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ANALYTICAL DETERMINATION OF FUROSEMIDE: THE LAST RESEARCHES

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ABSTRACT

Furosemide (FUR) is an anthranilic acid derivative which is a potent diuretic widely used in the treatment of congestive heart failure and edema. FUR works by blocking the absorption of salt and fluid in the kidney tubules, causing a profound increase in urine output (diuresis). Due to the considerable use of FUR, analytical determination is frequent. In this manuscript, a review of the methods developed for determination of FUR from the year 2008 is presented.

KEY WORDS

Furosemide; Review; Biological samples; Pharmaceutical

1. INTRODUCTION

Drugs that facilitate diuresis are widely used for the treatment of edematous conditions and in the management of hypertension and other conditions for which the increase in urinary flow can relieve symptoms. Diuretics are an important group of the drugs used in various clinical situations such as cardiac and renal insufficiency, nefrotic syndrome, edema, cirrhosis, and hypertension. Diuretics are substances that produce increase urinary excretion of bodily sodium bound to anions and water, thereby reducing its concentration in extracellular liquids. Furosemide (4-chloro-N-furfuryl-5-sulfamoyl-anthranillic acid, FUR), an effective diuretic, has been widely used in the treatment of chronic renal failure, hypertension, congestive heart failure and cirrhosis of the liver. FUR 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. FUR acts inhibiting the co-transportation of sodium, potassium and chloride, and further cause´s excretion of calcium, magnesium and bicarbonate ions. Intense and fast dieresis may also mask the ingestion of other doping agents by reducing their concentration in urine. For this reason, the Medical Commission of the International Olympic Committee banned the use of FUR among other diuretics in 1986.

Due to the clinical importance of FUR, a large number of analytical procedures to detect the presence of this drug in pharmaceutical and physiological samples have been developed. For example, several methods including titrimetric, optical and electrochemical detections and capillary electrophoresis have been used to quantify FUR. Also, liquid-liquid extraction, followed by solvent evaporation, has been used for sample preparation in the chromatographic analysis of diuretics in biological samples. Several reports addressed unresolved issues of precision, time consuming and loss of compounds of interest. Other researchers have proposed the use of micellar liquid chromatography for the determination of diuretics, such as FUR in pharmaceutical preparations. The literature survey revealed that HPLC has been employed to detect FUR in blood, urine, or perilymph. So far, HPLC is generally the method of choice for diuretics quantitation, due to the required time and cost of the analysis. In our previous work [1], we present the most relevant analytical methodologies used in its determination from the nineties decade at the year 2008.

This work consists of papers mainly reported from 2009 until today about the analytical methodology for FUR determination. In **Table 1** are summarized the more important aspects of the methods proposed for the determination of FUR alone and in mixture with other compounds based in different analytical techniques.

Table 1: Analytical determinations of furosemide

Compounds	Technique	Procedure	Linear range	RSD (%)	Applications	Ref.
-	UV/VIS	Method A: 1 ml 5M H_2SO_4 + 1 ml 500 µg ml^{-1} $KMnO_4$; at 550 nm Method B: with 0.1M NaOH +1 ml 2 mg ml^{-1} $KMnO_4$; at 610 nm	Method A: 3.0-24.0 µg ml^{-1} Method B: 1.25-20.0 µg ml^{-1}	Method A: 2.58 Method B: 2.63	Tablets, injection and combination tablet	2
-	UV/VIS	Acid hydrolysis of FUR and coupling with: Method A: N-1-napthylethylene diamine; at 520 nm Method B: 4,5-dihydroxynaphthalene-2,7-disulfonic acid; at 500 nm	Method A: 1.75-21.0 µg ml^{-1} Method B: 2.5-30.0 µg ml^{-1}	-	Spiked human urine and pharmaceutical formulations	3
-	UV/VIS	1 ml 0.3% CAS + 1ml 1M H_2SO_4 Method A: + 1 ml 0.05% XC; at 612 nm Method B: 2 ml 0.01% SAF; at 526 nm	Method A: 20-30 µg ml^{-1} Method B: 6-16 µg ml^{-1}	Method A: 0.064 Method B: 1.8345	Two different branded tablets	4
Spironolactone	UV/VIS	Method A: 1st deriv. Spironolactone: D1 amplitude at zero crossing point of FUR at 250.80 nm; FUR D1 at zero crossing point of spironolactone at 350 nm Method B: Absorbance ratio, from overlay spectra, FUR and spironolactone show an isoabsorptive point at 261.21 nm, second wavelength is 276 nm, λ_{max} of FUR	FUR: 2-10 µg ml^{-1}; Spironolactone: 5-25 µg ml^{-1}	FUR: 0.29-1.37 spironolactone: 0.8-1.62	Combined tablet dosage form	5
Spironolactone	UV/VIS	Method A: 2nd deriv. at 238 and 277 nm Method B: area under curve at 228-248 and 267-287 nm	FUR: 0.8-4.0 µg ml^{-1} Spironolactone: 2-10 µg ml^{-1}	<2 for both methods	Bulk drug and pharmaceutical formulations	6

