Journal of Chromatography, 533 (1990) 97–110
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO 5463

Simultaneous screening and quantitative analysis of benzodiazepines by dual-channel gas chromatography using electron-capture and nitrogen–phosphorus detection

P. LILLSUNDE* and T SEPPÄLÄ

Laboratory of Pharmacology and Toxicology, Department of Biochemistry, National Public Health Institute, Mannerheimintie 166, SF-00300 Helsinki (Finland)

(First received February 7th, 1990, revised manuscript received July 9th, 1990)

ABSTRACT

A rapid twin-column gas chromatographic method for the simultaneous screening and determination of commonly prescribed benzodiazepines from plasma and whole blood is presented Identical fused-silica SE-54 columns were inserted in a common split–splitless injector and connected to nitrogen–phosphorus and electron-capture detectors By combining these specific and sensitive detectors considerable and accurate chromatographic information was obtained in a single run. The drugs were extracted from 1 ml of buffered plasma or blood with *n*-hexane–dichloromethane (70:30) and analysed without derivatization Flurazepam was used as internal standard The method was reproducible enough to permit reliable quantitation of plasma diazepam, nordiazepam, oxazepam, lorazepam, chlordiazepoxide, temazepam, midazolam, alprazolam, clobazam, norclobazam, adinazolam, flunitrazepam, bromazepam, triazolam, nitrazepam and clonazepam within 12 min

INTRODUCTION

Benzodiazepines (Fig. 1) are widely prescribed for the treatment of anxiety, epilepsy and insomnia. Notwithstanding their low toxicity compared with many central nervous system (CNS)-active drugs, benzodiazepines are often involved in drug intoxication and they are liable to abuse and capable of affecting human skilled performance [1]. Therefore screening and quantitation of benzodiazepines from different specimens is widely indicated 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 (TLC) [2]. However, triazolam, alprazolam and clobazam do not produce benzophenones at all and since some benzodiazepines produce the same benzophenones, positive results must be confirmed by an alternative method. Radioreceptor assay [3], enzyme immunoassay [4], immunofluoropolariza-

