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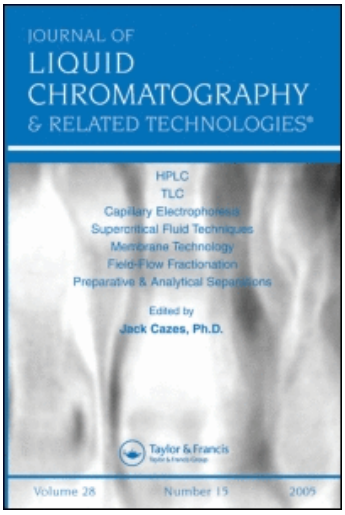

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DETERMINATION OF THE NON-THIAZIDE DIURETIC XIPAMIDE IN PHARMACEUTICALS AND URINE BY HPLC WITH AMPEROMETRIC DETECTION

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ABSTRACT

A high performance liquid chromatographic method with amperometric detection has been developed for the determination of the diuretic xipamide using a μ-Bondapak C_{18} column. The mobile phase consisted of a mixture water:acetonitrile 50:50, 5 mM in KH_2PO_4/K_2HPO_4, pH 4.3. The compound is monitored at +1325 mV with an amperometric detector equipped with a glassy carbon working electrode. A liquid-liquid or solid-liquid extraction prior to chromatographic analysis was done to avoid the interferences found in urine matrix. Percentages of recovery were (99.3±4.7) and (99.4±4.2) for liquid-liquid and solid-liquid extraction, respectively. The method developed has a linear concentration range from 0.05 to 0.50 μg/mL, with a reproducibility in terms of relative standard derivation (RSD) for a concentration level of 1 μg/mL of 4% and a quantitation limit of 0.50 ng/mL. The method was applied to the determination of xipamide in tablets and urine obtained from hypertensive patients after the ingestion of Demiax (xipamide 20 mg).

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INTRODUCTION

4-Chloro-5-sulfamylsalicyloyl-2´,6´-dimethylamilide (xipamide) is a non-thiazide diuretic with a greater natriuretic effect than the thiazides and a less abrupt onset and longer duration of action than furosemide. It is an effective antihypertensive drug, appears to be a more effective diuretic than the thiazides, and may cause a lower potassium loss relative to sodium excretion than these drugs. Xipamide offers a suitable alternative to other diuretics in the treatment of patients with mild to moderate hypertension and of patients with oedema due to a variety of causes.[1]

The common dose of xipamide is 20 mg/day and the maximum diuresis is between 3 and 4.5 hours. Xipamide is conjugated with glucuronide and during the first 24 hours, 26% of the administered dose is excreted in the conjugated form and 40-55% as unchanged drug. [2,3]

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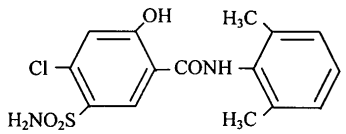
Xipamide, as the rest of diuretics, is included by the International Olympic Committee in the list of banned substances in sports.[4]



Xipamide

The determination of xipamide in pharmaceutical formulations has been carried out by voltammetry[5] and HPLC with photometric detection,[6] while its analysis in biological fluids involves the use of radioimmunoassay,[3] thin-layer chromatography coupled with fluorimetry,[7] and liquid chromatography with photometric detection;[8-10] this last technique being the most commonly used.

Electroanalytical methods have rarely been applied to diuretic analysis. Oxidative properties of several diuretics (loop, non-thiazide) have been studied in our laboratory[11-15] and chromatographic methods with amperometric detection have been developed for the analysis of torasemide,[16] clopamide,[17] and the simultaneous determination of furosemide and triamterene,[18] furosemide and piretanide.[19]

Liquid-liquid extraction is the most commonly used procedure for the separation of diuretics from endogenous compounds of urine matrix. Tisdall et al.[20] and Fullinfaw et al.[21] have described liquid-liquid extraction methods for several acidic diuretics. Cooper et al.,[22] Tsai et al.,[23] and Herraez et al.[24]

proposed similar procedures for the diuretics extraction which consisted of two liquid-liquid extraction steps: in acidic (pH 5-5.5) and alkaline medium (pH 9-9.5) with ethyl acetate. Park et al.[25] carried out a comparative study of the efficiency of solid-liquid extraction and liquid-liquid extraction at different pH values. On the other hand, Campins et al.[26] made a most exhaustive study on the possibility of solid-liquid extraction for the separation of acidic, basic, and neutral diuretics, using different extraction columns: C_{18}, C_8, C_2, cyclohexyl, phenyl and cianopropyl. Ventura has described the influence of pH, salt effect, and use of different extractants: ethyl ether or ethyl acetate on the recovery of diuretics.[27,28]

Taking into account that xipamide is electrooxidable at a glassy carbon electrode[5] and the literature consulted, the aim of this work is the development of a HPLC method with amperometric detection for the analysis of this diuretic in pharmaceuticals and urine, using the solid-liquid extraction as alternative procedure of sample treatment to liquid-liquid extraction commonly used.