-	FL	In a buffer solution of KCl/HCl 50 mM (pH 2.0); at λ_{exc} 280 nm; λ_{em} 410 nm	4.5- 5000 ng ml^{-1}	<0.8	Solid pharmaceutical forms	7
-	FL	50 µl Tris/HCl buffer (pH 7.80, 0.05 M), 50 µl 0.01M bix ethanol solution, 50 µL 0.01M $ZnSO_4$, suitable amounts of FUR and 160 µL 0.01M NaCl; after that, the mixture was diluted to 500 µl with ultra-pure water, shaking several seconds using a vortex mixer; mixture was left to stand 20 min at 37 ºC before measuring	0.06–7.2 µM	3.9	Tablets	8
Amiloride	FL	FUR: λ_{ex} 237 nm, λ_{em} 415 nm Amiloride: λ_{ex} = 365, λ_{em} = 406 nm	FUR: $1.33x10^{-3}$-4 µg ml^{-1} Amiloride: $3.2x10^{-4}$-0.8 µg ml^{-1}	FUR: <1.23 Amiloride: <1.36	Pharmaceutical formulations	9
Amiloride	FL	FUR: λ_{ex} 237 nm, λ_{em} 415 nm Amiloride: λ_{ex} = 365, λ_{em} = 406 nm	FUR: $1.2x10^{-3}$-4 µg ml^{-1} Amiloride: $3.7x10^{-4}$-0.8 µg ml^{-1}	In spiked human urine FUR: 1.56-5.21 Amiloride: 1.41-3.76	Urine	10
-	DRS	Onto 2.25 cm^2 filter papers (Whatman No. 1), 20 µL of the chromogenic eagent solution was spotted, followed immediately by addition of 20 µL of FUR; at 477 nm	$1.65\text{-}9x10^{-3}$ M	<2	Urine	11
-	DPV	In methanol:water (10:90) at pH 4.8 with 0.04M Britton-Robinson buffer as supporting electrolyte at 25 ºC	$6x10^{-6}$-$8x10^{-4}$ M	1.217	Tablet dosage form	12
-	Potenciometry	(-)Ag/AgCl/[NaCl]=0.01M; $[NaNO_3]$=0.490M//[NaFur] =xM;$[NaNO_3]$(0.500-x) M/Graphite/$Hg_2(Fur)_2$/Hg/Pt(+)	$5x10^{-7}$-$1x10^{-2}$ M	In tablets <2	Pharmaceuticals, urine, blood serum and bovine milk	13
-	DPV	In 5.0 pH with 0.04 M Britton–Robinson buffer as	$8x10^{-6}$-$2x10^{-4}$ M	3.54	Pharmaceuticals and	14