0378-4347/90/$03 50

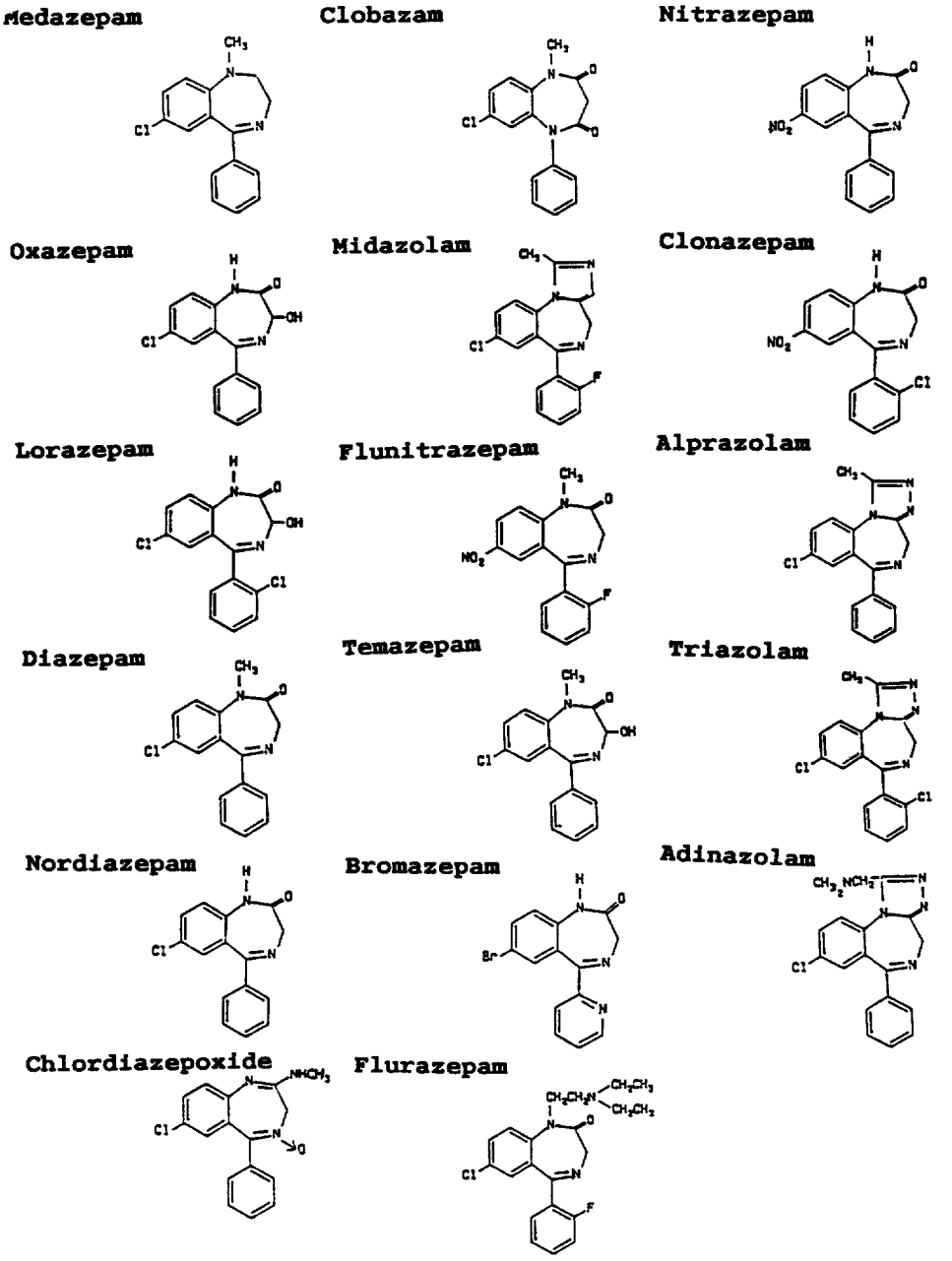


Fig. 1 Structures of benzodiazepines (in order of increasing retention times)

tion [5] and radioimmunoassay [6] measure small blood samples rapidly but give no indication of the identity of any benzodiazepines found.

The most commonly used methods for quantitation of benzodiazepines em-

ploy gas chromatography (GC) and high-performance liquid chromatography (HPLC) [7,8]. In GC methods, flame-ionization detection (FID) [9], electron-capture detection (ECD) [10–12] and nitrogen–phosphorus detection (NPD) [13,14] have been used. Since some benzodiazepines are thermally unstable, they have been derivatized [15] or converted into the corresponding benzophenones [16] prior to analysis. Benzodiazepines have been also analysed without chemical modification [12,17–19].

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 flame ionization detectors [20]. We have applied this principle to the simultaneous screening and quantitation of benzodiazepines using dual-channel capillary GC with two fused-silica columns attached to electron-capture and nitrogen–phosphorus detectors. These specific and sensitive detectors have not previously been reported to be combined in dual-column systems for drug analysis although together they are a powerful tool, especially for detecting and determining benzodiazepines. The method has proved to be reliable and practical when simultaneous screening and quantitative analysis is required. It permits analysis of several substances from small sample amount in a short time.

EXPERIMENTAL

Reagents

Diazepam, nordiazepam, temazepam, flurazepam, desalkylflurazepam, chlordiazepoxide, desmethylchlordiazepoxide, demoxepam, nitrazepam, clonazepam, medazepam, desmethylmedazepam, flunitrazepam and bromazepam were obtained from Hoffmann-La Roche (Basel, Switzerland). Lorazepam and oxazepam were from Wyeth (Philadelphia, PA, U.S.A.), triazolam, adinazolam and alprazolam were from Upjohn (Kalamazoo, MI, U.S.A) and clobazam as well as norclobazam from Hoechst (Frankfurt, F.R.G.). Dichloromethane, *n*-hexane, ethyl acetate, Na_3PO_4 and Na_2HPO_4 were from Merck (Darmstadt, F.R.G.) and toluene from BDH (Poole, U.K.).

Extraction procedure

Samples of 1 ml of plasma, control solutions and working standards were shaken with 1 ml of 0.5 *M* Na_2HPO_4 (pH 9) and 5 ml of *n*-hexane–dichloromethane (70:30) containing flurazepam (100 μg/l) as internal standard. Flurazepam was chosen as internal standard because it is not marketed in Finland. It can be replaced by several other benzodiazepine derivatives. After centrifugation, the aqueous layer was discarded and the organic layer evaporated. The residue was dissolved in 100 μl of ethanol.

The validity of the extraction was studied by examing blood samples from drivers suspected of driving under the influence of psychotropic drugs. Ethyl

acetate was used as a solvent for lorazepam (pH 11), nitrazepam and clonazepam (pH 9), and other benzodiazepines were extracted in toluene (pH 9). These solvents have traditionally been used for benzodiazepines in our laboratory. Forty successive samples were analysed in parallel by using (1) *n*-hexane–dichloromethane and (2) a traditional extraction solvent. The coefficients of variation (C.V.) were calculated for parallel results obtained from these two extraction procedures. In addition, C.V. were estimated for duplicate analysis using only the traditional solvent.

