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GC–MS procedure for the analysis of zipeprol

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Abstract: A sensitive and specific quantitative method for the determination of zipeprol, a newly abused antitussive, in human fluids is described. Zipeprol and an internal standard, levallorphan, are isolated by a basic extraction and back-extraction process. The final extract is derivatizated with BSTFA + 1% TMCS and separated on a 12-m HP-1 capillary column. Drugs are detected by selected ion monitoring at *m/z* 335 and *m/z* 355 for zipeprol and the internal standard, respectively. The minimum detectable quantities are 0.6 and 0.4 ng ml^{-1}, for zipeprol in plasma and urine, respectively. Relative standard deviations for within-run data are less than 6%.

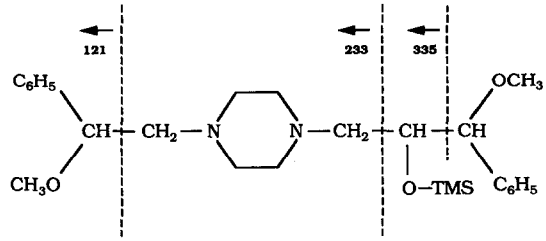
Keywords: *Zipeprol; drug of abuse; gas chromatography–mass spectrometry; determination in urine and plasma.*

Introduction

Zipeprol, a piperazine ethanol derivative with a non-phenantrenic chemical structure is a non-essential but widely used antitussive, which is stated to have a peripheral action on bronchial spasm. A 150–300 mg dose may be given daily by mouth in divided doses. As the drug is a non-opioid cough suppressor agent, it is not legally considered as being capable of creating dependance or abuse liability. In March 1990, the French Ministry of Health decided to withdraw the tablet form of zipeprol from the market. Since that date, only the syrup (0.3 and 0.5%) is available for oral dosage. However, several reports, particularly from Italy have shown that zipeprol in high doses has a definite abuse liability and dependence potential. Many of its effects were identical to those induced by opiates, including hallucinogenic effects, convulsions, cerebral oedema or sudden loss of consciousness [1–4]. Two cases of lethal intoxication involving or due to oral ingestion of zipeprol have been reported [5].

Gas chromatography with nitrogen–phosphorus detection was recently proposed for the assay of zipeprol [6] but this technique lacks specificity, particularly in addict populations where many drug combinations are ingested.

Therefore, a gas chromatography combined with mass spectrometry method was developed for the analysis of zipeprol in human biofluids.



Scheme 1
Structure of TMS-zipeprol.

Experimental

Chemicals and reagents

Zipeprol dichlorhydrate (Sterling-Winthrop, France) and the internal standard (I.S.) levallorphan bitartrate (Theta Corporation, USA) were generous gifts of the respective companies. *N,O*,bis-(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) as catalyst was supplied by Pierce (USA). Chloroform, 2-propanol, *n*-heptane, and methanol were HPLC grade. All other chemicals were analytical grade (Merck). Stock solutions or zipeprol (1 mg ml^{-1}, free base) and I.S. were prepared in methanol and stored at 4°C. The zipeprol standard concentrations, obtained by dilution with methanol, were 20, 50, 100, 500, 1000 and 2000 ng ml^{-1}. Phosphate buffer was prepared with a saturated solution of dipotassium hydrogen phosphate, diluted 40% with deionized water and adjusted to pH 9.2.

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Chromatography

A model 5890 Series II Hewlett–Packard GC coupled with a model 5971 mass spectrometer was used. Data acquisition and manipulation were performed using standard software (Chemstation). Splitless injection was employed (split valve off-time of 0.75 min) and the mass selective detector was used in the electron impact mode at 70 eV. The injection port and transfer line temperatures were 270 and 280°C, respectively. A 12 m × 0.20 mm fused-silica capillary column HP-1 (dimethylsiloxane) was used. The initial GC oven temperature was 100°C held for 3 min, then programmed at 30°C min^{-1} to 280°C and then held for a further 3 min. The flow of carrier gas (helium, purity N 55) through the column was 1.8 ml min^{-1} and the head pressure on the column was maintained at 10 psi. The instrument was autotuned daily with perfluorotributylamine. For sample analysis, the electron multiplier voltage of the detector was set in the range 200–400 V above the autotune voltage.

The determination of zipeprol was performed by plotting peak-area ratios of the selected ions (drug–I.S.) against the concentration of standards to produce standard curves and by comparing the results for the case samples with the calibration plot.

