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ANALYSIS OF MEDAZEPAM, DIAZEPAM, AND METABOLITES IN PLASMA BY GAS–LIQUID CHROMATOGRAPHY WITH ELECTROLYTIC CONDUCTIVITY DETECTION

BRIAN E. PAPE and MICHAEL A. RIBICK

Department of Pathology, Toxicology Laboratory, School of Medicine, University of Missouri, Columbia, Mo. 65201 (U.S.A.)

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SUMMARY

An improved electrolytic conductivity detector allowed the gas–liquid chromatographic analysis of medazepam, diazepam, and major metabolites in 2 ml plasma at concentrations of 20 μg/l. The detector had a sensitivity limit of less than 1 ng (or 100 pg nitrogen) when operated in the nitrogen-selective mode and a nitrogen/carbon elemental selectivity ratio of greater than 100,000 compared to octadecane and cholesterol. Detector response when operated in various element-selective chemical modes was investigated, and its application to the analysis of the title compounds was compared to electron capture and flame ionization detection systems.

INTRODUCTION

The gas–liquid chromatography (GLC) of 1,4-benzodiazepines and 1,4-benzodiazepin-2-ones (1,4-benzodiazepin/-2-ones) and the determination of these drug and drug-metabolite concentrations in body fluids have received considerable interest in recent years[1,2]. Quantitative GLC procedures have usually involved a liquid–liquid extraction at selected pH, followed by evaporation or clean-up of the organic phase using a back-extraction, and quantitation of the drug (a chemical derivative) or an acid-hydrolysis product by GLC–electron capture detection (GLC–ECD)[3–11].

De Silva and coworkers have made major contributions to the development of clinical laboratory procedures for the quantitation of diazepam and metabolites. Their original procedure involved selective extraction into diethyl ether followed by back-extraction and subsequent acid hydrolysis to the respective substituted benzophenones, and analysis by GLC–ECD[3]. This method was later modified to allow the GLC–ECD quantitation of intact 1,4-benzodiazepin/-2-ones after extraction and clean-up[4] and more recently de Silva and coworkers developed a simplified analytical scheme for 1,4-benzodiazepin/-2-ones which involved protein precipitation, solvent extraction and GLC–ECD analysis of the intact drugs or their respective N_1-methyl derivatives[12]. Although ECD is widely used in the analysis of 1,4-benzodiazepin/-2-ones to obtain good analytical sensitivity, the selectivity or relative electron affinity

TABLE I

E1CD ELEMENT-SELECTIVE FURNACE CHEMISTRY MODES, FURNACE REACTION CONDITIONS, AND CELL CHEMISTRY

Selectivity: a non-selective mode indicates the absence of a post-furnace abstractor. Under such conditions, the resin tube (IRN-77 or IRN-78–IRN-150) and liquid pH will determine cell neutralization and ionization processes (see Results and discussion). All reaction gas flow-rates were 75 ml/min, unless otherwise noted. Reaction catalyst: —, Empty quartz reaction tube, 16 cm × 4 mm I.D. (Tracor); Ni, approx. 50 cm of fine nickle wire (Tracor) was coiled into a 3-cm length and positioned in the quartz reaction tube, in the center of the furnace heating zone. Abstractor: Fiberfax matrix (20 mg) containing the $Sr(OH)_2$ abstractor (Tracor) was positioned in the post-furnace portion of the quartz reaction tube approx. 0.5 cm in front of the PTFE transfer line. Acid abstractor: the boric acid abstractor employed by Hailey *et al.*[15] was not used to trap NH_3. Ion-exchange resins (IRN-77, -78, and -150 from Rohm & Haas (Philadelphia, Pa., U.S.A., or from Tracor) were soxhlet-extracted for 8 h in methanol followed by 8 h in distilled water prior to use. Stacked bed resins consisted of approx. 70% acidic IRN-77 or basic IRN-78 resin on the pump side and 30% mixed-bed IRN-150 resin on the cell side of the resin tube (pump-side resin–cell-side resin). Conductivity solvents were isopropanol–distilled water (15:85 v/v) or 100% *n*-butanol (AR grade). Liquid flow-rate was 0.5 ml/min unless otherwise noted.

Furnace mode	*Selectivity*	*Reaction gas*	*Reaction catalyst*	*Abstractor*	*Resin tube*	*Conductivity solvent*	*pH*
Pyrolytic	N	He	—	$Sr(OH)_2$	IRN-78–150	Isopropanol–water	7.5–8.0
	Cl	He	—	Acid	IRN-77–150	*n*-Butanol	6.0–6.5
Catalytic pyrolysis	N	He	Ni	$Sr(OH)_2$	IRN-78–150	Isopropanol–water	7.5–8.0
	Cl	He	Ni	Acid	IRN-77–150	*n*-Butanol	6.0–6.5
Reductive	N	H_2	—	$Sr(OH)_2$	IRN-78–150	Isopropanol–water	7.5–8.0
	Cl	H_2	—	Acid	IRN-77–150	*n*-Butanol	6.0–6.5
Catalytic reductive	N	H_2	Ni	$Sr(OH)_2$	IRN-78–150	Isopropanol–water	7.5–8.0
	Cl	H_2	Ni	Acid	IRN-77–150	*n*-Butanol	6.0–6.5
Oxidative		O_2	—	—	IRN-77–150	*n*-Butanol	6.0–6.5
Catalytic oxidative		O_2	Ni	—	IRN-77–150	*n*-Butanol	6.0–6.5

of the ECD for 1,4-benzodiazepin/-2-ones *vs.* real or "model" interference compounds has not been investigated.