Instrumentation

The instrument was a Hewlett-Packard 5880 capillary gas chromatograph with electron-capture and nitrogen–phosphorus detectors. Two 25 m × 0.31 mm I.D. fused-silica capillary columns, coated with SE-54 (5% phenyl methyl silicone, film thickness 0.17 μm, Hewlett-Packard, Avondale, PA, U.S.A.), were placed in the same injector. Injection (2 μl) was done in splitless mode at 280°C.

The detectors were maintained at 300°C. The carrier gas was helium (flow-rate 2–3 ml/min) and the make-up gases were argon–methane for the electron-capture detector (60 ml/min) and nitrogen for the nitrogen–phosphorus detector (30 ml/min). The flow-rate of air was 80–100 ml/min and that of hydrogen 3 ml/min. Signals from the two detectors were recorded by two HP 5880 keyboards. The oven temperature was held initially at 200°C for 1 min and increased thereafter up to 290°C at 10°C/min. The chromatography time was 12 min.

The reliability of the results from the capillary technique was compared with that from the packed-column technique using an HP 5770 gas chromatograph with a electron-capture detector. Reference analyses of nitrazepam and clonazepam were carried out on a column (2 m × 2 mm I.D.) packed with 2% SP 2510 DA (Supelco, Bellefonte, PA, U.S.A.) and those of other benzodiazepines by a similar column packed with 3% OV-1 on Gas Chrom Q (Applied Science Labs., State College, PA, U.S.A.). In these analyses argon–methane (40 ml/min) was used as a carrier gas. The detector and injector were maintained at 300°C. Oven temperatures were 240°C for oxazepam, lorazepam, diazepam and nordiazepam, 250°C for temazepam, 260°C for clobazam, norclobazam, nitrazepam and clonazepam and 275°C for triazolam and alprazolam.

Calibration

A stock solution containing 1 mg/ml of each drug was prepared in ethanol The working standards were prepared in human plasma to contain 0.1, 0.075, 0.05, 0.025, 0.01, 0.005 and 0 mg/l lorazepam, alprazolam, clonazepam, nitrazepam, midazolam and triazolam, and 1, 0.75, 0.5, 0.25, 0.1, 0.05 and 0 mg/l oxazepam, diazepam, nordiazepam, chlordiazepoxide, temazepam, clobazam, norclobazam, adinazolam and medazepam.

Precision, limit of detection, selectivity and recovery

Within-run precision was calculated from repeated analysis during one working day. The precision of the instrument was tested by repeating injections from the same sample. Day-to-day precision was calculated from repeated analyses of quality control samples on successive working days. The limits of detection at a signal-to-noise ratio of 5:1 were calculated.

Retention times of *ca.* 200 psychotropic compounds were investigated in order to check their interference with the method. These compounds included phenothiazines, antidepressants, analgesics, stimulants, sedatives, etc.

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

RESULTS

Comparison of the two detectors

Oxazepam, diazepam and nordiazepam produced single peaks in both the electron-capture and the nitrogen–phosphorus channel, and they were easily detectable in both channels, although the former was more sensitive (Figs. 2 and 3). Further, the electron-capture detector was clearly preferable for lorazepam, nitrazepam, clonazepam, alprazolam and triazolam. The nitrogen–phosphorus detector was found to be required for detecting chlordiazepoxide, desmethylchlordiazepoxide, medazepam and normedazepam. These compounds did not produce peaks in the electron-capture channel at moderate serum levels. The retention times of benzodiazepines are listed in Table I.

Detection limit and stability: a comparison between capillary and packed columns

The detection limit of the capillary column procedure was *ca.* 50 ng/ml for oxazepam and 10 ng/ml for lorazepam, diazepam, nordiazepam, chlordiazepoxide, temazepam and triazolam. By comparing detection limits obtained with capillary and packed columns, oxazepam, diazepam and nordiazepam were found to give similar detection limits on both columns. The packed-column (OV-1) technique was more sensitive for lorazepam, alprazolam and triazolam (detection limits 5, 5 and 2 ng/ml, respectively) and less sensitive for chlordiazepoxide (50 ng/ml).

Interference

Of the drugs tested for interference (Table II), levomepromazine was found to have a retention time (6.13 min) very similar to that of chlordiazepoxide (6.16 min). Normal serum components or other drugs tested did not interfere with the analysis of benzodiazepine derivatives. Unfortunately, several metabolites of the parent drugs were not available for interference testing.

