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Simultaneous screening and quantitation of alpidem, zolpidem, buspirone and benzodiazepines by dual-channel gas chromatography using electron-capture and nitrogen–phosphorus detection after solid-phase extraction

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ABSTRACT

A rapid twin-column gas chromatographic (GC) method for simultaneous screening and determination of commonly prescribed benzodiazepines and other new anxiolytics from plasma is described. Identical fused-silica Ultra 2 (5% phenyl methyl silicone) columns were connected to nitrogen–phosphorus and electron-capture detectors. The drugs were isolated from 1 ml of plasma by solid-phase extraction (SPE) onto a C_8 reversed-phase sorbent and recovered with 0.5% acetic acid in methanol. The eluate was reconstituted with isopropanol which was found suitable for on-column injection. Prazepam was used as internal standard. The method was found appropriate for the quantification in a single run of alpidem, alprazolam, buspirone, chlordiazepoxide, clobazam, clotiazepam, diazepam, estazolam, flunitrazepam, lorazepam, midazolam, oxazepam, tofisopam, triazolam, and zolpidem within 30 min. Limits of quantification allow toxicological or pharmacological determinations, except for buspirone: only toxic blood levels can be quantified by this method. This first SPE of imidazopyridines (alpidem and zolpidem) provides faster, more efficient and cheaper sample preparation than the traditional liquid–liquid procedure. This GC analysis of alpidem and zolpidem is also the first described procedure for simultaneous quantification of all different classes of anxiolytics.

INTRODUCTION

Benzodiazepines

The benzodiazepines (Fig. 1) are an important class of sedative hypnotic drugs and are widely prescribed throughout the world for the treatment of anxiety, epilepsy and insomnia [1–3]. The trace analysis of benzodiazepines in plasma is important in toxicology since these compounds are frequently involved in drug intoxications or traffic accidents and are also used by criminals to incapacitate their victims [4,5]. Therefore screening for and quantitation of benzodiazepines is widely used for medical and jurisdictional purposes.

A large number of analytical methods have been published for the determination of benzodiazepines. They can be screened as benzophenones by thin-layer chromatography [6]. Radioreceptor assay [7], enzyme immunoassay [8], immunofluoropolarization [9] and radioimmunoassay [10] can measure small blood samples rapidly but they give no indication on the identity of the benzodiazepines found.

The most commonly used methods for quantitation of benzodiazepines employ gas chroma-

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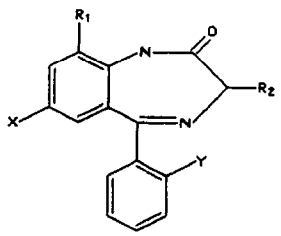
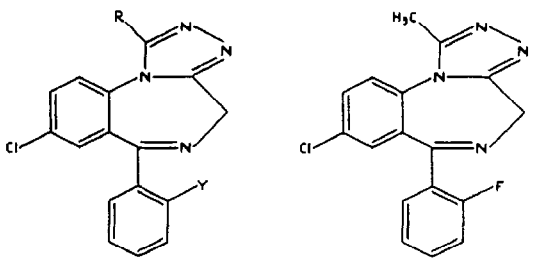
(a)



	X	Y	R_1	R_2
a-Diazepam	Cl	H	CH_3	H
b-Nordiazepam	Cl	H	H	H
c-Oxazepam	Cl	H	H	OH
d-Lorazepam	Cl	Cl	H	OH
e-Nitrazepam	NO_2	H	H	H
f-Flunitrazepam	NO_2	F	CH_3	H
g-Clonazepam	NO_2	Cl	H	H

(b)



	R	Y
Estazolam	H	H
Triazolam	CH	Cl

Midazolam

Fig. 1. Chemical structures of some (a) 1,4-benzodiazepines, (b) imidazobenzodiazepines.

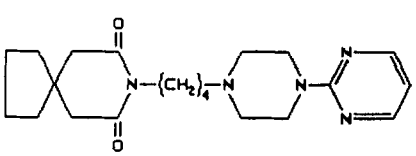
tography (GC) and high-performance liquid chromatography (HPLC) [11–13]. In GC methods, flame ionization detection (FID) [14], electron-capture detection (ECD) [15,16] and nitrogen–phosphorus detection (NPD) [17,18] have been used. In order to increase the number of drugs detected in a single run, a dual-column analysis has been used by installing two columns in an injector and connecting the column ends to nitrogen–phosphorus and electron-capture detectors [19]. The combination of these specific and sensitive detectors is a powerful tool when simultaneous screening and quantitative analysis is required.

Buspirone

Buspirone (Buspar) (Fig. 2) is a recently introduced anti-anxiety drug, whose properties have been extensively reviewed by Goa and Ward [20]. It is equipotent with the benzodiazepines but it does not exhibit adverse side-effects such as sedation, muscle relaxation and anticonvulsion. A radioimmunoassay method for plasma quantification has been developed [21]. On the other hand, GC or HPLC methods are numerous [22–25]. Solid-phase extraction (SPE) procedures for sample clean-up have already been proposed [22,25]. The extraction recovery of the latest published method is 66% on a C_{18} SPE column [25].

