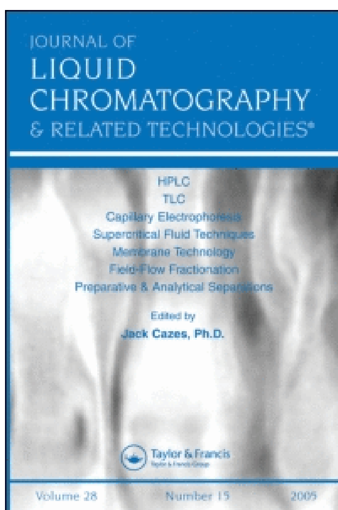
This article was downloaded by:
On: *24 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Analysis of Diuretics in Urine by Column-Switching Chromatography and Fluorescence Detection

Pilar Campíns-Falcó[a]; Rosa Herráez-Hernández[a]; Maria Dolores Pastor-Navarro[a]
[a] Department of Analytical Chemistry, University of Valencia, Burjassot, València, Spain

To cite this Article Campíns-Falcó, Pilar , Herráez-Hernández, Rosa and Pastor-Navarro, Maria Dolores(1997) 'Analysis of Diuretics in Urine by Column-Switching Chromatography and Fluorescence Detection', Journal of Liquid Chromatography & Related Technologies, 20: 12, 1867 — 1885

To link to this Article: DOI: 10.1080/10826079708005549

URL: http://dx.doi.org/10.1080/10826079708005549

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of this material.

J. LIQ. CHROM. & REL. TECHNOL., 20(12), 1867–1885 (1997)

ANALYSIS OF DIURETICS IN URINE BY COLUMN-SWITCHING CHROMATOGRAPHY AND FLUORESCENCE DETECTION

Pilar Campíns-Falcó,* Rosa Herráez-Hernández,
Maria Dolores Pastor-Navarro

Department of Analytical Chemistry
University of Valencia
Dr. Moliner 50
46100 Burjassot (València) Spain

Downloaded At: 11:59 24 January 2011

ABSTRACT

The potential of column-switching chromatography and fluorescence detection for the analysis of diuretics in urine is evaluated. Sample cleanup and chromatographic parameters have been optimized to achieve maximum sensitivity for the detection and quantification of some relevant diuretics. On the basis of these studies, an on-line procedure for the simultaneous determination of amiloride, furosemide, bumetanide and triamterene is presented. The linearity, precision, accuracy and sensitivity of the method are discussed. The utility of the described approach has been tested by analysing urine samples obtained after administration of bumetanide.

Copyright © 1997 by Marcel Dekker, Inc.

INTRODUCTION

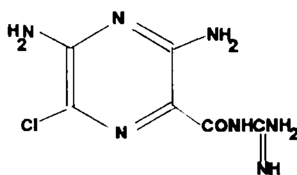
The rapid and sensitive identification and quantification of diuretics in biological fluids are often required in therapeutic drug monitoring and in doping control tests. In this respect, Gas Chromatography (GC) coupled to Mass-Spectroscopy (MS) is the most reliable technique for diuretics caracterization.[1,2] However, owing to the time and cost involved when using the GC-MS approach, Liquid Chromatography (LC) is the method of choice for the analysis of diuretics.[3] Moreover, recent technological advances in LC have resulted in sophisticated systems which allow the detection of these drugs at ng/ml levels.[4] In contrast, the advances in the area of sample preparation (including cleanup and preconcentration of the analytes) have been more modest, and many analytical procedures still involve time-consuming manual methods. Whereas separation and identification of the interesting compounds can require a few minutes, sample preparation time can be one or even two orders of magnitude longer.[5] Therefore, the development of sample conditioning procedures that can reduce analysis times and improve sample throughput is an area of major interest.