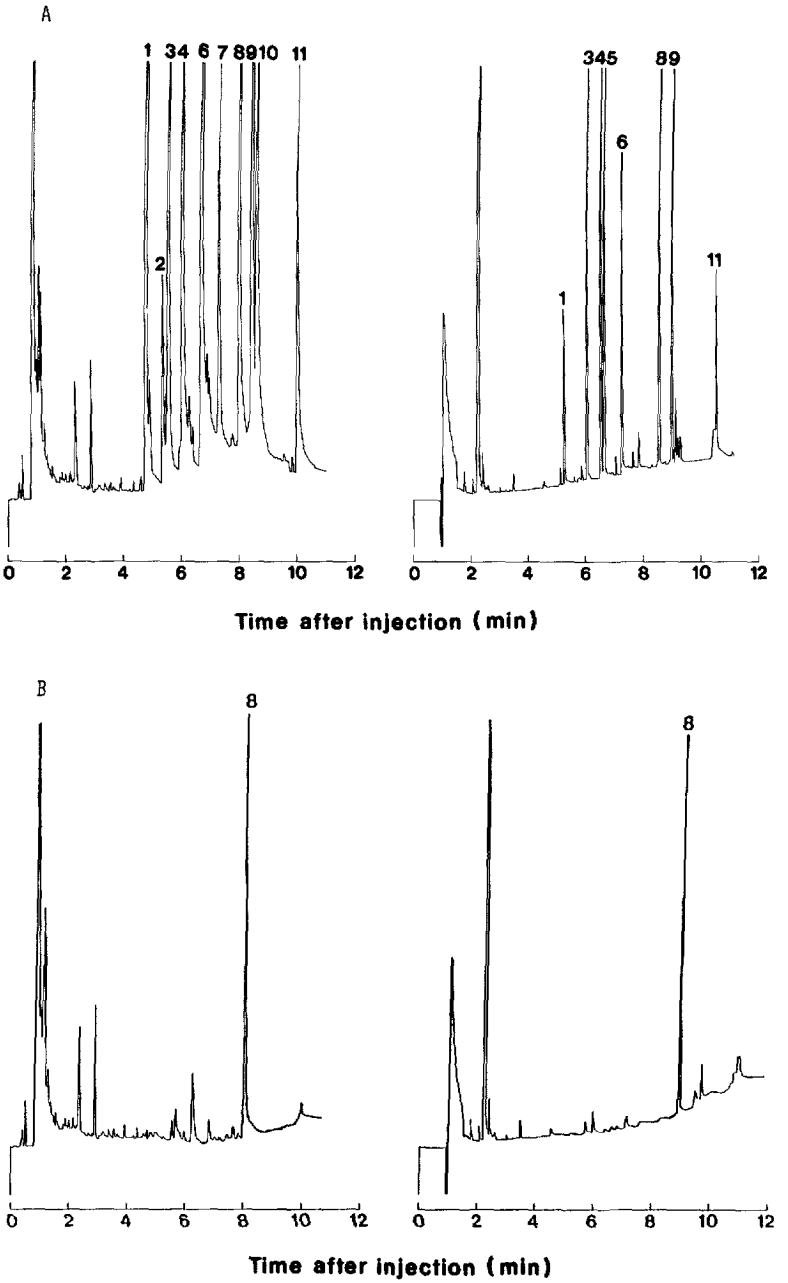
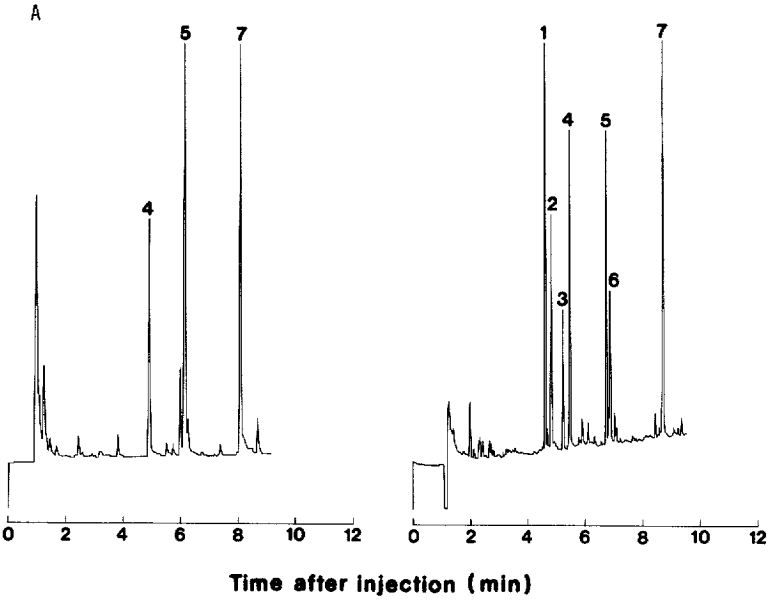


Fig 2 (A) ECD (left) and NPD (right) chromatograms of an extracted plasma standard containing a mixture of benzodiazepines Conditions as in Experimental Peaks 1 = oxazepam (1 mg/ml); 2 = lorazepam (0 1 mg/l), 3 = diazepam (1 mg/l); 4 = nordiazepam (1 mg/l), 5 = chlordiazepoxide (1 mg/l); 6 = temazepam (1 mg/ml) (first peak), 7 = temazepam (second peak), 8 = flurazepam, 9 = nitrazepam (0 1 mg/l), 10 = clonazepam (0 1 mg/l), 11 = triazolam (0 1 mg/l). (B) ECD (left) and NPD (right) chromatograms of an extracted plasma blank containing flurazepam as internal standard.