Imidazopyridines: alpidem and zolpidem

Alpidem (Ananxyl) and zolpidem (Stilnox) are two compounds of the imidazopyridine chemical series (Fig. 3) developed for their psychoactive properties [26,27]. At clinical level, zolpidem is a



Buspirone

Fig. 2. Chemical structure of buspirone.

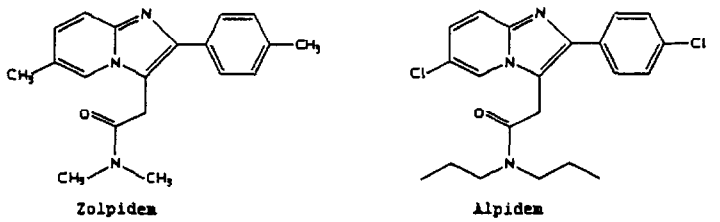


Fig. 3. Chemical structures of imidazopyridines.

potent, short acting hypnotic devoid of residual effects on daytime activities [28], while alpidem possesses an interesting anxiolytic activity, virtually devoid of sedative and myorelaxant properties [29].

The methodology for the determination of zolpidem and alpidem in body fluids has been described [30,31]. The methods used were high-performance liquid chromatography and fluorimetric detection. A recent paper by Tracqui *et al.* [32] described the first toxicological screening of zopiclone, zolpidem, suriclone and alpidem by high-performance liquid chromatography with diode array detection. No GC method or SPE procedure has been reported yet.

EXPERIMENTAL

Instrumentation

The equipment used was a Hewlett-Packard 5890 series II (Les Ulis, France) capillary gas chromatograph with electron-capture and nitrogen–phosphorus detectors. The HP 5890 and an HP 7673 automatic injector were interfaced and monitored by the software HP Chemstation 3365.

Two 25 m × 0.32 mm I.D. fused-silica capillary columns, coated with Ultra 2 (5% phenyl methyl silicone, film thickness 0.52 μm, Hewlett-Packard) were connected to the two different detectors. Deactivated fused-silica tubing (5 m × 0.53 mm I.D.) from SGE (Villeneuve Saint-Georges, France) was used as precolumn (retention gap). The precolumn was connected to the analytical columns with a metal fixed inlet splitter for capillary columns from SGE. On-column injection (2 μl) in the oven track mode was done at 213°C. The detectors were maintained at 300°C. The carrier gas was helium, flow-rate 2.1 ml/min (constant flow mode, *i.e.* 120 kPa at 210°C). The make-up gases were nitrogen (60 ml/min) for the electron-capture detector and helium (23 ml/min) for the nitrogen–phosphorus detector. The flow-rate of air was 80–100 ml/min and that of hydrogen 3 ml/min. The oven temperature was 210°C and was increased to 285°C at 2.5°C/min. The chromatography time was 30 min. The relative retention times are listed in Table I.

For SPE, we used C_8 100 mg/1 ml (Amprep) cartridges from Amersham International plc (Bucks, UK), and a Vac Elut sample processing station (Analytichem International) from Prolabo (Paris, France).

TABLE I

RELATIVE RETENTION TIMES OF THE COMPOUNDS

Compound	Relative retention time[a]
Oxazepam	0.599
Lorazepam	0.688
Diazepam	0.722
Clotiazepam	0.813
Clobazam	0.872
Midazolam	0.922
Flunitrazepam	0.946
Prazepam	1
Zolpidem	1.230
Chlordiazepoxide	1.283
Estazolam	1.402
Alprazolam	1.477
Triazolam	1.631
Tofisopam	1.645
Alpidem	1.773
Buspirone	2.025

[a] Relative to the internal standard prazepam.

Reagents

The drugs were gifts from the manufacturers: chlordiazepoxide, diazepam, flunitrazepam and midazolam from Hoffmann-La Roche (Neuilly sur Seine, France); lorazepam and oxazepam from Sanofi Pharma Industrie (Massy, France); prazepam from Parke Davis France (Orléans, France); tofisopam from Biogalénique (Paris, France); estazolam from Takeda (Puteaux, France); alprazolam and triazolam from Upjohn (Sussex, UK); clotiazepam from Latema (Suresnes, France); clobazam from Diamant (Puteaux, France); buspirone from Bristol-Myers Squibb (Paris, France); alpidem and zolpidem from Synthelabo (Le Plessis-Robinson, France). Methanol and acetonitrile RPE-ACS were from Carlo Ebra (Milano, Italy). Isopropanol and acetic acid of HPLC-grade, potassium bicarbonate of analytical-grade were from Merck (Darmstadt, Germany).