In the last years, column-switching chromatography has gained popularity in the context of sample preparation,[6] because switching devices allow the selective retention of the analytes in a primary column (or precolumn), whereas, matrix components are flushed-out; the enriched analytes are subsequently transferred to an analytical column, where they are separated and detected. Although, different configurations may be required depending on the polarity of the analyte, satisfactory recoveries can be obtained for most diuretics by using precolumns packed with apolar stationary phases (usually C_{18}).[7] Moreover, by selecting an appropriate switching device, additional dispersion of the analytes into the chromatographic system is minimized, so the sensitivity achieved is comparable to that obtained under a conventional approach. Successful applications of column-switching chromatography and UV detection to the analysis of diuretics have already been reported, the limits of detection being typically in the 0.002 - 2.0 μg/ml range.[7,8] Unfortunately, the analysis of these compounds by switching techniques is limited by the presence of apolar matrix components, which are also trapped in the precolumn. Therefore, more selective and sensitive detection may be required for the determination of some diuretics at therapeutical levels.[9]

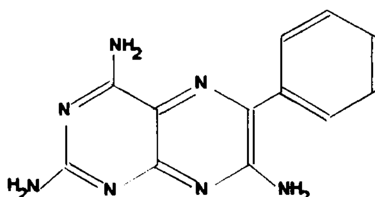
This work was aimed to the evaluation of column-switching, in combination with fluorescence detection, for improving the selectivity and sensitivity in the analysis of diuretics. Experimental conditions have been optimized for the detection and identification of some native fluorescent diuretics (Figure 1).

Downloaded At: 11:59 24 January 2011

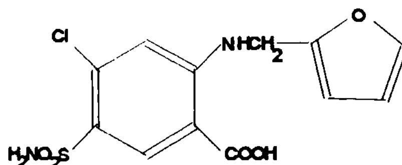
Amiloride



Triamterene



Furosemide



Bumetanide

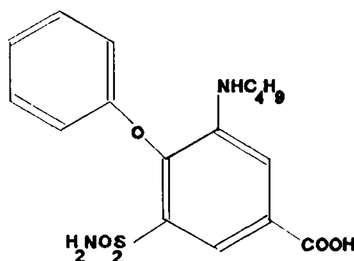


Figure 1. Chemical structures of the diuretics assayed.

A simple and rapid method for the simultaneous determination of amiloride, triamterene, furosemide and bumetanide is described. The usefulness of the described approach has been tested by determining bumetanide in real samples.

Downloaded At: 11:59 24 January 2011

MATERIALS

Apparatus

The chromatographic system used consisted of two quaternary pumps (Hewlett-Packard, 1050 Series, Palo Alto, CA, USA), an automatic sample injector (Hewlett-Packard, 1050 Series) with a sample loop injector of 100 μL, and a high pressure six-port valve (Rheodyne model 7000). For detection a fluorescence detector (Hewlett-Packard, 1046 Series) and a diode-array detector (Hewlett-Packard, 1040 series) were coupled in series. The detectors were linked to a data system (Hewlett-Packard, HPLC Chem. Station) for data acquisition and storage. The precolumn and the analytical column have been combined by means of a switching arrangement in a back-flush configuration. A schematic set-up has been published previously.[7] All assays were carried out at ambient temperature.

Downloaded At: 11:59 24 January 2011

Reagents

All the reagents were of analytical grade. Acetonitrile and methanol (Scharlau, Barcelona, Spain) were of HPLC grade. Water was distilled, deionized and filtered in 0.45 μm nylon membranes (Teknokroma, Barcelona, Spain). Amiloride hydrochloride was obtained from ICI-Pharma (Pontevedra, Spain), triamterene from Sigma (St. Louis, MO, USA), furosemide from Lasa (Barcelona, Spain) and bumetanide from Boehringer Ingelheim (Barcelona, Spain). Sodium dihydrogen phosphate monohydrate (Merk, Darmstadt, Germany), propylamine hydrochloride (Fluka, Busch, Switzerland) and phosphoric acid (Probus, Barcelona, Spain) were also used.

METHODS

Preparation of Solutions

Stock standard solutions of the diuretics (at a concentration of 2000 μg/mL for amiloride, furosemide and bumetanide, and at a concentration of 400 μg/mL for triamterene) were prepared by dissolving the pure compounds in methanol. Working solutions were prepared by dilution of the stock solutions with water. All solutions were stored in the dark at 2°C.

