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Review article

Sample preparation and RPHPLC determination of diuretics in human body fluids

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This article describes reverse phase high-performance liquid chromatography (RPHPLC) methods for determination of diuretics in different human body fluids (whole blood, plasma, serum or urine). Sample preparation procedures, including solid-phase extraction, liquid-liquid extraction, dilution, precipitation as well as automated RPHPLC procedures, are discussed in order to present the advantages and disadvantages of each type of sample preparation. Also, values of analytical recovery of each procedure used for sample preparation are summarized. The most important RPHPLC parameters (detection mode, stationary phase, mobile phase, sensitivity, *etc.*) are also summarized and discussed.

Keywords: diuretics, RPHPLC, determination, human body fluids

INTRODUCTION

By definition, diuretics are drugs that increase the rate of urine flow. However, clinically useful diuretics also increase the rate of excretion of Na^+ and an accompanying anion, usually Cl^-. NaCl content in the body is the major determinant of the extracellular fluid volume, and most clinical applications of diuretics are directed toward reducing the extracellular fluid volume by decreasing the total body NaCl (1). In particular, diuretics promote excretion of water and electrolytes by the kidneys. They are used in the treatment of heart failure or in hepatic, renal, or pulmonary diseases when salt and water retention has resulted in oedema or ascites (2). Diuretics are also used, either alone or in association with other drugs, in the treatment of hypertension.

Diuretics have been misused and abused in sports where weight categories are involved, such as weight-lifting, wresting and boxing, in order to reduce body weight rapidly. Also, some athletes have used diuretics as masking agents to increase the urine volume, thereby diluting the concentration of a banned doping under the detection limit. Not only for ethical reasons, but also because of serious health risks, use of these compounds was prohibited by the International Olympic Committee (3).

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Since 1980, numerous analytical methods for determination of a large number of diuretics using liquid chromatography have been reported. Chromatographic procedures for determination of different diuretics in biological fluids are necessary for pharmacokinetic studies, including identification and quantification of metabolites, bioequivalence/bioavailability studies, and doping control. It is necessary to establish accurate and specific analytical techniques, that allow measurement of diuretics in biological fluids at different therapeutic levels. There are liquid chromatographic methods for quantification of some diuretics, but most of them are not sensitive enough for the pharmacokinetic analysis of the dosage forms of investigated drugs.

In order to increase the throughput and reduce costs, the speed of analysis has become of paramount importance in many application areas of reverse phase high-performance liquid chromatography (RPHPLC), especially when bioequivalence/bioavailability studies are conducted. Hence, reliable, rapid and sensitive methods for the determinations of diuretics in biological fluids (blood, serum) are required.

Some analytical problems of the detection and identification of diuretics and their metabolites are due to the wide variety of their chemical structures and functional groups, wide differences in pK_a values, low volatility and lack of metabolic studies in several cases. On the other hand, liquid chromatography has emerged as one of the most accepted and widely employed techniques for determination of diuretics in biological fluids owing to its high-efficiency, reliability, versatility and being able to be combined with other tools, such as mass spectrometry.

Reverse phase high-performance liquid chromatographic methods for the determination of different diuretics in biological samples such as plasma, serum, blood or urine are reviewed. Different methods for sample preparation are also discussed.

SAMPLE PREPARATION

Some of the analytical methods developed for the determination of diuretics in biological fluids use direct injection of urine samples into liquid chromatograph (4–9). Determination of some diuretics in urine samples without their preconcentration is possible because concentration levels are sufficiently high. Therefore, some authors (4, 9) propose direct injection of urine samples after filtration through cellulose filters. In some cases, these assays yield poor separation of the investigated drug from urine endogenous interferences.

Table I. Sample preparation methods for RPHPLC determination of carbonic-anhydrase inhibitors

Drug	Sample	Sample preparation	Recovery (%)	Ref.
Dorzolamide	P	LLE with EtAc/toluene/*i*-propanol (50:40:10), BE with H_3PO_4	70 ± 5	47, 48
Dichlorfenamide	U	LLE with EtAc	100 ± 7.2	29
Methazolamide	B	LLE with EtAc, BE with glycine buffer	52	41
Methazolamide	P	LLE with EtAc, BE with glycine buffer	46	41
Methazolamide	U	LLE with EtAc, BE with glycine buffer	34	41

P – plasma; U – urine; B – blood; LLE – liquid liquid extraction; BE – back extraction; EtAc – ethyl acetate

The remarkable success RPHPLC technology encouraged researchers to apply the principles of chromatography to separate the analytes of interest from other interfering substances while treating test samples. In most of the reviewed papers, solid-phase or liquid-liquid extraction techniques were used to separate and selectively elute analytes from plasma, serum, urine or blood endogenous interfering species. Recently, the on-line solid-phase extraction using column-switching and automated techniques were proposed for determination of diuretics in biological fluids. According to the techniques used, different procedures for sample preparation are discussed. In Tables I–IV, the recovery

Table II. Sample preparation methods for RPHPLC determination of loop-diuretics

Drug	Sample	Sample preparation	Recovery (%)	Ref.
Bumetanide	U	D with SDS (1:25)	88.7, 99.9	14, 15
Bumetanide	P, U	PRE with ACN	80–100	21, 83
Bumetanide	U	LLE with EtAc, Acidic extraction	93 ± 5, 90 ± 1.5	29, 30
Bumetanide	P	LLE with diethyl ether	85–95	43
Bumetanide	U	LLE	71	58
Bumetanide	P, U	SPE (C18)	80–100	21
Bumetanide	U	SPE	84.2	58
Ethacrynic acid	U	D with SDS (1:25)	100.5	15
Ethacrynic acid	U	LLE with EtAc	64 ± 3.5	29
Ethacrynic acid	P	LLE with dichloromethane	95	49
Ethacrynic acid	P, U	SPE (C18)	58–60	67
Ethacrynic acid	U	CST	99	85
Furosemide	U	D with SDS (1:25)	103.8	15
Furosemide	U	D with water (1:50)	100.6	17
Furosemide	U	D with MeOH (1:1)	91.6–103.3	18
Furosemide	P, S	PRE with ACN	91.5, 100.5	17, 19
Furosemide	U	DI	99.4–101.6	6
Furosemide	P	LLE with EtAc	80–97.1	28, 32, 37
Furosemide	U	LLE with EtAc	69 ± 2.1	29
Furosemide	U	LLE with EtAc, Acidic extraction	85 ± 2.2	31
Furosemide	S, U	LLE with EtAc and ACN	85–92	50
Furosemide	U	LLE with EtAc	> 90	38
Furosemide	P, U	LLE with diethyl ether	71.1–107.2	44
Furosemide	P	LLE with diethyl ether:hexane (35:35)	90.75	27
Furosemide	P	CST	76.32	27
Furosemide	P	LLE	99.8–101.6	61
Furosemide	P, U	SPE (C18)	94–102	69
Furosemide	P	SPE (C18)	> 90	70
Furosemide	P	SPE (C18)	> 90	77
Torasemide	P	LLE with EtAc	> 80	59
Torasemide	U	SPE	60	75

P – plasma; U – urine; B – blood; S – serum; LLE – liquid liquid extraction; BE – back extraction; D – dilution; SPE – solid phase extraction; CST – column-switching technique; PRE – precipitation; ACN – acetonitrile; EtAc – ethyl acetate; SDS – sodium dodecyl sulfate; DI – direct injection

Table III. Sample preparation methods for RPHPLC determination of potassium sparing diuretics

