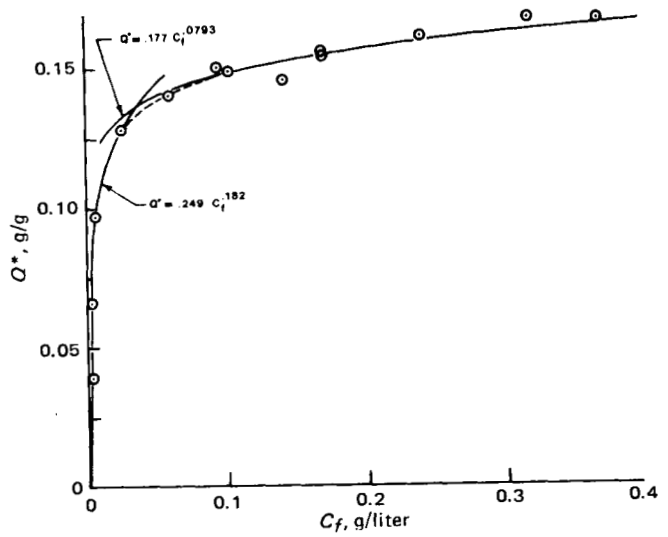

Figure 1—*Adsorption of emetine by powdered activated charcoal from simulated gastric fluid.*

DISCUSSION

On the basis that the usual dose of syrup of ipecac is 1 tablespoonful, or 15 ml (7), and that ipecac syrup yields 123–157 mg of ether-soluble alkaloids/100 ml of syrup (7), an average dose of ipecac syrup would contain about 21 mg of alkaloids. If this dose mixes with V liters of gastric contents upon ingestion, it would produce a concentration, C_0, equal to $0.021/V$ g/liter of alkaloids in the gastric contents. If one were then to administer W g of activated charcoal, adsorption of some of the alkaloids would occur, lowering their concentration to a value of C_f at equilibrium.

One can show by a simple mass balance that $Q^* = V(C_0 - C_f)/W$. If it is assumed that C_f will be lower than 0.03 g/liter (as will nearly always be the case) and if it is assumed that all alkaloids present behave like emetine in terms of adsorption, then $Q^* = 0.249C_f^{0.182}$ will pertain. Therefore, $0.249C_f^{0.182} = V(0.021/V - C_f)/W = (0.021 - VC_f)/W$. Now, it can be shown that for *any* reasonable choices of V and W, C_f will be very small. As one example, for $V = 0.5$ liter and $W = 1$ g, $C_f = 1.25 \times 10^{-6}$ g/liter, or 0.003% of the initial concentration.

This result indicates that the extent of emetine binding by any reasonable dose of activated charcoal is extremely high *in vitro*. Although *in vivo* data on emetine binding are lacking, the present results strongly suggest that there may be no acceptable dose level for syrup of ipecac that would leave enough free alkaloids in solution, after an adsorption equilibrium is attained, to cause emesis. Moreover, since the quantity of charcoal that *should* be administered to counteract poisonings effectively is as high as 120 g (8), even an extremely large dose of ipecac syrup might be inactivated under *in vivo* circumstances.

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Determination of Desmethyldiazepam in Plasma by Electron-Capture GLC: Application to Pharmacokinetic Studies of Clorazepate

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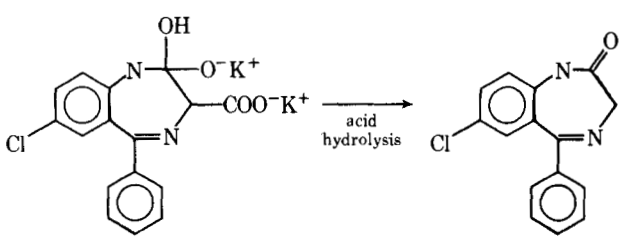
Abstract □ Plasma desmethyldiazepam concentrations were quantitated by a rapid and sensitive technique using electron-capture GLC. Following addition of diazepam as the internal standard, plasma is extracted at physiological pH into benzene–isoamyl alcohol. The extract is evaporated to dryness and reconstituted with toluene–isoamyl alcohol prior to chromatography. Both diazepam and desmethyldiazepam are quantitatively extracted. The variation of identical samples is 5%, and the sensitivity is 5 ng of desmethyldiazepam/ml of original sample. The method is applicable to pharmacokinetic studies of clorazepate, a benzodiazepine derivative transformed to desmethyldiazepam prior to absorption.

