a fluorescent dye (rhodamine 6G), for the determination of FUR in pharmaceutical formulation [43].

Also, as the most popular example of Ru(II) complex chemiluminescence reagents, tris-(2,2′-bipyridine)ruthenium(II) ($Ru(bipy)_3^{2+}$) has been studied in detail and widely applied for the past four decades, including FUR [44]. On the basis of studying the chemiluminescence properties of $Ru(bipy)_3^{2+}$, several novel ruthenium(II) complexes with diphenylsubstituted bipyridine and phenanthroline as ligands were synthesized and exhibited increased quantum efficiencies compared to $Ru(bipy)_3^{2+}$. Based in this fact, Xi et al. investigated the chemiluminescence mechanism of tris-(4,7-diphenyl-1,10-phenanthrolinedisulfonic acid)ruthenium(II) (RuBPS)–Ce(IV) system and the effects of two diuretics, hydrochlorothiazide and furosemide on its chemiluminescence intensity [45]. Under the optimum experimental conditions, the linear range and detection limit of hydrochlorothiazide were 2.5×10^{-3} to 6.0×10^{-1} μg mL^{-1} and 1.0×10^{-3} μg mL^{-1}, respectively; those of FUR were 1.0×10^{-2} to 4.0 μg mL^{-1} and 8.8×10^{-3} μg mL^{-1}, respectively.

3.5. Other optical methods

Salem described simple and accurate methods for the quantitative determination of FUR and diclofenac acid utilizing precipitation reactions with Ag(I), Cu(II) and Fe(III). FUR and diclofenac acid were precipitated from their neutral alcoholic solutions with silver nitrate, copper acetate or ferric chloride standard solutions followed by direct determination of the ions in the precipitate or indirect determination of the ions in the filtrate by atomic absorption spectroscopy (AAS). The optimal conditions for precipitation were carefully studied. Statistical analysis of the results revealed equal precision and accuracy to the results of the official methods. The validity of the suggested procedures was verified by the determination of FUR and diclofenac acid in pharmaceutical preparations [46]. Latter, the investigation group of Salem proposed a method for the quantitative determination of FUR, flufenamic acid, mefenamic acid, tranexamic acid, diclofenac sodium and thiaprofenic acid by precipitation reactions with Ni(II) and the same procedure exposed above with similar results [47].

For many years, the use of reflectance spectroscopy as an analytical technique was limited to paints and pigments, paper, textile areas, ceramics, dye-stuffs and printing inks to evaluate properties such as colour, whiteness, gloss, covering power, etc. Little attention has been given to diffuse reflectance spectroscopy as a quantitative technique as it was not possible to attain highly precise measurements from conventional spot tests. However, with the development of optical devices including optical fibres and reflectance spheres, this situation has changed and the quantification from spot tests by diffuse reflectance, on inert support, yield good precision and selectivity. The aim of study realized by Gotardo et al. was to develop an analytical method employing diffuse reflectance spectroscopy for the routine analysis of FUR in pharmaceuticals. The proposed method is based on the reflectance measurements in the visible region of the spectrum of the violet compound produced from the spot test reaction between FUR and *p*-dimethylaminocinnamaldehyde using a filter paper as solid support, in acid medium, after heating to 80 °C for 5 min. The results obtained from this study showed the good performance of this technique, suggesting its use as a reliable and advantageous alternative to most other previously reported method in the routine control of FUR in pharmaceutical formulations [48].

4. Electrochemical methods

Electrochemical methods have been rarely applied to determination of pharmaceutical compounds in plasma and urine. Barroso et al. investigated the determination of the loop diuretics piretanide (PIR) and FUR in pharmaceuticals and urine by differential pulse voltammetry (DPV) and square wave voltammetry (SWV) [49]. Oxidation was studied in methanol–water (10:90) in the pH range 0.3–13 (PIR) and 0.5–13 (FUR), with 0.04 mol L^{-1} Britton–Robinson buffers as supporting electrolytes and 0.5 mol L^{-1} KCl as ionic medium at a glassy carbon electrode. Voltammetric methods have been developed for the determination of PIR at pH 5.0 and FUR at pH 4.5 using differential pulse and square wave voltammetry. The detection limit obtained (50 ng L^{-1}) allows the application of these methods to urine samples. The recoveries obtained for pharmaceutical formulations and urine samples show the applicability of these techniques to control analysis of these drugs.

Recently, potentiometric sensors have became important and viable devices for use in chemical and pharmaceutical analyses. The advantages of these methods are their fast and linear responses over wide concentration and pH ranges and good limit of detection with high accuracy and reproducibility. Several papers have related the minimal interference in this method by associated and related species; the possibility of direct application to turbid and coloured drug solutions without any pretreatment, low cost and easy of construction and handling. Tescarollo Dias et al. describes the development and application of a FUR selective electrode based on the poly(vinyl-chloride) (PVC) membrane for direct determination of this diuretic in pharmaceutical preparations [50].

5. Liquid chromatography

A variety of HPLC techniques were developed for determination of FUR in plasma and other samples for 30 years approximately. Some of these required 1–2 mL of plasma. Other methods required lengthy sample extraction and have very long elution times for the

drug and the internal standard. Farthing et al. [51] used an external standard and solid-phase extraction, while Reeuwijk et al. [52] used the lengthy and tedious technique of reversed-phase ion pair chromatography. On the other hand, Saugy et al. [53] used gas chromatography–mass spectrometry with different types of ionization to confirm the occurrence of FUR after per-methylation of the extract eluted by HPLC. Most of the HPLC methods have not achieved the complete separation of FUR from endogenous substances with a minimum detectable concentration of less than 20 ng mL^{-1}, which would allow their application to the measurement of low FUR concentrations in biological fluids. Most of the previously reported methods published the precision of the calibration data but usually omitted the accuracy for concentrations at the lower end of the concentration range found in pharmacokinetic and bioavailability/bioequivalence studies. Furthermore, the minimum quantifiable concentrations are rarely reported. Potential interference from the major FUR metabolite, FUR glucuronide, and the hydrolytic product 4-chloro-5-sulfamoyl anthranilic acid was also not reported for the majority of these methods. Abou-Auda et al. report a rapid, sensitive and selective reversed-phase HPLC assay that meets the acceptable criteria for analytical method validation and that is suitable for the processing of a large number of FUR samples [54].

HPLC has been employed to detect FUR in blood, urine and perilymph, as can be see in Tables 3 and 4. However, detection of FUR from tissues using HPLC has not been initially described. In an effort to examine the influence of changes in the pharmacokinetics and metabolism of FUR in developing verses juvenile rats, Mills et al. developed an assay using HPLC for the detection of FUR and FUR glucuronide in serum, perilymph, renal and hepatic tissues [57]. The assay proved sensitive enough to detect FUR in as little as 3 μL of perilymph from 10-day-old rats.

HPLC is generally the method of choice for the analysis of diuretics due to the time consumed, cost of the analysis, and some limitation involving the GC/MS technique to detect diuretics in urine (low volatility of the compounds and the necessity of the additional step of derivatization).

Liquid–liquid extraction followed by solvent evaporation is the traditional method for sample preparation in the chromatographic analysis of diuretics and probenecid in biological samples. These procedures are usually very time consuming and often imprecise, as many sample manipulations are usually involved. In addition, multi-step extractions under different pH conditions may be required for screening tests, due to the wide differences in the polarities of these drugs. Solid-phase extraction on disposable cartridges has been reported to simplify chromatographic quantification of different diuretics. The employment of apolar (C_8 or C_{18}) solid-phase extraction columns is clearly advantageous over liquid–liquid extraction procedures because a unique extraction can provide acceptable recoveries for the most common diuretics. In last years, an increasing number of HPLC methods incorporating on-line sample cleanup by solid-phase extraction using column switching have been developed for the assay of several drugs. Switching devices permit the off-line multi-step methods for sample treatment to be transformed into single-step procedures by the on-line purification of the samples. Since rapid and sensitive methods are required for screening of diuretics, especially in therapeutic drug monitoring and in control of doping, Campíns-Falcó et al. have evaluated the potential of column-switching techniques for sample cleanup and enrichment of these compounds in urine samples with UV detection [63]. Probenecid, a uricosuric acid, has also been included in this study because it has a weak diuretic activity and has been used as a masking agent in sports to decrease urinary excretion of anabolic steroids. Subsequently, the potential of column-switching chromatography and fluorescence detection for the analysis of diuretics in urine is evaluated for the same authors [77].