Drug	Sample	Sample preparation	R (%)	Ref.
Amiloride	U	D with SDS (1:25)	110.9, 101.3	14, 15
Amiloride	U	DI	99.5–102	4
Amiloride	P, U	PRE with $HClO_4$	97.6	20
Amiloride	P	LLE with EtAc	74	28
Amiloride	U	LLE with EtAc, basic extraction	25 ± 2.3, 23 ± 4.6	29, 31
Amiloride	P	LLE with EtAc	92.4–114	30
Amiloride	P	LLE with EtAc	60.7	32
Amiloride	P, U	LLE wit diethyl ether: dichloro-methane (2:1), BE with TBAH	82	32
Amiloride	P	SPE (C8)	100.9	29
Spironolactone	U	LLE with EtAc	95 ± 3.8	29
Spironolactone	U	LLE with EtAc, acidic extraction	65 ± 4.7	31
Spironolactone	P, S	LLE with tetrachloromethane	80, 89	52, 53
Spironolactone	U	CST	100	89
Spironolactone	P, U	SPE (C18 and CN)	72–80	14, 72
Triamterene	U	D with SDS (1:25)	106.4, 105.1	14, 15
Triamterene	U	D with methanol (2:1)	98.7–109.7	12
Triamterene	P	PRE with $HClO_4$	97.6–122	12
Triamterene	U	LLE with EtAc, basic extraction	81 ± 1.5, 88 ± 4	29, 31
Triamterene	S, U	CST	102	90, 92

P – plasma; U – urine; B – blood; S – serum; D – dilution; PRE – precipitation; LLE – liquid liquid extraction; BE – back extraction; SPE – solid phase extraction; CST – column-switching technique; SDS – sodium dodecyl sulfate; EtAc – ethyl acetate; TBAH – tetrabutylammonium hydroxide

values obtained after different sample preparation procedures are summarized. Extraction recoveries were usually calculated by comparing the peak height of the investigated drug obtained from spiked samples and those resulting from the direct injection of the theoretical amount of drug, or in cases where the internal standard method was used, calculations were based on comparison of the analyte/internal standard peak height or area ratios obtained after the extraction of spiked samples and the peak height or area ratio of an equal amount of the analyte standard solution.

Dilution

Some authors have proposed methods for the determination of metolazone (10), triamterene (11–15), hydrochlorothiazide (16), furosemide (14, 15, 17–19), amiloride, bendroflumethiazide, bumetanide, benzthiazide, chlorthalidone, ethacrynic acid, torasemide, trichlormethiazide (14, 15) in unextracted urine using fluorescent (FL), ultraviolet (UV) or electrochemical (EC) detection. Prior to direct injection into the liquid chromatograph, urine samples were diluted with water (10, 13, 16, 17, 19), methanol (MeOH) (12, 18) or sodium dodecyl sulfate (SDS) (14, 15) in different volume ratios, ranging from 1:1 to 1:50.

Table IV. Sample preparation methods for RPHPLC determination of thiazide diuretics

Drug	Sample	Sample preparation	Recovery (%)	Ref.
Bendroflumethiazide	U	D with SDS (1:25)	101.1, 98.1	14, 15
Bendroflumethiazide	U	LLE with EtAc	87 ± 3.6	29
Bendroflumethiazide	U	LLE with EtAc, acidic extraction	98 ± 1.1	31
Bendroflumethiazide	U	D with SDS (1:25)	97–100	84
Benzthiazide	U	D with SDS (1:25)	101.5	15
Benzthiazide	U	LLE with EtAc, basic extraction	89 ± 1.7, 88 ± 5	29, 31
Chlorothiazide	U	LLE with EtAc	78 ± 3.2	29
Chlorothiazide	U	LLE with EtAc, acidic extraction	97 ± 0.2	31
Chlortalidone	U	D with SDS (1:25)	105.1	15
Chlortalidone	B	PRE with acetonitrile	86	22
Chlortalidone	U	LLE with EtAc	93 ± 2.1	29
Chlortalidone	U	LLE with EtAc, basic extraction	72 ± 4.2	31
Chlortalidone	P	LLE with dichloro-methane:isopropanol (75:25)	92.7	46
Chlortalidone	U	on-line SPE	88.2	92
Cyclothiazide	U	LLE with EtAc	86 ± 0.6	29
Cyclothiazide	U	LLE with EtAc, acidic extraction	97 ± 0.2	31
Hydrochlorothiazide	U	DI	98.2–98.4	6
Hydrochlorothiazide	U	D with water (1:8)	94.6–103.3	16
Hydrochlorothiazide	U	LLE with EtAc, acidic extraction	88 ± 1, 96 ± 0.2	29, 31
Hydrochlorothiazide	P	LLE with EtAc	94.3–99	30, 39
Hydrochlorothiazide	S	LLE with *tert*-butylmethyl ether	90	51
Hydrochlorothiazide	P	SPE (RP select B)	97.2–104.1	73
Hydrochlorothiazide	P	fully automated	83–90	58
Hydrochlorothiazide	U	fully automated	20–25	58
Hydroflumethiazide	U	D with SDS (1:25)	95.9	14
Hydroflumethiazide	U	LLE with EtAc	91 ± 2.6	29
Hydroflumethiazide	P	96-well LLE	–	62
Indapamide	U	LLE with EtAc	< 87	11
Indapamide	U	LLE with EtAc	99.5	40
Indapamide	B	LLE with diethyl ether	87.4	45
Indapamide	B	SPE (RP select B)	80.1–83.5	78
Indapamide	S	SPE (RP select B)	96.9–100.7	78
Indapamide	B	LLE with methyltertarybuthyl ether	–	63
Methyclothiazide	U	LLE with EtAc	93 ± 2	29
Metolazone	U	D with water (1:3)	99.5–104.5	10
Metolazone	U	LLE with EtAc	92 ± 3.6	29, 35
Metolazone	B, P	on-line SPE	90–93	94
Polythiazide	U	LLE with EtAc	93 ± 3.6	29
Quinethazone	U	LLE with EtAc	53 ± 2.1	29, 35
Trichlormethiazide	U	D with SDS (1:25)	96.4	15
Trichlormethiazide	U	LLE with EtAc, acidic extraction	89 ± 2.3, 85 ± 1.2	29

P – plasma; U – urine; B – blood; S – serum; D – dilution; D LLE – liquid liquid extraction; I – direct injection; SPE – solid phase extraction; RP – reversed phase; PRE – precipitation; SPE – solid phase extraction; CST – column-switching technique; SDS – sodium dodecyl sulfate; EtAc – ethyl acetate; DI – direct injection

Precipitation

As mentioned, prior to determination of diuretics in plasma, blood or serum, samples must be treated in order to separate analytes from interfering species. For this reason, precipitation of plasma, serum or blood proteins is often the first step in sample treatment and has to be carried out to clean the sample in some way. This serves to cleave the union between protein and drug in order to determine the total drug concentration in plasma, serum or blood samples. Precipitant agents such as strong acids or organic solvents for analytes stable at acid pH values are commonly used. These agents must have two characteristics: no loss of analyte and maximal clean-up must be achieved following the process.

In the reviewed papers, perchloric acid and acetonitrile (ACN) were used as precipitating agents in different proportions (12, 19–25). Following protein precipitation, supernatants were directly injected into a RPHPLC column and the investigated drugs were determined using a fluorescent, ultraviolet, or electrochemical detector. Ng *et al*. (26) proposed a quantitative clean-up procedure for determination of amiloride in plasma and urine by salts precipitation using zinc sulfate and barium hydroxide. Some of these methods yielded poor separation of investigated diuretics from the blood, plasma and serum endogenous interfering species.

Liquid-liquid extraction

Liquid-liquid extraction technique is the most commonly used method for isolation of diuretics from plasma, serum or urine samples (11, 27, 28–63). For this purpose, different organic solvents have been used: ethyl acetate, diethyl ether, dichloromethane, tetrachloromethane, *tert*-butylmethyl ether, or mixtures of diethyl ether and dichloromethane, dichloromethane and *i*-propanol, ethyl acetate and acetonitrile, diethyl ether and hexane, and ethyl acetate, toluene and *i*-propanol. Also, some of these methods involve extraction of drugs with organic solvents and back-extraction into glycine buffer (41), tetrabutylammonium hydroxide (42) and phosphoric acid (47, 48) for determination of methazolamide, amiloride and dorzolamide, respectively.