GLC–flame ionization detection (GLC–FID) has been used in the analysis of medazepam, diazepam, and nitrazepam in whole blood[13]. However, the minimum analytical sensitivity of 80 μg/l for 5 ml of specimen did not allow its application to pharmocokinetic studies or the development of a micromethod. Additionally, FID is not selective and its use required specimen clean-up to assure a reasonable degree of analytical selectivity[13].

Mallach *et al.* used a nitrogen-selective thermionic detector to quantitate medazepam and metabolites in serum or urine[14]. However, back-extraction was required to obtain the necessary selectivity at the 2.5–20 μg/l range and GLC column temperature-programming appeared to cause a baseline rise and peak integration problems.

Hailey *et al.* applied the Coulson electrolytic conductivity detector (ElCD) to the GLC analysis of medazepam and metabolites[15]. The ElCD detection limit, defined as a detector signal-to-noise response ratio of 2:1 for an "on-column" injection, was 30 ng of medazepam (I), N-desmethylmedazepam (II), diazepam (III), and N-desmethyldiazepam (IV). Their reported GLC–ElCD analysis of 2 mg/l of compounds I–IV required 25 ml of plasma when 1/100 of the volume of the final residue was injected on column. We have recently reported the use of an improved ElCD in the analysis of 1,4-benzodiazepin/-2-ones in body fluids[16].

The ElCD is an element-selective detector which was first commercially introduced by Cou'son to allow the selective detector response to halogen-, nitrogen-, and sulfur-containing compounds[17]. The effluent from the gas chromatographic column undergoes thermal decomposition under predetermined conditions of furnace temperature, reaction gas, reaction catalyst, and chemical abstractors, and the reaction products are then combined with a deionized liquid in a gas–liquid contactor. The electrical conductivity of the liquid is then measured using a bridge circuit and auxiliary recorder. The instrumental evolution and application of the ElCD have been reviewed by Selucky[18], and David[19], and Ševčík[20]. Hall[21] recently designed an improved microelectrolytic conductivity detector which is now commercially available[22]. The present work reports the application of the Hall ElCD in the GLC analysis of compounds I–IV in biological fluids and compares ElCD response to ECD and FID using standard solutions of pure drugs and extracts of biological specimens.

MATERIALS AND METHODS

Apparatus

The GLC–ElCD analyses were done on a Model MT 222 gas chromatograph equipped with a Model 310 Hall ElCD (Tracor, Austin, Texas, U.S.A.). Furnace reaction conditions (*i.e.*, reaction gas, reaction catalyst, reaction temperature, and post-furnace chemical abstractor) and cell chemistry (*i.e.*, conductivity solvent, resin, and liquid pH) for ElCD element-selective modes are summarized in Table I. Helium carrier gas (25 ml/min) was used because of the slight reduction of N_2 to NH_3 when the ElCD is operated in the catalytic reduction mode. Unless stated otherwise, the conductivity attenuation was set at $1 \times 100\ \mu\Omega^{-1}$ full scale deflection (f.s.d.) in

The GLC–ECD and GLC–FID analyses were done on Varian Model 2445 gas chromatographs equipped with the respective Varian detectors (Varian, Palo Alto, Calif., U.S.A.). Gas chromatographs were equipped with detector and carrier gas flow controllers and were operated at a detector temperature of 325°. GLC–ECD analyses utilized a 100 mCi scandium tritide (Sc^3H) source operated at a constant polarization potential of 90 V (d.c.). GLC–FID utilized the conventional FID system, with detector gases optimized to give a detector response of approximately 100% f.s.d. with the on-column analysis of 100 ng of diazepam at an electrometer attenuation of 8×10^{-11} A. Nitrogen was used as carrier gas in ECD and FID analyses.

GLC columns and column packings

GLC was done on 180 cm × 2 mm I.D. glass columns containing 3% OV-17 on Gas Chrom Q, 60–80 mesh (30 g liquid phase per kg support; Applied Science Labs., State College, Pa., U.S.A.).

Chemicals

Authentic medazepam HCl, N-desmethylmedazepam (II), diazepam (III), and N-desmethyldiazepam (IV) were obtained from Hoffmann-La Roche (Nutley, N.J., U.S.A.). Medazepam HCl was extracted from aqueous alkaline solution and evaporated to dryness to yield medazepam. *n*-Octadecane (V), azobenzene (VI), caffeine (VII), and cholesterol (VIII) were obtained from Aldrich (Milwaukee, Wisc., U.S.A.). Other chemicals were AR grade.