Keyphrases □ Desmethyldiazepam—electron-capture GLC analysis in plasma □ GLC, electron capture—analysis, desmethyldiazepam in plasma □ Clorazepate—pharmacokinetic study by GLC analysis of desmethyldiazepam in plasma □ Tranquilizers—clorazepate, pharmacokinetic study by GLC analysis of desmethyldiazepam in plasma

Clorazepate dipotassium (I) is a 1,4-benzodiazepine derivative extensively used as a sedative and antianxiety agent (1, 2). Previous studies (3) suggested that clorazepate is transformed to desmethyldiazepam (II) by hydrolysis and decarboxylation in the acidic stomach contents (Scheme I). Desmethyldiazepam is subsequently absorbed from the proximal small bowel and appears to account for most or all of the clinical effects attributable to clorazepate.



I *Scheme I* II

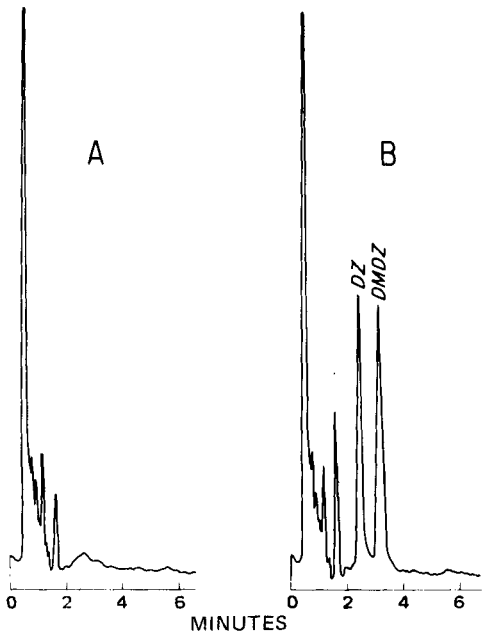


Figure 1—*A: sample chromatogram of a drug-free control plasma extract. B: chromatogram of the same plasma sample to which were added diazepam (DZ) and desmethyldiazepam (DMDZ) in concentrations of 100 ng/ml.*

Reported GLC procedures for desmethyldiazepam in plasma involved chromatography of the intact substance following extensive cleanup procedures (4–7) or acid hydrolysis of the compound to a benzophenone derivative prior to chromatography (8–10). Some studies, however, suggested that desmethyldiazepam may be analyzed using simple extraction without extensive cleanup or derivatization (11–19). The present report describes a rapid and sensitive method for analysis of intact desmethyldiazepam in plasma and illustrates its applicability to clinical pharmacokinetic studies of clorazepate.

EXPERIMENTAL

Instrumental Conditions—The gas chromatograph[1] was equipped with a 2-mCi ^{63}Ni-electron-capture detector operated at 320° in the pulsed mode, with a pulse interval of 150 μsec. The purge gas[2] was argon–methane (95:5) at a flow rate of 80 ml/min. Detector settings were: range, 1; and attenuation, 128.

The column was coiled glass[3], 1.8 m (6 ft) long × 4 mm i.d., packed with 3% OV-17 on 80–100-mesh Chromosorb WHP[3]. Carrier gas[2] was ultrapure helium at a flow rate of 50 ml/min. The column was conditioned at 325° using a low flow of carrier gas for 48 hr prior to its attachment to the detector. Temperature settings during operation were: injection port, 300°; and column, 280°.

Assay—Stock solutions of diazepam and desmethyldiazepam, 1 μg/ml, were prepared in benzene–acetone–methanol (80:15:5) as described previously (13). Diazepam served as the internal standard; a fixed quantity of the diazepam stock solution (usually 100–200 μl) was added to conical 40-ml centrifuge tubes equipped with polytef-lined screw caps. The solution was evaporated to dryness at 40° using a mild vacuum. Calibration standards were prepared by addition of at least four known quantities (usually 100, 200, 300, and 400 ng) of desmethyldiazepam to consecutive tubes. These solutions also were evaporated to dryness.