Other common question is that the patients are treated simultaneously with a few drugs representing different groups. Therefore, it is necessary to develop one chromatographic system which could provide simultaneous determination of concurring drugs in the shortest time. In a study realized by Baranowska et al., the selection of drugs was made following the frequency of their use in an environment of a postoperative cardio-surgical ward. The objects of analysis were imipenem, paracetamol, dipyrone, vancomycin, amikacin, fluconazole, cefazolin, prednisolone, dexamethasone, furosemide and ketoprofen belonging to four different groups (antibiotics, analgesic, demulcent and diuretic) in urine. The separation of analyzed compounds was conducted by means of a LiChroCART® Purospher® C18e (125 mm × 3 mm, particle size 5 μm) analytical column with LiChroCART® LiChrospher® C_{18} (4 mm × 4 mm, particle size 5 μm) pre-column with gradient elution; mobile phase was comprised of various proportions of methanol, acetonitrile and 0.05% trifluoroacetic acid in water and detection carried out for DAD and FD [78]. The same investigation group also proposed a HPLC system for separation and determination of teophylline, 1-methylxanthine, 3-methylxanthine, 1,3-dimethyluric acid, caffeine, paracetamol, furosemide, dexamethasone, prednisolone, cefazolin and imipenem in urine samples by using RP-18e column with a RP-18 pre-column and a DAD detector applying gradient elution with 0.05% TFA aqueous solution with acetonitrile at the flow-rate of 0.8 mL min^{-1} [79].

The organic solvent content and the pH in the mobile phase are the usual main factors in reversed-phase liquid chromatographic separations, owing to their strong effects on retention and/or selectivity. Temperature is often neglected. However, even in cases where the impact of this factor on selectivity is minor, the reduction in analysis time is still an interesting reason to consider it. In addition, ionisable compounds may exhibit selectivity changes, owing to the interaction of organic solvent and/or temperature with pH. Recently, a retention model accounting all these interactions, valid for wide ranges in the experimental factors, is presented and tested with a set of 11 compounds (nine diuretics, including FUR and two β-blockers), sampling diverse acid–base behaviours [80]. The compounds were tested in a Zorbax SB C_{18} column under a wide range of conditions: 25–45% (v/v) acetonitrile, pH 3–7 and 20–50 °C. Models considering two factors (organic solvent/pH and temperature/pH), and three factors (organic solvent/temperature/pH) were developed from a previously reported equation, which considers the polarity contributions of solute, stationary and mobile phases.

5.1. Micellar liquid chromatography

In the last 20 years, reported applications of micellar liquid chromatography (MLC) have increased. MLC, which employs solutions of surfactants as the mobile phases, is a mode of liquid chromatography that can be considered as an alternative to classical partition chromatography.

The use of aqueous solutions of surfactants at concentrations above the critical micelle concentration as mobile phases for reversed-phase chromatography has advantages over traditional hydro-organic mobile phases. These advantages include the ability to simultaneously chromatograph hydrophilic and hydrophobic compounds, the lower cost and greater safety of micellar mobile phases as compared with conventional mobile phases containing organic solvents, and the greater solubility of solutes that can be used to control ionic strength, pH and buffering capacity in micellar mobile phases. In this way, Cline Love and Fett investigated the determination of FUR, hydrochlorothiazide and propranolol in urine by direct injection micellar chromatography [81]. The type of

surfactant used in the mobile phase and the pH of the mobile phase have been identified as two key parameters that can be varied to obtain the required resolution between a drug and the components of urine. In this case, non-ionic surfactant, polyoxyethylene 23 lauryl ether (Brij 35), was found to be the surfactant of choice for the separation of these drugs from urine, in mobile phases adjusted between pH 5.5 and 7.5.

The research group of Bonet Domingo et al. proposed the use of MLC for the determination of diuretics, including FUR, in pharmaceutical preparations [82] and urine [83,84] by direct injection of the sample. Micellar mobile phases containing sodium dodecyl sulfate (SDS), with and without different alcohols, are considered in order to determine the most appropriate combination and the effect of a varying pH, in the range 3–7, on the retention of diuretics

Table 3
HPLC methods with UV detection

Experimental conditions	Results	Applications	Ref.
RP Accurasil ODS C_{18} column (250 mm × 4.6 mm, 5 μm) using mobile phase of acetonitrile–0.1% orthophosphoric acid (pH 3) (60 + 40) at flow-rate of 1.0 mL min^{-1}; at 233 nm	Linear range of 1.01–121.8 μg mL^{-1}; detection limit 0.3 μg mL^{-1}	Bulk drug and formulations	[18]
In a C_{18} home-made column (50 mm × 4.6 mm, 3 μm) with methanol:phosphate buffer (10 mM, pH 5.5) (30:70) as the mobile phase, at a flow-rate of 1.0 mL min^{-1}; DAD monitored signals between 190 and 380 nm, with special attention to 237 nm	Linear range of 8–12 μg mL^{-1}	Tablets	[55]
On-line SPE based on column switching (hart-cutting) for direct injection; at 271 nm	Linear range of 5–1000 ng mL^{-1}	Human serum	[56]
15 μL of prepared sample injected onto the column; mobile phase, 0.01 M KH_2PO_4 (pH 5.5) with 37% MeOH; column, C_{18} reversed phase, ODS, particle size 5 μm, 250 mm × 4.6 mm ID; flow-rate 1.5 mL min^{-1}; at 235 nm; retention times: FUR-glucuronide conjugate 4.4 min, FUR 5.6 min, *p*-nitrophenol (IS) 8.3 min	Linear range of 0–500 μg mL^{-1}; detection limit 0.1 μg mL^{-1}	Serum and tissues	[57]
RP; using gradient elution with acetonitrile and phosphate buffer on a Hypersil-ODS column; for spiked urine extraction recovery of SPE using Sep-Pak C_{18} cartridge compared with LLE with diethyl ether at various pH; DAD; confirmation analysis performed by GC/MS following methylation	Linear range of 0.2–20 μg mL^{-1} for all diuretic agents except amiloride, 1–20 μg mL^{-1}, detection limit about 0.2 μg mL^{-1} for 3 mL of urine, except amiloride 1.0 μg mL^{-1}	Analysis of diuretic doping agents in urine	[58]
RP; using gradient elution with acetonitrile and phosphate buffer containing propylamine hydrochloride on a Bondclone-ODS (10 μm) column; DAD; confirmation analysis performed by GC–MS	Lower limit of detection ranges from 0.5 to 1.5 μg mL^{-1} in urine	14 diuretics in human urine	[59]
Column Spherisorb ODS (125 mm × 4 mm ID) 5 μm particle size; gradient elution with mobile phase 0.05 M buffer solution of ammonium acetate adjusted to pH 3 with concentrated orthofosforic acid and acetonitrile; DAD	Limits of detection estimated for 10 diuretic compounds from 0.09 to 0.75 μg mL^{-1}; 0.125 μg mL^{-1} for FUR	10 diuretics in human urine	[60]
RP; using alkaline extraction (pH 9.5) with ethyl acetate and the salting-out effect (NaCl); mobile phase with acetonitrile and 0.1 M ammonium acetate (adjusted to pH 3), with gradient elution	Detection limit for FUR 0.05 μg mL^{-1}	Diuretics, probenecid and other agents of doping interest (morazone, mesocarb, caffeine) in urine	[61]
Column HP-Hypersil ODS-Crs, particle size 5 μm, 250 mm × 4 mm ID; mobile phase acetonitrile–0.05 M phosphate (pH 3) or acetate buffer (pH 4); detector was set to collect a spectrum every 640 ms (over the range 200–400 nm); identity of each compound established by comparing retention times and UV spectra in real samples with those previously obtained by injection of standards	Detection limit 3 ng mL^{-1} for FUR	Diuretics and probenecid in urine	[62,63]
RP; column was C_{18} Spherisorb column (150 mm × 4.6 mm ID, 5 μm particle size); mobile phase was prepared by mixing methanol and water (acetic acid 3%) in a ratio of 40:60 (v/v); at 280 nm	Linear range of 0.750–250 μg mL^{-1}; detection limit 0.05 μg mL^{-1} and 0.2 μg mL^{-1} in the mobile phase and urine	Rabbit and human urine	[64]