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EXPERIMENTAL

Reagents, Chemicals and Standard Solutions

Xipamide was kindly supplied by Lacer S.A. (Barcelona, Spain). HPLC grade solvents were purchased from Lab-Scan (Bilbao, Spain), and water obtained from the Milli-RO and Milli-Q Waters systems. Potassium dihydrogenphosphate, and dipotassium hydrogenphosphate were Merck Suprapur (Bilbao, Spain). All the reagents used were Merck Suprapur (Bilbao, Spain).

A stock solution of xipamide (1000 µg/mL) was prepared in methanol and stored in the dark under refrigeration. Working solutions were obtained by appropriate dilution, just before use.

Procedure for Tablets

The tablets were pulverised in a mortar. The powder was weighed and treated with methanol. After shaking for 5 min, the mixture was centrifuged at 1800 g for 5 min and the supernatant was filtered with Albet 242 paper in order to avoid plugging the column. The precipitate was washed several times with the solvent. The filtered solution was made up to 100 mL with methanol, and an aliquot of this solution was diluted with mobile phase to provide the concentration required for the injection. The procedure was repeated for different tablets and the measurements were made by duplicate.

Procedure for Urine Samples

Solid-phase extraction

C_{18} Waters extraction cartridges (500 mg) were inserted into a vacuum manifold and activated by washing with 15 mL methanol and 15 mL deionized water, and conditioned with 1 mL phosphate buffer pH= 3.0. 2 mL of buffered urine samples at the same pH were poured into each cartridge reservoir and drawn slowly through the cartridge. The cartridges were washed with 5 mL of deionized water, 1 mL of hexane, and dried with air for 2 min. Elution of the analyte was performed with 2 mL of ethyl ether. The eluate was evaporated to dryness at 40ºC under a stream of nitrogen, using a Zymark Turbo Vap evaporator (Barcelona, Spain). The residue was dissolved in 1 mL of mobile phase.

Liquid-liquid extraction

4 mL of urine samples were acidified with 4 mL of 1 M KH_2PO_4 (pH 4.3) and 8 mL of ethyl acetate were added. Tubes were shaken for 20 min and centrifuged at 1800 g for 5 min. The organic phase was transferred to a second tube containing 8 mL of 0.1 M KH_2PO_4/K_2HPO_4 (pH 7.5) and were shaken for 20 min. After that, the mixture was centrifuged and the organic layer was separated and evaporated to dryness at 40ºC under a stream of nitrogen. The residue was dissolved in 1 mL of mobile phase.

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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 sample loop of 20 µL. The PAR Model 400 electrochemical detector was equipped with a glassy carbon electrode (EG&G Princeton Applied Research, Madrid, Spain). It was operated at +1325mV 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. Barcelona, Spain) column with a µBondapak C_{18} precolumn module (Waters Assoc., Barcelona, Spain) was used. To keep the column temperature constant, a Waters TMC temperature control system was used. The mobile phase was a mixture of acetonitrile-water (50:50) containing 5 mM potassium dihydrogenphosphate/dipotassium hydrogenphosphate. pH was adjusted to 4.3 and the buffer is also used as the supporting electrolyte. The flow rate utilized was 1.0 mL/min and the injection volume was 20 µL. The chromatographic separation was made at 30±0.2°C.