		supporting electrolyte at 25 ºC at a CNTPE			urine	
-	HPLC-UV	IS: nitrazepam; Lichrospher C_{18}; A: KH_2PO_4 10 mM, pH 2.5 (with H_3PO_4 85%) B: Acetonitrile Gradient elution: 0.00-18.00 min 77% A, 23% B 18.01-25.00 min 10% A, 90% B 25.01-29.00 min 77% A, 23% B; at 2 ml min^{-1}; column temperature 45^0C; injection volume 100µl; at 230 nm	0.020-10.00 µg ml^{-1}	-	Rat plasma	15
-	HPLC-UV	Spherisorb C_{18}; acetonitrile:10mM KH_2PO_4 (70:30) pH 3.85; at 1 ml min^{-1}; at room temperature; at 233 nm	5.2-25000 ng ml^{-1}	<2	Tablets and nanoparticles	16
-	HPLC-UV	Spherisorb C_{18}; 10 mM KH_2PO_4 (pH 5.5):methanol (70:30); at 1 ml min^{-1}; at 235 nm	0.5-50 µg ml^{-1}	<2	Solubility studies	17
Amiloride	HPLC-UV	HIQ SIL C_{18}; 50mM phosphate buffer:acetonitrile (50:50) pH 3.0; at 1 ml min^{-1}; at 25ªC; at 283 nm	FUR: 20-200 µg ml^{-1} Amiloride: 10-100 µg ml^{-1}	FUR: 0.072 Amiloride: 0.754	Tablets	18
Amiloride	HPLC-UV	Inertsil C_{18}; methanol:0.1% glacial acetic acid (43:57) pH 5.05 adjusted with NaOH; at 1 ml min^{-1}; at 226 nm	FUR: 800-2800 ng ml^{-1} Amiloride: 100-350 ng ml^{-1}	FUR: <2.6 Amiloride <2.1	Human plasma	19
Spironolactone	HPLC-UV	Hiber C_{18}; acetonitrile:water; at 1 ml min^{-1}; at room temperature; at 237 nm	FUR: 2-10 µg ml^{-1} Spironolactone: 5-25 µg ml^{-1}	FUR: 0.09-1.181 Spironolactone: 0.863-1.585	Combined pharmaceutical dosage form	20
Spironolactone	HPLC-UV	Inertsil C_{18}; methanol:water (70:30) pH 3.20 adjusted with o-	FUR: 10-60 µg ml^{-1} Spironolacton	FUR: 0.41-0.99 Spironolac	Combined pharmaceutical dosage	21

		phosphoric acid; at 1 ml min^{-1}; at 236 nm	e: 25-150 µg ml^{-1}	tone: 0.60-0.99	form	
Spironolactone	Micellar HPLC-UV	IS: indapamide; Shim-pack VP-ODS C_{18}; 0.1M sodium dodecyl sulphate, 0.3% triethylamine, 10% n-propanol in 0.02 M orthophosphoric acid at pH 4.0; at 1 ml min^{-1}; at 238 nm	FUR: 0.1-10 µg ml^{-1} Spironolactone: 0.4-20 µg ml^{-1}	FUR: 0.57 Spironolactone:0.72	Combined pharmaceutical dosage form and human plasma	22
Gemifloxacin Hydrochlorothiazide	HPLC-UV	Purospher Start C_{18}; methanol: water: acetonitrile (70:25:5) at pH 3.0 with phosphoric acid 85%; at 0.8 ml min^{-1}; at room temperature; at 232 nm	FUR: 0.025-0.5 µg ml^{-1} Gemifloxacin: 0.5-10 µg ml^{-1} Hydrochlorothiazide: 0.125-2.5 µg ml^{-1}	<0.16 in all cases	Bulk, pharmaceutical dosage form and human serum	23
Ethacrynic acid	HPLC-UV	OptimaPak C_{18}; methanol:H_2O (70:30); at 0.5 ml min^{-1}; at 228 nm	FUR: 0.5-400 ng ml^{-1} Ethacrynic acid: 1.2-200 ng ml^{-1}	FUR: 3.26 Ethacrynic acid: 3.74	Human urine	24
Spironolactone Terbinafine Vancomycin and their metabolites	UHPLC-UV	Hypersil GOLD C_{18}; acetonitrile: formic acid 0.1% in gradient; at different λ	0.1-20 µg ml^{-1}	-	Human plasma and urine	28
Antipyrine Carbamazepine Phenytoin	HPLC-UV	C18, water pH 3 and 50:50 methanol: acetonitrile (58:42); at 1 ml min^{-1}; at 40^0C; at 205 nm carbamazepine and phenytoin; at 230 nm FUR and antipyrine	5-100 µM	<2	In determining Caco-2 permeability of markers	29
23 selected drugs	HPLC-DAD	Merck LiChroCART C_{18}; gradient elution with mobile phase at various proportions of acetonitrile:methanol:0.05 % trifluoroacetic acid; in the range 200-450 nm	0.05-45 µg ml^{-1} for all drugs	1.35-5.41	Human urine	30
Metoprolol Verapamil	MLC-UV MLC-	ODS-3 MZ C_{18}; buffer (0.15M SDS, 25 mM Na_2HPO_4):	FUR: 0.5-4 µg ml^{-1} Metoprolo	<9.5	Human plasma	31