Table 3 (*Continued*)

Experimental conditions	Results	Applications	Ref.
Propranolol and pindolol (IS) first extracted from alkaline plasma into diethyl ether; followed by extraction of FUR into acidified ether:hexane (65:35); two extracts combined and evaporated under nitrogen, and reconstituted residues analysed on a C_{18}/SCX RP/cation exchange column with mobile phase of acetonitrile:0.1 M sodium acetate pH 4 (33:67); drugs also extracted from plasma by column-switching technique utilizing a ten-port valve, drug compounds were retained on a C_{18} pre-column; at 230 nm	Linear range of 25–300 ng mL^{-1} for FUR and 50–400 ng mL^{-1} for propranolol	FUR and propranolol in human plasma	[65]
RP; drugs eluted through a Nucleosil C_{18} column with mobile phase composed of 0.02 M potassium dihydrogen phosphate and acetonitrile (80:20, v/v) adjusted to pH 4.5; at 235 nm	Linear of 0.1–200 and 5–200 μg mL^{-1} for FUR and propranolol, respectively	FUR and propranolol in raw materials and in pharmaceutical formulations	[66]
Extracted with ethyl acetate; FUR at pH 1 and amiloride at pH 12; chromatographic separation conditions, i.e., column, mobile phase and flow-rate were the same for both investigated drugs, FUR detected using UV detector, whereas amiloride, because of its very low therapeutic range, detected with spectrofluorimetric detector	Linearity of FUR and amiloride assays were confirmed over the range of 30–3000 ng mL^{-1} and 0.5–30 ng mL^{-1}, respectively	FUR and amiloride in human plasma	[67]
Dissolved in methanol and 20 μL of mixture of the drugs injected onto a hypersil BDS C_{18} (150 mm × 4.6 mm) 5 μm column; mobile phase consisting of equivolume mixture of acetonitrile and 0.01 M tetrabutyl ammonium hydrogen sulfate at a flow-rate of 1 mL min^{-1}; at 238 nm	Linear in the dynamic range 13–400 ng mL^{-1} for FUR and 32–1000 ng mL^{-1} for spironolactone	FUR and spironolactone in pharmaceutical dosage form	[68]
Simple reversed phase with mobile phase acetonitrile–water–triethylamine–glacial acetic acid (41.5 + 57.4 + 0.1 + 0.9, adjusted pH 5.6) at a flow-rate of 1 mL min^{-1}; run time 9 min; at 280 nm	Linear range of 7.93–125 μg mL^{-1} for phenol red and 6.25–100 μg mL^{-1} for hydrochlorothiazide and FUR; limits of quantitation 7.2, 8.9 and 6.8 μg mL^{-1} for FUR, hydrochlorothiazide, and phenol red, respectively	FUR and hydrochlorothiazide along with phenol red as a nonabsorbable marker for in situ permeability studies in anaesthetized rats	[69]
RP; by gradient elution on Symmetry Shield C_{18} column; mobile phase consisting of potassium dihydrogen *ortho*-phosphate (pH 5.5; 0.01 M) and methanol at a flow-rate of 1.5 mL min^{-1}; retention times of antipyrine, metoprolol, ketoprofen, phenol red and furosemide were about 9, 12, 13, 16 and 17 min, respectively; data acquisition carried out using photo diode-array detector in range 210–600 nm	–	FUR, antipyrine metoprolol, ketoprofen, and phenol red, as a tool for standardization of rat in situ intestinal permeability studies	[70]
RP; on C_{18} column with mobile phase water (pH 3.0) and acetonitrile gradient pumped at a flow-rate of 1 mL min^{-1}	Linear range of 1–50 μM for topotecan and 2–100 μM for the others; detection limits were found to be maximum 0.002 μM	Topotecan and four intestinal permeability markers (atenolol, antipyrine, propranolol and FUR)	[71]

eluted with a SDS micellar mobile phase and with UV detection was also studied.

Later, the same group investigated different aspects of MLC related with the determination of FUR and other diuretics in urine and pharmaceutical preparations [85–89]. Of this mode, a chromatographic procedure that uses SDS and propanol at pH 3 is reported for the determination, in urine samples, of diuretics. Pre-column derivatization with sodium nitrite and *N*-(1-naphthyl)ethylenediamine, to form the coloured azo dyes of the hydrolyzed diuretics, decreased the polarity of the compounds and, consequently, their retention in the micellar eluents, and permitted detection in the visible region, eliminating thus the high background of the urine matrix and increasing the selectivity. Limits of detection were, approximately, 2 μg mL^{-1} [85]. An interpretive methodology is applied to optimise the resolution of a mixture of 15 diuretics of diverse polarity and acid–base behaviour using pH and concentrations of surfactant and organic modifier in the mobile phase as separation factors [86] and a comparative study on the performance of two reversed-phase liquid chromatography (RPLC) modes on the separation of 18 diuretics was carried out [89]. A conventional octadecylsilane column and acidic acetonitrile–water mobile phases, in the absence and presence of micelles of SDS, were used in this study.

In like manner, the authors demonstrated that FUR solutions are stable in SDS at pH 3–5 protected from light [87]. These conditions should be used to keep FUR standard solutions and samples in the analytical laboratory and reported a chromatographic procedure with micellar mobile phases containing SDS, which was applied to the assay of the diuretic in numerous pharmaceuticals commercialised in several dosage forms. Under the optimised exper-