Extraction procedure

Plasma or urine (2 ml) was pipetted into a 15-ml Pyrex centrifuge tube followed by 1 ml phosphate buffer (40% w/v, pH 9.2), 20 µl levallorphan (50 µg ml^{-1}), and 10 ml chloroform–isopropanol–*n*-heptane (50:17:33, v/v/v). After agitation and centrifugation, the organic phase was purified by an additional acidic extraction (5 ml 0.2 M HCl). Then the aqueous layer was re-extracted after addition of 2 ml phosphate buffer (40% w/v, pH 9.2), 0.5 ml concentrated ammonia solution, and 5 ml chloroform. After agitation and centrifugation, the organic phase was removed and evaporated to dryness at 45°C in a Speed Vac concentrator (Savant Instruments). BSTFA +1% TMCS (20 µl) was added to the dry extract, which was then stoppered and incubated at 70°C for 25 min. After cooling, a 2 µl volume of the reaction mixture was injected directly into the GC column.

Results and Discussion

Following extraction and silylation, samples spiked with zipeprol were found to yield only one chromatographic peak. No other by-products or impurities were observed. The TMS derivative showed good peak symmetry. Once the samples were derivatized, they were stable for a few hours.

The electron impact mass spectrum of zipeprol is shown in Fig. 1. The ion chosen for monitoring were the base peaks at *m/z* 335 (zipeprol) and 355 (internal standard). To be considered positive for zipeprol, the selected ion monitoring analysis must show coincident peaks in the *m/z* 335, 121 and 233 ion current profiles. Retention times of the internal standard and zipeprol were 9.19 and 10.52 min, respectively. The retention times were reproducible as long as the carrier gas flow remained constant. The retention times of the internal standard varied by less than 0.11 min over a 4-month period.

Levallorphan, chosen as the internal standard, was clearly separated from zipeprol. Moreover, the compound also is separated from opiates which are sometimes co-abused: codeine (RT 9.54 min, *m/z* 371), ethylmorphine (RT 9.66 min, *m/z* 385), morphine (RT 9.78 min, *m/z* 429), 6-monoacetylmorphine (RT 10.07 min, *m/z* 399) and nalbuphine (RT 10.39 min, *m/z* 446). This is of particular interest since several opiates can be identified in the same sample, especially in addicted subjects. A single ion chromatogram for a patient plasma sample is shown in Fig. 2.

Chloroform–isopropanol–*n*-heptane (50:17:33, v/v/v) was chosen as the extraction solvent on the basis of its ability to minimize emulsion formation during the extraction and to give a suitable recovery (86.3% ± 4.6, n = 6). Concentration versus response curves for zipeprol were linear from 20 to 2000 ng ml^{-1}. Correlation coefficients ranged from 0.9978 to 0.9995 for five independently established calibration curves.

Within-run precision and accuracy data for spiked plasma and urine samples are presented in Table 1. The assay was accurate to within 6% of the target concentrations. The relative standard deviations for within-run data were less than 6% at the three concentrations.

Minimum detectable quantities at *m/z* 335, for a signal-to-noise ratio of 3:1, are found to be 0.6 and 0.4 ng ml^{-1} for plasma and urine, respectively. These detection limits are suitable for forensic and clinical analyses.

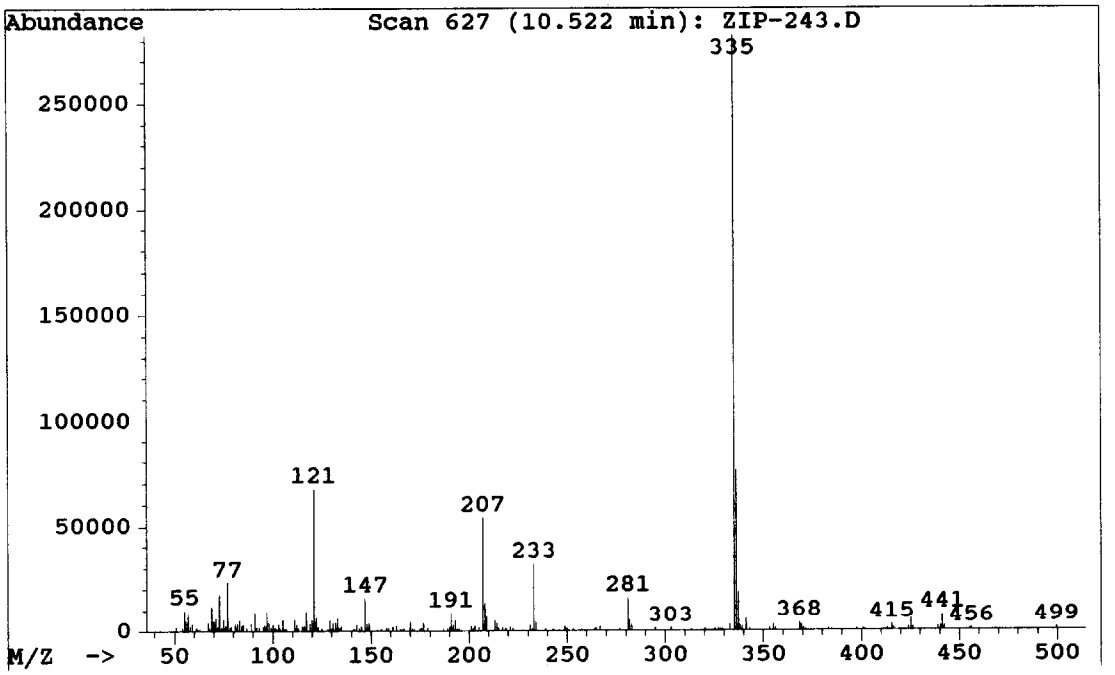

Figure 1
Electron impact mass spectrum of TMS-zipeprol.