Determination of detector response characteristics

GLC–ElCD. The minimum detectable quantity (MDQ) was defined as the weight or millimolar quantity of compounds I–IV which gave a signal-to-noise ratio greater than 2:1. The detector signal was measured in terms of peak height or percent of f.s.d., while noise was defined as short-term fluctuation in a defined bandwidth and was measured as average peak-to-peak height or percent of f.s.d. Long-term noise, defined as detector fluctuation having a time period of 5–15 sec, was less predictable in amplitude and frequency and is discussed separately (see Results and discussion).

Peak height measurements were used to describe ElCD response to I–IV when operated under different element-selective modes (Table I). When GLC–ElCD analyses were done using column temperature-programming (200° isothermal for 2 min, followed by temperature-programming at 5°/min to 275°), peak heights of I–IV were directly related to peak area. Detector response was measured for the on-column injection of 100 ng of I–IV at a furnace temperature of 800–900°, a conductivity cell flow-rate of 0.5 ml/min, and electrometer attenuation of $1 \times 100\ \mu\Omega^{-1}$ f.s.d. The N-selective ElCD response to I–IV under catalytic reductive conditions was determined at furnace temperatures of 650–1000°.

GLC–ElCD selectivity. The selectivity of the ElCD when operated in the N-selective catalytic reductive mode was determined under defined conditions (see Table I and above). The ElCD relative response or molar selectivity ratio was calculated from the detector response to the on-column injection of 100 ng of I–IV and 1000 ng of V, 50 ng of VI, 25 ng of VII, and 2000 ng of VIII at $2 \times 100\ \mu\Omega^{-1}$ and $2 \times 10\ \mu\Omega^{-1}$ f.s.d. From peak height data, ElCD response expressed on an equimolar

or elemental basis was calculated for I–IV *vs.* V–VIII, with the 1,4-benzodiazepin/-2-ones and relative standards considered as individual pairs. An example of such a molar selectivity ratio for I/V would be calculated as in eqn. 1.

$$\text{ElCD molar selectivity}_{I/V} = \frac{\text{peak height}_I}{\text{peak height}_V} \times \frac{\text{molarity}_V}{\text{molarity}_I} \quad (1)$$

Eqn. 1 may be modified to obtain elemental selectivity data for N *vs.* C response (*e.g.*, eqn. 2).

$$\text{ElCD N/C selectivity}_{I/V} = \frac{\text{peak height}_I}{\text{peak height}_V} \times \frac{\text{molarity}_V}{\text{molarity}_I} \times \frac{(N)}{(C)} \quad (2)$$

where (N) and (C) represent the number of atoms of nitrogen and carbon in I and V, respectively. Since ElCD response to the hydrocarbons is negligible[21], the contribution of the carbon content of I–IV was disregarded. This equation would then represent the ElCD response to NH_3 *vs.* CH_4 when the ElCD was operated in the N-selective catalytic reductive mode and complete reductive pyrolysis and 100% product throughput from furnace-to-cell was assumed. When no detectable ElCD signal was recorded for the on-column injection of relative standards V and VIII, the detector response was arbitrarily defined as equal to the short-term noise level (see Results and discussion).

GLC–ECD. The relative molar selectivity of I–III *vs.* V–VIII was determined for the on-column injection of 20 ng of I, 22 ng of II, and 5 ng of III *vs.* 2 μg of V, 40 ng of VI, 8 μg of VII, and 4 μg of VIII under isothermal GLC conditions (injector, column, and detector temperatures of 270°, 240°, and 315°, respectively; electrometer attenuation of 8×10^{-9} A f.s.d.). The selectivity ratios based on peak areas were obtained by dividing the ECD molar response for a selected 1,4-benzodiazepin/-2-one by the molar response for a selected relative standard (*e.g.*, eqn. 3).

$$\text{ECD molar selectivity}_{I/V} = \frac{\text{peak area}_I \times \text{attenuation/molarity}_I}{\text{peak area}_V \times \text{attenuation/molarity}_V} \quad (3)$$

GLC analysis of I–IV in plasma

The analysis of I–IV in 5 ml plasma at concentrations of 200, 300, 40, 80 and 40, 40, 40, 80 μg/l were done according to a modified back-extraction procedure of de Silva and Puglisi[4] and the more recent procedure of de Silva *et al.*[12]. The modified method of de Silva and Puglisi[4] consisted of the extraction of buffered plasma (pH 9.0) with diethyl ether, followed by back-extraction into acid, and a final re-extraction into chloroform instead of diethyl ether[16]. The procedure of de Silva *et al.*[12] was modified to reflect our larger sample size (*i.e.*, buffering with 10 ml Na_3PO_4, extraction with 30 ml benzene–methylene chloride, and final dissolution of the dried residue with 50 μl of methyl alcohol).

