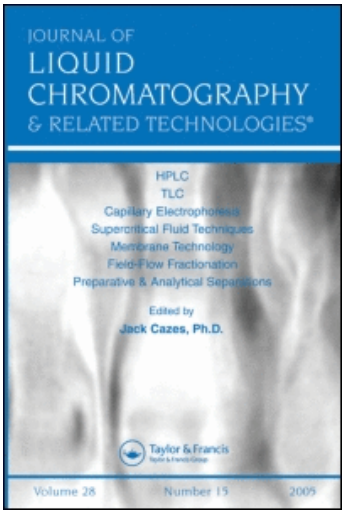

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QUANTITATIVE ANALYSIS OF THE LOOP DIURETIC TORASEMIDE IN TABLETS AND HUMAN URINE BY HPLC-EC

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ABSTRACT

A High Performance Liquid Chromatographic method with amperometric detection for the determination of the loop diuretic 1-Isopropyl-3-[4 - (3-methylphenylamino)-3-pyridinesulphonyl] urea, torasemide, is reported using a μ-Bondapak C_{18} column and a mobile phase water:acetonitrile (65:35), 5 mM in potassium dihydrogenphosphate/dipotassium hydrogenphosphate at pH 5.3. Eluant is monitored at +1300 mV with an amperometric detector equipped with a glassy carbon working electrode. The method showed a determination limit of 8 ppb and a reproducibility in terms of relative standard deviation lower than 2% in intra-day assays and 5 % in inter-day assays. The HPLC-EC method was applied to urine samples obtained from a healthy volunteer. Concentration levels of torasemide at different time intervals were monitorized and results were in agreement with the pharmacokinetic parameters of this diuretic. The determination of torasemide in urine required a liquid-liquid extraction prior to chromatographic analysis due to the interferences found in urine matrix. With this simple clean-up

procedure, recoveries around 70% were achieved. Torasemide was directly determined in tablets after dissolution of the powder in a methanolic solution.

INTRODUCTION

1-Isopropyl-3-[4-(3-methyl phenyl amino)-3-pyridinesulphonyl]urea, torasemide is a high-ceiling loop diuretic of the pyridil sulfonylurea class, structurally related to furosemide, azosemide and bumetanide[1]. This long-acting loop diuretic has a bioavailability of about 90 %[2,3] and undergoes oxidative biotransformation, while furosemide is excreted unchanged. In healthy volunteers given torasemide 20 mg, about 25 % was recovered unchanged in urine within 24 hours[3-6]. Due to its diuretic properties, torasemide is a compound forbidden in sports, and it is included by the International Olympic Committee in the list of banned substances in sports.[7]

Since torasemide is a relatively new diuretic (J. Delarge and C. L. Lapière 1978)[8], in comparison with other loop such as furosemide, only a few papers can be found in literature dealing with its analysis. Methods reported for the determination of torasemide include preferently chromatographic procedures with photometric detection[9-12] and voltammetric analysis on different electrodes[13,14].

The aim of this work is the application of an HPLC system with amperometric detection for the determination of the diuretic torasemide in tablets and real urine samples.

MATERIALS AND METHODS

Reagents, Chemicals and Standard Solutions

Pure torasemide and tablets containing 10 mg of torasemide, were very kindly supplied by Boehringer Mannheim (Barcelona, Spain). HPLC grade solvents were purchased from Lab-Scan (Dublin, Ireland), and water obtained from the Milli-RO and Milli-Q Waters systems. All the reagents used were Merck Suprapur (Bilbao, Spain).

A stock solution of torasemide 1000 μg/mL was prepared in pure acetonitrile and stored in the dark under refrigeration.

Procedure for Tablets and Urine Samples

The tablets were pulverized and dissolved in methanol. The resulting solution was filtered through the 0.45 μm membrane and an aliquot diluted with the mobile phase to provide the concentration required for the injection.

The clean-up procedure for urine samples was a liquid-liquid alkaline extraction with NaCl and ethyl acetate based on the method proposed by Ventura et al[15].