As diuretics are drugs with different pK_a values, ranging from 3.5 to 10.7, the isolation steps in the reviewed papers were performed either at alkaline or acidic pH, usually after addition of aqueous NaOH, Na_2CO_3, HCl, CH_3COOH, KH_2PO_4, H_2SO_4 or a mixture of KH_2PO_4 and Na_2HPO_4, $KHCO_3$ and K_2CO_3 buffers, *etc*. Namely, some diuretics are acidic and some are basic and a single liquid extraction step will not permit simultaneous recovery of the drugs. Therefore, some authors proposed double extraction methods for simultaneous determination of some diuretics, which have different molecular structures, using a combination of different separation mechanisms in order to separate them within an acceptable total analysis time (27–29, 31, 32). These methods involve extraction of acidic drugs from acidified biological fluids and extraction of basic drugs from alkaline samples.

Although some of these liquid-liquid extraction methods are sufficiently sensitive, the drugs have been measured utilizing either a large sample volume or extraction procedures yielding poor separation of investigated drugs from the blood plasma, serum and urine endogenous interferences and gave highly variable and relatively low recoveries (28, 29, 31, 32, 35, 41, 44, 47, 48, 58).

There are also several investigations concerning the determination of hydrochlorothiazide in serum, plasma or urine (64, 65) by fully automated high performance liquid chromatography methods. These methods provided equal or improved accuracy and precision compared to manual procedures, as well as capability of analyzing over 100 samples per day. The high price of the equipment is the only disadvantage of these methods.

Solid-phase extraction

Solid-phase extraction (SPE), which is emerging as a very important sample preparation technique, is preferred to other traditional extraction procedures, such as liquid-liquid extraction, mainly because it is more efficient and much less time-consuming (66). By integrating SPE with RPHPLC, complete automation of sample analysis can be achieved. Numerous publications have described the determination of amiloride, bumetanide, ethacrynic acid, furosemide, hydrochlorothiazide, torsemide, spironolactone and polythiazide concentrations in plasma or urine by RPHPLC using, fluorescence or electrochemical detection after solid-phase extraction on different cartridges such as silica, C_8 and C_{18}-bonded silica, reversed phase (RP) select B and cyano (CN) solid phases (4, 64, 67–77).

The methods using solid-phase extraction are sensitive but need an internal standard. Also, these methods overcome problems caused by endogenous compounds, but some of the methods gave relatively low recoveries (67, 75). In order to obtain satisfactory recovery values, the solid-phase extraction method should be optimized. For this purpose, some authors suggest testing different types of cartridges for solid phase extraction (71, 72, 74, 78–82). To improve the extraction procedure, cartridges for solid phase extraction have been conditioned with different buffers or samples have been buffered before being introduced into SPE columns. Lower eluent volume was advantageous by avoiding the need for evaporation at low serum, plasma and blood drug levels (4, 79), thus simplifying the extraction methods.

RPHPLC ANALYSIS

Some authors (5–8, 83, 84) proposed micellar chromatographic procedures for determination of some diuretics in urine by direct injection. They reported that direct injection of the urine sample into a chromatographic system, with a SDS mobile phase, has the drawback of the strong urine matrix band at the beginning of the chromatogram. Therefore, optimization of the mobile phase composition was proposed in order to improve separation selectivity between the drug and interfering compounds. Specific mobile phase parameters, including pH and the type of surfactant used were identified and examined. Namely, optimal control of these parameters can simplify the development of micellar chromatographic procedures for the determination of diuretics in human urine. Also, the advantages of direct injection techniques such as micellar chromatography include reduced analysis time, increased sample throughput, ability to simultaneously chromatograph hydrophilic and hydrophobic compounds, lower cost and greater safety

of micellar mobile phases compared to conventional mobile phases containing organic solvents, improved accuracy and precision, *etc*.

Some papers offered a short analytical run time and achieved an excellent baseline resolution between the peak of drugs and endogenous substances (10, 15, 18). Some authors (14, 15) proposed the RPHPLC method for determination of diuretics in human urine using micellar mobile phases and direct injection of samples after dilution with SDS. These methods are useful for simultaneous determination of more than seven diuretics in urine. Farthing *et al*. (16) reported a method for determination of hydrochlorothiazide, which did not require mobile phase modifiers, such as SDS, but, which employed narrowbore column technology in order to achieve adequate sensitivity. The authors chose to use a simple dilute and shoot procedure, which eliminated the need for sample extraction, thus making the method cost effective. On the other hand, the only disadvantage of this method is that the use of the narrowbore column required two minor modifications to the RPHPLC system (small injection volume of 2 mL, and detector cell volume of 4.5 μL).

In the last decade, an increasing number of publications have demonstrated the potential of column-switching techniques for determination of drugs in biological fluids (27, 85–95). However, direct injection is often accomplished by automation of the sample pretreatment operations through the use of different columns in conjunction with column-switching valves. HPLC determination based on direct injection of biological fluids has been increasingly used due to the more widespread use of the column-switching technique. Namely, this method allows direct injection of various biological fluids (serum, plasma, blood, urine, intestinal aspirates) into the HPLC system and on-line clean--up and sample enrichment by a column-switching technique. Recently, special column packing materials that allow direct and repetitive injection of untreated samples, *i.e.*, automated liquid chromatography (LC) integrated sample preparation, were developed. Using a special extraction column, the sample is first fractionated and the analyte fraction is transferred in a back-flush mode onto an analytical column where it is separated and quantified. However, these automated direct injection procedures are complicated in that they require additional instrumentation in the form of precolumns, switching valves, pumps, accurate and precise timing of valve switching for the separation to be successful. Another disadvantage of this method is that it requires high reagent consumption and an HPLC system with two pumps.

Chromatographic procedures for determination of diuretics in biological fluids are given in Tables V–VII, which contain the basic information about each procedure to facilitate rapid selection of a suitable method. The procedures are listed according to drug names. Information about stationary and mobile phases, as well as detection or quantification limits is summarized in the tables as well.

RPHPLC column

Some authors performed a series of studies in order to develop a convenient and easy-to-use method for quantitative analysis of diuretics, alone or in combination with other diuretics, in biological fluids (32, 72, 78, 94). Namely, method variables with respect to their effect on the RPHPLC separation of diuretics from the matrix were investi-

Table V. RPHPLC methods for the determination of carbonic-anhydrase inhibitors in biological samples

Drug	Sample	Stationary phase	Mobile phase	Detection	LOD[a]/LOQ[b] (ng mL^{-1})	Ref.
Dorzolamide	P	Hypersil CN 100 x 3.0 mm, 5 μm	ACN: (10 mmol L^{-1} CH_3COONH_4 and 0.1 % CF_3COOH) (35:65, *V:V*, %), 0.6 mL min^{-1}	MS/MS	0.5[b]	47
Dorzolamide	P	RP-8 250 x 4.6 mm, 5 μm RP-18 50 x 4.6 mm, 3 μm	ACN/water containing 5.5 mmol L^{-1} of octanesulfonic acid in 0.085 % H_3PO_4 (25:75, *V/V*, %), 1 mL min^{-1}	UV	5[b]	49
Dichlorphenamide	U	HP Hypersil ODS 200 x 4.6 mm, 5 μm	0.05 mol L^{-1} NaH_2PO_4 (pH 3) containing propylamine hydrochloride and ACN, gradient	UV (230 and 275 nm)	1000[a]	29
Methazolamide	B, P	Altima C-18 250 x 4.6 mm, 5 μm	ACN: 0.1 mol L^{-1} CH_3COONa (pH 4.0), (20:80, *V:V*, %), 1 mL min^{-1}	UV (285 nm)	1000[b]	41
Methazolamide	U	Altima C-18 250 x 4.6 mm, 5 μm	ACN: 0.1 mol L^{-1} CH_3COONa (pH 4.0), (20:80, *V:V*, %), 1 mL min^{-1}	UV (285 nm)	100000[b]	41

P – plasma; U – urine; B – blood; ACN – acetonitrile; MS – mass detector
[a] LOD (limit of detection)
[b] LOQ (limit of quantification)

gated. In their extensive preliminary experiments, a set of column packing materials of different lengths and particle sizes were tested.