Calibration and reagent solutions

A stock solution containing 1 g/l of each drug was prepared in acetonitrile. A plasma standard was prepared containing 2 mg/l of alpidem, chlordiazepoxide, clobazam, clotiazépam, diazepam, lorazepam, midazolam, oxazepam, tofisopam, zolpidem and 0.2 mg/l of alprazolam, buspirone, estazolam, flunitrazepam and triazolam. This standard was diluted to 1:4 and 1:19 with drug free plasma to get the proper calibration range.

The working internal standard solution is prepared by diluting 200 μl of the stock solution of prazepam (1 g/l in acetonitrile) in 100 ml of distilled water.

The reagent for conditioning the solid-phase columns was prepared by dissolving 1 g of potassium bicarbonate in 100 ml of 10% acetonitrile in distilled water.

Drug free plasma was collected from drug free volunteers. Plasma was pooled, extracted and analysed by our method. No peaks corresponding to compounds of interest were observed (Fig. 4).

Extraction procedure

Solid-phase columns were positioned in luerlock fittings on the cover of the vacuum box. The

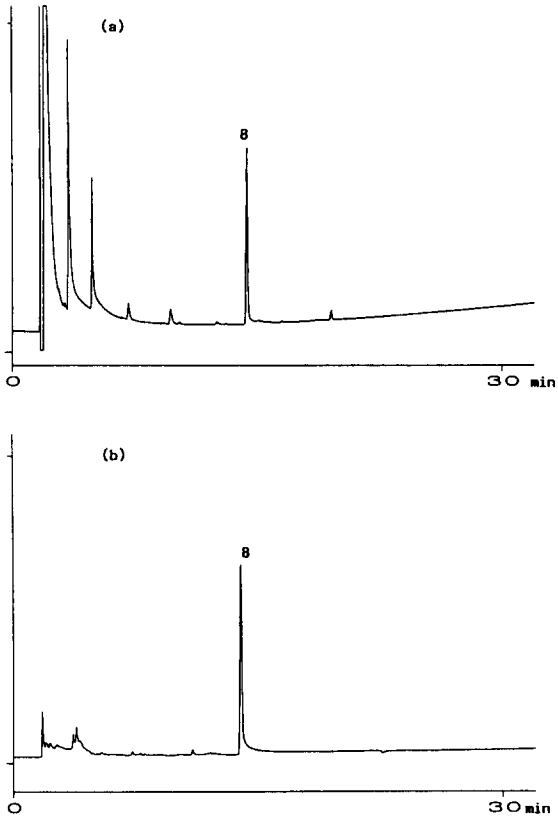


Fig. 4. Chromatograms by (a) NPD, and (b) ECD of an extract of 1 ml of drug free plasma. Peak: 8 = prazepam.

columns were conditioned with 2 × 1 ml of methanol and then with 1 ml of the conditioning reagent. A 1-ml volume of standard, control or patient sample diluted with 1 ml of internal standard working solution was then applied and allowed to drain under vacuum. The columns were washed with 500 μl of 10% acetonitrile in distilled water and left under vacuum for 1 min.

The analytes were eluted with three volumes of 200 μl of 0.5% acetic acid in methanol. The eluate was evaporated under a stream of nitrogen at 40°C. Next, the residue was dissolved in 50 μl of isopropanol and 2 μl were injected.

METHOD VALIDATION PARAMETERS

Precision and recovery

Within-run precision was calculated from repeated analysis ($n = 10$) during one working day. Day-to-day precision was calculated from re-

peated analyses of quality control samples on ten successive working days.

The recovery of benzodiazepines and other anxiolytics was calculated by comparing peak-area ratios of the drugs to internal standard with and without extraction. In both cases the internal standard was added just before chromatographic analysis.

Linearity

Range. The linearity of a test procedure is defined as its ability (within a given range) to produce results which are directly proportional to the concentration of the analyte in the sample [33]. The FDA guidelines [34] recommend a concentration range equivalent to 80–120% of the theoretical content of the active compound. In the literature often a range of even 0–200% is examinated. In practice the study should be designed to be appropriate for the intended analytical method. In toxicology the range needs to be wide enough to determine in a single injection if the patient is at therapeutic or toxic plasma levels.

Evaluation of results. Often, data are processed by linear least-squares regression and authors quote values obtained for the regression coefficient "a" and "b" of the linear equation $Y = aX + b$, together with a correlation coefficient. Following Carr and Wahlich [33], this is inappropriate because:

– the value of "a" does not provide any information on the linearity of the procedure or on the goodness of fit to a regression line.

– The value of "b" is very important as it is the value of the intercept of the regression line. This is often presented as the number computed in the regression calculation and will not have any meaning to the reader. This is easily remedied by expressing "b" as a percentage of the value of the analytical response at a 100% analyte level. It is suggested that this percentage should normally be within the range −2.0 to +2.0% for the validation to be considered satisfactory.

Analysis of variance in linear regression [35]. Analysis of variance (ANOVA) is a powerful and very general method which separates the contributions to the over-all variation in a set of experimental data and tests their significance. The sources of variation are each characterized by a sum of squares (SS), *i.e.* the sum of a number of squared terms representing the variation in question, a number of degrees of freedom (DF), and a mean square, which is the former divided by the latter and which can be used to test the significance of the variation contribution by means of the F-test.