	FLD	1-butanol: triethyl amine (93:6:1); at 40°C; at 2 ml min^{-1}; verapamil and metoprolol detected by spectrofluorimeter (excitation 230 nm and emission 311 nm) and FUR by UV detector at 240 nm	and Verapamill: 0.1-0.8 µg ml^{-1}			
-	HPLC-FLD	IS: Imipramine Inersil C_{18}; 5 mM NaH_2PO_4 buffer:acetonitrile (30:70) pH 3; at 1.5 ml min^{-1}; at λ_{ex} 270 and λ_{em} 470 nm	0.03-150 µM	-	Paediatric samples of plasma and urine	32
Canrenone Spironolactone	LC/MS/MS	Reversed phase mechanism, on a 250 mm length column packed with octadecyl modified silicagel and thermostated at 35 ºC; under isocratic conditions (3:7 aqueous 0.1% formic acid and methanol); at 0.8 ml min^{-1}	FUR: 20-1600 ng ml^{-1} Canrenone and Spironolactone: 2-100 ng ml^{-1}	<15	Human plasma	33
17 diuretics	HPLC-UV LC/MS/MS	Capcell-pak C_{18}; mobile phase: trifluoroacetic acid 0.15% (A) and acetonitrile (B); initial composition 90% A - 10% B; different gradient elution for UV and MS ; at 254 nm for UV; electrospray ionisation in either positive- or negative ion mode	UV: 2.5-60 µg ml^{-1} MS: 7-70 ng ml^{-1}	UV: <3 MS:<13	Drug adulteration screening of dietary supplements	34
Spironolactone	HPTLC	HPTLC plates precoated with 0.25 mm layer of silica gel mixture [Silica GF254] on aluminum sheets; ethyl acetate: hexane (80:20); UV Scanning densitometer set at 254 nm	FUR: 0.016-0.064 mg ml^{-1} Spironolactone: 0.040-0.160 mg ml^{-1}	<2	Tablet formulation	35

2. OPTICAL METHODS

2.1. Spectrophotometric methods

Due to its inherent simplicity, sensitivity and cost-effectiveness, visible spectrophotometry is a technique of choice employed in quality control laboratories of many developing countries. Therefore, developing a selective and sensitive methods using visible spectrophotometry is of paramount

importance. As can be seen in the previous work [1], several spectrophotometric methods has been reported for the determination of FUR in bulk, pharmaceutical dosage forms, and/or biological fluids.

Tharpa et al. [2] describes two visible spectrophotometric methods based on the reduction of $KMnO_4$ in acid and basic mediums. In method A, FUR was treated with a measured excess of permanganate in acid medium and the unreacted oxidant was measured at 550 nm, whereas in method B the reaction was carried out in alkaline medium and the resulting manganate was measured at 610 nm. Simplicity, sensitivity, wide linear ranges, mild experimental conditions and above all cost-effectiveness characterize the proposed methods. Further, the methods were found to possess adequate accuracy and precision. Method A is simpler and can be extended successfully to the quantification of FUR present in the injections and combination tablets without interference from the other active ingredients. However, method B entails an extraction step when applied to tablets to overcome the interference from some inactive ingredients. Later, the same research group describes two simple, sensitive, and selective spectrophotometric methods for the determination of FUR [3]. The methods are based on acid hydrolysis of FUR to free primary aromatic amine and diazotization followed by coupling with N-1-napthylethylene diamine (method A) or 4,5-dihydroxynaphthalene-2,7-disulfonic acid (method B). The coloured reaction product can be measured spectrophotometrically at 520 nm (method A) or 500 nm (method B).

Narayana and Ashwini proposed the determination of FUR using a measured excess of ceric ammonium sulphate (CAS) followed by the determination of unreacted oxidant using xylene cyanol FF (XC) (method A) and safranin O (SAF) (method B) [4]. The reaction mixture exhibited maximum absorbance at 612 nm (method A) and 526 nm (method B).