As shown in Tables V–VIII, most of the papers presented (5, 6, 10, 12, 14–16, 19, 21, 24, 28–30, 32, 37, 39–41, 45–48, 51, 53, 54, 60, 61, 64, 69, 72, 77, 78, 84, 96) describe reversed phase chromatography on 5 μm C18, C8 or CN columns, protected with appropriate precolumns, usually at ambient temperature, with isocratic elution.

On the other hand, Oertel *et al*. (91) report that for a direct measurement of drugs in serum or plasma, a drug has to be more lipophilic for better retention on the ADS (alkyl--diol-silica) column. These columns, in comparison with conventional silica-based reversed-phase materials, have a very long lifetime and are more robust.

Table VI. RPHPLC methods for the determination of loop-diuretics in biological samples

Drug	Sample	Stationary phase	Mobile phase	Detection	LOD[a]/LOQ[b] (ng mL^{-1})	Ref.
Bumetanide	U	ODS2, 120 x 4.6 mm, 5 μm	0.0550 mol L^{-1} SDS : propanol (92:8, *V/V*), 1 mL min^{-1}	FL, Ex/Em (270/430 nm)	1.4[a]	14
Bumetanide	U	ODS2, 120 x 4.6 mm, 5 μm	0.055 mol L^{-1} SDS : 1-propanol pH 3, (94:6, *V/V*), 1 mL min^{-1}	UV (274 nm)	32[a]	15
Bumetanide	U	HP Hypersil ODS 200 x 4.6 mm, 5 μm	0.05 mol L^{-1} NaH_2PO_4 (pH 3) containing propylamine hydrochloride and ACN, gradient	UV (230 and 275 nm)	1000[a]	29
Bumetanide	U	Bondclone ODS 300 x 3.9 mm, 10 μm	0.05 mol L^{-1} NaH_2PO_4 (pH 3) containing propylamine hydrochloride and ACN, gradient	UV (230 and 275 nm)	1000[a]	31
Bumetanide	P	LC-8-DB 75 x 4.6 mm, 3 μm	0.03 mmol L^{-1} Na_3PO_4 (pH 3) : ACN (200:125, *V/V*), 1 mL min^{-1}	FL, Ex/Em (340/440 nm)	0.1[a]	43
Bumetanide	P, U	Radial Pak C18 100 x 8 mm, 5 μm	MeOH : H_2O : glacial acetic acid (66:34:1, *V/V/V*), 1.2 mL min^{-1}	FL, Ex/Em (228/418 nm)	5[a]	21
Bumetanide	U	μBondapak C18	ACN : water mixture containing 5 mmol L^{-1} KH_2PO_4-K_2HPO_4 (pH 4), (50:50, *V/V*)	AMP (+1350 mV)	0.25[a]	58
Ethacrynic acid	U	ODS2, 120 x 4.6 mm, 5 μm	0.055 mol L^{-1} SDS : 1-propanol pH 3, (94:6, *V/V*), 1 mL min^{-1}	UV (274 nm)	40[a]	15
Ethacrynic acid	U	Spherisorb ODS-2 120 x 4.6 mm, 5 μm	MeOH : SDS (5:95, *V/V*), pH 6.9	UV	1000–5000[a]	5
Ethacrynic acid	U	HP Hypersil ODS 200 x 4.6 mm, 5 μm	0.05 mol L^{-1} NaH_2PO_4 (pH 3) containing propylamine hydrochloride and ACN, gradient	UV (230 and 275 nm)	5000[a]	34
Ethacrynic acid	P, U	Supelcosil LC-8 75 x 4.6 mm, 3 μm	0.05 mol L^{-1} H_3PO_4 : ACN (55:45, *V/V*), 1 mL/min	UV (235 nm)	50–150[a]	49
Ethacrynic acid	P	Spherisorb ODS2, 125 x 4.6 mm	0.2% H_3PO_4 : ACN : MeOH : THF (50:32:13:1.5, *V/V/V/V*), 0.9 mL min^{-1}	UV (275 nm)	20[b]	67

Table VI. continued

Drug	Sample	Stationary phase	Mobile phase	Detection	LOD[a]/LOQ[b] ($ng\ mL^{-1}$)	Ref.
Ethacrynic acid	U	Spherisorb ODS2, 125 x 4.6 mm,	0.2% H_3PO_4 : ACN : MeOH : THF (50:32:13:2, *V/V/V/V*), 0.8 mL min^{-1}	UV (275 nm)	20[b]	67
Ethacrynic acid	U	HP-LiChrospher RP 18, 125 x 4 mm, 5μm	ACN : acetate buffer (pH 4) gradient elution	UV (275 nm)	6[a]	68
Ethacrynic acid	U	HP-Hypersil ODS C18,	ACN : phosphate buffer (pH 3) gradient elution	UV		97
Furosemide	P	Nucleosil C18, 250 x 4.6 mm, 5 μm	ACN : 0.3 mol L^{-1} CH_3COONa, pH 5 (69:31, *V/V*), 1.5 mL min^{-1}	UV (280 nm)	30[b]	28
Furosemide	U	ODS2, 120 x 4.6 mm, 5 μm	0.055 mol L^{-1} SDS : 1-propanol pH 3, (94 : 6, *V/V*), 1 mL min^{-1}	UV (274 nm)	15.5[a]	15
Furosemide	U	Spherisorb ODS-2 120 x 4.6 mm, 5 μm	MeOH : SDS (5:95, *V/V*), pH 6.9	UV	1000–5000[a]	5
Furosemide	U	HP Hypersil ODS 200 x 4.6 mm, 5 μm	0.05 mol L^{-1} NaH_2PO_4 (pH 3) containing propylamine hydrochloride and ACN, gradient	UV (230 and 275 nm)	500[a]	29
Furosemide	U	Bondclone ODS 300 x 3.9 mm, 10 μm	0.05 mol L^{-1} NaH_2PO_4 (pH 3) containing propylamine hydrochloride and ACN, gradient	UV (230 and 275 nm)	500[a]	31
Furosemide	P	Nucleosil 100 C18 100 x 3 mm, 5 μm	ACN:0.125 mol L^{-1} SDS:0.01 mol L^{-1} $HClO_4$ (pH 2), (234.6:35:665, *w/w*), 0.6 mL min^{-1}	FL, Ex/Em (360/413 nm)	0.3[b]	32
Furosemide	U	Hypersil C18 100 x 4.6 mm, 5 μm	0.02 mol L^{-1} Brij 35 and 0.01 mol L^{-1} Na_2HPO_4, pH 3, 1 mL min^{-1}	FL, Ex/Em (235/320 nm)	30[a]	6
Furosemide	P	Shim-pack GLC-CN 150 x 6 mm, 5 μm	ACN : 20 mmol L^{-1} CH_3COONH_4, pH 7 (4:1, *V/V*), 1 mL min^{-1}	MS, APCI SIM (*m/z* 329.2)	10[a]	37
Furosemide	S, U	Nova-Pak C18 150 x 3.9 mm, 4 μm	ACN:0.08 mol L^{-1} H_3PO_4 gradient	FL, Ex/Em (233/389 nm)	5[a]	50