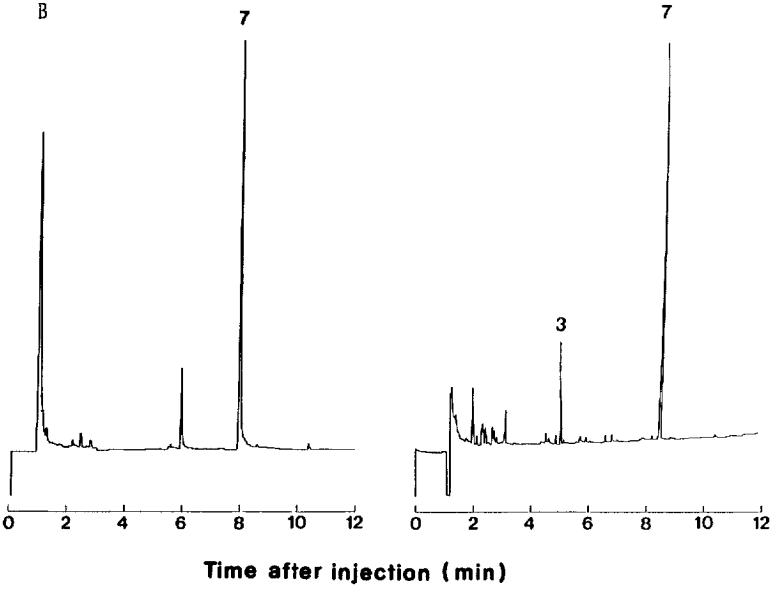


Fig 3. (A) ECD (left) and NPD (right) chromatograms of an extracted whole blood sample obtained from a driver suspected from driving under the influence of drugs. Conditions as in Experimental Peaks 1 = trimipramine (0 30 mg/l); 2 = a metabolite of trimipramine, 3 = a background peak, 4 = oxazepam (0.77 mg/l); 5 = nordiazepam (0.25 mg/l), 6 = levomepromazine, 7 = flurazepam (B) ECD (left) and NPD (right) chromatograms of an extracted blood blank containing flurazepam as internal standard

TABLE I

RETENTION TIMES OF BENZODIAZEPINES

Substance	Retention time (min)
Medazepam	4 10
Oxazepam	4 85
Lorazepam	5 40
Diazepam	5 57
n-Desalkylflurazepam	5 66
n-Desmethylchlordiazepoxide	5 97
Nordiazepam	6.10
Demoxepam	6 10
Chlordiazepoxide	6 16
Clobazam	6 28
Midazolam	6 37
Flunitrazepam	6.55
Temazepam (major peak)[a]	6 68
Norclobazam	6 76
Bromazepam	6 99
Temazepam (minor peak)[a]	7 07
Flurazepam	7 65
Nitrazepam	7 87
Clonazepam	8 26
Alprazolam	9 26
Triazolam	9 30
Adinazolam	9 46

[a] Thermally unstable, producing two peaks.

Linearity

The standard curves of benzodiazepines were linear on both channels: Fig. 4 shows the calibration curves. The procedure produced linear curves for oxazepam, diazepam, nordiazepam, chlordiazepoxide, temazepam and triazolam up to 20 mg/l and for lorazepam up to 1.0 mg/l.

Variation

The accuracy data, within-run and day-to-day variations on capillary and packed columns, are listed in Tables III and IV, respectively. The precision of the instrumental part of the assay was also tested, and the variation caused by the capillary GC itself was found to be of the same magnitude as the within-run variation of the whole procedure.

When the reproducibilities on the capillary and packed columns were compared, the precisions on the packed column were found to be better for nitrazepam, clonazepam, triazolam, lorazepam, diazepam, nordiazepam and oxazepam.