The phosphate buffer solutions were prepared by dissolving the appropriate amount of sodium dihydrogen phosphate monohydrate in 500 mL of water containing 0.7 mL of propylamine hydrochloride. The pH was then adjusted by adding the minimum amount of concentrated phosphoric acid.

Columns and Mobile-Phases

The precolumn (20 mm x 2.1 mm I. D.) was dry-packed with a Hypersil ODS-C_{18}, 30 μm stationary phase. A LiChrospher 100 RP 18, 125 mm x 4 mm I. D., 5 μm column (Merck, Darmstadt, Germany) or a Hypersil ODS-C_{18}, 250 mm x 4 mm I. D., 5 μm column (Hewlett-Packard) were used as an analytical column. A 0.05 M phosphate buffer of pH 3 was used as washing solvent to eliminate matrix components from the precolumn. Different phosphate buffer-acetonitrile mixtures (at a flow rate of 1.0 mL/min) were used as a mobile-phase; the ionic strength and pH of the buffers were varied from 0.001 to 0.1 M, and from 3.0 to 5.0, respectively. Solvents were filtered with 0.45 μm nylon membranes (Teknokroma) and degassed with helium before use.

Column-Switching Operation

At the beginning of each assay 5 μL of the samples were injected into the precolumn. Matrix components were flushed-out of the precolumn by pumping 0.05 M phosphate buffer (pH = 3) at a flow rate of 1.0 mL/min. Simultaneously, the analytical column was being reequilibrated with the mobile-phase. At t = 1.0 min, the switching valve was rotated, so the precolumn was incorporated into the flow-scheme of the analytical column. At t = 9.0 min, the valve was turned back to the original position to regenerate and requilibrate both, the precolumn and the analytical column. Rotation of the valve was manually performed.

Recovery Studies

Blank urine samples were spiked with the stock solutions of the diuretics, reproducing different concentrations in their respective therapeutical intervals.[10] The percentage of drug recovered was calculated by comparing the peak areas obtained for each compound in the spiked samples, with those obtained for direct injections of 5.0 μL of standards containing the same concentration of diuretic. Each concentration was assayed in triplicate.

Downloaded At: 11:59 24 January 2011

Preparation of Standards for Calibration

Untreated urine (plasma in some instances) samples were spiked with the appropriate volumes of the stock solutions of the diuretics, placed in injection glass vials and processed as described above. Peak areas obtained for each compound were plotted versus analyte concentration, and the resulting calibration curves were used to calculate the concentration of the diuretics in unknown samples. Each concentration was assayed in triplicate.

Human Studies

Urinary excretion studies were performed with a human volunteer after a single dose administration of bumetanide (5.0 mg). Urine samples were collected at appropriate time intervals post-dose, and analyzed as described above. Each sample was assayed in triplicate.

Downloaded At: 11:59 24 January 2011

RESULTS AND DISCUSSION

Chromatographic Separation and Detection of Diuretics

Initially, we investigated the chromatographic conditions necessary to achieve a satisfactory resolution of the compounds of interest by direct injection of aqueous standard solutions of the diuretics into the analytical column. Two different columns (LiChrospher 100 RP C_{18}, 125 mm x 4 mm I. D., 5 μm, and Hypersil ODS-C_{18}, 250 mm x 4 mm I. D., 5μm) in combination with different phosphate buffer/acetonitrile mixtures were tested. Best resolution of the diuretics assayed in a short time was obtained when using the LiChrospher column and a 0.05 M phosphate buffer of pH 3.0 as the aqueous component of the mobile-phase.

According to previous studies,[7] the precolumn was packed with a Hypersil C_{18}, 30 μm stationary phase for trapping the diuretics. In order to minimize base line drifts in the chromatograms, a 0.05 M phosphate buffer of pH 3 was also selected as washing solvent for flushing the precolumn during the cleanup stage. The fluorescence detector was programmed as listed in Table 1 (the UV signal was monitored at 254 nm). Table 1 summarizes the conditions finally selected for analysis of diuretics in urine.