Drug-free control plasma, 1 ml, was added to each calibration sample, and 1 ml of "unknown" plasma was added to the other tubes. A 7.5-ml aliquot of benzene (containing 1.5% isoamyl alcohol) was added to all tubes, and the mixtures were agitated gently on a vortex mixer for 60 sec.

[1] Model 5750, Hewlett-Packard, Avondale, Pa.
[2] Matheson Gas Products, Gloucester, Mass.
[3] Supelco, Inc., Bellefonte, Pa.

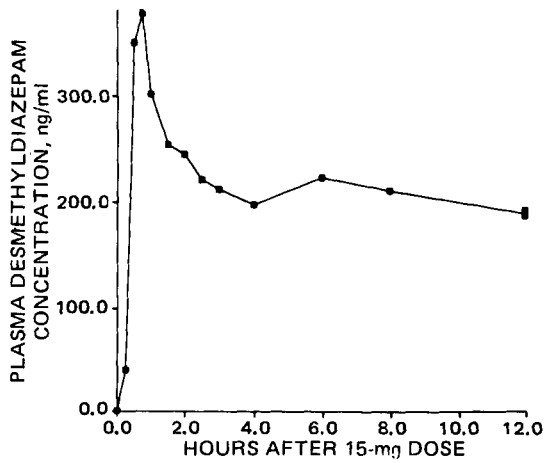


Figure 2—*Plasma desmethyldiazepam concentrations measured in a healthy volunteer during the first 12 hr after ingestion of 15 mg of clorazepate dipotassium.*

The tubes were centrifuged for 10 min at 2500 rpm, and an aliquot of the organic layer (~6 ml) was transferred to a conical 13-ml centrifuge tube.

The organic extracts were evaporated to dryness at 40° using a mild vacuum. The residue was redissolved in 100–200 μl of toluene (containing 15% isoamyl alcohol), of which 1–2 μl was injected into the chromatograph. Under these chromatographic conditions, the retention times were: diazepam, 2.5 min; and desmethyldiazepam, 3.25 min (Fig. 1).

Calculations—Calibration lines were prepared daily using the peak height ratio of desmethyldiazepam to diazepam *versus* the desmethyldiazepam concentration in the calibration samples. Least-squares regression analysis was used to determine the slope of the "best" line passing through the origin. Desmethyldiazepam concentrations in unknown samples were determined from the standard curve using the ratio of the desmethyldiazepam peak height to that of the internal standard.

Clinical Study—A healthy female volunteer ingested 15 mg of clorazepate dipotassium[4] with 100 ml of water after an overnight fast. Venous blood samples were drawn from an indwelling butterfly cannula or by separate venipuncture prior to drug administration and at 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3, 4, 6, 8, 12, 24, 36, 48, 73, 95, 120, and 144 hr after drug administration. Plasma samples were separated and frozen until analyzed. Desmethyldiazepam concentrations were determined by the described method. Samples drawn from 12 hr after ingestion through the end of the study were analyzed in duplicate.

The time course of plasma desmethyldiazepam concentrations following clorazepate ingestion in this subject appeared consistent with a two-compartment open pharmacokinetic model, with first-order drug absorption from the GI tract into the "central compartment," with a "lag time" prior to drug absorption (20). Accordingly, data points were analyzed with a computer, using iterative nonlinear least-squares regression analysis (21), and fitted to a function of the form:

$$C = -(A + B)e^{-k_a(t-t_0)} + Ae^{-\alpha(t-t_0)} + Be^{-\beta(t-t_0)} \quad \text{(Eq. 1)}$$

where C represents the plasma desmethyldiazepam concentration at time t after the dose; t_0 is the lag time prior to the start of absorption; A and B are "hybrid" intercept terms; and k_a, α, and β are hybrid exponents consistent with the absorption, distribution, and elimination phases, respectively, of the plasma concentration curve (22). The hybrid exponents were used to calculate the apparent first-order rates of drug absorption, $t_{1/2a}$, and elimination, $t_{1/2\beta}$, as follows:

$$t_{1/2a} = (\ln 2)/k_a = 0.693/k_a \quad \text{(Eq. 2)}$$

$$t_{1/2\beta} = (\ln 2)/\beta = 0.693/\beta \quad \text{(Eq. 3)}$$

RESULTS AND DISCUSSION

Assay Reproducibility and Sensitivity—Quantitative recovery of diazepam and desmethyldiazepam is achieved by this procedure. The coefficient of variation for 72 identical samples containing 25 ng of