Table 4
HPLC methods with fluorescence detection

Experimental conditions	Results	Applications	Ref.
FUR with its acyl glucuronide by means of direct gradient without enzymic deglucuronidation; column was Cp Spherisorb ODS 5 μm, 250 mm × 4.6 mm ID; mobile phase was acetonitrile and 0.5% orthophosphoric acid (98%) pH 2.1; flow-rate 1.2 mL min^{-1}; at 345 nm excitation and 405 nm emission wavelength	Detection limit 0.005 μg mL^{-1} for FUR in plasma and urine	Human plasma and urine	[72]
Using a μBondapak C_{18} reversed-phase column (15 cm × 3.9 mm ID, 10 μm particle size) for plasma and a resolve spherical C_{18} column (15 cm × 3.9 mm ID, 5 μm particle size) for urine; mobile phase containing 0.01 M potassium dihydrogen (62:38, v/v) adjusted to pH 3.0 with phosphoric acid (1:3, v/v); mixture filtered through a 0.22 μm membrane filter under vacuum; flow-rate 1.5 mL min^{-1}; at excitation and emission wavelengths 225 and 389 nm, respectively	Linear range of 0.05–2.00 μg mL^{-1}; detection limit 5 ng mL^{-1}	Plasma and urine	[54]
Whole milk defatted by initial centrifugation at room temperature, resulting skim milk deproteinated with acetonitrile and centrifuged again, acetonitrile from supernatant evaporated, and the remaining aqueous portion directly analyzed by LC, using Spherisorb 5 ODS 2 column, a phosphate/acetonitrile buffer (pH 3); at 272 and 410 nm excitation and emission wavelengths, respectively	–	Bovine milk	[73]
Plasma samples spiked with the drug in presence of propranolol hydrochloride (IS), purified using liquid–liquid extraction; RP using a Kromasil 100-5 C_{18} column with mobile phase of acetonitrile and 0.02 M potassium dihydrogen phosphate (34:66, v/v) adjusted pH 3.0; excitation at 268 nm, emission at 410 nm	Linear range of 0.005–1.5 μg mL^{-1}; detection limit 0.001 μg mL^{-1}	Plasma	[74]
Using a Chromolith® RP 18e (100 mm × 4.6 mm) monolithic silica rod HPLC column. After liquid–liquid extraction with diethylether, plasma or urine samples separated with a gradient consisting of solvent A (20% acetonitrile) and solvent B (80% acetonitrile), both in 0.25% acetic acid, flow-rate 3.5 mL min^{-1}; excitation at 230 nm and emission at 410 nm	Linear range of 7.8–1000 ng mL^{-1}	Plasma and urine	[75]
Mixture containing 5 mmol dihydrogenphosphate buffer and acetonitrile 30:70 (v/v) pH 3 was used as mobile phase with 1.5 mL min^{-1} flow-rate; reverse phase is C_{18} column; excitation at 270 nm; emission at 470 nm	Linear range of 0.03–150 μM	Paediatric samples	[76]

imental conditions FUR was resolved from its photo-degradation products.

Finally, the same authors uses SDS, propanol and phosphate buffer at pH 3, and fluorimetric detection (λ(exc) = 270 nm, λ(em) = 430 nm) for determination of mixtures of seven diuretics, including FUR, in urine samples [90].

Recently, a simple, high-throughput, highly selective and sensitive HPLC–FD method for isolation and determination of FUR and/or norfloxacin in human plasma samples following a simple organic solvent deproteinization step with acetonitrile as sample 'cleanup' procedure is reported by Galaon et al. [91]. One of the two drug substances plays the internal standard role for the determination of the other. Separation of analyte and internal standard was achieved in less than 5.3 min on a Chromolith Performance RP-18e column, using an aqueous component containing 0.015 M sodium heptanesulfonate and 0.2% triethylamine brought to pH 2.5 with H_3PO_4. The mobile phase was acetonitrile–methanol–aqueous component 70:15:15 (v/v/v) and the flow-rate was set up to 3 mL min^{-1}. The chromatographic method applied to the determination of FUR relies on fluorescent detection parameters of 235 nm for the excitation wavelength, and 402 nm for the emission wavelength.

5.2. Electrochemical detection

Such as described above, most of the proposed methods in the literature for the determination of FUR employ LC with UV or fluorimetric detection, and they have been applied to the determination of this diuretic in urine, serum or plasma and pharmaceuticals; only one application has been found for the determination of FUR in bovine milk. On the other hand, electrochemical detection has been very scarcely applied, probably due to the adsorption of this compound on the surface of carbon electrodes [49]. However, an HPLC method with amperometric detection at a glassy carbon electrode using a detection potential of +1.20 V versus Ag/AgCl, and electrochemical or chemical pretreatment of the electrode surface has been reported [92]. This method was applied to the determination of FUR in pharmaceuticals and urine. Moreover, an improved electrochemical detection of FUR and other diuretics based on post-column on-line photolysis, and applying a detection potential of +0.20 V versus Pd has been also described [93]. Guzman et al. using flow-injection and HPLC for determination of FUR utilizing pulsed amperometric detection (PAD) at cylindrical carbon fibre microelectrodes [94]. Repetitive flow-injection amperometric measurements at +1.25 V for FUR showed a continuous decrease in the peak current, probably as a consequence of the microelectrode surface fouling. However, a suitable amperometric detection of FUR was achieved using a PAD program consisting of a two-step potential waveform with alternating anodic and cathodic polarization. In Table 5 are summarized the principal experimental conditions of proposed methods that using HPLC with electrochemical detection.

5.3. Liquid chromatography–tandem mass spectrometry

For clinical studies, it is necessary to establish an accurate and specific analytical technique which permits measurement of FUR in biological specimens at different therapeutic levels. Chromatographic resolution and mass spectrometric sensitivity are important considerations for all analytical methods. HPLC was extensively applied for the determination of FUR in biological fluids using UV, fluorescence and electrochemical detectors. Sample preparation for HPLC analysis was done by applying liquid–liquid extraction, protein precipitation and solid-phase extraction procedures. In the majority of HPLC methods, a complete resolution of FUR from the endogenous plasma constituents is necessary to avoid erroneous results. On the other hand, LC is the most commonly used approach for separating analytes from sample matrix prior to mass spectrometry (MS) analysis. Tandem MS (MS/MS), specifically using triple–quadrupole detection, has also demonstrated its utility

Table 5
HPLC methods with electrochemical detection

Experimental conditions	Results	Applications	Ref.
Serum proteins precipitated with acetonitrile containing IS and clear supernatant separated; urine diluted with water to 50 times; RP, 8MB C_{18} column, using 35% ethanol solution containing 5 mM tetrabutylammonium phosphate (pH 7.50) as mobile phase (1 mL min^{-1}); detected at 0.90 V	Detection limit 16 and 9 ng mL^{-1} serum and urine, respectively	Serum and urine	[95]
On μBondapak C_{18} column with mobile phase acetonitrile–water (40:60) and 5 mM KH_2PO_4/K_2HPO_4; flow-rate 1 mL min^{-1}; amperometric detector equipped with glassy carbon electrode operated at +1200 mV versus Ag/AgCl in the direct current mode	Detection limit 15 ppb for both compounds	FUR and piretanide in pharmaceuticals and human urine	[92]
On μBondapak C_{18} column, mobile phase water:acetonitrile, 30:70, 5 mM in KH_2PO_4/K_2HPO_4, pH 5.5; flow-rate 1 mL min^{-1}; amperometric detector, equipped with glassy carbon electrode operated at +1300 mV	Detection limits 15 ng mL^{-1} for FUR and 0.1 ng mL^{-1} for triamterene	FUR and triamterene in pharmaceutical formulations and urine	[96]
On Kromasil C_{18} column (150 mm × 4.6 mm ID, 5 μm), mobile phase 25:75 acetonitrile:5 M × 10 M NaH_2PO_4 (pH 5) mixture; flow-rate 1.4 mL min^{-1}; PAD using a detection potential, E_1, of +1.25 V (t_1 = 0.1 s), and a cleaning potential, E_2, of +0.2 V (t_2 = 0.2 s)	Detection limits 0.55 μM	FUR in presence of several thiouracil drugs and oxytetracycline in spiked commercial milk samples	[94]
Analytical column was Brownlee Lab RP-18, Spheri 5 μm, 100 mm × 4.6 mm ID, including postcolumn on-line UV irradiation; mobile phase prepared by mixing methanol and water (80:20) and adding 2 g L^{-1} of $LiClO_4$; coulometric detection at a working potential as low as +200 mV (for FUR)	Detection limit 2 ng per 6 μL for FUR	Quantification of diuretics	[93]

as a highly selective and sensitive method for MS quantitation, for example of enzymatic reactions. There have been many techniques for increasing the throughput of LC/MS/MS analyses, especially in the drug metabolism and pharmacokinetics areas of expertise. One example is staggered parallel HPLC, in which four LC runs are staggered in time allowing peaks to be sequentially introduced to the MS/MS to reduce cycle times to 1.4 min per sample with potential for even shorter times. In this way, multiplexed (MUX) electrospray is a different approach which has a four- or eight-channel electrospray ionization source interface for analyzing streams from four or eight parallel HPLC systems. This device has been used to analyse both single components, including FUR and mixtures by LC/MS as well as synthetic samples prepared by automated procedures [97]. The method provides better efficiency in the detection and quantitation processes, particularly when the analytes have poor UV absorption properties and when the analytes are not completely resolved or contaminated with endogenous plasma constituents. The high specificity and sensitivity with marked short time of analysis are prominent advantages of LC–MS.