The GLC-detector parameters were as follows.

GLC–ElCD. Injector temperature, 225°; column temperature, 200° for 2 min followed by temperature-programmed analysis to 275° at 5°/min; ElCD furnace temperature 850° while operated in the N-selective catalytic reductive mode; electrometer attenuation of $1 \times 100\ \mu\Omega^{-1}$ f.s.d.

GLC–ECD. Injector temperature, 275°; column temperature, 220° followed by temperature programmed analysis to 250° at 4°/min; detector temperature, 325°; electrometer attenuation of 64×10^{-10} A f.s.d.

GLC–FID. Injector temperature, 275°; column temperature, 200° followed by temperature-programmed analysis to 320° at 4°/min; detector temperature, 325°; electrometer attenuation, 8×10^{-11} A f.s.d.

RESULTS AND DISCUSSION

ElCD furnace chemistry and detector response

N-Selective catalytic reductive mode. Confirming earlier observation[16], ElCD response to I–IV reached a maximum at a furnace temperature of 950° when operated in the N-selective catalytic reductive mode. ElCD response to I–III was directly related to the nitrogen content, and differences due to molecular chemistry were not noted at furnace temperatures of >750°. A decreased detector response for IV was noted under non-ideal GLC conditions, where column sorption resulted in decreased GLC throughput[4,12]. As suggested by Hailey *et al.*[15], NH_4Cl was formed *via* post-furnace gas-phase reaction unless $Sr(OH)_2$ was used to abstract HCl. Without HCl abstraction, NH_4Cl formation in our system resulted in increased peak tailing or the appearance of a "ghost" peak after each 1,4-benzodiazepin/-2-one (Fig. 1). The more

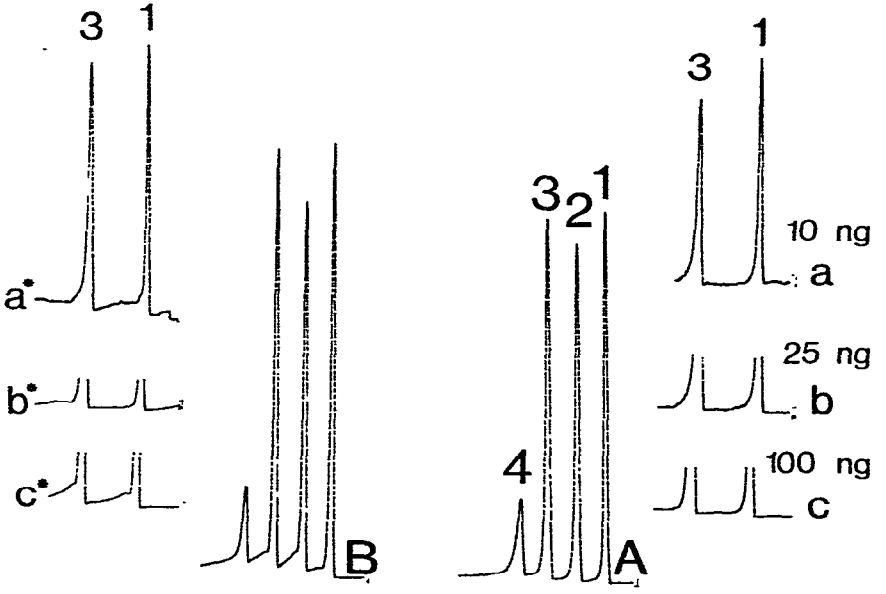

Fig. 1. GLC–ElCD of medazepam (1), N-desmethylmedazepam (2), diazepam (3), and N-desmethyldiazepam (4) under N-selective and non-selective catalytic reductive conditions. Chromatograms A, a, b, c were obtained under N-selective conditions [$Sr(OH)_2$], while chromatograms B, a*, b*, c* were obtained under non-selective conditions [no $Sr(OH)_2$]. Chromatograms A and B represent the on-column injection of 100 ng of I–III and 40 ng of IV. ElCD conductivity solvent was isopropanol–water (15:85 v/v) at a cell flow-rate of 1.0 ml/min and pH 7.5 (IRN-78–IRN-150 resin bed); electrometer attenuations were: A and B, $1 \times 100\,\mu\Omega^{-1}$; a and a*, $2 \times 3\,\mu\Omega^{-1}$; b, $4 \times 3\,\mu\Omega^{-1}$, b*, $8 \times 3\,\mu\Omega^{-1}$; c and c*, $32 \times 3\,\mu\Omega^{-1}$.