Chromatographic Conditions

The HPLC system consisted of a Model 2150-LKB (Pharmacia, Barcelona, Spain) HPLC pump, and a Rheodyne (Pharmacia) Model 7125 injector with a loop of 20μL. The electrochemical detector (PAR Model 400) equipped with a glassy carbon cell (EG&G Princeton Applied Research, Madrid, Spain). It was operated at +1300 mV vs a Ag/AgCl electrode, in the DC mode with a 5-s low-pass filter time constant, and a current range between 0.2 and 100 nA. Chromatograms were recorded using an LKB Model 2221 integrator. A 125Å μBondapak C_{18}, 30 cm x 3.9 mm I.D., 10-μm, (Waters Assoc.) column with a μBondapak C_{18} precolumn module (Waters Assoc.) were used. To study the influence of the temperature, a Waters TMC temperature control system was used.

The mobile phase was a mixture acetonitrile-water (35:65) containing 5 mM potassium dihydrogenphosphate/dipotassium hydrogenphosphate. pH was adjusted to 5.3 and the buffer served as the supporting electrolyte. The μBondapak C_{18} column head-pressure was 69 bar at a flow rate of 1.0 mL/min. The injection volume was 20 μL. The work was made at room temperature.

RESULTS AND DISCUSSION

In order to choose the optimum potential value for the amperometric detection of this diuretic, hydrodynamic voltammetry of the compound was carried out. An oxidative potential of 1300 mV was chosen as the working potential, since it was the potential which provided the maximum sensitivity for torasemide.

The study of the influence of pH gave an optimum value of 5.3 which

allowed the separation of torasemide from the electrooxidable interferences found in urine, keeping a low retention time.

The buffer potassium hydrogen phosphate/dipotassium hydrogen phosphate was used as supporting electrolite providing the best signal to noise ratio at a concentration of 5mM.

A variation in temperature from 26 to 55 °C produced small variations on the peak area of chromatograms. A linear relationship between k'-log 1/T was obtained. Since the influence of the temperature was not very relevant, the work was carried out at room temperature and with a flow rate of 1 mL/min.

When optimum chromatographic conditions had been established, a quantitative method for the determination of torasemide was developed at two concentration levels: ppm and ppb. The calibration curves for both concentration levels showed that the detector response was lineal up to 12 mg/l. The reproducibility studies (intra-day and inter-day assays) made on n=10 solutions for both concentration levels gave rise to the following results in terms of relative standard deviations: 1.1% (at day) and 5.6% (inter-day) at ppm level and 0.7% (at day) and 2.9% (inter-day) at ppb level. The experimental quantitation limit, defined as the minimum concentration of torasemide which gives rise to a signal able to be quantified for the integrator, was 8.5 ng/mL.

Analytical Applications

The method developed was applied to the determination of torasemide in tablets. Since torasemide was not commercially available in Spain, the pharmaceutical Company (Boehringer Mannheim) supplied the tablets containing 10 mg of torasemide (torasemide 10 mg tablets GA-1/3. Sample for research). Values obtained were in accordance with those certificated, as can be seen in Table 1, with errors lower than 1%, which demonstrate the accuracy of the method.

In order to calculate the percentages of recovery, the method was applied to urine samples spiked with this diuretic. Quantitative recoveries calculated from urine samples spiked with 0.5μg/mL and 1μg/mL were (71.02±2.9)% and (70.1±3.0)% respectively.

The chromatographic method has been applied to the analysis of torasemide in real urine samples obtained from a healthy volunteer after a