In analytical calibration experiments, only variation in the Y-direction is considered (Fig. 5). This variation is expressed as the sum of the squares of the distances of each calibration point from the mean Y value: Y_{mean}. This is the total SS about Y_{mean}: S_t. There are two contributions to this over-all variation. One is the SS due to the regression: S_1, and the second source of variation is the SS about regression, *i.e.* residual: S_0.

Quality of the model

Source of variation	DF	Mean square
S_1 (regression)	1	$s_1^2 = S_1$
S_0 (residual)	$n-2$	$s_0^2 = S_0/n-2$
S_t (total)	$n-1$	$s_t^2 = S_t/n-1$

$S_1 = \varepsilon(Y_{cal} - Y_{mean})^2$

$S_0 = \varepsilon(Y_i - Y_{cal})^2$

$S_t = \varepsilon(Y_i - Y_{mean})^2$

where Y_i are the individual values of Y, Y_{cal} is the value of Y_i calculated by the regression and Y_{mean} is the mean value of Y_i.

If $F_{cal} = s_1^2/s_0^2 > F_{the}$ F_{the} is for $(1, n-2)$ DF (where DF is degree of freedom) so, the source of variation is well described by the regression,

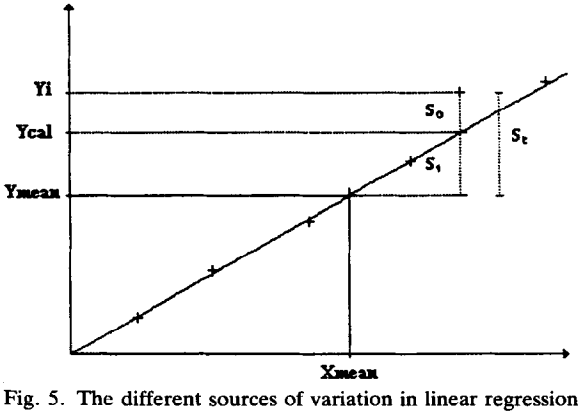


Fig. 5. The different sources of variation in linear regression for analysis of variance.

and the model (linear regression) can be considered as correct.

Quality of the fit

The residual variation can be divided into two contributions: the lack of fit and the analytical pure error:

Source of variation	DF	Mean square
S_{LF} (lack of fit)	$(n-2)-n/2$	$s^2_{LF} = S_{LF}/(n/2)-2$
S_{PE} (pure error)	$n/2$	$s^2_{PE} = S_{PE}/n/2$
S_0 (residual)	$n-2$	$s^2_0 = S_0/n-2$

$S_{PE} = \varepsilon(y_i - y_{mean})^2$ where y_i are the values of Y_i for the same concentration (within groups variation), and y_{mean} is the mean value of y_i for the same concentration.

$S_{LF} = S_0 - S_{PE}$

If $F_{cal} = s^2_{LF}/s^2_{PE} > F_{the}$ F_{the} for $[(n-2)-n/2, n/2]$ DF

so, the lack of fit represents the main source of variation, the fit of the linear regression must be rejected.

Accuracy

Firstly, a plasma standard of known concentration is prepared and measured n times. From the values of the peak-areas, we can calculate the mean (m) and the standard deviation (S.D.) which we consider as being the true values [36].

Secondly, from the equation of the linear regression: $Y = aX + b$, we can calculate the measured peak-area corresponding to this point: Y_t. Then, we test the accuracy by the mean of a t-statistic, by comparing a mean to a point as follows:

$$t_{observed} = (m - Y_t)/(\text{S.D.}/(n^{1/2}))$$

If $t_{observed} < t_{table}$, the null hypothesis is accepted, *i.e.* the measured value and the true value are not different; t_{table} is given with $(n-1)$ DF.

We must mention that by that this procedure, only internal errors were tested. Since our way for standard preparation is always the same, we cannot estimate the lack of accuracy due to external errors (incorrect weight or volumes of standards or samples, or inaccurate dilution of primary standards).

In our study, we have tested accuracy at the lowest and the highest point of the calibration range.

Limit of quantification (LOQ)

The limit of detection is a parameter of limit tests and may be defined as the smallest quantity of analyte which may be expected to produce a response which is significantly different from that of a blank [33,37].

The limit of quantification is a parameter of determination tests for minor components and may be defined as the smallest quantity of analyte which can be determined with acceptable precision.