Table VI. continued

Drug	Sample	Stationary phase	Mobile phase	Detection	LOD[a]/LOQ[b] (ng mL^{-1})	Ref.
Furosemide	P, U	Cp Spherisorb ODS, 250 x 4.6 mm, 5 μm	ACN : 0.5% H_3PO_4, pH 2.1, gradient, 1.2 mL min^{-1}	FL, Ex/Em (345/405 nm)	7.100[b]	19
Furosemide	U	μBondapak C18 30 x 3.9 mm, 10 μm	ACN : water (40:60) containing 5 mmol L^{-1} KH_2PO_4/K_2HPO_4	EC (+1200 mV)	15[b]	38
Furosemide	S	CST: Guard-pak μBondapak C18, 10 μm, YMC Pack ODS 150 x 4.6 mm, 5 μm	MP1: 0.02 mol L^{-1} phosphate buffer pH 7 MP2: ACN MP3: 0.002 mol L^{-1} phosphate buffer: ACN (65:35, *V/V*) containing 15 mmol L^{-1} TBAB	UV (271 nm)	5[b]	90
Furosemide	P, U	μBondapak C18 150 x 3.9 mm, 10 μm	0.01 mol L^{-1} KH_2PO_4 : ACN (62:38, *V/V*), pH 3, 1.5 mL min^{-1}	FL, Ex/Em (225/389)	5[b]	44
Furosemide	U	Spherisorb C18 150 x 4.6 mm, 5 μm	MeOH : acetic acid (3%) (40:60, *V/V*), 0.75 mL min^{-1}	UV (280 nm)	0.75[b]	18
Furosemide	P	C18/SCX 250 x 4.6 mm, 5 μm	100 mmol L^{-1} CH_3COONa (pH 4): ACN, (67:33, *V/V*), 1 mL min^{-1}	UV (230 nm)	25[b]	27
Furosemide	P, U	LiChrosorb RP-18 150 x 4.6 mm, 5 μm	MeOH:0.02 mol L^{-1} phosphate buffer pH 3 (48:52, *V/V*), 1 mL min^{-1}	FL, Ex/Em (275/400)	10[a]	64
Furosemide	P	PRP-1 250 x 4.1 mm, 10 μm	3% H_3PO_4 : ACN (37:33, *V/V*), 1.4 mL min^{-1}	FL, Ex/Em (268/410)	1[a]	65
Furosemide	S, U	8MB C18	35% ethanol containing 5 mmol L^{-1} TBAP, pH 7.5, 1 mL min^{-1}	EC (0.9 V)	9[a]	17
Furosemide	U	μBondapack C18	ACN: water (70:30), 5 mmol L^{-1} KH_2PO_4/K_2HPO_4, pH 5.5	EC (+1300 mV)	15[a]	54
Furosemide	P	Kromasil 100 C18, 5 μm	ACN : 0.02 mol L^{-1} KH_2PO_4 (34:66, *V/V*), pH 3	FL, Ex/Em (268/410)	1[a]/3[b]	61
Torasemide	U	μBondapack C18	H_2O : ACN (80:20, *V/V*, pH 3)	EC (1300 mV)	8[b]	70

P – plasma; U – urine; B – blood; S – serum; MP – mobile phase; LC – liquid chromatography; CST – Column-switching technique; PRP – Polymeric Reversed Phase; SDS – sodium dodecyl sulfate; ACN – acetonitrile; ODS – octadecylsilane; FL – fluorescence detector; AMP – amperometric detector; EC – electrochemical detector

[a] LOD (limit of detection)

[b] LOQ (limit of quantification)

Table VII. RPHPLC methods for the determination of potassium sparing diuretics in biological samples

Drug	Sample	Stationary phase	Mobile phase	Detection	LOD[a]/LOQ[b] (ng mL^{-1})	Ref.
Amiloride	U	ODS2, 120 x 4.6 mm, 5 μm	0.055 mol L^{-1} SDS:propanol (92:8, *V/V*), 1 mL min^{-1}	FL, Ex/Em (270/430 nm)	9.7[a]	14
Amiloride	P, U	Waters C18, 160 x 5 mm, 10 μm	ACN : 4 mol L^{-1} acetic acid, pH 4.5, (12:88, *V/V*), 2 mL min^{-1}	UV (365 nm)	1[b]	4
Amiloride	P	Nucleosil C18, 250 x 4.6 mm, 5 μm	ACN : 0.3 mol L^{-1} CH_3COONa, pH 5 (69:31, *V/V*), 1.5 mL min^{-1}	UV (280 nm)	0.5[b]	28
Amiloride	U	ODS2, 120 x 4.6 mm, 5 μm	0.055 mol L^{-1} SDS: 1-propanol pH 3, (94 : 6, *V/V*), 1 mL min^{-1}	UV (274 nm)	136[a]	15
Amiloride	U	Spherisorb ODS-2 120 x 4.6 mm, 5 μm	MeOH : SDS (5:95, *V/V*), pH 6.9	UV	1000–5000[a]	5
Amiloride	U	HP Hypersil ODS 200 x 4.6 mm, 5 μm	0.05 mol L^{-1} NaH_2PO_4 (pH 3) containing propylamine hydrochloride and ACN, gradient	UV (230 and 275 nm)	5000[a]	29
Amiloride	P	Spherisorb ODSII 125 x 4.6 mm, 5 μm	ACN : MeOH : TEAP, pH 2.8 (10:9:100), 1.2 mL min^{-1}	FL, Ex/Em (368/415 nm)	0.5[a]	30
Amiloride	U	Bondclone ODS 300 x 3.9 mm, 10 μm	0.05 mol L^{-1} NaH_2PO_4 (pH 3) containing propylamine hydrochloride and ACN, gradient	UV (230 and 275 nm)	1500[a]	31
Amiloride	P	Nucleosil 100 C18 100 x 3 mm, 5 μm	ACN : 0.125 mol L^{-1} SDS:0.01 mol L^{-1} $HClO_4$ (pH 2), (234.6:35:665, *w/w*), 0.6 mL min^{-1}	FL, Ex/Em (360/413 nm)	0.03[b]	32
Amiloride	P	reversed phase C18	ACN : 0.4% acetic acid (pH 4.5), gradient, 1.2 mL min^{-1}	UV (360 nm)	1[b]	8
Amiloride	P, U	Nucleosil C18	MeOH : 0.1 mol L^{-1} $HClO_4$, (45:55, *V/V*)	FL, Ex/Em (286/418 nm)	0.5[a]	20

Table VII. continued

Drug	Sample	Stationary phase	Mobile phase	Detection	LOD[a]/LOQ[b] (ng mL^{-1})	Ref.
Amiloride	P, U	reversed phase C18	ACN : 0.5% TEA (pH 3), (11:89, *V/V*)	FL, Ex/Em (366/418 nm)	0.2[a]	42
Spironolactone	U	HP Hypersil ODS 200 x 4.6 mm, 5 μm	0.05 mol L^{-1} NaH_2PO_4 (pH 3) containing propylamine hydrochloride and ACN, gradient	UV (230 and 275 nm)	1000[a]	29
Spironolactone	U	Bondclone ODS 300 x 3.9 mm, 10 μm	0.05 mol L^{-1} NaH_2PO_4 (pH 3) containing propylamine hydrochloride and ACN, gradient	UV (230 and 275 nm)	1000[a]	31
Spironolactone	P	S5 ODS2 Kontron, 500 x 4.6 mm	ACN : H_3PO_4, pH 3.4 (89:11, *V/V*, %),	UV (245 nm)	10[b]	52
Spironolactone	S	Partisil Chrompak, 150 x 4.6 mm, 5 μm	diisopropil ether : MeOH (98.25:1.75, *V/V*, %), 2.2 mL min^{-1}	UV (240 nm)	5[a]	53
Triamterene	U	ODS2, 120 x 4.6 mm, 5 μm	0.055 mol L^{-1} SDS : propanol (92:8, *V/V*), 1 mL min^{-1}	FL, Ex/Em (270/430 nm)	1.7[a]	14
Triamterene	U	ODS2, 120 x 4.6 mm, 5 μm	0.055 mol L^{-1} SDS : 1-propanol pH 3 (94:6, V/V), 1 mL min^{-1}	UV (274 nm)	32[a]	15
Triamterene	U	Spherisorb ODS-2 120 x 4.6 mm, 5 μm	MeOH : SDS (5:95, *V/V*), pH 6.9	UV	1000–5000[a]	5
Triamterene	U	HP Hypersil ODS 200 x 4.6 mm, 5 μm	0.05 mol L^{-1} NaH_2PO_4 (pH 3) containing propylamine hydrochloride and ACN, gradient	UV (230 and 275 nm)	1000[a]	29
Triamterene	U	Bondclone ODS 300 x 3.9 mm, 10 μm	0.05 mol L^{-1} NaH_2PO_4 (pH 3) containing propylamine hydrochloride and ACN, gradient	UV (230 and 275 nm)	1000[a]	31
Triamterene	S, U	CST: SPE:LiChroCART Lichrospher RP 18 ADS, 250 x 4 mm, 25 μm; LiChroCART RP 18, 125 x 4 mm, 5 μm	MeOH-phosphate buffer (0.02 mol L^{-1}, pH 4), (38:62, *V/V*), 1 mL min^{-1}	UV (245 nm)	–	91