TABLE II

DRUGS TESTED FOR INTERFERENCE

Acebutolol	Carbromal	Dipipanon	Mefenamic acid
Acetazolamide	Carisoprodol	Diprophylline	Mefenorex
Acetophenazine	Cathine	Dipropylacetate	Melperone
Acetylprocainamide	Chlorcyclizine	Disopyramide	Meprobamate
Adrenaline	Chlordisopyramide	Disulfiram	Mepyramine
Ajmaline	Chloroquine	Dixyrazine	Mescaline
Alimemazine	Chlorpheniramine	Doxepin	Methadone
Alcuronium	Chlorpromazine	Droperidol	Methamizol
Allobarbital	Chlorprothixene	Emepron	Methamphetamine
Allypropymal	Chlorzoxazone	Ephedrine	Methaqualone
Alprenolol	Cimetidine	Etilefrin	Methixene
Amantadine	Cinnarizine	Ethambutanol	Methocarbamol
Amfepramone	Cinchonidine	Ethenzamide	2,5-Methylenedioxy-amphetamine
Aminophenazone	Clemastine	Ethinamate	
Aminosalicylic acid	Clobutinol	Ethionamide	Methylephedrine
Amiloride	Clomethiazole	Ethosuximide	Methylphenidate
Amitriptyline	Clomipramine	Ethylamphetamine	Metoprolol
Amobarbital	Clonidine	Ethylbenztropine	Metronidazole
Amphetamine	Clopentixol	Ethylmorphine	Mexiletine
Anileridine	Clozapine	Etodroxizine	Mianserin
Antazoline	Cocaine	Etoxeridine	Minoxidil
Apronal	Codeine	Fencamphamine	Moperone
Atenolol	Cotinine	Fenfluramine	Morphine
Azathioprine	Cyclizine	Fentanyl	Naproxen
Baclophen	Cyclobarbital	Fluphenazine	Nicotine
Barbital	Cyclopentolat	Furosemide	Nomifensine
Belladonnine	Cyproheptadine	Glipizide	Norclomipramine
Benorylate	Cyheptamide	Glutetimide	Nordoxepin
Benzhexol	Dapsone	Glycopyrron	Norephedrine
Benzilon	Debrisoquin	Guaifenesin	Nortriptyline
Benzocaine	Desipramine	Guanethidine	Norpseudoephedrine
Benztropine	Dexamethasone	Guanoxan	Noscapine
Bethanidine	Dextrometorphan	Haloperidol	Opipramol
Biperiden	Dextromoramide	Heptabarbital	Orphenadrine
Brallobarbital	Dextropropoxyphene	Hexobarbital	Oxprenolol
Bromazepam	Diazoxide	Hydralazine	Oxycodone
Bromisoval	Dibenzepin	Hydrochlorothiazide	Oxymorphone
Brompheniramine	Dichlorphenamide	Hydrocodone	Paracetamol
Buclizine	Diclofenac	Hyoscylamine	Penfluridol
Bupivacaine	Diflunisal	Imipramine	Pentazocine
Busbirone	Digitoxin	Labetalol	Pentobarbital
Butalbital	Dihydralazin	Levomepromazine	Pentoxyverine
Butenemal	Dihydrocodein	Lidocaine	Perphenazine
Butobarbital	2,5-Dimethoxy-amphetamine	Loperamide	Pethidine
Butobarbitone		LSD	Phenazocine
Caffeine	Diphenhydramine	Maprotiline	Phenazone
Carbamazepine	Diphenylpyraline	Meclozine	Phencyclidine

(Continued on p. 106)

TABLE II *(continued)*

Phendimetrazine	Phenylbutazone	Propranolol	Theophylline
Phenethylamine	Phenytoin	Propyphenazone	Thiopental
Phenformin	Pindolol	Proquazone	Timolol
Pheniramine	Primidone	Protriptyline	Tioridazine
Phenmetrazine	Prilocaine	Pseudoephedrine	Tolfenamic acid
Phenobarbital	Procainamide	Pyribenzamine	Trimethoprime
Phenprobamate	Prolintane	Secobarbital	Trimipramine
Phensuximide	Promazine	Sulpiride	Verapamil
Phentermine	Prometazine	Sultiam	Warfarin

The long-term (six months, $n = 24$) analytical error variabilities (indicated as C.V.) on packed column (2% SP 2510 DA) were 6.2 and 6.6% for nitrazepam (100 ng/ml) and clonazepam (65 ng/ml), respectively.

Extraction

In the present study the selection of the extraction solvent was based on the optimal extractability of the whole group investigated. The extraction procedure with *n*-hexane–dichloromethane (70:30) at pH 9 gave an acceptable yield (70–97%) for all substances examined, with a short evaporation time.