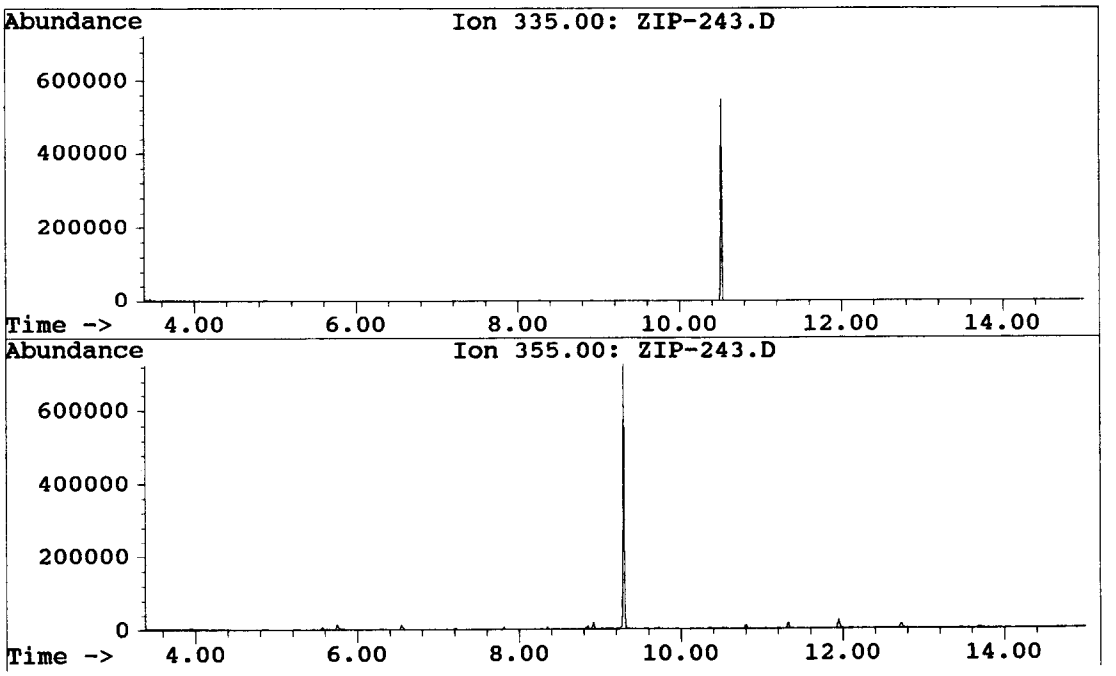

Figure 2
Single ion chromatogram for a patient plasma sample (*m/z* 335 zipeprol, concentration = 187 ng ml^{-1}; *m/z* 355 internal standard).

Table 1
Within-run precision and accuracy of GC–MS assay for zipeprol in plasma and urine (n = 6)

Fluid	Conc. given (ng ml^{-1})	Conc. found (ng ml^{-1})	RSD (%)	Error (%)
Plasma	20	21.1	5.9	5.5
Plasma	100	104.9	5.2	4.9
Plasma	500	521.6	4.8	4.2
Urine	100	96.2	5.8	−3.8
Urine	500	482.7	4.7	−3.6
Urine	1000	1057.3	5.2	5.7

Experimental conditions as given in the text.

Application

A 21-year-old man (subject V) was admitted in April 1992 to the intensive care unit Pavillon Pasteur of the CHRU Hospital of Strasbourg after self ingestion of approximately one bottle of Respilene® (zipeprol, 0.5%, 200 ml) corresponding to 1 g of zipeprol dichlorhydrate. On arrival, he presented drowsiness and a normal ECG. Heart rate and blood pressure were 104 beats min^{-1} and 145/80 mm Hg. Gastric lavage was performed (10 l). The next day, the subject was discharged, fully recovered 24 h after the suicide attempt.

Toxicological analyses were performed on blood sample at the time of admission (T0) and two hours later (T2) and on the gastric lavage liquid (GL). Zipeprol concentrations were found to be 1.06 μg ml^{-1}, 277 ng ml^{-1} and 511 ng ml^{-1} at T0, T2 and GL, respectively. No ethanol was found.

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