[4] Tranxene, Abbott Laboratories, North Chicago, Ill.

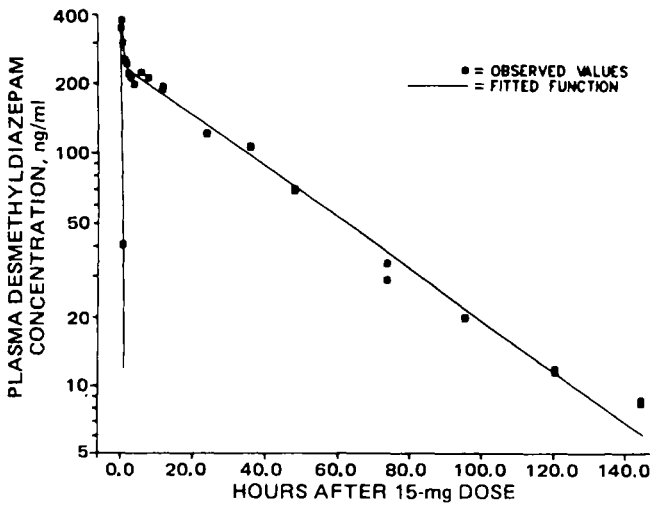


Figure 3—*Plasma desmethyldiazepam concentrations in the same volunteer during 144 hr after ingestion of 15 mg of clorazepate dipotassium. Replicate determinations were performed on samples drawn at 12 hr and thereafter. The solid line is the computer-fitted function.*

desmethyldiazepam/ml was 5.3%. The limits of sensitivity are approximately 5 ng of desmethyldiazepam/ml of original sample.

Pharmacokinetic Results—Following oral administration of 15 mg of clorazepate dipotassium to the healthy volunteer subject, a peak plasma desmethyldiazepam concentration of 379 ng/ml was measured in the sample drawn at 0.75 hr (Fig. 2). Following the achievement of peak levels, concentrations declined with an apparent biexponential pattern. The following function was generated by the computer following 50 iterative steps:

$$C = -2448.2e^{-4.19(t-t_0)} + 2208.0e^{-3.28(t-t_0)} + 240.2e^{-0.0257(t-t_0)} \quad \text{(Eq. 4)}$$

The lag time value, t_0, was 0.24 hr. Apparent half-life values were: $t_{1/2\alpha}$, 9.9 min; and $t_{1/2\beta}$, 27.0 hr (Fig. 3).

The described method enables rapid, reproducible, and sensitive quantitation of desmethyldiazepam in plasma. There is no evidence that desmethyldiazepam undergoes *N*-methylation to form diazepam in humans (2); chromatograms of plasma samples, containing no internal standard, from patients receiving clorazepate reveal no measurable amounts of diazepam. Thus, diazepam serves as a suitable internal standard. Addition of this compound to the original biological sample prior to extraction eliminates the need for precise volumetric measurements during subsequent steps.

Both desmethyldiazepam and diazepam are extracted quantitatively into the organic solvent at physiological pH using a single extraction. Chromatograms of these extracts are consistently free of any interfering peaks having retention times close to those of diazepam and desmethyldiazepam. Therefore, further cleanup procedures are not necessary. Since both compounds yield symmetrical, Gaussian peaks under the described conditions, peak heights rather than peak areas can be used for reliable quantitation of detector response (23). Thus, an electronic integrator is not necessary.

The method is readily adaptable to pharmacokinetic studies of desmethyldiazepam in human blood or plasma following ingestion of therapeutic doses of clorazepate dipotassium.

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