Table VII. continued

Drug	Sample	Stationary phase	Mobile phase	Detection	LOD[a]/LOQ[b] (ng mL^{-1})	Ref.
Triamterene	P, U	Nova-Pak C18 150 x 4.6 mm, 5 μm	phosphate buffer (pH 2.8) : ACN : MeOH (70:14:8, *V/V/V*), 0.8 mL min^{-1}	FL, Ex/Em (340/400 nm)	1[b]	12
Triamterene	P, U	Spherisorb NH_2	buffer	FL	1[a]	13
Triamterene	U	CST: SPE precolumn: Hypersil ODS C18, 20 x 2.1 mm, 30 μm; HP-LiChrospher RP 18 125 x 4 mm, 5 μm	ACN : phosphate buffer gradient elution	FL, Ex/Em (230/430 nm)		90
Triamterene	U	μBondapack C18	ACN : water (70:30), 5 mmol L^{-1} KH_2PO_4/K_2HPO_4, pH 5.5	EC (+1300 mV)	0.1[a]	54

P – plasma; U – urine; S – serum; ODS – octadecylsilane; SPE – solid phase extraction; ADS – alkyl diol silica; SDS – sodium dodecyl sulfate; ACN – acetonitrile; TEAP – tetraethylammonium phosphate; FL – fluorescence detector; EC – electrochemical detector

Mobile phase

To optimize the RPHPLC conditions, some authors (5, 14, 19, 32, 37, 38, 72, 93) investigated a series of aqueous mobile phases of different pH values in combination with different modifiers, including acetonitrile, methanol, propanol, tetrahydrofurane (THF) and triethylamine (TEA) with different volume fractions. Optimization of the mobile phase should take into account not only the retention of the compound to be analyzed, but also the retention of the matrix.

Mobile phases were usually a mixture of phosphate buffer of variable pH and an organic modifier such as MeOH or ACN (10, 12, 19, 24, 38, 39, 43, 44, 49–52, 54, 58, 61, 69, 70, 83, 87–93, 95–97). Sodium acetate, acetic acid, phosphoric acid or perchloric acid were also used as aqueous components. TEA is frequently added to conventional reversed--phase mobile phases to bind to the available silanols and reduce the tailing of basic compounds.

Some diuretics were eluted using mobile phases containing an ion-pairing reagent such as octanesulfonic acid, tetrabutylammonium bromide (TBAB), tetrabutylammonium phosphate (TBAP), tetraethylammonium phosphate (TEAP) or tetrabutylammonium hydroxide (TBAH) (17, 39, 48, 64, 65, 93). Okuda *et al*. (93) proposed the column-switching technique and reported that furosemide was enriched at the top of the analytical column by using ion-pairing chromatography with TBAB. Hsieh *et al*. (64) found that the LC

Table VIII. RPHPLC methods for the determination of thiazide diuretics in biological samples

Drug	Sample	Stationary phase	Mobile phase	Detection	LOD[a]/LOQ[b] (ng mL^{-1})	Ref.
Bendroflumethiazide	U	ODS2, 120 x 4.6 mm, 5 μm	0.055 mol L^{-1} SDS : propanol (92:8, *V/V*), 1 mL min^{-1}	FL, Ex/Em (270/430 nm)	10[a]	14
Bendroflumethiazide	U	ODS2, 120 x 4.6 mm, 5 μm	0.055 mol L^{-1} SDS : 1-propanol pH 3, (94:6, *V/V*), 1 mL min^{-1}	UV (274 nm)	16[a]	15
Bendroflumethiazide	U	Spherisorb ODS-2 120 x 4.6 mm, 5 μm	MeOH : SDS (5:95, *V/V*), pH 6.9	UV	1000–5000[a]	5
Bendroflumethiazide	U	HP Hypersil ODS 200 x 4.6 mm, 5 μm	0.05 mol L^{-1} NaH_2PO_4 (pH 3) containing propylamine hydrochloride and ACN, gradient	UV (230 and 275 nm)	1000[a]	29
Bendroflumethiazide	U	Bondclone ODS 300 x 3.9 mm, 10 μm	0.05 mol L^{-1} NaH_2PO_4 (pH 3) containing propylamine hydrochloride and ACN, gradient	UV (230 and 275 nm)	1000[a]	31
Bendroflumethiazide	U	Kromasil C18 125 x 4.6 mm, 5 μm	0.05 mol L^{-1} SDS, 0.01 mol L^{-1} NaH_2PO_4, H_2), pH 3	UV (274 nm)	330[b]	84
Benzthiazide	U	ODS2, 120 x 4.6 mm, 5 μm	0.055 mol L^{-1} SDS : 1-propanol pH 3, (94:6, *V/V*), 1 mL min^{-1}	UV (274 nm)	60[a]	15
Benzthiazide	U	HP Hypersil ODS 200 x 4.6 mm, 5μm	0.05 mol L^{-1} NaH_2PO_4 (pH 3) containing propylamine hydrochloride and ACN, gradient	UV (230 and 275 nm)	1000[a]	29
Benzthiazide	U	Bondclone ODS 300 x 3.9 mm, 10 μm	0.05 mol L^{-1} NaH_2PO_4 (pH 3) containing propylamine hydrochloride and ACN, gradient	UV (230 and 275 nm)	1000[a]	31
Chlorothiazide	U	HP Hypersil ODS 200 x 4.6 mm, 5 μm	0.05 mol L^{-1} NaH_2PO_4 (pH 3) containing propylamine hydrochloride and ACN, gradient	UV (230 and 275 nm)	1000[a]	29
Chlorothiazide	U	Bondclone ODS 300 x 3.9 mm, 10 μm	0.05 mol L^{-1} NaH_2PO_4 (pH 3) containing propylamine hydrochloride and ACN, gradient	UV (230 and 275 nm)	1000[a]	31

Table VIII. coninued

Drug	Sample	Stationary phase	Mobile phase	Detection	LOD[a]/LOQ[b] (ng mL^{-1})	Ref.
Chlortalidone	U	ODS2, 120 x 4.6 mm, 5 μm	0.055 mol L^{-1} SDS : 1-propanol, pH 3, (94:6, *V/V*), 1 mL min^{-1}	UV (274 nm)	127[a]	15
Chlortalidone	U	Spherisorb ODS-2 120 x 4.6 mm, 5 μm	MeOH : SDS (5:95, *V/V*), pH 6.9	UV (224 nm)	1000–5000[a]	5
Chlortalidone	U	HP Hypersil ODS 200 x 4.6 mm, 5 μm	0.05 mol L^{-1} NaH_2PO_4 (pH 3) containing propylamine hydrochloride and ACN, gradient	UV (230 and 275 nm)	1000[a]	29
Chlortalidone	U	Bondclone ODS 300 x 3.9 mm, 10 μm	0.05 mol L^{-1} NaH_2PO_4 (pH 3) containing propylamine hydrochloride and ACN, gradient	UV (230 and 275 nm)	1500[a]	31
Chlortalidone	B	μBondapack CN 300 x 3.9 mm, 10 μm	THF : ACN : H_2O containing 10 mmol L^{-1} dibutylamine phosphate pH 5, (2:0.5:97.5, *V/V*), 2.5 mL min^{-1}	UV (214 nm)	200[b]	22
Chlortalidone	P	Supelcosil LC-18 250 x 4 mm, 5 μm	0.05 mol L^{-1} SDS in phosphate buffer (pH 5.8)-*n*-propanol (95:5, *V/V*), 1.3 mL min^{-1}	UV (225 nm)	10[b]	46
Chlortalidone	U	CST: SPE precolumn: Stable Bond-CN 125 x 4 mm, 5 μm; C 18 Ultrasphere 250 x 4.6 mm, 5 μm	ACN : 0.01 mol L^{-1} phosphate buffer pH 7 (28:80, *V/V*), 2 mL min^{-1}	UV (214 nm)	20[a]	82
Cyclothiazide	U	HP Hypersil ODS 200 x 4.6 mm, 5 μm	0.05 mol L^{-1} NaH_2PO_4 (pH 3) containing propylamine hydrochloride and ACN, gradient	UV (230 and 275 nm)	1000[a]	29
Cyclothiazide	U	Bondclone ODS 300 x 3.9 mm, 10 μm	0.05 mol L^{-1} NaH_2PO_4 (pH 3) containing propylamine hydrochloride and ACN, gradient	UV (230 and 275 nm)	1000[a]	31
Hydrochlorothiazide	U	Spherisorb ODS-2 120 x 4.6 mm, 5 μm	MeOH : SDS (5:95, *V/V*), pH 6.9	UV	1000–5000[a]	5