Abdel-Hamid presents a new investigation to elucidate the potential of LC–MS in the determination of FUR, using diclofenac as IS, in plasma with one-step extraction procedure [98]. Both compounds were extracted from human plasma with ethyl acetate at pH 1 and were chromatographed using Shim-Pack GLC-CN column and a mobile phase consisting of acetonitrile and 20 mM ammonium acetate buffer solution pH 7, 4:1 (v:v) at a flow-rate 1 mL min^{-1}. FUR and diclofenac (IS) were detected by mass spectrometer operated in the negative single ion monitoring mode using APCI as an ionization process at m/z 329.2 and 294.1, respectively. Detection limit for FUR in plasma was 10 ng mL^{-1}.

Electrospray ionization mass spectrometry (ESIMS) has been widely used for the characterization and determination of biomolecules available for biomedical research. ESI spectra show typically protonated molecules and less structural information than EI spectra, which are searchable in libraries, but it is one of most popular ionization techniques, because of its ability to analyse large, thermally labile bio-molecules, using mass analysers with limited range as quadrupoles. Sanz-Nebot et al. establish a versatile method for the characterization and determination of FUR and other diuretics in human urine samples by LC coupled to pneumatically assisted ESIMS [99]. The diuretics extracted from spiked urine samples by a liquid extraction and cleanup procedure at basic pH, using ethyl acetate as solvent and the salting-out effect (NaCl), are analysed using previously optimized operational parameters of electrospray, such as counter electrode voltage, capillary voltage, sample cone voltage and source temperature, in order to obtain the best signal stability and the highest sensitivity for the greatest number of diuretic agents.

The possibility of automated sample preparation increases the potential for faster and simpler analysis. A robust LC–MS screening procedure for the detection of 32 diuretics and masking agents has been reported [100]. The analytical procedure is reduced to a single XAD extraction step for sample preparation, followed by reversed-phase liquid chromatography in combination with atmospheric pressure ionisation/tandem mass spectrometry. This method was based on liquid–liquid extraction, which is difficult to automate, and required two injections per sample. The duplication of analysis was performed because of the need for both positive and negative ionisation in order to detect all the diuretics with sufficient sensitivity. Goebel et al. developed a procedure for the routine detection of diuretics in urine samples collected from athletes. The method uses automated SPE with analysis of the extracts by HPLC using electrospray ionisation tandem mass spectrometry. It requires only one injection per sample and is currently capable of detecting 35 diuretics and related compounds at the rate of five samples per hour [101].

Deventer et al. presented a fast and selective LC/MS/MS method for the screening of 18 diuretics and probenecid in human urine [102]. Analyses were performed on an instrument equipped with ESI interface using scan by scan polarity changing. All diuretics and probenecid were separated in less than 20 min after liquid–liquid extraction with ethyl acetate. Detection limit for all substances was 100 ng mL^{-1} or better. Later, this method was substantially extended with 21 β-blockers and 8 other diuretics allowing simultaneous determination of diuretics and beta-adrenergic blocking agents in human urine. Analysis was performed using an ion trap instrument with an ESI interface after liquid/liquid extraction with ethyl acetate. Full-scan MS and full-scan MS/MS were applied in combination with scan-to-scan polarity switching. All compounds were separated in less than 22 min [103].

Castiglioni et al. describes an improved analytical method to measure an extended list of 30 drugs, including FUR, belonging to several therapeutic classes, at low concentrations in surface waters (about 1 ng L^{-1}). Pharmaceuticals were divided in two groups, extracted by different SPE methods, and analysed by reversed-phase liquid chromatography tandem mass spectrometry [104].

The aim of a recent study realized by Politi et al. is develop a screening procedure for the detection of 24 diuretics by direct injection of diluted urine in the LC–MS/MS system, exploiting the information dependent acquisitions (IDA) feature for combining selected reaction monitoring (SRM) and product ion scan detection. In particular, the production scan was performed using the third quadrupole of a mass spectrometer as a linear ion trap, thus enhancing scan sensitivity [105].

6. Capillary electrophoresis

Riekkola and Jumppanen reviewed the application of capillary electrophoresis to the screening, identification and determination of diuretics and probenecid [106]. The number of publications is in these years still limited, but the studies already published clearly show that capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) are excellent alternatives for the investigation of diuretics. High accuracy identifications of diuretics and probenecid, even in urine samples, can be obtained when CZE is used with the marker techniques. Previously, these authors developed a method that screens diuretics in urine and blood serum. Two successive runs were required because of the heterogeneity of this group of compounds. Screening for diuretics that contained sulfonamide and/or carboxylic groups was done at pH 10.6 with 3-(cyclohexylamino)-1-propanesulfonic acid (0.06 M) as buffer. Diuretics that contained primary, secondary or tertiary amine groups were investigated at pH 4.5 with acetate (0.07 M)–betaine (0.5 M) buffer system. Hydrostatic injection mode for 5 s gave the best efficiency. Detection limits at the low femtomole level are achievable for most compounds with a UV–vis detector operating at 220 and 215 nm [107].

CZE has been used for the determination of the binding of a β2-blocker drug, FUR and ceftriaxone to serum proteins [108]. The analyses were carried out by injecting a solution of sα1,-acidic glycoprotein (α1-AGP) or human serum albumin in 70 mM NaH_2PO_4/Na_2HPO_4 (pH 7.4) buffer into an uncoated fused-silica capillary filled with the same buffer.

Sadecka and Polonsky present a capillary isotachophoretic (ITP) method for the determination of β-blockers (metoprolol, deacetylmetipranolol and labetalol) and diurectics (amiloride and furosemide) in serum and urine [109]. FUR was separated using the anionic electrolyte system histidine hydrochloride buffer (pH 6.2) (10 mM)-morpholinopropanesulfonic acid. Endogenous and the possible exogenous compounds were almost totally removed from serum and urine by SPE using a Separon SGX C_{18} cartridge.

MEKC, a mode of capillary electrophoresis, was initially conceived for the electrokinetic analysis of neutral compounds. By adding micelle-forming surfactants to the background electrolyte, separations resembling reversed-phase liquid chromatography are achieved with the benefits of high efficiency and speed of analysis. MEKC, however, can equally well be applied to the analysis of ionic compounds. Moreover, it was shown that it is possible to perform sample stacking in MEKC of ionic compounds. Injection of larger sample volumes is thus feasible hereby drastically improving detectability. Lalljie et al. evaluated the possibility of using MEKC for screening and quantitative analysis of FUR and piretanide in urine samples. A simple sample preparation step, involving liquid–liquid extraction, followed by fast MEKC analysis is described. The analytical parameters have been optimized to separate the target compounds from the urine matrix solutes [110].

FUR, chlorthalidone, hydrochlorothiazide and triamterene were separated in 5 min by MEKC using a carrier containing sodium dodecylsulfate as surfactant, and were subsequently detected spectrophotometrically using a diode-array detector. The limits of detection were in all cases less than 1.2 μg mL^{-1} for all compounds [111].

Caslavska and Thormann reports the first capillary electrophoresis–laser induced fluorescence (CE–LIF) and capillary electrophoresis–mass spectrometry (CE–MS) data of FUR and CE–LIF and capillary electrophoresis–ion trap tandem mass spectrometry ($CE–MS^2$) based assays for analysis of FUR in human urine [112].