direct furnace-to-cell transfer line in the Hall ElCD system apparently allowed NH_4Cl solid → gas sublimation to produce the observed "ghosting". NH_4Cl solution phase ionization at pH 7 resulted in a lower effective change in conductance, $\Delta c_{sp(tot)}$, than that due to an equivalent amount of NH_3. This was due to the difference in the limiting equivalent ionic conductances ($\lambda°$) of NH_4Cl *vs.* NH_3 ionization products ($\lambda°$ for NH_4^+, Cl^-, and OH^- are 74, 76, and 199 Ω^{-1} cm^2 equiv.$^{-1}$, respectively)[23]. An increase in the amount of $Sr(OH)_2$ scrubber (0, 5, 10, 20 mg) resulted in less peak tailing and the disappearance of the "ghosting" phenomenon[16]. Peak tailing increased at furnace temperatures >900° when operated in the N-selective catalytic reductive mode. This may be due to less efficient HCl abstraction by $Sr(OH)_2$ resulting in post-furnace NH_4Cl formation, to changes in product distribution due to high-temperature reaction mechanisms, or to activation of sorption sites on the wall of the quartz reaction tube. With no abstractor and furnace temperatures <750°, peak tailing and ghosting increased, suggesting a shift in the reaction kinetics (*i.e.*, activation energies) to favor the greater equivalent production of HCl *vs.* NH_3. ElCD response with a conductivity solvent pH > 7 and non-selective catalytic pyrolysis was characteristic of neutralization and neutralization → ionization processes when $Sr(OH)_2$ abstractor was removed. Neutralization (*e.g.*, negative detector response) with a conductivity solvent pH > 7 was due to HCl production ($OH^- + HCl \rightarrow H_2O + Cl^-$, $\lambda°$ 199 and 76 for OH^- and Cl^-, respectively); while neutralization → ionization process was due to a greater equivalent production of HCl ($xOH^- + (x+n)HCl \rightarrow xH_2O + (x+n)Cl^- + nH^+$) ($\lambda°$ for H^+ equal to 350 Ω^{-1} cm^2 equiv.$^{-1}$). Therefore, these neutralization and neutralization → ionization conductivity processes support the contention that dehydrochlorination is favored over NH_3 production under "less reactive" furnace conditions.

The detector can be routinely optimized in the N-selective catalytic reductive mode to detect less than 1 ng of I–IV, representing less than 100 pg nitrogen (Fig. 2). A lower MDQ can be obtained by optimizing one or a combination of GLC–ElCD parameters. Since effective conductance of ionizable products is inversely proportional to conductivity solvent volume, a decrease in cell flow-rate to 0.1 ml/min would increase detector sensitivity[21]. Likewise, change in GLC carrier gas flow-rate or column temperature which sharpens chromatographic peaks will increase detector signal-to-noise ratio and lower the MDQ. The use of a "reactive" solute in the conductivity liquid may also be used to achieve a greater sensitivity to NH_3. A 3-ppm

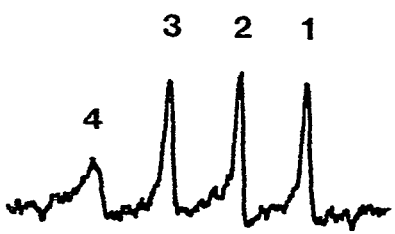

Fig. 2. GLC–ElCD N-selective catalytic reductive analysis of 1 ng medazepam (1), N-desmethyl-medazepam (2), diazepam (3), and N-desmethyldiazepam (4) at 900° furnace temperature. ElCD conductivity cell flow-rate was 0.25 ml/min and conductivity electrometer attenuation was 1 × 1 $\mu\Omega^{-1}$.

HCl solution of HCl–ethanol has been recommended for the highly sensitive detection of nitrogen compounds[18,19,21]. While Hailey *et al.*[15] used a "reactive" conductivity solute of 3-ppm HCl, their detection limit of 30 ng of I–IV for the Coulson ElCD was most likely due to a combination of factors including (1) HCl through-put with NH_4Cl formation, (2) a greater transfer line "dead-volume" with resultant product loss through sorptive processes, (3) the use of a large volume conductivity cell, and (4) the relatively poor optimization of conductivity cell voltage and electrode geometry[19,21].

ElCD response to I–IV in the N-selective catalytic reductive mode was non-linear below 10 ng. This may have been due to pre-furnace transfer line degradation, to sorptive loss of NH_3 on the abstractor's fiberfax matrix or in the post-furnace transfer line, or to the effect of trace neutralization or ionization processes associated with conductivity solvent pH or resin "bleed". GLC–ECD on-column analysis of I–III at 1–10 ng was linear, and suggested that a transfer line or post-column detector phenomenon was operating to cause ElCD non-linearity. GLC–ECD and GLC–ElCD linearity for IV can be improved by forming a deviative[4,12].