Table VIII. coninued

Drug	Sample	Stationary phase	Mobile phase	Detection	LOD[a]/LOQ[b] (ng mL^{-1})	Ref.
Hydrochlorothiazide	U	HP Hypersil ODS 200 x 4.6 mm, 5 μm	0.05 mol L^{-1} NaH_2PO_4 (pH 3) containing propylamine hydrochloride and ACN, gradient	UV (230 and 275 nm)	500[a]	29
Hydrochlorothiazide	P	Spherisorb ODSII 125 x 4.6 mm, 5 μm	ACN : MeOH : TEAP, pH 2.8 (10:9:100), 1.2 mL min^{-1}	UV (271 nm)	10[a]	30
Hydrochlorothiazide	U	Bondclone ODS 300 x 3.9 mm, 10 μm	0.05 mol L^{-1} NaH_2PO_4 (pH 3) containing propylamine hydrochloride and ACN, gradient	UV (230 and 275 nm)	500[a]	31
Hydrochlorothiazide	P, U	Beckman Octyl Ultrasphere 250 x 4.6 mm, 5 μm	12% (*V/V*) ACN : 88% of either an ion-pairing reagent (plasma) or 0.1% CF_3COOH (urine)	UV (271 nm)	2[b] (P) 100[b] (U)	64
Hydrochlorothiazide	S	LiChroCART RP 18e 125 x 4 mm, 5 μm	phosphate buffer 7.5 mmol L^{-1} (pH 7.3) : ACN (90:10, *V/V*)	EC (+630 mV)	5000[b]	51
Hydrochlorothiazide	U	Hypersil C 18 100 x 4.6 mm, 5 μm	0.02 mol L^{-1} brij 35, 0.004 mol L^{-1} SDS, 0.01 mol L^{-1} Na_2HPO_4, pH 6.5, 1 mL min^{-1}	UV (271 nm)	280[a]	6
Hydrochlorothiazide	P	Supelcosyl C18 150 x 4.6 mm, 5 μm	10 mmol L^{-1} KH_2PO_4 : MeOH : ACN, pH 2.5 (5:80:15, *V/V/V*), 1 mL min^{-1}	UV (275 nm)	2980[b]	39
Hydrochlorothiazide	U	Hypersil C 18 150 x 2 mm, 5 μm	% CH_3COOH : ACN, pH 3 (97:3, *V/V*) 0.3 mL min^{-1}	UV (272 nm)	2000[a]	16
Hydrochlorothiazide	P	Hibar Lichrospher 100 RP 8, 250 x 4 mm, 5 μm	25 mmol L^{-1} phosphate buffer (pH 5 with TEA) : ACN (85:15, *V/V*), 1.2 mL min^{-1}	UV (230 nm)	10[b]	72
Hydrochlorothiazide	S, P	RP C18	20% ACN in 0.01 mol L^{-1} phosphate buffer with 0.05 mol L^{-1} TBAH (pH 7.5)	UV (272 nm)	10[a]	66
Hydrochlorothiazide	S	RP C18	0.01mol L^{-1} KH_2PO_4 : ACN (65:35, *V/V*) pH 3.1	UV (232 nm)	15[b]	97
Hydrochlorothiazide	P	Supelcosyl C18 150 x 4.6 mm, 5 μm	10 mmol L^{-1} KH_2PO_4 : MeOH : ACN (2:80:18, *V/V/V*), pH 2.5, 1 mL min^{-1}	UV (260 nm)	6.75[b]	24

Table VIII. coninued

Drug	Sample	Stationary phase	Mobile phase	Detection	LOD[a]/LOQ[b] (ng mL^{-1})	Ref.
Hydroflumethiazide	U	ODS2, 120 x 4.6 mm, 5 μm	0.055 mol L^{-1} SDS : propanol (92:8, *V/V*), 1 mL min^{-1}	FL, Ex/Em (270/430 nm)	7.1[a]	14
Hydroflumethiazide	U	HP Hypersil ODS 200 x 4.6 mm, 5 μm	0.05 mol L^{-1} NaH_2PO_4 (pH 3) containing propylamine hydrochloride and ACN, gradient	UV (230 and 275 nm)	500[a]	14
Indapamide	U	μBondapak C18	ACN : water mixture (45:55, 5 mmol L^{-1}) in KH_2PO_4-K_2HPO_4 (pH 4)	AMP (+1200 mV)	1[b]	58
Indapamide	S	ODS 5 μm	ACN : CH_3COONa (pH 3.72), (45:55, *V/V*), 1.2 mL min^{-1}	UV	40[b]	40
Indapamide	B	Nucleosil C18, 5 μm	80 mmol L^{-1} CH_3COONH_4:ACN : 2-propanol (65:30:5, *V/V/V*)	UV	10[b]	45
Indapamide	B	YMC (R) ODS – A 150 x 4.6 mm, 5 μm	ACN : 2-propanol : 0.1% (*V/V*) TEA in water (35:5:60, *V/V/V*)	UV	5[b]	63
Indapamide	S	Supelcosyl LC-8-DB, 250 x 4.6 mm, 5 μm	0.1% (*V/V*) TEA in water (pH 3.5) : ACN (63:37, *V/V*),	UV (240 nm)	10[b]	78
Indapamide	B	Supelcosyl LC-8-DB, 250 x 4.6 mm, 5 μm	0.1% (*V/V*) TEA in water (pH 3.5) : ACN (63:37, *V/V*),	UV (240 nm)	50[b]	78
Methyclothiazide	U	HP Hypersil ODS 200 x 4.6 mm, 5 μm	0.05 mol L^{-1} NaH_2PO_4 (pH 3) containing propylamine hydrochloride and ACN, gradient	UV (230 and 275 nm)	500[a]	29
Metolazone	U	HP Hypersil ODS 200 x 4.6 mm, 5 μm	0.05 mol L^{-1} NaH_2PO_4 (pH 3) containing propylamine hydrochloride and ACN, gradient	UV (230 and 275 nm)	1000[a]	29
Metolazone	B, P	CST: C_2 ethyl sorbent, Spherisorb ODS C18, 100x4.6 mm, 3 μm	K_2HPO_4 (pH 3) : ACN (73:30, *V/V*), 1 mL min^{-1}	FL, Ex/Em (235/410 nm)	1[b]	94

