GAS CHROMATOGRAPHIC IDENTIFICATION AND QUANTIFICATION OF HYDROXYZINE: APPLICATION IN A FATAL SELF-POISONING

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Summary

A method for the identification and quantification of hydroxyzine in human fluids by GC/NPD is presented. The method employs acepromazine as the internal standard and requires no derivatization. After a single alkaline extraction in n-heptane isoamylalcohol (98.5:1.5, v/v), analysis is achieved in 7 min. The lower limit of detectability was 0.8 ng/ml in plasma. Capillary gas chromatography mass spectrometry assay was developed for confirmation. Toxicological findings, after a fatality involving hydroxyzine are presented as an application of the procedure.

Key words: Hydroxyzine; Gas chromatography; Mass spectrometry; Poisoning

Introduction

Hydroxyzine, 2-(2-(4-(4-chlorophenyl)phenylmethyl)-1-piperazinyl) ethoxy) ethanol, is an antihistamine prescribed for sedation and treatment of anxiety [1]. In adults, therapeutic plasma hydroxyzine concentrations are about 20—100 ng/ml [2]. Only few analytical references seem to be available for hydroxyzine, most of them being suitable for pharmaceutical forms [3,4]. Therefore, we have developed a new, direct GC method that has permitted the analyses of more than 240 clinical specimens without column reconditioning. As an application, we have described the identification and quantification of hydroxyzine in a fatal case. Very few data seem to be available about hydroxyzine intoxication [5,6].

Experimental

Reagents

All organic solvents were HPLC grade. All other chemicals were analytical grade. Hydroxyzine dihydrochloride and acepromazine maleate were generously offered by UCB Laboratories (Nanterre, France) and Sanofi
Pharma Industrie (Evreux, France), respectively. Extraction buffer was prepared with a saturated solution of sodium carbonate in deionized water.

**Standard solution**

All stock solutions of the drug and the internal standard (acepromazine) were prepared using methanol and stored at 4°C. Concentrations were based on the molecular weight of their free bases. Standard solutions containing 10, 50, 100, 200 and 500 ng/ml of hydroxyzine were prepared by proper dilution of a 100 μg/ml primary standard solution.

**Gas chromatography**

An 8500 Perkin-Elmer gas chromatograph equipped with a nitrogen-phosphorus detector (NPD) was used. The glass column (1.8 m × 2 mm i.d.) was an Alltech 3% OV-17 Gas Chrom Q, 100—120 mesh. The operating conditions were as follows: column, injector port and detector temperatures of 240, 300, and 310°C, respectively. N₂ was used as the carrier gas, with an operating head column velocity of 40 ml/min. The parameters of the rubidium bead in the nitrogen detector were adjusted to optimum conditions before use.

**Mass spectrometry confirmation**

A model 8500 (Perkin-Elmer) gas chromatograph with Ion Trap Detector (ITD), a capillary column and a splitless inlet injection system was employed. The data system used was an Epson PC AX computer. Data acquisition and manipulation were performed using standard software. The ITD was operated in electron impact mode at 70 eV with an ion source temperature of 200°C to 220°C and m/z range from 40 to 500 amu to determine suitable selected ion monitoring. The electron multiplier voltage of the detector was set at 1850 V. A fused silica capillary column (SGE), BP-1 (methylmethylsiloxane) 25 m × 0.22 mm, was used. The flow of carrier gas (helium purity N 55) through the column was 3.2 ml/min and the head pressure on the column was maintained at 18 psi. The column and injector port temperature were 260°C and 290°C, respectively. Splitless injection with a split valve off-time of 0.9 min was employed.

**Extraction**

Five standards were prepared in 15-ml screw-top tubes by adding 10, 50, 100, 200 and 500 ng of hydroxyzine to 1 ml of fresh human plasma. Next, 20 μl of acepromazine (10 μg/ml), 500 μl of saturated carbonate buffer and 5 ml of n-heptane isoamylalcohol (98.5:1.5, v/v) were added, in that order, to all tubes. After vortexing, phase separation was effected by centrifugation. The solvent was transferred then evaporated to dryness at 45°C in a Speed Vac Concentrator (Savant Instrument, Inc.). The residue was dissolved in 20 μl of methanol and 1 μl was injected into the column.

**Calculations**

Quantification was done by plotting peak area ratios (drug/internal stand-
and) against the concentration of standards, to produce standard curves and by comparing the results for the case samples with the curves.

Results

Under the chromatographic conditions used, there was no interference with the hydroxyzine or the internal standard by any extractable endogenous materials present in human fluids (plasma, whole blood, urine and bile). A typical GC tracing of plasma extract from a medical subject under hydroxyzine treatment is shown in Fig. 1. Under the experimental conditions, the retention times were 3.53 and 5.50 min for acepromazine and hydroxyzine, respectively.

Detector response was linear over the range of 10—500 ng/ml for hydroxyzine. The mathematical expression and the correlation coefficient were $y = 1.18x - 0.08$ and $r = 0.991$, respectively. These results indicated a good linear proportionality between the NPD response and the concentration of hydroxyzine in the range used.

Extraction recovery was determined for hydroxyzine by comparing ($n = 6$) the representative peak areas of extracted plasma samples at a concentration of 100 ng/ml with the peak area of the methanolic standard at the same concentration. The peak area of the extracted plasma samples was $81.3 \pm 5.6\%$ of that of the standard solution.

The detection limit was evaluated with decreasing concentrations of hydroxyzine, until a response equivalent to 3-times the background noise was observed. By this method, concentration as low as 0.8 ng/ml could be quantified.

Within-run precision was evaluated by 12 analyses of blank plasma spiked to contain 100 ng/ml of hydroxyzine. The reproducibility was 4.8%. Day-to-day precision was also studied, using blank plasma loaded with hydroxyzine at a concentration of 100 ng/ml. Analyses were performed every day over a period of 3 weeks. The precision was found to be 6.1%.

The electron impact mass spectra of hydroxyzine is shown in Fig. 2. The ions chosen for monitoring were the base peaks at $m/z: 201$ (hydroxyzine) and 58 (acepromazine, internal standard). To be considered positive for hydroxyzine, the selected ion monitoring analysis must show coincident peaks in the $m/z$ 201, 165, 166, 203 and 56 ion current profiles.

Case report

An 18-year-old woman was found in February 1990 in an isolated forest lying on the floor of a bunker. Containers of hydroxyzine (Atarax®) were found near the body. At the autopsy, the X-ray did not show any bullets. No external trauma was noted. Post-mortem examination showed edematous lungs with abundant frothy fluid on sectioning.

Postmortem samples, including blood, urine, bile and stomach contents were collected for toxicological investigations.
Fig. 1. Chromatogram obtained from a medical subject under hydroxyzine treatment (3.53 min, acepromazine, I.S.; 5.50 min, hydroxyzine, 81 ng/ml)

Fig. 2. Electron impact mass spectrum of hydroxyzine.
Hydroxyzine was identified and quantified in all samples (Table 1). Confirmation was obtained by mass spectrometry.

No ethanol was detected in blood and urine. The blood hydroxyzine concentration, was within the range of fatal concentrations previously described [5,6].

Since the blood concentration was about 40 times greater than even high therapeutic plasma concentrations [2], one can rule the cause of death to be hydroxyzine intoxication.

**Discussion**

A method for identification and quantification of hydroxyzine is described. A simple alkaline extraction step, the absence of derivatization and the introduction of a suitable internal standard provided a reliable and sensitive method. A case report is presented in which blood, urine, bile and stomach contents were analyzed for hydroxyzine.

**References**