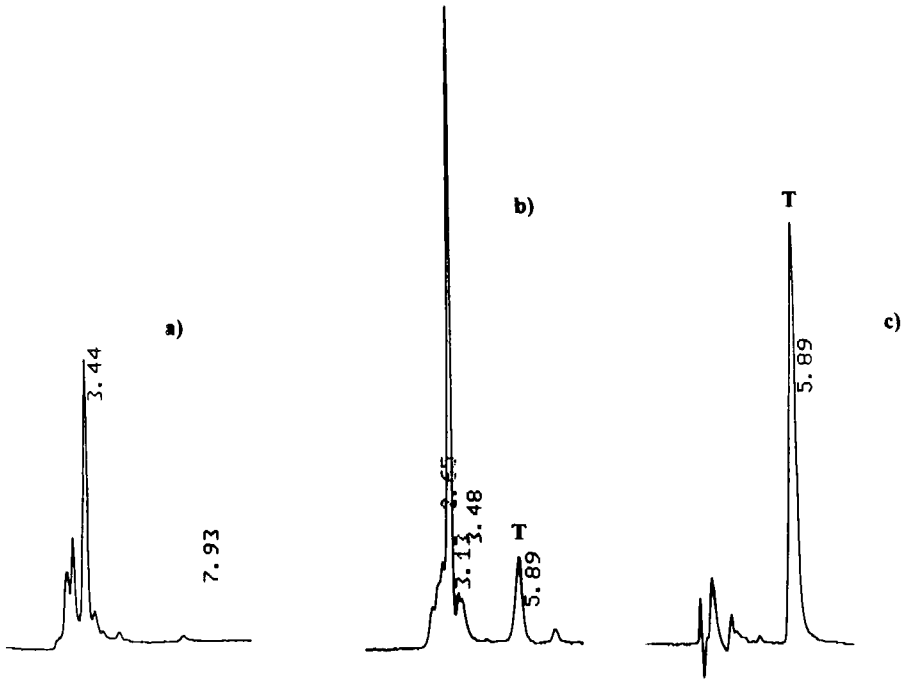

Figure 1. Chromatograms obtained from an extract of: a) blank urine sample, b)urine sample 2-8 hours after oral administration of 1 tablet of torasemide 10 mg to a healthy male volunteer and c) a diluted solution of a tablet containing 10 mg of torasemide. Potential +1300 mV, full current scale: 50nA. Mobile phase: acetonitrile-water (35:65), 5mM KH_2PO_4/K_2HPO_4, pH 5.3.

Table 1

Determination of Torasemide in Pharmaceutical Formulations and Urine

Tablet Formulation	Component	Found (mg)[a]	Nominal (mg)
Torasemida GA-1/3 Sample for Research	torasemide	9.98 ± 0.02	10.00
Urine Samples			
Time interval	0-2 hours	2-8 hours	8-24 hours
Torasemide conc. (μg/mL):	0.84	0.89	0.12

[a] amount ± ts/√n. n=4 different samples and 3 replicates of each sample.

single dose of a tablet containing torasemide 10 mg. Urine was collected at different time intervals for the quantitative determination of the diuretic: 0-2 hours, 2-8 hours and 8-24 hours. The compound was easily detected at the different times and the concentrations determined, collected in Table 1, were in agreement with the pharmacokinetic data[3]. Urine samples were treated following the clean-up procedure described in the experimental section and Fig. 1 shows the chromatograms corresponding to a blank urine, a real urine sample obtained 2-8 hours after administration of a single dose of torasemide 10 mg and a diluted solution of a tablet (Torasemida GA-1/3 Sample for research).

CONCLUSIONS

In a previous work we described a static voltammetric method for the determination of torasemide based on its oxidation peak[14]. Since the diuretic was not commercialized in Spain, the method was applied to standard solutions and spiked urine samples. The static method allowed the determination of torasemide without problems using the standard aditions method.

But when this method was applied to real urine samples obtained after the oral ingestion of a tablet of torasemide, some matrix interferences found made impossible the determination of the diuretic and led us to develop a chromatographic method.

The clean-up procedure used in static conditions was an acid extraction using ethyl acetate. Although this method allowed a good recovery, the chromatographic peak of torasemide was very close to the peaks from endogenous compounds, and the quantification of the diuretic was very difficult. So other procedures had to be tried. Changing the organic solvent for the extraction lower recoveries were obtained and the interferences could not be eliminated.

A method based on the one proposed by Ventura et al.[15] allowed the elimination of most of the interferences . Although the percentage of recovery was lower than with the acid extraction, it was enough for the quantitation of torasemide at the usual levels found in urine samples.

The chromatographic method with amperometric detection developed present some advantages over other reported methods: the low retention time for the diuretic, as well as the low detection limit achieved. The compound is eluted in less than 6 min, while other authors[9-12] have reported methods ranging from 10 to 25 min. The determination limit achieved for torasemide in

urine with the HPLC-EC method developed (8 ng/mL with an injection volume of 20 μL) is lower than the one achieved with the HPLC-UV system (10 ng/mL with an injection volume of 50 μL), reported by March et al.[12], and makes possible the application of the method developed to the monitoring of low levels of torasemide for pharmacokinetic and pharmacodynamic purpouses, as well as for doping control.

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