Downloaded At: 11:59 24 January 2011

Table 1

Time Schedule and Conditions Used in the Determination of Diuretics in Urine

Sample injection (t = 0 min)	5 μL of untreated urine			
Sample cleanup	precolumn: 20 mm x 2.1 mm I.D., packed with a Hypersil, ODS-C18, 30 μm phase washing solvent: 0.05 M phosphate buffer (pH = 3) at a flow-rate of 1.0 mL/min duration of the flushing: 1 min			
(valve rotation) (t = 1 min)				
Analytical separation	column: 125 mm x 4 mm I.D. Lichrospher RP 18, 5 μm mobile phase: 0.05 M phosphate buffer pH = 3)/acetonitrile, at a low-rate of 1.0 mL/min at 1 min: 100% phosphate buffer at 8 -m 9 min: 40% phosphate buffer/60% acetonitrile			
Detection wavelengths		$\lambda_{excitation}$ (nm)	$\lambda_{emission}$(nm)	
	amiloride:	286	418	(t = 0 - 4.9 min)
	triamterene:	365	440	(t = 4..9 - 6.0 min)
	furosemide:	233	389	(t = 6.0 - 8.0 min)
	bumetanide:	228	418	(t = 8.0 - 9.0 min)

(continued)

Downloaded At: 11:59 24 January 2011

Table 1 (continued)

Time Schedule and Conditions Used in the Determination of Diuretics in Urine

End of the run (valve rotation) (t = 9.0 min)	
Reequilibration (t = 9.0 - 11.0 min)	precolumn and analytical column: 0.05 M phosphate buffer (pH = 3) at a flow-rate of 1.0 mL/min

Downloaded At: 11:59 24 January 2011

Table 2

Analytical Data for Diuretics in Urine

Diuretic	Conc'n. Interval	Recovery* (n = 3)	Linearity (n = 10)		Intra-day Precision* (n = 8)	Inter-day Precision* (n = 6)
	(μg/mL)	(%)	A = a + bC	r	(%)	(%)
Amiloride	2.5 - 25.0	91 ± 1	a = 1.5 ± 0.3 b - 1.24 ± 0.01	0.9996	4	7
Triamterene	1.0 - 20.0	97 ± 3	a = -5 ± 5 b = 11.7 ± 0.2	0.998	4	5
Furosemide	5 - 100	93 ± 2	a = -2 ± 5 b = 1.66 ± 0.05	0.997	3	10
Bumetanide	0.10 - 3.50	106 ± 5	a = 0 ± 2 b = 12.5 ± 0.5	0.996	3	5

*Determined at half of concentration in tested range.

Analysis of Urine Samples

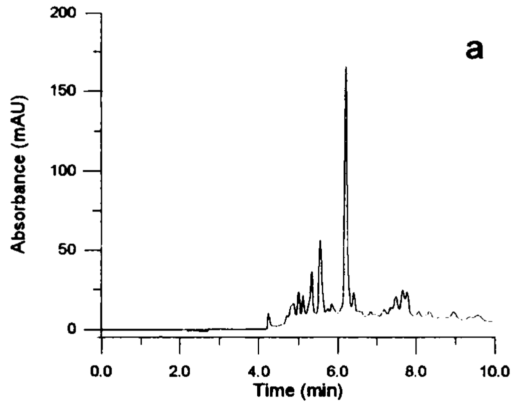
Selectivity

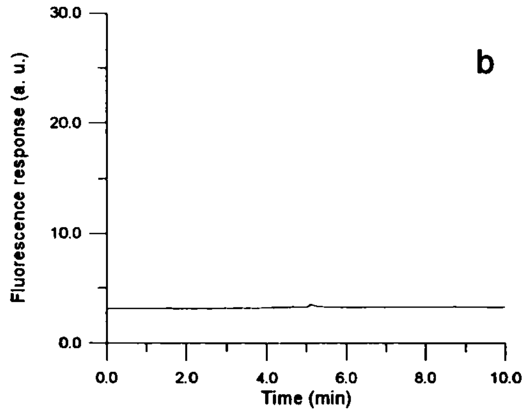
UV chromatograms showed that a vast majority of the urinary endogenous compounds were flushed-out from the precolumn with the first 1.0 mL fraction eluting from the precolumn. However, several apolar components can not be eliminated, even when flushing the precolumn with up to 10.0 mL of washing solvent. Phosphate buffers of different pH and water were also tested as washing solvents, but no significant improvements in selectivity were observed. In most instances, interferences due to the apolar matrix components can be avoided by optimizing the gradient elution profile. However, matrix components significantly increase background noise; in addition, recoveries for some diuretics are overestimated due to the contribution of the matrix to the analytical signal.[6]