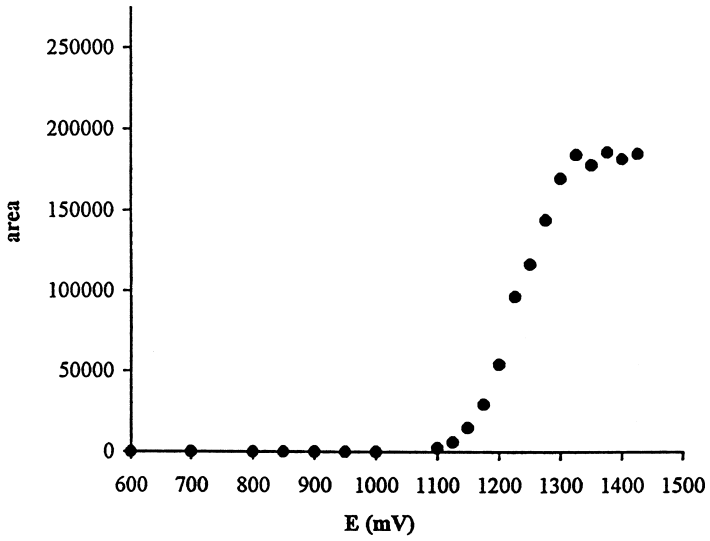

Figure 1. Hydrodynamic voltammogram of xipamide. Drug concentration: 1 µg/mL, mobile phase: water:acetonitrile 50:50 containing 5 mM KH_2PO_4/ K_2HPO_4 pH 4.3, flow rate 1 mL/min and T^a 30±0.2ºC.

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RESULTS

In order to choose the optimum potential value for the amperometric detection of xipamide, the hydrodynamic voltammogram of the compound was done (Figure 1). An oxidative potential of 1325 mV was chosen as the working potential, since it was the potential which provided the maximum sensitivity for xipamide.

Upon the basis of the studies carried out in our laboratories for the separation of other sulfonamidic diuretics,[16-19] acetonitrile was used as organic modifier in the mobile phase.

A study of the influence of the organic modifier proportion and pH of the mobile phase on the retention times and resolution of chromatographic peaks was carried out. As was expected, an increase of the mobile phase polarity gave rise to an increase of retention time, due to low polarity and hydrofility of xipamide. A mobile phase acetonitrile:water (50:50) was used for the study of pH influence. A decrease of retention times is observed with the increase of pH. A ratio of acetonitrile-water, 50:50, containing potassium hydrogen phosphate/dipotassium hydrogen phosphate 5 mM buffer, pH 4.3 was used

throughout this work, since the retention time of xipamide was 7.4 min under these conditions, which allows its determination free of electrooxidable interferences from urine matrix.

Once the optimum chromatographic conditions had been established, a quantitative method for the determination of xipamide in urine samples was developed.

A solid-liquid procedure was used for the treatment of urine samples. A study of the different stages of the procedure of extraction was carried out: conditioning of the cartridge, introduction of the sample, elimination of interferences, and elution of the diuretic. The developed HPLC-EC method was utilized, for the evaluation of each step of the procedure as a function of compound recovery. The pKa value of the diuretic and the retention of the compound in C_{18} columns were kept in mind.

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The percentages of recovery of xipamide were calculated for comparison of the area of chromatographic peak obtained with the corresponding to a standard solution of same concentration. Several volumes of methanol:water were assayed for the conditioning of cartridge. This variable does not considerably affect the recovery of this diuretic.

The optimization of the adequate pH for the extraction of xipamide was carried out in the pH range 3.0-9.0, using urine samples spiked with 1 µg/mL of the diuretic. The recovery of this compound decreases with the pH increment, although at higher pH values the interferences extracted are lower.

Due to these facts, a study of recovery percentages and reproducibility of the method was done at two pH values: 3.0 and 7.0. A recovery of 98.0% (pH= 3.0) and 62.7% (pH = 7.0) with a reproducibility in terms of %RSD of 2.33 and 13.0, respectively, were obtained.

Different elution solvents: ethyl ether, ethyl acetate, methanol, and acetonitrile were used. Ethyl ether was chosen as optimum due to fewer interferences for the extract obtained. The possibility of re-using the cartridge after its regeneration with water and methanol was checked. The percentages of recovery were kept practically constant after at least five different extraction assays.

In optimal conditions, collected in the Experimental section, the percentage of recovery for urine samples spiked with 1 µg/mL of xipamide was 99.4±4.3. This recovery was compared with the one obtained (99.3±4.7), applying the well-established liquid-liquid procedure described by Fullinfaw et al.[21]

Table 1

Quantitative Determination of Xipamide in Urine

Retention time (min)	7.4 ± 0.1
Linear concentration range (ng/mL)	50-500
Slope of calibration graph	221463 ± 5842[a]
Intercept	-1031 ± 1782
Correlation coefficient, r^2	0.998
Reproducibility (%RSD)	4%[b]
Quantitation limit (ng/mL)	0.5

[a] area/concentration (ng/mL).
[b] seven determinations at the 1μg/mL level.