The combination of FUR and spironolactone is very useful in the treatment of heart failure. Spironolactone prevents hypokalaemia due to FUR in their combined dosage forms. In the view of the need in the industry for routine analysis of FUR and spironolactone in formulation, attempts are being made to develop simple and accurate analytical methods for simultaneous estimation of FUR and Spironolactone and extend it for their determination in formulation. In this way, spectrophotometry method has been reported for the simultaneous estimation of FUR and spironolactone in combined dosage form and no method is available in the pharmacopoeias [5,6]. In both cases, the proposed methods use derivative spectroscopy.

2.2. Spectrofluorimetric methods

Buitrago et al. develops and validate an alternative analytic method by spectrofluorometry for the quality control of FUR in solid pharmaceutical forms based on the native fluorescence of the molecule in acidic medium [7].

Liu et al. found that Zn (II)-1, 4-bis (imidazol-1-ylmethyl) benzene (bix) complexes can greatly increase the fluorescence emission of FUR at 400 nm, and the enhanced fluorescence intensity is directly proportional to the concentration of FUR [8]. Based on this phenomenon, a novel spectrofluorometric method for the determination of FUR was developed, which has the advantages of simplicity, rapidity and sensitivity.

The association of FUR and amiloride furnishes a valuable natriuretic agent with a diminished kaliuretic effect, minimizing the risk of alkalosis in the treatment of refractory oedema associated with hepatic cirrhosis or congestive heart failure. Although, FUR and amiloride are usually found in commercial pharmaceutical formulations containing both drugs, in the literature there are only a few reports about its simultaneous determination. In this way, the investigation group of Peralta et al. proposed two spectrofluorimetric methods for their quantification [9,10]. In the first work, a method to separate and precisely determine both drugs is proposed; FUR and amiloride present an important fluorescence spectral interference. The approach is to separate amiloride, extracting it from the sample matrix by chemofiltration with a nylon membrane, while FUR is maintained in the filtered aqueous solution. Amioride shows low native fluorescence, but its separation and preconcentration by immobilization (solid-phase

extraction) on nylon membrane surface provides a considerable enhancement in fluorescence intensity. This study demonstrates the feasibility of using a membrane as a novel support for solid-phase extraction procedures, focused on AMI and FUR separation and determination. This nylon property is an important point to be considered when water samples are filtered via nylon discs before chromatographic determinations of AMI, especially if low concentrations are analyzed. The different behaviour of both studied drugs respect to the membrane constitutes an economic and simple separation methodology that permits to resolve spectral interferences and determine FUR and amiloride with high sensitivity and precision. The second method [10] for the sequential determination of FUR and amiloride in urine by fluorimetry is simple, efficient and allows resolving the mixture by using a solid phase extraction to suppress the background fluorescence of urine and avoid the overlap between the FUR and amiloride spectra. The use of a solid-support, combined with the use of fluorimetry technique, contributes to enhancing both sensitivity and selectivity, so performing simultaneously the separation (retention), preconcentration and detection of the analytes.

2.3. Diffuse reflectance spectroscopy

Spot tests using filter papers, coupled with diffuse reflectance spectroscopy (DRS), is a technique that uses very small quantities of reagents and solvents, making waste treatment easier and reducing costs, in accordance with the principles of Green Chemistry. The fact that spot tests are especially rapid and simple, and that they minimize use of reagents/solvents makes them very useful for the development of analytical methods that address the notion of Green Chemistry, which aims to develop methods and techniques that reduce or eliminate the use and generation of substances hazardous to human health or to the environment. The procedure is very simple, practical, and fast, and can be performed using portable equipment (which can be homemade) for in situ analyses. Filter paper provides a good surface for spot tests because it is composed of cellulose fibers, is very inexpensive, and is manufactured in nearly every part of the world from renewable and recyclable resources. In this sense, Marques Luiz et al. describes the development and application of a simple, portable, and environmentally friendly method for the rapid determination of FUR in urine [11]. The method is based on the complexation reaction of furosemide (5-(aminosulfonyl)-4-chloro-2-((furanylmethyl)amino)benzoic acid, dissolved in ethanol, with $FeCl_3$ and the surfactant dodecyltrimethylammonium bromide (DTAB) in aqueous solution, yielding a coloured compound on the surface of a filter paper.