LOQ was determined by diluting successively the lowest point of calibration and by performing

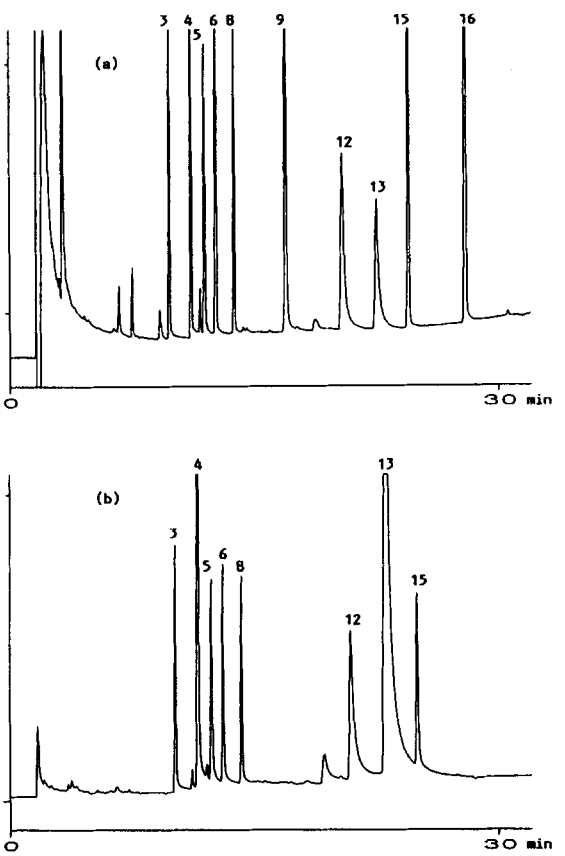


Fig. 6. Chromatograms by (a) NPD, and (b) ECD of an extract of 1 ml of a plasma standard containing 500 μg/l of each drug. Peaks: 3 = diazepam, 4 = clotiazepam, 5 = clobazam, 6 = midazolam, 8 = prazepam, 9 = zolpidem, 12 = alprazolam, 13 = triazolam, 15 = alpidem, 16 = buspirone.

a within-day precision study. As long as C.V. was <10% ($n = 10$), a further dilution was made and a precision study executed.

Interferences

Retention times of psychotropic compounds were investigated in order to check their interfer-

TABLE II

WITHIN- AND BETWEEN-DAY PRECISION STUDY ($n = 10$)

Compound	Concentration (μg/l)	Coefficient of variation (%)	
		Within-day	Between-day
Oxazepam	2000	5.2	8.8
	500	4.1	8.7
	100	7.3	8.2
Lorazepam	2000	6.0	8.0
	500	3.8	8.6
	100	9.0	4.3
Diazepam	2000	6.0	2.2
	500	4.1	3.2
	100	5.4	3.3
Clotiazepam	2000	2.9	3.7
	500	1.7	3.8
	100	3.8	3.0
Clobazam	2000	5.3	4.0
	500	5.8	6.1
	100	6.3	5.3
Midazolam	2000	4.4	3.1
	500	4.4	4.9
	100	4.1	3.4
Zolpidem	2000	7.3	0.1
	500	2.7	4.5
	100	4.1	3.2
Chlordiazepoxide	2000	4.1	7.6
	500	3.7	4.8
	100	4.8	7.8
Tofisopam	2000	6.0	7.3
	500	3.9	6.6
	100	1.8	6.3
Alpidem	2000	2.6	3.2
	500	3.2	7.4
	100	2.3	4.7
Flunitrazepam	2000	2.8	8.9
	500	7.2	6.3
	100	5.5	7.9
Estazolam	2000	3.2	7.5
	500	4.2	6.9
	100	5.9	7.8
Alprazolam	2000	5.2	7.3
	500	6.5	6.8
	100	5.7	5.9
Triazolam	2000	4.1	6.5
	500	4.2	5.8
	100	4.8	7.7
Buspirone	2000	3.9	4.6
	500	3.4	8.3
	100	5.9	9.6

ence with the method. This included phenothiazines, antidepressants, sedatives and some major metabolites of benzodiazepines.

RESULTS AND DISCUSSION

Comparison of the two detectors

Since ECD affords a higher degree of selectivity and sensitivity towards chlorinated drugs as compared with other endogenous substances which may be present in the sample extract, it was found to be clearly preferable for alpidem and benzodiazepines. All compounds could be detected with the nitrogen–phosphorus detector and it was found to be required for detecting buspirone and zolpidem. These compounds did not produce peaks on the electron-capture channel (Fig. 6).

Precision and recovery

The within-run and day-to-day variations are listed in Table II, recoveries are given in Table III. The extraction efficiency for the imidazopyridines is better than 93%, and those for the benzodiazepines are between 63 and 105%.

TABLE III

RECOVERIES OF THE COMPOUNDS FOR EACH LEVEL OF CALIBRATION

Compound	Recovery (%)		
	100 μg/l	500 μg/l	2000 μg/l
Oxazepam	64.8	64.7	63.3
Lorazepam	76.5	70.7	70.5
Diazepam	83.2	83.6	82.9
Clotiazepam	95.0	96.4	95.8
Clobazam	101.0	100.7	101.4
Midazolam	95.1	95.7	97.1
Zolpidem	96.3	97.1	94.9
Chlordiazepoxide	97.8	98.9	100.2
Tofisopam	86.8	91.4	83.7
Alpidem	94.5	93.0	93.9
	10 μg/ml	50 μg/ml	200 μg/ml
Flunitrazepam	97.4	100.1	99.2
Estazolam	99.3	98.6	98.5
Alprazolam	103.8	102.9	101.5
Triazolam	98.0	97.7	97.7
Buspirone	88.6	89.9	90.2

Our extraction procedure enhances the extraction efficiency for buspirone to 89% as compared to 66% with the previous published method [25]. Moreover the extraction columns can be used twice without the occurrence of interferences.