Other ElCD modes. Compared to the N-selective catalytic reductive analysis of I–IV, the ElCD response to I–IV using other furnace chemistry modes (see Table I) was not as sensitive (Fig. 3). Under catalytic conditions, N-selective ElCD response to I–IV was decreased with helium reaction gas (Fig. 3A and B) or low hydrogen reaction gas flow-rates. Without $Sr(OH)_2$ abstractor, neutralization processes sug-

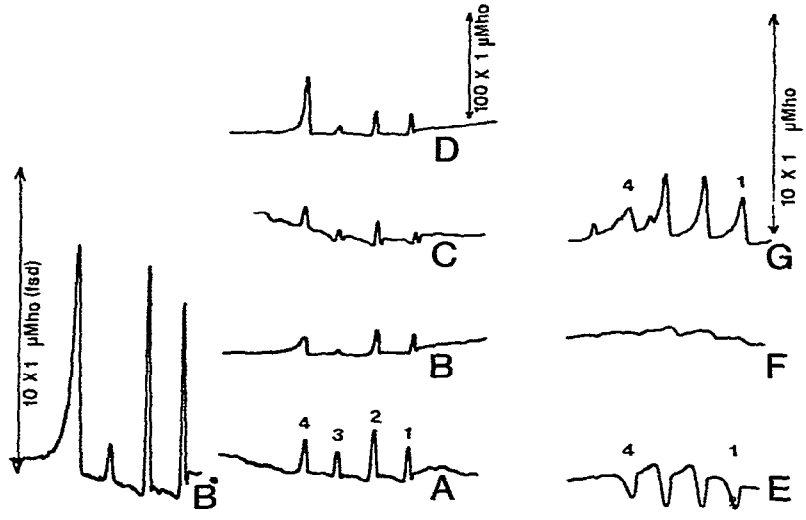

Fig. 3. GLC–ElCD of medazepam (1), N-desmethylmedazepam (2), diazepam (3), and N-desmethyldiazepam (4) under different detector modes. Chromatograms A and B: analysis of 200 ng of compounds 1–4 with N-selective catalytic pyrolysis chemistry at 1000° and 800°, respectively. B* (same as B) was determined at $10 \times 1\ \mu\Omega^{-1}$ (neutralization process). C and D: analysis of 200 ng of 1–4 under non-selective catalytic pyrolysis chemistry with conductivity solvent pH >7 (IRN-78–IRN-150 resin bed) at 1000° and 800°, respectively. E: non-selective catalytic reductive chemistry mode at 900° with a conductivity solvent pH <7 (IRN-77–IRN-150 resins) and electrometer attenuation of $30 \times 1\ \mu\Omega^{-1}$. F: non-selective catalytic pyrolysis furnace chemistry (other conditions same as in E.). G: analysis of 100 ng of 1–4 under catalytic oxidative furnace chemistry at 900°.

gested HCl through-put to the conductivity cell (Fig. 3C and D). With *n*-butanol as the conductivity solvent and a liquid pH < 7, ElCD response was also diminished under non-selective pyrolytic or oxidative conditions. Reaction under non-selective catalytic reductive conditions with a conductivity solvent of pH < 7 produced cell neutralization reactions (Fig. 3E) due to NH_3. Catalytic pyrolysis resulted in an acidic ionization process, supporting the hypothesis of an increased production of HCl relative to NH_3 under "less reactive" furnace conditions (Fig. 3F). Catalytic oxidation resulted in a greater change in effective cell conductance (Fig. 3G), but ElCD response was still considerably less than that noted for an equivalent analysis of I–IV under N-selective catalytic reductive conditions (Fig. 1A).

Hailey *et al.*[15] suggested that the catalytic oxidative mode would yield good detector response, presumably due to the production of acids of N and Cl which would avoid gas-phase neutralization reactions. In addition to a diminished response, ElCD long-term noise was greatest when the detector was operated in the catalytic oxidative mode. As a general rule, both long term detector noise and detector response to the operation of the by-pass valve were increased at furnace temperatures of $>900°$. A study of furnace chemistry parameters and conductance parameters allowing the optimization of an oxidative furnace chemistry mode is presently underway in this laboratory.

ElCD selectivity. The selectivity of ElCD response to I–IV *vs.* relative standards V–VIII was determined in the N-selective catalytic reductive mode at a furnace temperature of 850°. No ElCD response was noted for the on-column injection of 1 μg of V and 2 μg of VIII at $1 \times 10\ \mu\Omega^{-1}$ f.s.d. and a noise level of 1% f.s.d. The ElCD molar selectivity ratio for I–IV *vs.* V and VIII was $>100{,}000$ (see eqns. 1 and 2). The molar selectivity ratios for I–IV *vs.* V and VIII were approximately 1.0 and 2.0, respectively; while elemental selectivity ratios (N/N) were equivalent.

ECD selectivity. The ECD molar selectivity ratios for I–IV *vs.* V–VIII are summarized in Table II. The wide range in the molar selectivity for 1,4-benzodiazep in/2-ones *vs.* relative standards was primarily due to differences in ECD response to V–VIII. The ECD molar response to II and III, relative to I, was 1.5 and 22, respectively. Differences in the electron affinity for I–IV and analogs have been reported by de Silva and Puglisi[4], although the influence of specific functional groups was not discussed. The suggestion that the carbonyl group contributes to the enhanced electron affinity of III and IV, relative to I and II, was supported by the ECD relative molar response of 6:1 for 2-amino-5-chlorobenzophenone *vs.* I. The differences in electron affinity between V–VIII were much greater than the benzodiazepin/-2-one analogs.