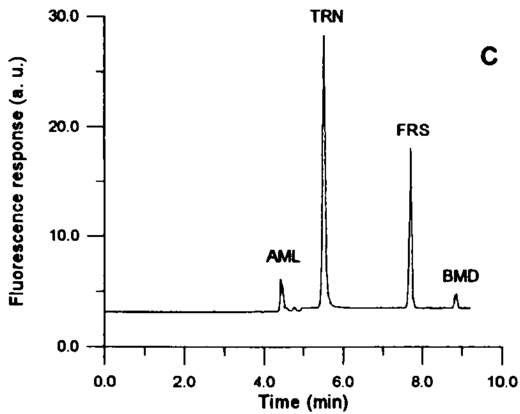
Downloaded At: 11:59 24 January 2011

As expected, fluorescence provided much better selectivity, even when flushing the precolumn with a volume of washing solvent as small as 0.25 mL. No significant differences in the fluorescence chromatograms were observed when flushing the precolumn with volumes of buffer in the 0.25 - 5.0 interval. However, UV signals showed that an important amount of matrix components was transferred to the analytical column when using volumes of phosphate buffer lower than 0.5 mL in the cleanup stage. In order to ensure an adequate performance of the analytical column, a volume of 1.0 mL was chosen as the best option for flushing the precolumn. In Figure 2 are shown the UV and fluorescence chromatograms obtained for blank urine under the conditions selected. This figure also shows a chromatogram obtained for urine spiked with a mixture of the diuretics assayed. As can be deduced from this figure, the analysis of interesting compounds can be performed with excellent selectivity when using fluorescence detection. On the other hand, no significant improvement in the signal-to-noise ratios was observed when volumes of urine in the 5 - 25 μL interval were processed. Since the injection of larger volumes would require longer cleanup periods to prevent deterioration of the analytical column, a sample volume of 5.0 μL was chosen as the best option for analysing urine samples.

Figure 2. (right) Chromatograms obtained for urine samples: (a) UV detection and blank urine, (b) fluorescence detection and blank urine and (c) fluorescence detection and urine spiked with a mixture of the diuretics assayed: amiloride (AML), 10.0 μg/mL; triamterene (TRN), 8.0 μg/mL; furosemide (FRS), 40 μg/mL; bumetanide (BMD), 0.4 μg/mL. For experimental details, see text.







Downloaded At: 11:59 24 January 2011

Table 3

Accuracy for Diuretics in Urine

Diuretic	Sample Number	Added Concentration (μg/mL)	Determined Concentration (μg/mL)	E_r (%)
		5.0	4.81 ± 0.05	- 4
	1	10.0	10 ± 1	0
		17.5	17 ± 2	- 3
		5.0	4.7 ± 0.4	- 6
Amiloride	2	10.0	8.9 ± 0.3	- 11
		17.5	17 ± 2	- 3
		5.0	5.0 ± 0.4	0
	3	10.0	9.8 ± 0.1	- 2
		17.5	17 ± 2	+ 3
		4.0	3.7 ± 0.3	- 8
	1	8.0	7.5 ± 0.5	- 6
		14.0	13 ± 1	- 7
		4.0	3.86 ± 0.12	- 3
Triamterene	2	8.0	8.0 ± 0.1	0
		14.0	13.49 ± 0.06	- 4
		4.0	4.2 ± 0.2	+ 5
	3	8.0	8.5 ± 0.5	+ 6
		14.0	15.1 ± 0.5	+ 8
		20.0	21.5 ± 0.2	+ 8
	1	40.0	41 ± 4	+ 3
		70.0	69.9 ± 0.4	- 0.1
		20.0	21 ± 2	+ 5
Furosemide	2	40.0	41 ± 2	+ 3
		70.0	70 ± 5	0
		20.0	19.4 ± 0.1	- 3
	3	40.0	38.8 ± 0.8	- 3
		70.0	68 ± 3	- 3