Linearity

Linearity studies are resumed in Table IV. The values of "r" are between 0.9991 and 0.9999 for each compound. The Y-intercept expressed as a percentage of the value of analytical response at the 100% analyte level was found to be within the range −1.2 to +0.4% which can be considered satisfactory for the validation.

The analysis of the variance consists of the following:

– *Quality of the model.* In the case of alpidem:

$S_1 = 14.087417$ so $s_1^2 = 14.087417$
$S_0 = 0.108353$ so $s_0^2 = 0.013544$
$S_t = 14.195770$

$F_{cal} = s_1^2/s_0^2 = 1040.10$ F_{the} S.D.(1.8) = 5.32

In this case $F_{cal} \gg F_{the}$, linear regression represents a good model. Analysis of the variance was realized in the same way for each compound and F_{cal} was always found to be higher than 950.

– *Quality of the fit.* In the case of alpidem:

$S_{LF} = 0.059673$ so $s_{LF}^2 = 0.019891$
$S_{PE} = 0.048679$ so $s_{PE}^2 = 0.009736$
$S_0 = 0.108353$

$F_{cal} = s_{LF}^2/s_{PE}^2 = 2.04$ F_{the} (3.5) = 5.41

In this case $F_{cal} < F_{the}$, the lack of fit is not the main cause of variation, and we can accept this fit as being of good quality. The same analysis was performed for each compound, F_{cal} was always found to be lower than 3.50.

Accuracy

Table V gives the different values of m, S.D., Y_t, and t_{obs} for each drug; t_{table} ($\alpha = 5\%$, $n - 1$ DF) = 2.26. In these conditions the hypothesis of equality must be rejected for the concentration of 100 μg/l for alpidem and tofisopam, and for the concentration of 10 μg/l for alprazolam.

Limit of quantification

The limit of quantification is the lowest con-

TABLE IV

LINEARITY STUDY FOR LINEAR REGRESSION: $y = ax + b$

Compound	Slope (mean ± S.D., $n = 10$)	C.V. (%)	y-Intercept (%)[a]
Oxazepam	0.000374 ± 0.000035	9.3	−0.25
Lorazepam	0.000631 ± 0.000053	8.4	−0.03
Diazepam	0.000593 ± 0.000013	2.2	0.19
Clotiazepam	0.002943 ± 0.000113	3.8	0.09
Clobazam	0.000684 ± 0.000028	4.2	0.32
Midazolam	0.000581 ± 0.000019	3.2	0.04
Flunitrazepam	0.001296 ± 0.000118	9.1	−0.15
Zolpidem	0.001200 ± 0.000064	5.4	−1.18
Chlordiazepoxide	0.001409 ± 0.000113	8.0	−0.6
Estazolam	0.001346 ± 0.000112	8.3	−0.29
Alprazolam	0.001299 ± 0.000080	6.2	−0.32
Triazolam	0.004578 ± 0.000233	4.7	−0.08
Tofisopam	0.000899 ± 0.000069	7.7	−0.05
Alpidem	0.000841 ± 0.000028	3.3	0.12
Buspirone	0.001664 ± 0.000103	6.2	−0.85

[a] y-intercept (%): y-intercept expressed as a percentage of the value of analytical response at the 100% analyte level.

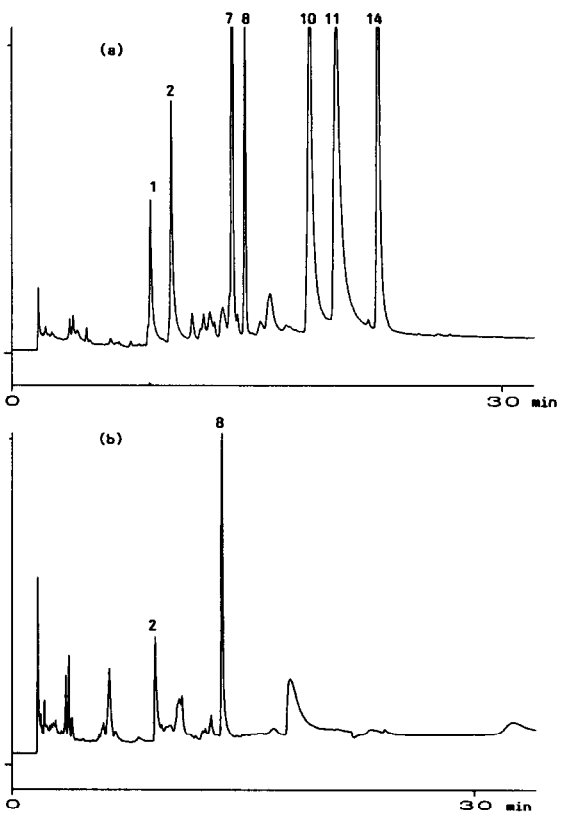


Fig. 7. Chromatograms by ECD of (a) an extract of 1 ml of a plasma standard containing 500 μg/l of each drug. Peaks: 1 = oxazepam, 2 = lorazepam, 7 = flunitrazepam, 8 = prazepam, 10 = chlordiazepoxide, 11 = estazolam, 14 = tofisopam. (b) An extract of 1 ml of a plasma from a patient. Peaks: 2 = lorazepam (966 μg/l), 8 = prazepam.