3. Electrochemical methods

In recent years, the electrochemical techniques have led to the advancement in the field of analysis because of their sensitivity, low cost and relatively short analysis time, as compared with other techniques. Electrochemical have proven to be useful for development of very sensitive and selective methods for the determination of organic molecules including drugs. In addition application of electroanalytical techniques includes the determination of electrode mechanisms. Redox properties of drugs can give insights into their metabolic fate or their in vivo redox processes or pharmaceutical activity. On the other hand, surfactants even in trace quantities can exert a strong effect on the electrode process and the other, the gold electrode has been widely used in electrochemical studies and electro analysis for various substrates for a long time because of its stability, wide potential window and fast electron transfer rate. Shetti et al. investigated an electrochemical oxidation process of FUR on gold electrode [12]. Further, differential pulse voltammetric (DPV) method with good precision and accuracy was developed by the authors for the determination of FUR in pharmaceutical formulations. Potentiometric methods with ion-selective electrodes have proved to be effective for the analysis of pharmaceuticals and biological samples, because these sensors offer the advantages of simple design, construction, and manipulation, reasonable selectivity, fast response time, applicability to coloured and turbid solutions and possible interfacing

with automated and computerized systems. Santini et al. describes the development and application of a simple, and low-cost potentiometric 4-cloro-*N*-furfuryl-5-sulphamoyl-anthranilate ion sensor immobilized in a graphite matrix (Pt|Hg|$Hg_2(Fur)_2$|Graphite [13]. The proposed potentiometric sensor is easy to prepare, exhibits long lifetime, shows high sensitivity and wide dynamic range. Good selectivity, very low detection limit, rapid response and low-cost of fabrication make this electrode suitable for analysis of FUR in pharmaceutical and biological samples.

Malode et al. develop a convenient and sensitive method for the determination of FUR by multi-walled carbon nanotubes-paraffin oil paste electrode (CNTPE) [14]. The authors report the electrochemical oxidation of FUR on CNTPE. The ability of the modified electrode for voltammetric response of selected compound was also evaluated. Finally, this modified electrode was used for the analysis of FUR in pharmaceutical and urine samples. The resulted biosensor exhibits high sensitivity, rapid response, good reproducibility and freedom of other potentially interfering species.

4. Liquid chromatography

The literature survey revealed that HPLC has been employed to detect FUR in different samples [1]. Also, FUR has been simultaneously determined with other compounds using HPLC [1]. So far, HPLC is generally the method of choice for diuretics determination, due to the required time and cost of the analysis. In **table 1** are presented a resume of principal characteristics of methods based on HPLC.

Determination of solubility class of a drug substance according to FDA guidance is very important for the pharmaceutical companies which are taking bio waiver for the immediate release oral solid drug products. The aim of the study carried out by Kaynak and Sahin was to develop and validate an HPLC method for quantification of FUR in the samples obtained from the in vitro solubility studies performed at five different pH values (pH 1.0, 2.9, 3.9, 4.9 and 7.5). Developed and validated method was proved to be simple, reliable and also suitable as a single method for studying the solubility of FUR as a function of pH. Finally, based on the results obtained by the authors, solubility of FUR was dependent on pH. Its solubility was low between pH 1.0 and 4.9, and was high at pH 7.5.

Kumar et al. develop a simple and sensitive direct estimation RP-HPLC method for the simultaneous determination of FUR and amiloride in plasma, for their pharmacokinetic studies and therapeutic drug monitoring [19]. Reported HPLC method involves simple single step rapid extraction procedure, economic isocratic mobile phase, and single detection wavelength for simultaneous estimation of analytes, also the run time was less than 11 minutes which allows minimal mobile phase consumption with analysis of a large number of plasma samples in a short time period. The stability of FUR and amiloride was excellent, with no evidence of degradation during sample proceeding.

Dispersive liquid–liquid microextraction (DLLME) was developed for the simultaneous determination of FUR and ethacrynic acid in human urine by HPLC [24]. DLLME is one of the most recently developed techniques of liquid phase microextraction sample preparation [25-27]. It is based on a ternary solvent system. It requires the appropriate aqueous mixing of extraction and dispersing solvents. The method is simple, fast, and low-cost and has high recovery and high enrichment factor. DLLME has many merits: fast operation, no need of large amounts of extraction solvent, high recovery, low cost, and it has easy linkage to most analytical methods.