TABLE II

ECD SELECTIVITY (RELATIVE MOLAR RESPONSE) FOR BENZODIAZEPIN/-2-ONES *VS.* RELATIVE STANDARDS

1,4-Benzodiazepin/-2-one (generic compound)	*Octadecane*	*Azobenzene*	*Caffeine*	*Cholesterol*
Medazepam	$>10^4$	1	300	125
N-Desmethylmedazepam	$>10^4$	2	480	200
Diazepam	$>10^5$	27	6300	2650

GLC analysis of I–IV in plasma

The GLC analyses of I–IV in plasma using FID, ECD, and ElCD systems were compared in order to allow the estimation of biological interferences seen in routine analyses (Figs. 4–6). Since the back-extraction procedure offered no real advantage in terms of the elimination of biological interference, the more simplified Na_3PO_4 buffered extraction method is recommended. Analyses by GLC–FID were sufficiently sensitive, but detector selectivity did not allow accurate quantitation when faced with variable patterns of biological interferences. GLC–ECD provided the lowest ultimate sensitivity limit for quantitative analysis and was successfully temperature-programmed over a limited range when the GLC was equipped with carrier gas flow-controllers. The rather low electron affinity of ECD for I and II caused no real

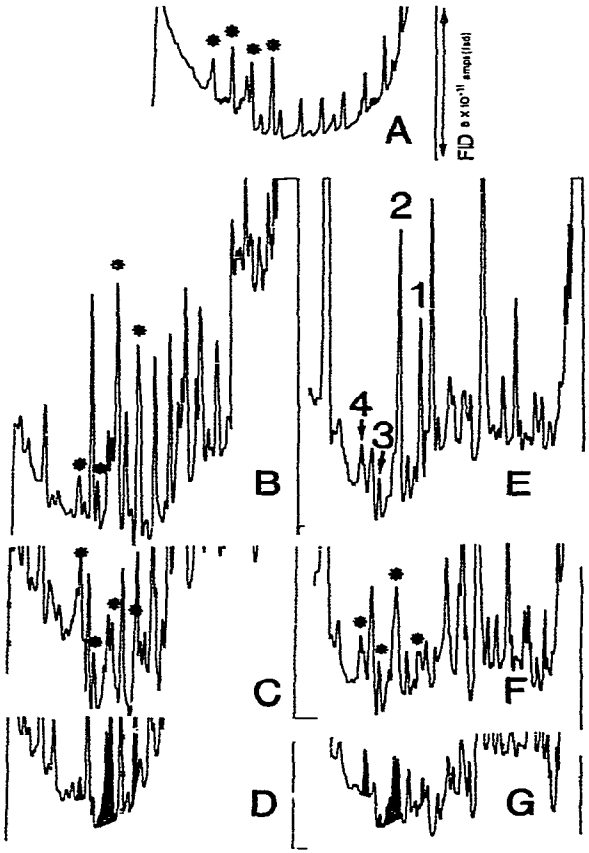

Fig. 4. GLC–FID of medazepam (1), N-desmethylmedazepam (2), diazepam (3), and N-desmethyldiazepam (4) in plasma. A: on-column analysis of 20 ng of 1–4 at 8×10^{-11} A f.s.d. B, C and D: analysis of (200, 300, 40, 80), (40, 40, 40, 80) and (0, 0, 0, 0) μg/l of compounds 1, 2, 3, and 4, respectively, in 5 ml plasma using the modified back-extraction procedure[16]. E–G, same concentrations of 1–4 in 5 ml plasma specimens analyzed using the Na_3PO_4 buffered extraction procedure (see Materials and methods). Cholesterol was confirmed by GLC. Co-extractable interferences in blank plasma are shaded (chromatograms D and G). * denotes GLC peaks for 1,4-benzodiazepin/-2-ones identified by retention time.