Downloaded At: 11:59 24 January 2011

Table 3 (continued)

Accuracy for Diuretics in Urine

Diuretic	Sample Number	Added Concentration (μg/mL)	Determined Concentration (μg/mL)	E_r (%)
		0.2	0.218 ± 0.012	+ 9
	1	0.4	0.41 ± 0.02	+ 3
		0.7	0.66 ± 0.02	- 6
		0.2	0.198 ± 0.001	- 1
Bumetanide	2	0.4	0.37 ± 0.01	- 8
		0.7	0.6466 ± 0.0005	- 8
		0.2	0.20 ± 0.01	0
	3	0.4	0.36 ± 0.02	- 10
		0.7	0.67 ± 0.02	- 4

n = 3

Downloaded At: 11:59 24 January 2011

Analytical Parameters

The reliability of the described assay was tested by determining diuretics in urine in their respective therapeutical intervals. Table 2 summarizes relevant analytical data of the method. Satisfactory recoveries of the analytes were observed, which is in agreement with previously published results.[7] Linearity was also suitable over the studied intervals. In all instances, the intra-day reproducibility was of about 3 - 4%, whereas the inter-day precision ranged from 5% to 10%. These values can be considered acceptable and comparable to those of most reported LC assays.[11,12]

Accuracy of the method was tested by determining the concentration of the analytes in different spiked urine samples. The results obtained are shown in Table 3. In most instances, the method provided concentrations close to the actual concentrations, the relative errors ranging from -11% (for amiloride in sample 2 at a concentration of 10.0 μg/mL) to +9% (for bumetanide in sample 1 at the lowest concentration tested).

Table 4 compares the limits of detection obtained with the described assay (calculated as the concentration required to generate a signal-to-noise ratio of 3) with those reported by other LC assays. Analyte detectability is comparable

Downloaded At: 11:59 24 January 2011

Table 4

Limits of Detection of the Different Methods Proposed for Analysis of Diuretics in Biological Samples

Detection	Sample Type	Sample Volume	Cleanup	Limit of Detection (ng/mL)		Reference
Fluorescence	urine & plasma	0.2mL	precipit'n. of proteins followed by solid-phase extraction	bumetanide:	5	11
Fluorescence	urine & plams	1 mL	liquid-liquid extraction	furosemide:	0.3	12
				amiloride:	0.03	
Fluorescence	urine & plasma	150 μL	precipit'n. of proteins	triamterene:	1	13
Fluroescence	urine	300 μL	liquid-liquid extraction	furosemide:	5	14
Fluorescence	urine	0.2 mL	precipit'n. of proteins	amiloride:	0.5	15
Fluorescence	urine & plasma	100 μL	precipit'n of proteins	furosemide:	5	16
UV	urine	50 μL	column-switching chromatography	amiloride:	20	7
				triamterene:	7	
				furosemide:	5	
				bumetanide:	4	
Fluorescence	urine	5 μL	column-switching chromatography	amiloride:	50	this work
				triamterene:	0.01	
				furosemide:	5	
				bumetanide:	0.1	

to that reported by other LC assays based in fluorescence detection, and in some instances, significantly better.[11,13] For amiloride, the limits of detection reported in references 12 and 15 are lower than that obtained in the present study. This is most probably due to the fact that in those methods, larger volumes of the (purified) samples were injected in the analytical column, and also due to the analyte preconcentration achieved during the sample preparation. Compared with the previously reported method based in column-switching, and UV detection previously reported,[7] the present assay provides comparable sensitivity for amiloride and furosemide; in the latter instance the volume of sample processed is ten times lower and, clearly, the described assay is more sensitive for triamterene and bumetanide.

Human Studies

The proposed method has been applied to the determination of bumetanide after a single dose administration of the lowest recommended dose (5.0 mg).[10] Figure 3 shows the chromatogram obtained 6 hours after dose, corresponding to a bumetanide concentration of (1.83 ± 0.07) μg/mL. No interferences from possible metabolites were observed.