The validity of the extraction was confirmed by extracting blood samples from drivers suspected of driving under the influence of drugs, both with *n*-hexane–dichloromethane (70:30) and a traditionally used solvent. The samples were found to contain alprazolam, oxazepam, diazepam, nordiazepam, midazolam, chlordiazepoxide and lorazepam, alone or combined The results after these two

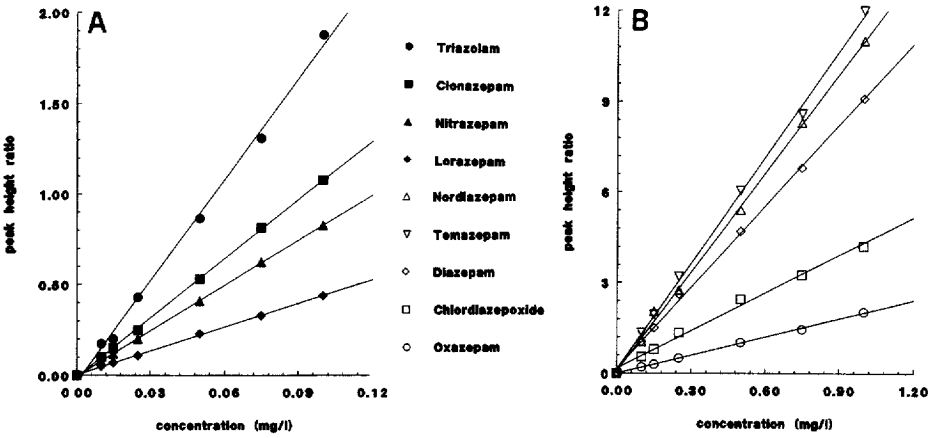


Fig 4 Typical standard curves for plasma samples containing known concentrations of nine benzodiazepines Flurazepam was employed as internal standard. Peak-height ratios of drug to internal standard were calculated for chlordiazepoxide in the nitrogen–phosphorus channel and for the others on electron-capture channel

TABLE III

WITHIN-RUN VARIATIONS AND ACCURACY

Compound	C V (%)	Concentration (mg/l)		*n*
		Expected	Obtained	
Capillary column				
Adinazolam	3 3	0 500	0 502	10
Chlordiazepoxide	6 1	0 100	0 098	15
Clobazam	8 2	0 400	0 410	10
Clonazepam	11 2	0 010	0 008	10
Desalkylflurazepam	12 5	0 050	0.054	15
Diazepam	10 0	0 185	0.184	10
Diazepam	7 7	0 100	0.104	15
Flurazepam	9.5	0 030	0.031	15
Lorazepam	9.5	0 050	0.048	15
Lorazepam	13.0	0.010	0 011	10
Nitrazepam	11.0	0.010	0 008	10
Norclobazam	7.9	0 75	0 74	10
Nordiazepam	10.9	0.185	0 184	10
Nordiazepam	8 5	0.10	0 09	15
Oxazepam	9 6	0.50	0 49	15
Oxazepam	12 5	0 15	0 16	10
Temazepam	5 0	0 50	0 51	15
Triazolam	10 4	0 020	0 019	14
Packed column				
Oxazepam	5 0	0 50	0 52	15
Lorazepam	5 2	0 050	0 049	15
Triazolam	3 5	0 0100	0 0098	15

extraction procedures were equal (C.V. 6.4%) and did not diverge more than those from duplicate analyses using only traditional extraction solvent (C.V. 7.4%).

DISCUSSION

Many benzodiazepines are thermolabile [21]. Temazepam was the only compound investigated that produced two well separated chromatographic peaks. Heat decomposes temazepam incompletely and the first peak is thought to result from the rearrangement product [21]. Chlordiazepoxide has also been described to undergo thermal rearrangement on the chromatographic column [22]. Obviously its thermal decomposition is complete because it produced only one peak in the nitrogen–phosphorous channel. In spite of the chlorine atom in the chlordia-

TABLE IV

DAY-TO-DAY VARIATIONS

Compound	C V. (%)	Mean concentration (mg/l)	*n*
Capillary column			
Diazepam	9.5	0 15	6
Nordiazepam	5.8	0 15	6
Oxazepam	17.0	0 15	6
Temazepam	8 8	0 15	6
Chlordiazepoxide	4.8	0.20	5
Lorazepam	14 6	0 016	15
Nitrazepam	15 0	0 013	5
Clonazepam	9 2	0 013	5
Triazolam	12 0	0 013	5
Packed column			
Diazepam	3 4	0 28	10
Nordiazepam	3 4	0 28	10
Oxazepam	5 0	0 50	15
Temazepam	5 0	0 50	15
Chlordiazepoxide	8 1	0 50	15
Lorazepam	5 2	0 048	15
Triazolam	3 5	0 010	15

zepoxide molecule it was not detectable in the electron-capture channel. The response stability and the precision of quantitative analysis of chlordiazepoxide, like those of other thermolabile compounds oxazepam and lorazepam, were comparable with those of the thermostabile benzodiazepines.

Demoxepam, a metabolite of chlordiazepoxide, had the same retention time as nordiazepam. This is due to the fact that demoxepam decomposes to nordiazepam very readily in the injection port of the gas chromatograph [21]. Thus, demoxepam and nordiazepam are indistinguishable by the present method.