7. Other techniques

Diverse methods were reported that allow the determination of FUR in plasma using thin-layer chromatography. These methods, published in years seventy, require complicated preparative steps before quantitation of the drug can be carried out. Later, Argekar et al. developed a method for simultaneous determination of FUR and amiloride hydrochloride in pharmaceutical preparation with ethyl acetate:methanol:18% ammonia (7.5:1:0.8, v/v/v), as the mobile phase and hydrochlorothiazide as IS. The quantification was done by densitometry at 275 nm [113].

Until recently to confirm the identity of diuretic compounds detected by HPLC, gas chromatography–mass spectrometry (GC–MS) with electron ionization (EI) is the standard method used. In order to improve the volatility of the diuretics either methylation or silylation has been required prior to GC–MS. The methylation of these polar drugs has been the most common multi-residue procedure and has allowed GC–MS to be used as a screen to replace the less selective HPLC procedures. The simplest process is an extractive alkylation one, where extraction and derivatization is carried out in a single step. Recently a rapid method has been published using microwaves to assist the methylation after extraction. Two problems with the methylation prior to GC–MS approach are the difficulty in methylating some diuretics, and the toxicity of the methyl iodide used in the derivatization process. Extractive methylation has been described for the determination of FUR [53,114], which has been also applied in screening procedures for diuretics including FUR, in physiological samples [115]. 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). In other procedure developed by Ptacek et al., after acidification the samples were extracted by ethyl acetate and methylated by methyl iodide. The chromatography was carried out on a fused-silica capillary column with SE-54 stationary phase. Detection was performed by selected ion monitoring (ions 81 and 372 for FUR and ions 363 and 406 for IS bumetanide). Limit of quantitation was 10 ng mL^{-1} for plasma and 40 ng mL^{-1} for urine [116].

More recent, Amándola et al. describes an alternative, rapid method to detect and confirm the presence of diuretics in human urine by GC–MS of the corresponding methyl derivatives. The performance of the method was verified for 18 representative diuretics. The novel aspect of the method is represented by the technique used for the derivatization reaction that is carried out by incubation of the purified urine extracts by methyl-iodide and potassium carbonate in acetone, supplying the energy transfer by microwave irradiation instead of by direct thermal heating. The proposed

approach allowed setting up a robust analytical procedure for the screening and confirmation analysis of diuretic agents searched by the anti-doping laboratories, requiring 10 min instead 3 h for the derivatization step [117].

A SPE procedure using BondElut®-LRC Certify columns was used by Margalho et al. to extract FUR from blood samples, using ketoprofen as IS. The extracts were analyzed by gas chromatography–electron ionization–mass spectrometry after on-column derivatization with trimethylanilinium hydroxide (0.2 M in methanol). The calculated limits of detection and quantitation were 0.010 and 0.045 $\mu g\,mL^{-1}$, respectively [118].

In official doping controls, about 300 drugs and metabolites have to be screened for each sample. Moreover, the number of determinations to be routinely processed increases continuously as the number of both samples and potential illicit drugs keeps growing. As a consequence, increasingly specific, sensitive, and, above all, fast methods for doping controls are needed. A research group of university of Torino presents two efficient fast-GC/MS approaches to the routine screening of two different classes of doping agents, namely β-adrenoceptor ligands and diuretics [119] and diuretics and masking agents [120]. Narrow bore columns (100 mm ID) of different lengths and coated with apolar stationary phases were successfully used to separate the derivatized analytes. The whole method involves three analytical steps, including (1) liquid/liquid extraction of the analytes from the matrix, (2) their reaction with methyl iodide at 70 °C for 2 h to form methyl derivatives, (3) analysis of the resulting mixture by fast gas chromatography/electron impact mass spectrometry (fast GC/EI-MS).

8. Conclusions

UV–vis detection is the technique most commonly used in determinations of pharmaceuticals. As its sensitivity and selectivity are very limited, better systems of detection are necessary in order to quantify low drug dosages. Fluorimetry, which meets these requirements, is usable only for a small group of drugs having native fluorescence. The same problem arises with electrochemical detection.

In the last years, the different working methods presented by the CE have become one of the most popular techniques for the analysis of drugs. In spite of chromatographic methods provide a similar range of applicability in the analysis of drugs, vitamins and excipients, the main advantage of CE in the pharmaceutical field lies in its low cost and short time of analysis. The introduction of micelles in the buffer solution where the electrophoretic process takes place allows the determination of neutral and charged analytes in a single injection. The most frequent CE techniques for analysing diuretics CZE and MEKC.

After being extracted from samples, diuretics have been screened and determined either by GC–MS as their methyl-derivatives, or by LC–MS(/MS). LC–MS allows the chromatographic separation of polar functions with no need for derivatization and, at least in the reversed-phase mode, a simplified sample preparation due to the compatibility between aqueous samples and the analytical system. By using LC–MS/MS, highly sensitive and selective analyses can be achieved in selected reaction monitoring (SRM). The specificity of the SRM analysis can be further increased by combination with the acquisition of the product ion spectrum of the analyte of interest. This combination of detections can be straightforwardly performed via the so-called information dependent acquisition (IDA) or data dependent acquisition (DDA), depending on the manufacturer: peaks found by SRM (survey scan) will instantly trigger the product ion scan (dependent acquisition) of the precursor ion of the eluting analyte. In this way, the high sensitivity of the SRM analysis is merged with the specificity of a product ion spectrum in a single analytical run.