Downloaded At: 11:59 24 January 2011

The difficulties in detecting or quantifying bumetanide in biological samples arise because this diuretic is rapidly metabolized and eliminated (the lifetime of elimination is 4 - 6 hours). Therefore, most proposed procedures could not be suitable for the analysis of this drug in real samples and, specially, for doping control tests.[9] Shown in Figure 4, is the urinary excretion profile obtained for this diuretic, with the described approach. The assay allows the quantification of bumetanide at least 30 hours after drug administration, and it can be detected for a longer time. Therefore, the sensitivity of the described approach can be considered satisfactory for most applications concerning to the determination of bumetanide in urine, as well as, for doping control practice.

Utility

The combined administration of diuretics has been proved to be effective in the treatment of some diseases such as congestive heart failure and essential hypertension.[10] In this sense, amiloride-furosemide and triamterene-furosemide are the most commonly used combinations. Self-evidently, the proposed method is valid for analysing samples obtained from subjects treated with such mixtures. Also, hydrochlorothiazide and atenolol, which are commonly coadministered with the diuretics assayed, have been tested for possible interferences. Under the present conditions, atenolol is completely resolved

Downloaded At: 11:59 24 January 2011

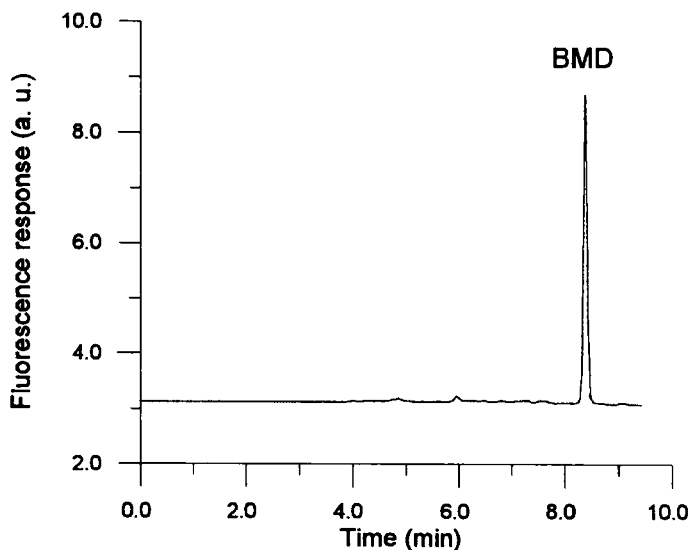


Figure 3. Chromatogram of a urine sample collected 6 h after administration of a single dose of 5.0 mg of bumetanide. For experimental details, see text.

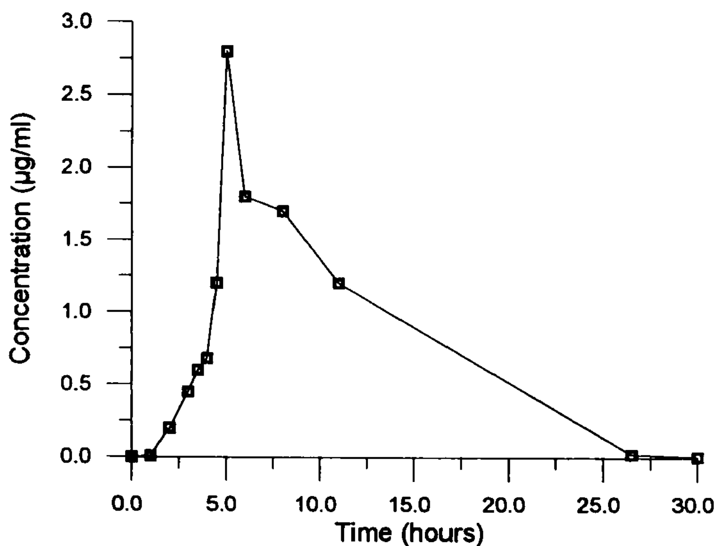


Figure 4. Urinary excretion time-profile of bumetanide. Dose administered, 5.0 mg/mL. For experimental details, see text.

from diuretics of interest. UV chromatograms showed that hydrochlorothiazide (which is also a diuretic) eluted at 6.0 min, thus, overlapping with triamterene. However, since hydrochlorothiazide does not exhibit native fluorescence, it does not interfere with the assay.