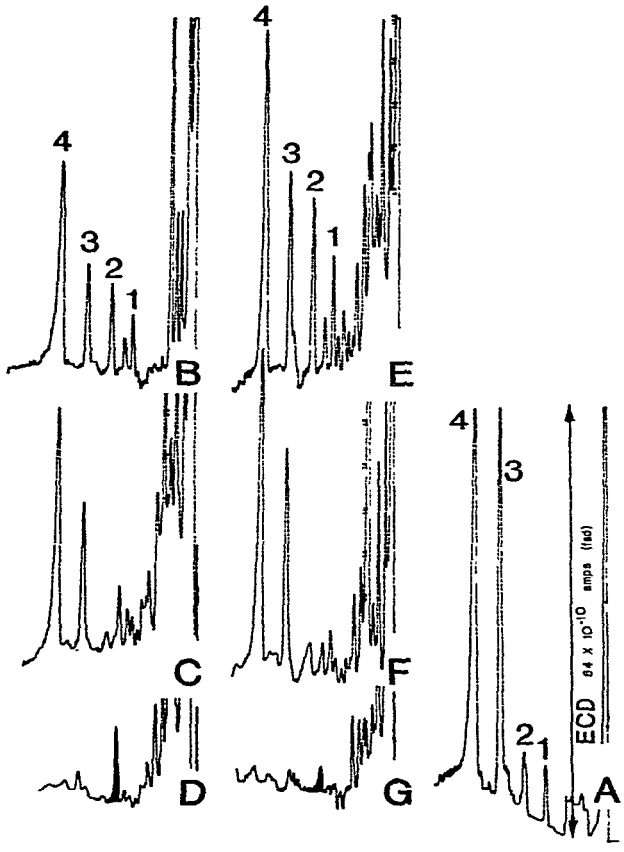

Fig. 5. GLC–ECD of the analysis of medazepam (1), N-desmethylmedazepam (2), diazepam (3), and N-desmethyldiazepam (4) in plasma. A: on-column analysis of 10 ng of 1–4 at 64 × 10^{-10} A f.s.d. B, C and D: analysis of (200, 300, 40, 80), (40, 40, 40, 80) and (0, 0, 0, 0) μg/l of compounds 1, 2, 3 4, respectively, in 5 ml plasma using the modified back-extraction procedure[16]. E–G, same concentrations of 1–4 in 5 ml plasma specimens analyzed using the Na_3PO_4 buffered extraction procedure. Co-extractable interferences in blank plasma are shaded (C and F).

problems in quantitative analyses, as the therapeutic drug concentrations of medazepam and metabolites are sufficiently high and GLC–ECD provides adequate analytical selectivity. Column temperature-programming over temperature ranges of greater than 40° caused significant baseline rise which effected computer integration capabilities and ECD linear range due to the depletion of ECD background current.

GLC–ElCD analysis in the N-selective catalytic reductive mode was free of significant interference peaks and detector sensitivity allowed the quantitation of I–IV in 2 ml plasma at concentrations of 20 μg/l. Temperature-programmed analysis from 200° to 300° using a well conditioned OV-17 column and an electrometer attenuation of 1 × 10 $\mu\Omega^{-1}$ f.s.d. routinely resulted in a baseline rise of less than 5% f.s.d. The preparation of a series of plasma standards over the relevant concentration range precluded any problems caused by detector non-linearity at low drug levels.

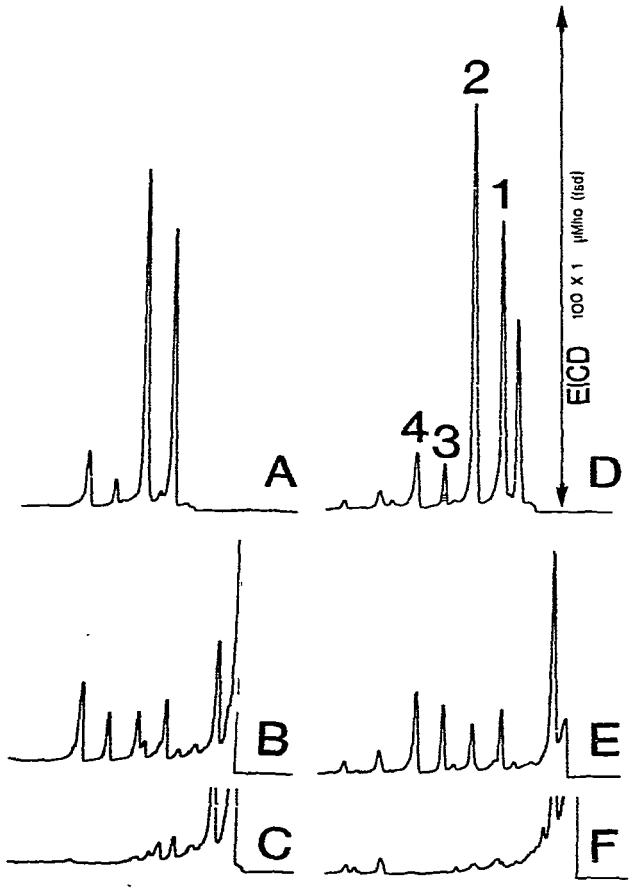

Fig. 6. GLC–ElCD of medazepam (1), N-desmethylmedazepam (2), diazepam (3), and N-desmethyldiazepam (4) in plasma. A, B and C: analysis of (200, 300, 40, 80), (40, 40, 40, 40), and (0, 0, 0, 0) µg/l of compounds 1, 2, 3, 4 respectively in, 5 ml plasma specimens analyzed using the Na_3PO_4 buffered extraction procedure. Co-extractable interferences in blank plasma are shaded (C and F).

CONCLUSIONS

The Hall ElCD may be used for the N-selective GLC detection and quantitation of benzodiazepin/-2-ones in biological fluids at therapeutic drug concentrations. ElCD sensitivity to nitrogen-containing compounds, its successful use in temperature-programmed GLC analyses, the choice of operation in different element-selective modes, and an unexpectedly modest cost are among its attractive features.

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