References

[1] R. Ventura, J. Segura, J. Chromatogr. B: Biomed. Appl. 687 (1996) 127–144.
[2] D. Zendelovska, T. Stafilov, Acta Pharm. 56 (2006) 115–142.
[3] M.J. Ruiz-Angel, A. Berthod, S. Carda-Broch, M.C. Garcìa-Álvarez-Coque, Sep. Purific. Rev. 35 (2006) 39–58.
[4] Vremennaya farmakopeinaya stat'ya (Temporary Pharmacopoeia Article) 42U-1-722-98: Furosemide.
[5] S.A. Kulichenko, S.A. Fesenko, J. Anal. Chem. 57 (2002) 231–234.
[6] S.A. Kulichenko, G.M. Shevchenko, Ukrainskij Khim. Z. 69 (2003) 99–103.
[7] S.A. Kulichenko, G.M. Shevchenko, Anal. Bioanal. Chem. 375 (2003) 255–258.
[8] K. Basavaiah, U. Chandrashekar, P. Nagegowda, Indian J. Chem. Technol. 12 (2005) 149–155.
[9] J. Barbosa, D. Barrón, S. Buti, Acta Chem. Scand. 51 (1997) 1078–1084.
[10] J. Barbosa, I. Marqués, G. Fonrodona, D. Barrón, R. Bergés, Anal. Chim. Acta 347 (1997) 385–393.
[11] L. Živanović, S. Agatonović, D. Radulović, Mikrochim. Acta 100 (1990) 49–54.
[12] S. Agatonovic-Kustrin, L. Zivanovic, D. Radulovic, D. Pecanac, J. Pharm. Biomed. Anal. 8 (1990) 983–986.
[13] H. Salem, Sci. Pharm. 68 (2000) 403–419.
[14] E. Sell, A. Chmielewska, L. Konieczna, Chem. Anal. 45 (2000) 249–255.
[15] C.S.P. Sastry, K. Rama Srinivas, K.M.M. Krishna Prasad, Indian J. Pharm. Sci. 58 (1996) 120–122.
[16] A. Sevillano-Cabeza, P. Campíns-Falcć, M. Serrador-García, Anal. Lett. 30 (1997) 91–107.
[17] J. Shah, M.R. Jan, M.A. Khan, J. Chin. Chem. Soc. 52 (2005) 347–352.
[18] K. Basavaiah, U. Chandrashekar, Indian J. Chem. Technol. 12 (2005) 401–406.
[19] A. Gölcü, J. Anal. Chem. 61 (2006) 748–754.
[20] J.S. Esteve-Romero, E.F. Simo-Alfonso, M.C. García-Álvarez-Coque, G. Ramis-Ramos, Talanta 40 (1993) 1711–1718.
[21] M.S. García, C. Sánchez-Pedreño, M.I. Albero, V. Ródenas, J. Pharm. Biomed. Anal. 15 (1997) 453–459.
[22] M.I. Evgen'ev, S.Y. Garmonov, L.S. Shakirova, E.V. Degterev, Pharm. Chem. J. 36 (2002) 559–564.
[23] F.S. Seeman, R.A. De Sousa, E.T.G. Cavalheiro, J. Flow Injet. Anal. 22 (2005) 34–37.
[24] F.S. Semaan, E.T.G. Cavalheiro, Anal. Lett. 39 (2006) 2557–2567.
[25] I.L.T. Dias, J.L.S. Martins, G.O. De Neto, Anal. Lett. 38 (2005) 1159–1166.
[26] M.C.F. Ferraro, P.M. Castellano, T.S. Kaufman, J. Pharm. Biomed. Anal. 26 (2001) 443–451.
[27] P. Parimoo, A. Bharathi, K. Padma, Indian J. Pharm. Sci. 57 (1995) 126–129.
[28] S. Gangwal, P. Trivedi, Indian Drugs 35 (1998) 412–414.
[29] H. Salem, M. El-Maamli, M. El-Sadek, A.A. Kheir, Spec. Lett. 24 (1991) 451–470.
[30] R. Panchagnula, K. Kaur, A. Sood, I. Singh, Pharm. Sci. 3 (1997) 425–429.
[31] J.S. Millership, C. Parker, D. Donnelly, Farmaco 60 (2005) 333–338.
[32] M.H. Abdel-Hay, Inter. J. Pharm. 99 (1993) 333–336.
[33] M.I. Toral, S. Pope, S. Quintanilla, P. Richter, Inter. J. Pharm. 249 (2002) 117–126.
[34] F.G. Sánchez, A.F. Gutierrez, C.C. Blanco, Anal. Chim. Acta 306 (1995) 313–321.
[35] M.L. Luis, J.M.G. Fraga, A.I. Jiménez, F. Jiménez, O. Hernández, J.J. Arias, Talanta 62 (2004) 307–316.
[36] E.J. Llorent-Martínez, P. Ortega-Barrales, A. Molina-Díaz, Anal. Bioanal. Chem. 383 (2005) 797–803.
[37] J. Huclová, D. Šatínský, T. Maia, R. Karlíček, P. Solich, A.N. Araújo, J. Chromatogr. A 1087 (2005) 245–251.
[38] F.S. Semaan, P.A. Nogueira, E.T.G. Cavalheiro, Anal. Lett. 41 (2008) 66–79.
[39] N. Zhou, Y.Z. Liang, P. Wang, J. Mol. Struct. 872 (2008) 190–196.
[40] S.V. Beltyukova, E.I. Tselik, A.V. Egorova, J. Pharm. Biomed. Anal. 18 (1998) 263–266.
[41] P.C. Ioannou, N.V. Rusakova, D.A. Andrikopoulou, K.M. Glynou, G.M. Tzompanaki, Analyst 123 (1998) 2839–2843.
[42] N. Arnaud, J. Georges, Anal. Chim. Acta 476 (2003) 149–157.
[43] Y. Rao, X. Zhang, G. Luo, W.R.G. Baeyens, Anal. Chim. Acta 396 (1999) 273–277.
[44] S. Zhang, J. Xi, Z. He, Fenxi Huaxue 33 (2005) 1269–1271.
[45] J. Xi, X. Ji, S. Zhang, X. Ai, Z. He, Anal. Chim. Acta 541 (2005) 193–198.
[46] H. Salem, Chin. Pharm. J. 47 (1995) 263–272.
[47] H. Salem, K. Kelani, A. Shalaby, Sci. Pharm. 69 (2001) 189–201.
[48] M.A. Gotardo, A.C. Gigante, L. Pezza, H.R. Pezza, Talanta 64 (2004) 361–365.
[49] M.B. Barroso, R.M. Alonso, R.M. Jiménez, Anal. Chim. Acta 305 (1995) 332–339.
[50] I.L. Tescarollo Dias, G. De Oliveira Neto, D.C. Vendramini, C. Sommer, J.L.S. Martins, L.T. Kubota, Anal. Lett. 37 (2004) 35–46.
[51] D. Farthing, T. Karnes, T.W.B. Gehr, C. March, I. Fakhry, D.A. Sica, J. Pharm. Sci. 81 (1992) 569–571.
[52] H.J.E.M. Reeuwijk, U.R. Tjaden, J. Van Der Greef, J. Chromatogr.: Biomed. Appl. 575 (1992) 269–274.
[53] M. Saugy, P. Meuwly, A. Munafo, L. Rivier, J. Chromatogr.: Biomed. Appl. 564 (1991) 567–578.
[54] H.S. Abou-Auda, M.J. Al-Yamani, A.M. Morad, S.A. Bawazir, S.Z. Khan, K.I. Al-Khamis, J. Chromatogr. B: Biomed. Appl. 710 (1998) 121–128.
[55] F.S. Semaan, A.J.D.S. Neto, F.M. Lanças, E.T. Gomes Cavalheiro, Anal. Lett. 38 (2005) 1651–1658.