Baranowska et al. propose the use of ultra high performance liquid chromatography (UHPLC) equipment for the fast simultaneous determination of FUR, vancomycin (antibacterial drug), spironolactone (diuretics) and terbinafine (antifungal drug) along with their metabolites in human urine and plasma [28]. The investigated metabolites were: saluamine, FUR metabolite; carnenone, active metabolite of spironolactone; *N*-desmethylcarboxy terbinafine, metabolite of terbinafine. The authors chosen this mixture because these drugs are often administrated to patients on Intensive Care Units. Thanks to the presented chromatographic conditions, the separations of FUR, terbinafine, spironolactone and vancomycin with the metabolites can be made in 5.3 min.

Assessment of *in-vitro* Caco-2 permeability requires simultaneous quantitation of high and low permeability markers to ascertain the suitability of the method. The study developed by Patil et al. [29] represents a simple, rapid and reliable validated RP-HPLC method for the quantification of FUR, antipyrine, carbamazepine and phenytoin in presence of Hanks balanced salt solution buffer with a relatively short run time. The developed method is highly specific, accurate and precise, making it suitable for the routine analysis of these permeability markers in Caco-2 permeability study.

The determination of the RP-HPLC-DAD method for accurate measurements of 23 analyzed drugs concentrations in human urine samples from patients suffering from different illnesses was elaborated by Baranowska et al. [30]. The investigators suggested a simple method for a simultaneous and quick analysis of 23 drugs for laboratories, which did not dispose of the MS detector. Simple, fast and effective sample preparation for analysis makes it an easy decision about applying the method in a medical laboratory. This method enables the determination of the one or several analyzed drugs in one run time in urine sample from treated patients.

A rapid and cost-effective benefit micellar liquid chromatography (MLC) method coupled with UV and fluorimetric detectors has been developed to separate and quantify three frequently used cardiovascular drugs (i.e., FUR, metoprolol and verapamil) from human plasma [31]. The developed method is applicable for pharmacokinetic, pharmacodynamic and therapeutic drug monitoring studies. The main advantages of the developed method are simple sample preparation and minimal use of organic solvents. In comparison with previous MLC methods for the analysis of mentioned drugs, the method has the advantage of being able to simultaneously analyze the studied drugs, in addition to the validation of the method according to FDA guidelines.

The aim of the work presented by Sora et al. consisted in the development and validation of an analytical method for assaying simultaneously FUR, spironolactone and canrenone at pharmacokinetic concentration levels in plasma samples, based on a programmed bimodal functioning of the MS/MS detection (negative ion mode for FUR, positive ion mode for spironolactone and canrenone) [33]. The method was successfully applied to a bioequivalence study for immediate release pharmaceutical oral dosage forms (capsules) containing 20 mg of FUR and 50 mg of spironolactone in fasting conditions.

In other aspect, most of the methods published are limited for assaying diuretics in urine, plasma and bovine milk; there are few methods for the simultaneous analysis of diuretics adulteration in foods and dietary supplements. In order to ban the sales of adulterated products effectively, it is necessary to develop a fast and reliable method for screening suspicious dietary supplements. In the study developed by Woo et al., the authors report validated methods for the simultaneous analysis of 17 diuretics, including FUR, in dietary supplements by HPLC-UV and LC-MS/MS [34]. Developed HPLC-UV and LC-MS/MS methods permit the simultaneous analysis of a large number of diuretics in dietary supplement matrices with a minimum of sample preparation.

5. Thin Layer Chromatography

Due to no reported HPTLC method estimation of FUR and spironolactone in tablet, taking into consideration the simplicity, cost effectiveness and reliability of HPTLC in the analysis of drugs, Kher et al. develop a new, simple and validated HPTLC method for simultaneous estimation of FUR and spironolatone dosage form tablets [35]. The HPTLC method was subjected to statistical validation and was applied for the determination of FUR and spironolactone from its combined dosage form. In this study, stability of FUR and spironolactone in present dosage form was established through employment of ICH recommended stress condition. The developed procedure has been evaluated over the specificity, linearity, accuracy, precision and robustness in order to ascertain the stability of the analytical method.

6. Stability indicating methods

The stability of a pharmaceutical preparation is defined by its resistance to the various chemical, physical, and microbiological reactions that may

change their original properties during transport, storage, and use. Chemical degradation may result in a loss of potency or an increase in drug toxicity, so that clinical use of a pharmaceutical preparation must be unacceptable if the degradation is relatively high. When a drug dosage form is altered (by dissolution, pulverization, or addition to other materials) or the environment of the drug is modified by changes in storage conditions, the stability of the drug may be affected.