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Table 2

Concentrations of Xipamide (μg/mL) Obtained for Urine Samples from Two Hypertensive Patients After the Application of Two Different Clean-up Procedures

	Solid-Liquid Extraction		Liquid-Liquid Extraction	
Time Interval	**0-2 h**	**2-6 h**	**0-2 h**	**2-6 h**
Patient 1	3.35	3.37	3.37	3.39
Patient 2	0.70	5.48	0.72	5.60

A calibration curve was made from urine solutions spiked with different concentrations of xipamide. The concentration range assayed for the determination of the diuretic was chosen upon the basis of its excretion percentages as unchanged form and the usual therapeutic dose of these antihypertensive agents. In Table 1, the quantitative characteristics of the method are collected. The quantitation limit defined as the minimum signal which the register identified as a peak, was 0.5 ng/mL.

The analytical method was applied to the determination of urine samples obtained from hypertensive patients, at different time intervals after the administration of Demiax (xipamide-20 mg). The results obtained, Table 2, demonstrates the applicability of the HPLC-EC method with a solid-liquid clean-up procedure for the determination of xipamide in real urine samples (Figure 2).

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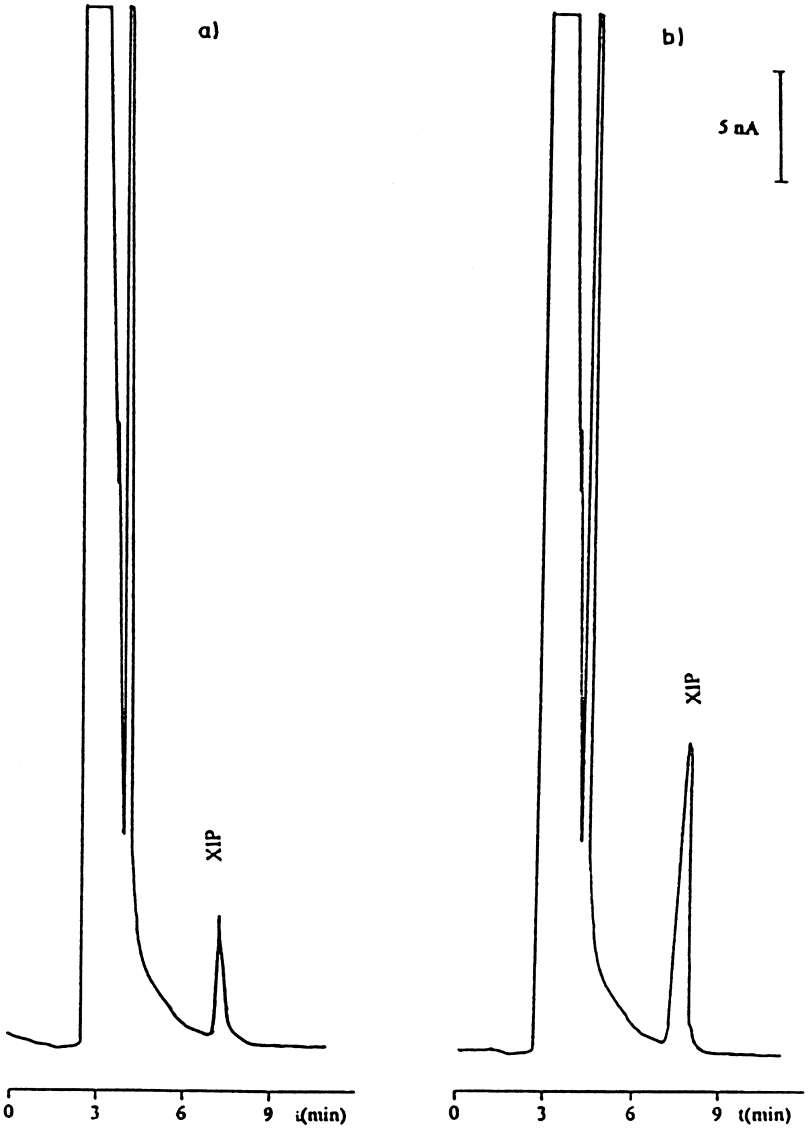

Figure 2. Chromatograms obtained from an extract of a) urine sample, 2h after oral administration of Demiax-20 mg (xipamide) to a hypertensive patient, and b) urine sample after the addition of 2 µg/mL of xipamide standard solution. Chromatographic conditions: mobile phase: water:acetonitrile 50:50 containing 5 mM KH_2PO_4/K_2HPO_4 pH 4.3, potential: 1325 mV, flow rate : 1 mL/min, and Tª 30±0.2ºC.