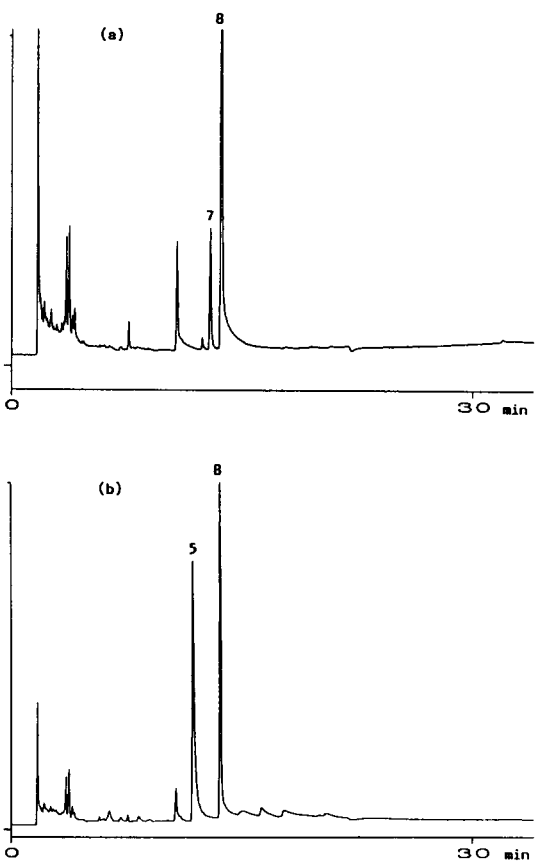


Fig. 8. Chromatograms by ECD of an extract of 1 ml of plasma from patients after an attempt at suicide. Peaks: (a) 7 = flunitrazepam (153 μg/l), 8 = prazepam (b) 5 = clobazam (1580 μg/l).

TABLE V

ACCURACY STUDY USING t-STATISTICS

y_t = value of area response given by the linear regression equation $y_t = ax + b$.

Compound	Concentration (μg/l)	Accuracy (n = 10)		y_t	t (observed)
		Mean	S.D.		
Alpidem	100	0.0722	0.0080	0.0860	5.45
	2000	1.6912	0.0514	1.6839	0.40
Alprazolam	10	0.0117	0.0005	0.0126	5.69
	200	0.2591	0.0130	0.2578	0.31
Buspirone	10	0.0142	0.0012	0.0136	1.58
	200	0.3301	0.0121	0.3296	0.13
Chlordiazepoxide	100	0.1241	0.0096	0.1239	0.07
	2000	2.8577	0.2204	2.8010	0.81
Clobazam	100	0.0752	0.0042	0.0727	0.41
	2000	1.3694	0.0550	1.3723	0.17
Clotiazepam	100	0.3047	0.0098	0.2994	1.71
	2000	5.8912	0.2199	5.8911	0.01
Diazepam	100	0.0603	0.0039	0.0614	0.89
	2000	1.1879	0.0311	1.1881	0.02
Estazolam	10	0.0131	0.0010	0.0128	0.95
	200	0.2591	0.0181	0.2646	0.96
Flunitrazepam	10	0.0132	0.0012	0.0126	1.58
	200	0.2588	0.0208	0.2571	0.26
Lorazepam	100	0.0629	0.0031	0.0626	0.30
	2000	1.2634	0.1112	1.2615	0.05
Midazolam	100	0.0644	0.0040	0.0625	0.15
	2000	1.1651	0.0250	1.1624	0.34
Oxazepam	100	0.0356	0.0036	0.0355	0.09
	2000	0.7440	0.0527	0.7461	0.12
Tofisopam	100	0.0862	0.0020	0.0889	4.26
	2000	1.7912	0.2417	1.7971	0.08
Triazolam	10	0.0439	0.0062	0.0448	0.46
	200	0.9195	0.0403	0.9168	0.21
Zolpidem	100	0.1055	0.0202	0.0917	2.16
	2000	2.4012	0.1354	2.3717	0.68

centration which can be reproducibly analysed (n = 10, C.V. < 10%). It was found to be 10 μg/l for oxazepam and zolpidem; 5 μg/l for buspirone, chlordiazepoxide, estazolam and lorazepam; 2 μg/l for alpidem, alprazolam, clobazam, diazepam, midazolam and tofisopam; 1 μg/l for flunitrazepam and triazolam; 0.5 μg/l for clotiazepam.