A stability indicating method is a quantitative test method that can detect possible degradants and impurities of drug substance (API) and drug products, normally using HPLC. Stability information is needed for regulatory submissions such as IND (Investigational New Drug Application) and NDA (New Drug Applications) and to set expiration dates for the API or drug product.

On the other hand, the forced degradation studies are another very important part of the validation of the stability indicating method. In forced degradation studies, samples are tested under extreme conditions (acid, base, peroxide, heat, light, humidity etc) in order to rapidly screen drug product stabilities.

In this way, diverse works have been published about studies of stability of FUR, alone or in mixture with other drugs, which use HPLC methods [36-39]. The principal aspects of this investigation are summarized in **Table 2**.

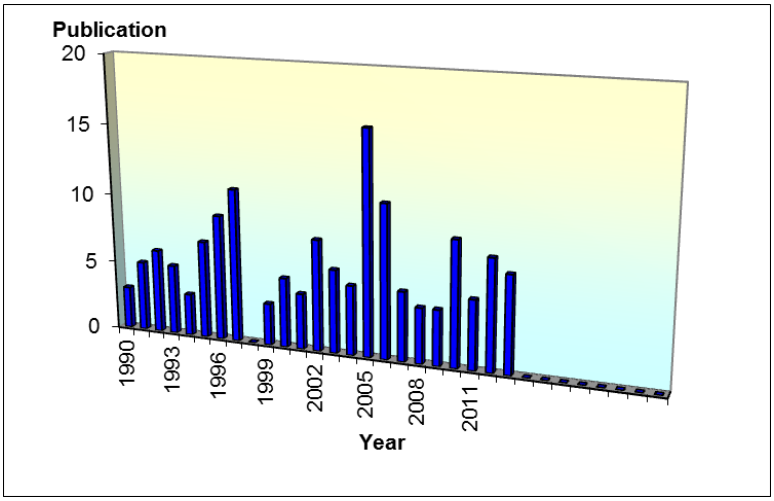
Table 2: Stability indicating HPLC methods

Compounds	Procedure	Conclusions	Ref.
FUR	Waters C_{18}; KH_2PO_4: methanol (70:30) and citric acid solution to adjust pH to 5.5; at 1ml min^{-1}; at 235 nm; injection volume 20 µl	Extemporaneously compounded FUR suspensions, 2 mg ml^{-1}, were stable for at least 60 days stored in glass bottles protected from light at 4, 30 and 45^{0}C	36
FUR Amiloride	C_{18}; acetonitrile:50 mM phosphate buffer (pH 3) (50:50); at 26°C; at 1 ml min^{-1}; at 283 nm; injection volume 10 µl	The stability studied indicated that the drugs are susceptible to wet heat, dry heat, day light, acidic and alkaline conditions with maximum degradation in oxidation degradation	37
FUR Aminophylline (APN)	Shim-pack VP–ODS C_{18}; acetonitrile:phosphate buffer (pH 7.0) (30:70); at 0.6 ml min^{-1}; at 228 nm and 275 nm; at 25 ^{0}C; injection volume 20 µL	After 20 h at room temperature, individually, FUR and APN added to 20% mannitol and 0.9% NaCl solutions had the highest stability at pH 10-11; when FUR and APN were combined in the same parenteral solutions, the behaviour of FUR was similar to the behaviour observed for the drug individually in the same solutions	38
FUR Spironolactone	SGE SS Wakosil II C_8; acetonitrile:ammonium acetate buffer pH 3.9 (50:50); at 1.0 ml min^{-1}; at 254 nm	The stability of FUR and spironolactone in present dosage forms was established through employment of ICH recommended stress conditions	39

7. CONCLUSIONS

This work is an actualized revision of the work published in 2008 [1], about analytical determination of FUR. The overview includes the most relevant analytical determinations classified in the following types: Optical methods, electrochemical methods, liquid chromatography and thin layer chromatography. Also, we are enclosed a section about stability indicating methods. As can be seen from the **Figure 1** a similar number of publication have appeared in last two decades, being the year 2005 where more determinations of furosemide were published in the literature.

Figure 1: Number of publications from last two decades



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