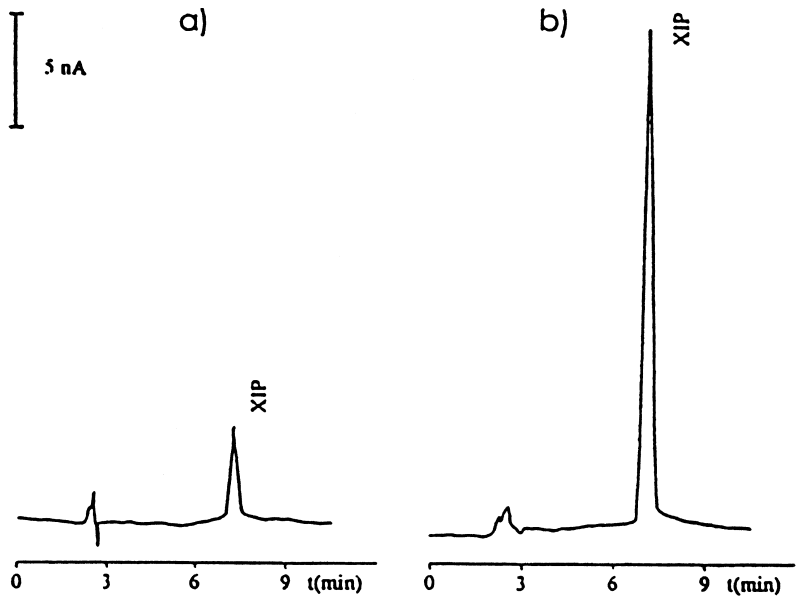

Figure 3. Chromatogram corresponding to a) diluted solution of a tablet of Demiax 20 mg and b) solution a) after the addition of 1 µg/mL of standard solution of xipamide. Chromatographic conditions: mobile phase: water:acetonitrile 50:50 containing 5 mM KH_2PO_4/K_2HPO_4 pH 4.3, potential: 1325 mV, flow rate: 1 mL/min, and T^a 30±0.2^{o}C.

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Table 3

Concentrations of Xipamide (µg/mL) Obtained for Urine Samples from Two Hypertensive Patients by Means of HPLC-DAD (Liquid-Liquid Extraction) and HPLC-EC (Solid-Liquid Extraction)

	HPLC-DAD (L-L)		HPLC-EC (S-L)	
Time Interval	**0-2 h**	**2-6 h**	**0-2 h**	**2-6 h**
Patient 1	3.35	3.29	3.35	3.37
Patient 2	0.72	5.60	0.70	5.48

The validation of the method was carried out comparing the HPLC-EC results with those obtained by HPLC - diode array detector (DAD) using liquid-liquid extraction procedure, following the method applied in the doping controls in the Olympic Games, 1992.[28]

In Table 3, concentrations of xipamide obtained by both methods are collected. The chromatographic method developed was applied to the determination of xipamide in tablets (Demiax, xipamide-20 mg) obtaining values (20.05±0.48) in accordance with those certified by the pharmaceutical company, with relative errors lower than 0.5 %. The chromatogram obtained for a solution of the pharmaceutical formulation was free of interferences (Figure 3). The quantitation of xipamide was made using the standard additions method.

DISCUSSION

A simple chromatographic method, with amperometric detection, has been developed which allows the determination of the diuretic xipamide in urine obtained from hypertensive patients at different time intervals, previously a solid-liquid extraction procedure, and in pharmaceutical formulations.

The quantitation limit, 0.5 ng/mL, is lower than that reported by Bodenan et al,[10] 10 ng/mL, by HPLC-photometric detection with liquid-liquid extraction procedure.

The clean-up procedure for urine samples developed using solid-liquid extraction, provides percentages of recovery for xipamide comparable to those obtained by means of liquid-liquid extraction, although the solid-liquid extraction makes possible the automation of the analysis.

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