These limits of quantification are valuable for toxicokinetic and pharmacokinetic studies and for monitoring drugs, except for buspirone of which the serum levels in patients receiving the drug on a regular basis are usually less than 5 μg/l. Thus, this method of assaying buspirone is only valuable for toxicokinetic studies.

Interferences

The retention times of *ca.* 100 psychotropic compounds were investigated in order to check their interference with our method. Of the drugs tested (Table VI), clomipramine was found to have a relative retention time (0.882) close to

TABLE VI

DRUGS AND METABOLITES TESTED FOR INTERFERENCES

Acebutolol	Diclofenac	Nortriptyline
Aceprometazine	Digitoxine	Opipramol
7-Acetamidoflunitrazepam	Digoxine	Papaverine
Alimemazine	Dosulepine	Paracetamol
Allobarbital	Doxepine	Pentobarbital
Amineptine	Etidocaïne	Phenobarbital
7-Aminoflunitrazepam	Ethambutol	Phenytoïne
7-Aminonorflunitrazepam	Flurazepam	Pipotiazine
Amisulpride	Fluoxetine	Primidone
Amitriptyline	Fluvoxamine	Prometazine
Amobarbital	Heptabarbital	Propericiazine
Apronal	Hexobarbital	Proxibarbal
Barbital	9-hydroxydemoxepam	Quinupramine
Benzocaïne	1-Hydroxymethyltriazolam	Salicylic acid
Brallobarbital	4-Hydroxytriazolam	Secobarbital
Bromazepam	Imipramine	Sulpiride
Bupivacaïne	Ketoprofene	Sultopride
Butalbital	Levomepromazine	Temazepam
Butobarbital	Lidocaïne	Theophylline
Caffeine	Loxapine	Thioproperazine
Carbamazepine	Lormetazepam	Thioridazine
Carbromal	Maprotiline	Tianeptine
Carisoprodol	Medifoxamine	Tiapride
Chlorpromazine	Meprobamate	Trifluoperazine
Chloroethazine	Metapramine	Trimipramine
Clomipramine	Metoclopramide	Viloxazine
Clorazepate	Mianserine	Vinylbital
Clonazepam	Nitrazepam	Zopiclone
Cyamemazine	Nomifensine	
Cyclobarbital	Nordazepam	
Demethylclomipramine	Nordoxepine	
Demoxepam	Norflunitrazepam	
Desipramine	Northiaden	

that of clobazam (0.872). Normal serum components or other tested drugs did not interfere with the analysis of benzodiazepines and the other anxiolytics.

CONCLUSION

Fused-silica capillary phenyl methyl silicone columns together with ECD and NPD detectors form an efficient equipment for the quantitative GC analysis of a large number of benzodiazepines and other anxiolytic substances: alpidem, zolpidem and buspirone, present in sample extracts (Figs. 7–9). The SPE of imidazopyridines described here for the first time provides a faster, more efficient and cheaper way of sample preparation than could be obtained with the traditional liquid–liquid procedure. This first GC analysis of alpidem and zolpidem is also the first described procedure for simultaneous quantification of all different classes of anxiolytics.

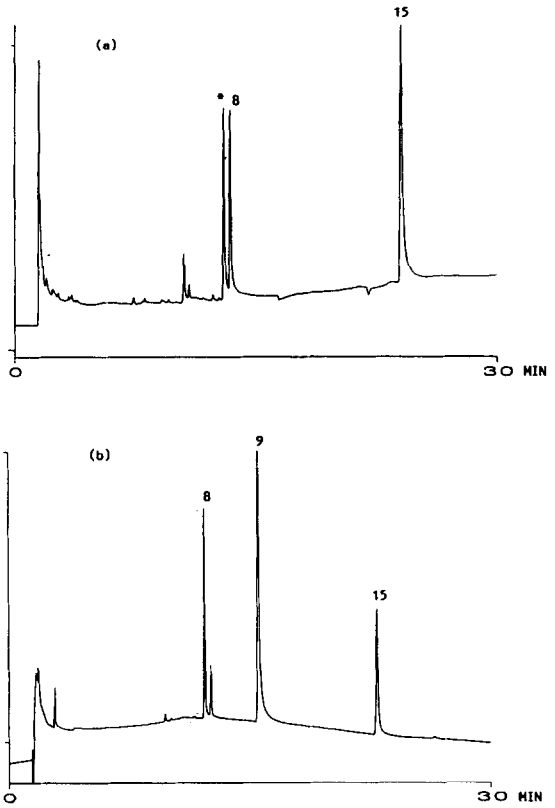


Fig. 9. Chromatograms of an extract of 1 ml of plasma from patients after an attempt at suicide. Peaks: (a) ECD, * = amoxapine, 8 = prazepam, 15 = alpidem (2350 μg/l). (b) NPD, 9 = zolpidem (845 μg/l), 15 = alpidem (420 μg/l).

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