We used an exceptionally high initial oven temperature for splitless mode to produce a short analysis time. However, this did not affect the precision.

The method was developed for a practical purpose, and the concentrations mentioned as detection limits were detected in routine analyses from day to day during several months without any extra instrumental adjustment. In optimal circumstances, the GC capillary method with *e.g.* electron-capture detection can yield better sensitivity [12] . The capillary columns tended to loose sensitivity to triazolam, alprazolam, nitrazepam and clonazepam with time. No changes in sensitivities in the course of time were noticed when packed columns were used. Thus, the packed-column technique seems to be preferable for triazolam, al-

prazolam, nitrazepam and clonazepam determinations. Besides for screening purposes, the capillary-column technique was also superior to the packed-column technique for quantitation of chlordiazepoxide and temazepam. Moreover, when a packed column was applied for temazepam assay the resolution of the drug, its rearrangement product and the internal standard was impaired. When benzodiazepines other than triazolam, alprazolam, nitrazepam and clonazepam were analysed on SE-54 capillary column, over 10 000 injections could be made before the column had to be replaced. The glass wool plug in the injector insert was changed after approximately every 1000 injections. The SP 2510 DA packed column and the OV-1 packed column had to be replaced after about 500 and 3000 analyses, respectively.

Several organic solvents have been used for extraction of benzodiazepines. Diethyl ether, ethyl acetate, toluene and dichloromethane are used most commonly for analysis of diazepam, chlordiazepoxide, bromazepam and flunitrazepam [23]. In the present work the selection of the extraction solvent was based on the optimal extractability of the whole group investigated The evaporation time of *n*-hexane–dichloromethane (70:30) was short and extraction gave acceptable yields (70–97%) for all substances. Alternatively, ethyl acetate can be used for lorazepam (pH 11), nitrazepam and clonazepam (pH 9) and toluene (pH 9) for other benzodiazepines.

In conclusion, the fused-silica capillary phenyl methyl silicone columns and ECD and NPD form an efficient method for the quantitative GC analysis of a large number of benzodiazepines and other substances present in the sample extract.

REFERENCES

1 J. H Woods, J L. Katz and G Winger, *Pharm Rev*, 39 (1987) 251.
2 E Roets and J. Hoogmartens, *J Chromatogr*, 194 (1980) 262.
3 P Hunt, J M. Husson and J P Raunaud, *Pharm. Pharmacol.*, 31 (1979) 448
4 R J. Bastiani, R C. Phillips, R S Schneider and E F Ullman, *Am J Med Techn*, 39 (1973) 211
5 M. E Jolley, *J. Anal Toxicol*, 5 (1981) 236
6 K Robinson, M G Rutterford and R N Smith, *J Pharm Phamacol.*, 32 (1980) 773
7 G L Lensmeyer, C Rajani and M. A Evenson, *Clin Chem.*, 28 (1982) 2274
8 R Gill, B Law and J P Gibbs, *J Chromatogr.*, 356 (1986) 37
9 F Marcucci, R Fanelli and E Mussini, *J Chromatogr*, 37 (1968) 318
10 B J. Hart and J Wilting, *J Chromatogr*, 424 (1988) 403.
11 J. M F Douse, *J Chromatogr*, 301 (1984) 137
12 H Bun, C Dechamp, Ph Coassolo and J. P Cano, *Anal Lett*, 22 (1989) 897
13 H. T Karnes, L A Beightol, R J. Serafin and D Farthing, *J Chromatogr*, 424 (1988) 398.
14 W A Joern and A B Joern, *J Anal Toxicol*, 11 (1987) 247
15 J A de Silva, I Bekersky, C. V Puglishi, M A Brooks and R E Weinfeld, *Anal. Chem.*, 48 (1976) 10.
16 K M Jensen, *J Chromatogr*, 111 (1975) 389
17 A. G. de Boer, J Rost-Kaiser, H Bracht and D D Breimer, *J Chromatogr*, 145 (1978) 105.
18 A. Lochmiskar and D. J Greenblatt, *J Chromatogr*, 337 (1985) 131
19 H. Schutz, *Z Rechtsmed.*, 100 (1988) 19

20 S Alm, S. Jonson, H Karlson and E G Sundholm, *J Chromatogr*, 254 (1983) 179
21 J. R. Joyce, T S. Bal, R E Ardrey, H. M. Stevens and A C Moffat, *Biomed Mass Spectrom*, 11 (1984) 284
22 A Frigerio, K M. Baker and G Belvedere, *Anal Chem*, 45 (1973) 1846
23 J J Himberg, *Dissertation*, University of Helsinki, Helsinki, 1982.