Table VIII. coninued

Drug	Sample	Stationary phase	Mobile phase	Detection	LOD[a]/LOQ[b] (ng mL^{-1})	Ref.
Metolazone	U	Nucleosil C18 150 x 4.6 mm, 5 μm	K_2HPO_4 (pH 3) : ACN (65:35, *V/V*), 1 mL min^{-1}	FL, Ex/Em (240/450 nm)	4.2[a]	10
Polythiazide	U	HP Hypersil ODS 200 x 4.6 mm, 5 μm	0.05 mol L^{-1} NaH_2PO_4 (pH 3) containing propylamine hydrochloride and ACN, gradient	UV (230 and 275 nm)	500[a]	29
Polythiazide	P	μBondapak CN	chloroform : MeOH (97:3, *V/V*, %)	UV (264 nm)	0.5[b]	68
Quinethazone	U	HP Hypersil ODS 200 x 4.6 mm, 5 μm	0.05 mol L^{-1} NaH_2PO_4 (pH 3) containing propylamine hydrochloride and ACN, gradient	UV (230 and 275 nm)	1500[a]	29
Trichlormethiazide	U	ODS2, 120 x 4.6 mm, 5 μm	0.055 mol L^{-1} SDS : 1-propanol pH 3, (94:6, *V/V*), 1 mL min^{-1}	UV (274 nm)	73[a]	15
Trichlormethiazide	U	HP Hypersil ODS 200 x 4.6 mm, 5 μm	0.05 mol L^{-1} NaH_2PO_4 (pH 3) containing propylamine hydrochloride and ACN, gradient	UV (230 and 275 nm)	1000[a]	29
Trichlormethiazide	U	Bondclone ODS 300 x 3.9 mm, 10 μm	0.05 mol L^{-1} NaH_2PO_4 (pH 3) containing propylamine hydrochloride and ACN, gradient	UV (230 and 275 nm)	1000[a]	31

P – plasma; U – urine; B – blood; S – serum; ODS – octadecylsilane; RP – reversed phase; SDS – sodium dodecyl sulfate; ACN – acetonitrile; TEAP – tetraethylammonium phosphate; TEA – triethylamine; TBAH – tetrabutylammonium hydroxide; FL – fluorescence detector; AMP – amperometric detector; EC – electrochemical detector

conditions must be changed for the assay in plasma, and the mobile phase utilized in the urine assay must be replaced with a mobile phase containing ACN and an ion-pairing reagent (tetramethylammonium chloride) in order to separate all plasma endogenous impurities from hydrochlorothiazide and internal standard.

Gradient elution was applied because of the wide variety of chemical structures, functional groups, polarity of the compounds or to improve the separation if several diuretics had to be detected (19, 29, 31, 50, 60, 68, 90, 96, 98).

As mentioned in the previous section, some authors proposed reverse-phase micellar liquid chromatographic procedures for simultaneous determination of some diuretics using a mobile phase containing SDS or polyoxyethylene 23 lauryl ether (5, 6, 14, 15, 23, 32, 46, 83, 84, 99–101). Carda-Broch *et al.* (15) used a three-factor interpretive optimization strategy to develop an isocratic chromatographic procedure for screening a mix-

ture of 15 diuretics using micellar-organic mobile phases of SDS and 1-propanol. They reported that good peak symmetry of weakly acidic or basic diuretics is only obtained in conventional reverse-phase LC if an organic competing amine or an ammonium salt is added to the acidic eluent. On the other hand, the surfactant coverage of the column makes the addition of amines less necessary, since sufficiently well shaped peaks are obtained in the acidic micellar mobile phase. Also, the range of elution strengths required to elute hydrophobic and hydrophilic compounds is narrower, which allows isocratic separation of the diuretics in a reasonable analysis time. Love and Fett (6) optimized selectivity in micellar chromatographic procedures through changes in specific mobile phase parameters. They found that retention of the urine matrix could be best minimized with the use of the non-ionic surfactant (Brij 35) in mobile phases adjusted between pH 5.5 and 7.5. They proposed that proper control of the parameters (type of surfactant, pH) offers a systematic and generalized approach for the development of an accurate and precise RPHPLC method for determination of structurally different drugs in urine.

Detection

As can be seen from Tables V–VIII, diuretics were usually determined using an ultraviolet detector for RPHPLC or an electrochemical detector for IPC. Using fluorescence or electrochemical detection, a lower concentration of the compound of interest can be detected. On the other hand, monitoring the eluates by connecting UV and fluorometric detectors in a series provided the immediate advantage of having two or more drugs detected simultaneously (6, 11, 28, 30). RPHPLC methods presented in Tables V–VIII, utilizing these three modes of detection, are sufficiently sensitive and can therefore be used for pharmacokinetic purposes or for conducting bioequivalence studies.

Constanzer *et al*. (47) report that utilization of tandem mass spectrometric instead of UV detection for the RPHPLC determination of dorzolamide in human plasma simplified chromatography and led to a significant improvement in assay sensitivity and speed of analysis.

Concentration of drugs in the sample may be a limiting factor in drug determination. Therefore, samples containing low levels of drugs can be confirmed by preconcentrating the sample and analyzing it by liquid chromatography-mass spectrometry (LC-MS) (37), LC-MS/MS (33, 34, 47, 62) or gas chromatography-mass spectrometry (GC-MS) (29, 31, 49, 50, 102–104). Namely, these techniques detect low levels of abused drugs in urine and could be very helpful in some doping control analyses of urine collected more than 24 hours after intake.

Methods presented in this paper have been validated for the determination of different diuretics by evaluating their selectivity, linearity, precision and accuracy. Also, some of them were used for determination of certain diuretics in the plasma, serum or blood samples obtained from healthy volunteers who participated in bioequivalence studies.

CONCLUSIONS

The paper describes high-performance liquid chromatographic procedures for quantification of different diuretics in biological fluids (plasma, serum, blood and urine).

The biomedical importance of the published procedures, different methods for sample preparation and chromatographic parameters are discussed. Information about the methods for sample preparation and values of analytical recovery of each procedure are summarized in four tables. Basic information about the stationary and mobile phases, detection mode and sensitivity of reviewed methods is given in additional tables. They are arranged according to the groups of diuretics.

Acronyms. – ACN – acetonitrile; ADS – alkyl diol silica; AMP – amperometric detection; APCI – atmospheric pressure chemical ionization; B – blood; BE – back extraction; CN – cyano; CST – column-switching technique; D – dilution; DI – direct injection; EC – electrochemical detector; EtAc – ethyl acetate; FL – fluorescence detector; GC – gas chromatography; GC-MS – gas chromatography-mass spectrometry; HPLC – high-performance liquid chromatography; RPHPLC – reverse phase high-performance liquid chromatography; LC – liquid chromatography; LC-MS – liquid chromatography-mass spectrometry; LLE – liquid liquid extraction; MeOH – methanol; MP – mobile phase; MS – mass detector; ODS – octadecylsilane; P – plasma; PRE – precipitation; PRP – polymeric reversed phase; R – recovery; RP – reversed phase; S – serum; SCX – strong cation exchange; SDS – sodium dodecyl sulfate; SIM – single ion monitoring; SPE – solid phase extraction; TBAH – tetrabutylammonium hydroxide; TBAB – tetrabutylammonium bromide; TBAP – tetrabutylammonium phosphate; TEA – triethylamine; TEAP – tetraethylammonium phosphate; THF – tetrahydrofuran; U – urine.

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S A Ž E T A K

Priprava uzoraka i određivanje diuretika u tjelesnim tekućinama čovjeka pomoću RPHPLC

DRAGICA ZENDELOVSKA i TRAJČE STAFILOV

U članku se opisuje metoda određivanja diuretika u različitim tjelesnim tekućinama čovjeka (punoj krvi, plazmi, serumu ili urinu) inverzno-faznom visokotlačnom tekućinskom kromatografijom (RPHPLC). Priprava uzoraka uključuje postupke čvrsto-tekuće ili tekuće-tekuće ekstrakcije, razrijeđivanje, taloženje i automatizirani RPHPLC postupak. Diskutiraju se prednosti i nedostaci različitih priprava uzoraka. Sažete su vrijednosti za analitički povrat svakog pojedinog postupka. Također se sumiraju i diskutiraju najvažniji RPHPLC parametri (način detekcije, stacionarna faza, mobilna faza, osjetljivost, itd.).

Ključne riječi: diuretici, RPHPLC, određivanje tjelesne tekućine

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