With the proposed procedure, the total analysis time takes about 11 min, and several hundred samples can be processed without replacement of the precolumn. Although, in principle, any biological fluid can be processed by switching-chromatography, samples containing a large fraction of proteins (e. g. blood, plasma or serum) are problematic, because irreversible adsorption of proteins results in an increase in back-pressure; moreover, the precolumn has to be frequently replaced in order to ensure an adequate analytical column performance.

With the present procedure, we observed good stability after the repetitive injection of plasma samples, which is most probably due to the small volume of sample injected. Experiments with plasma samples have confirmed the excellent selectivity of the described assay. Therefore, it can be easily adapted to the analysis of diuretics in this biofluid.

Downloaded At: 11:59 24 January 2011

CONCLUSIONS

The combination of column-switching chromatography and fluorescence detection is a useful tool for the analysis of diuretics in biological fluids. Quantification and detection at therapeutical levels can be easily achieved in less than 11 min, with satisfactory accuracy and reproducibility. The main advantage over other LC methods using fluorescence detection is the considerable simplification of the cleanup process. Compared with column-switching methods based on UV detection, the described assay provides better selectivity and, in some instances, significantly improves the sensitivity. Since only 5 μL of sample are required, the method performs properly during several injections of urine. Moreover, the system shows excellent compatibility with plasma samples.

ACKNOWLEDGMENT

The authors are grateful to the CICyT for financial support received for the realization of Project SAF 95-0586.

REFERENCES

1. H. Bi, S. F. Cooper, M. G. Côté, J. Chromatogr., **582**, 93-101 (1992).

2. A. M. Lisi, R. Kazlauskas, G. J. Trout , J. Chromatogr., **581**, 57-63 (1992).

3. R. Herráez-Hernández, P. Campíns-Falcó, A. Sevillano-Cabeza, Chromatographia, **33**, 177-185 (1992).

4. R. Ventura, T. Nadal, P. Alcalde, J. A. Pascual, J. Segura, J. Chromatogr., **655**, 233-242 (1993).

5. R. E. Majors, LC-GC Int., **4**, 10-14 (1991) .

6. P. Campíns-Falcó, R. Herráez-Hernández, A. Sevillano-Cabeza, J. Chromatogr., **619**, 177-190 (1993).

7. P. Campíns-Falcó, R. Herráez-Hernández, A. Sevillano-Cabeza, Anal. Chem., **66**, 244-248 (1994).

8. M. Saarinen, H. Siren, M. L. Riekkola, J. Liq. Chromatogr., **16**, 4063-4078 (1993).

9. P. Campíns-Falcó, R. Herráez-Hernández, A. Sevillano-Cabeza, J. Chromatogr., **612**, 245-251 (1993).

10. V. Rimbau, Offarm, **10**, 58-70 (1991).

11. T. G. Wells, I. R. Hendry, G. L. Kearns, J. Chromatogr., **570**, 235-242 (1991).

12. H. J. E. M. Reeuwijk, U. R. Tjaden, J. van der Greef, J. Chromatogr., **575**, 269- 274 (1992).

13. K. J. Swart, H. Botha, J. Chromatogr., **413**, 315-319 (1987).

14. M. Saugy, P. Meuwly, A. Munafo, L. Rivier, J. Chromatogr., **564,** 567-578 (1991).

15. D.-K. Xu, J.-H. Zhou, Y.-S. Yuan, X.-Q. Liu, S.-K- Huang, J. Chromtogr., **567**, 451-458 (1991).

Downloaded At: 11:59 24 January 2011

16. T. B. Vree, M. van den Biggelaar-Martea, C. P. W. G. M. Verwey-van Wissen, J. Chromatogr., **655**, 53-62 (1994).

Received September 1, 1996
Accepted October 30, 1996
Manuscript 4276

Downloaded At: 11:59 24 January 2011