[56] T. Okuda, K. Yamashita, M. Motohashi, J. Chromatogr. B: Biomed. Appl. 682 (1996) 343–348.
[57] C.D. Mills, C. Whitworth, L.P. Rybak, C.M. Henley, J. Chromatogr. B: Biomed. Appl. 701 (1997) 65–70.
[58] Song-Ja Park, Hee-Soo Pyo, Yun-Je Kim, Mi-Sook Kim, Jongsei Park, J. Anal. Toxicol. 14 (1990) 84–90.
[59] F.Y. Tsai, L.F. Lui, B. Chang, J. Pharm. Biomed. Anal. 9 (1991) 1069–1076.
[60] V. Moreira, R.L.M. Moreau, J. Liquid Chromatogr. Rel. Technol. 28 (2005) 2753–2768.
[61] R. Ventura, T. Nadal, P. Alcalde, J.A. Pascual, J. Segura, J. Chromatogr. A 655 (1993) 233–242.
[62] P. Campíns-Falcó, R. Herráez-Hernández, A. Sevillano-Cabeza, J. Chromatogr.: Biomed. Appl. 612 (1993) 245–251.
[63] P. Campíns-Falcó, R. Herráez-Hernández, A. Sevillano-Cabeza, Anal. Chem. 66 (1994) 244–248.
[64] A.A. Nava-Ocampo, E.Y. Velázquez-Armenta, H. Reyes-Pérez, E. Ramirez-Lopez, H. Ponce-Monter, J. Chromatogr. B: Biomed. Sci. Appl. 730 (1999) 49–54.
[65] M. Walshe, M.T. Kelly, M.R. Smyth, J. Pharm. Biomed. Anal. 14 (1996) 475–481.
[66] Y.S. El-Saharty, J. Pharm. Biomed. Anal. 33 (2003) 699–709.
[67] A. Jankowski, A. Skorek-Jankowska, H. Lamparczyk, J. Chromatogr. B: Biomed. Appl. 693 (1997) 383–391.
[68] V.V. Vaidya, M. Khanolkar, J.N. Gadre, Indian Drugs 39 (2002) 373–377.
[69] H. Valizadeh, P. Zakeri-Milani, Z. Islambulchilar, H. Tajerzadeh, J. AOAC Inter. 89 (2006) 88–93.
[70] S. Chawla, S. Ghosh, V. Sihorkar, R. Nellore, T.R.S. Kumar, N.R. Srinivas, Biomed. Chromatogr. 20 (2006) 349–357.
[71] T. Bansal, M. Singh, G. Mishra, S. Talegaonkar, R.K. Khar, M. Jaggi, R. Mukherjee, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 859 (2007) 261–266.
[72] T.B. Vree, M. Van den Biggelaar-Martea, C.P.W.G.M. Verwey-van Wissen, J. Chromatogr. B: Biomed. Appl. 655 (1994) 53–62.
[73] B. Shaikh, J. Agric. Food Chem. 43 (1995) 2117–2121.
[74] C. Gomez, C. Von Plessing, C.G. Godoy, R. Reinbach, R. Godoy, J. Chilean Chem. Soc. 50 (2005) 479–482.
[75] M. Wenk, L. Haegeli, H. Brunner, S. Krähenbühl, J. Pharm. Biomed. Anal. 41 (2006) 1367–1370.
[76] M.D. Blanco, A. Bennani, A. Alcaraz, P. Martínez, I. Tovar, F. Cañizares Hernández, Quim. Clin. 26 (2007) 45–48.
[77] P. Campíns-Falcó, R. Herráez-Hernández, M.D. Pastor-Navarro, J. Liquid Chromatogr. Rel. Technol. 20 (1997) 1867–1885.
[78] I. Baranowska, P. Markowski, J. Baranowski, Anal. Chim. Acta 570 (2006) 46–58.
[79] I. Baranowska, J. Płonka, J. Baranowski, Chem. Anal. 51 (2006) 751–760.
[80] S. Pous-Torres, J.R. Torres-Lapasió, J.J. Baeza-Baeza, M.C. García-Álvarez-Coque, J. Chromatogr. A 1163 (2007) 49–62.
[81] L.J. Cline Love, J.J. Fett, J. Pharm. Biomed. Anal. 9 (1991) 323–333.
[82] E. Bonet Domingo, M.J. Medina Hernandez, G. Ramis Ramos, M.C. Garcia Alvarez-Coque, Analyst 117 (1992) 843–847.
[83] E. Bonet Domingo, M.J. Medina Hernandez, G. Ramis Ramos, M.C. Garcia Alvarez-Coque, J. Chromatogr.: Biomed. Appl. 582 (1992) 189–194.
[84] E. Bonet-Domingo, J.R. Torres-Lapasi, M.J. Medina-Hernandez, M.C. Garcia-Alvarez-Coque, Anal. Chim. Acta 287 (1994) 201–210.
[85] S. Carda-Broch, M.C. García-Álvarez-Coque, E.F. Simó-Alfonso, J.S. Esteve-Romero, Anal. Chim. Acta 353 (1997) 215–226.
[86] S. Carda-Broch, J.R. Torres-Lapasió, J.S. Esteve-Romero, M.C. García-Alvarez-Coque, J. Chromatogr. A 893 (2000) 321–337.
[87] S. Carda-Broch, J. Esteve-Romero, M.C. García-Alvarez-Coque, J. Pharm. Biomed. Anal. 23 (2000) 803–817.
[88] S. Carda-Broch, J. Esteve-Romero, M.J. Ruiz-Angel, M.C. García-Alvarez-Coque, Analyst 127 (2002) 29–34.
[89] M.J. Ruiz-Angel, J.R. Torres-Lapasió, M.C. García-Alvarez-Coque, J. Chromatogr. A 1022 (2004) 51–65.
[90] S. Carda-Broch, J.S. Esteve-Romero, M.C. García-Alvarez-Coque, Anal. Chim. Acta 375 (1998) 143–154.
[91] T. Galaon, S. Udrescu, I. Sora, V. David, A. Medvedovici, Biomed. Chromatogr. 21 (2007) 40–47.
[92] M.B. Barroso, R.M. Jiménez, R.M. Alonso, E. Ortiz, J. Chromatogr. B: Biomed. Appl. 675 (1996) 303–312.
[93] M. Macher, R. Wintersteiger, J. Chromatogr. A 709 (1995) 257–264.
[94] A. Guzmaín, L. Agüí, M. Pedrero, P. Yáñez-Sedeño, J.M. Pingarrón, J. Pharm. Biomed. Anal. 33 (2003) 923–933.
[95] H.Z. Li, H. Kubo, Y. Kobayashi, T. Kinoshita, Acta Pharm. Sinica 26 (1991) 923–927.
[96] M.B. Barroso, R.M. Alonso, R.M. Jimeĭnez, J. Liquid Chromatogr. Rel. Technol. 19 (1996) 231–246.
[97] V. De Biasi, N. Haskins, A. Organ, R. Bateman, K. Giles, S. Jarvis, Rapid Commun. Mass Spectrom. 13 (1999) 1165–1168.
[98] M.E. Abdel-Hamid, Il Farmaco 55 (2000) 448–454.
[99] V. Sanz-Nebot, I. Toro, R. Bergés, R. Ventura, J. Segura, J. Barbosa, J. Mass Spectrom. 36 (2001) 652–657.
[100] D. Thieme, J. Grosse, R. Lang, R.K. Mueller, A. Wahl, J. Chromatogr. B: Biomed. Sci. Appl. 757 (2001) 49–57.
[101] C. Goebel, G.J. Trout, R. Kazlauskas, Anal. Chim. Acta 502 (2004) 65–74.
[102] K. Deventer, F.T. Delbeke, K. Roels, P. Van Eenoo, Biomed. Chromatogr. 16 (2002) 529–535.
[103] K. Deventer, P. Van Eenoo, F.T. Delbeke, Rapid Commun. Mass Spectrom. 19 (2005) 90–98.
[104] S. Castiglioni, R. Bagnati, D. Calamari, R. Fanelli, E. Zuccato, J. Chromatogr. A 1092 (2005) 206–215.
[105] L. Politi, L. Morini, A. Polettini, Clin. Chim. Acta 386 (2007) 46–52.
[106] M.L. Riekkola, J.H. Jumppanen, J. Chromatogr. A 735 (1996) 151–164.
[107] J. Jumppanen, H. Siren, M.L. Riekkola, J. Chromatogr. 652 (1993) 441–450.
[108] M.G. Quaglia, E. Bossù, C. Dell'Aquila, M. Guidotti, J. Pharm. Biomed. Anal. 15 (1997) 1033–1039.
[109] J. Sádecká, J. Polonský, J. Chromatogr. A 735 (1996) 403–408.
[110] S.P.D. Lalljie, M. Begoña Barroso, D. Steenackers, R.M. Alonso, R.M. Jiménez, P. Sandra, J. Chromatogr. B: Biomed. Appl. 688 (1997) 71–78.
[111] M.L. Luis, S. Corujedo, D. Blanco, J.M.G. Fraga, A.I. Jiménez, F. Jiménez, J.J. Arias, Talanta 57 (2002) 223–231.
[112] J. Caslavska, W. Thormann, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 770 (2002) 207–216.
[113] A.P. Argekar, S.V. Raj, S.U. Kapadia, Indian Drugs 32 (1995) 166–171.
[114] H.W. Hagedorn, R. Schulz, J. Anal. Toxicol. 16 (1992) 194–198.
[115] J.D. Ehrhardt, Rapid Comm. Mass Spectrom. 6 (1992) 349–351.
[116] P. Ptaĭček, O. Vyhnaĭlek, H.P. Breuel, J. Macek, Arzneimittel-Forschung/Drug Res. 46 (1996) 277–283.
[117] L. Amendola, C. Colamonici, M. Mazzarino, F. Botré, Anal. Chim. Acta 475 (2003) 125–136.
[118] C. Margalho, D. De Boer, E. Gallardo, M. Barroso, D.N. Vieira, J. Anal. Toxicol. 29 (2005) 309–313.
[119] C. Brunelli, C. Bicchi, A. Di Stilo, A. Salomone, M. Vincenti, J. Sep. Sci. 29 (2006) 2765–2771.
[120] V. Morra, P. Davit, P. Capra, M. Vincenti, A. Di Stilo, F. Botrè, J. Chromatogr. A 1135 (2006) 219–229.