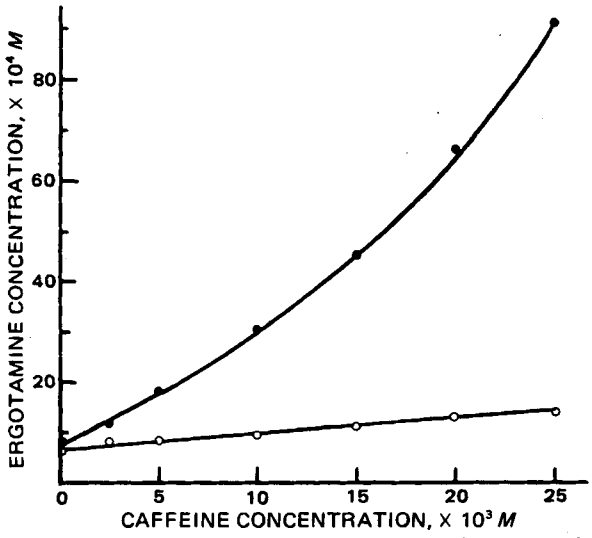

Figure 4—*Solubilizing effect of caffeine on ergotamine tartrate in 0.01 M HCl (●) and 0.50 M sulfuric acid (○).*

fuse from the solid–liquid interface into the bulk of the solution.

The inclusion of 0.025 *M* caffeine in a pH 5 acetate buffer increased the dissolution rate of ergotamine tartrate four times, whereas the inclusion of 0.15 *M* citric acid depressed the rate by a factor of five. The inclusion of 0.15 *M* citric acid in the acetate buffer that contained 0.025 *M* caffeine reduced the rate by a factor of eight.

The rate-enhancing effect of caffeine on ergotamine dissolution at pH 5.0 is consistent with the formation of a soluble molecular complex at this pH as well as at pH 1.0. These studies provided evidence of complex formation at pH 1.0, and Zoglio *et al.* (5) demonstrated complex formation at pH 6.65.

The results in Fig. 2 show that citric acid depressed ergotamine solubility in a pH 5 sodium acetate buffer by a factor of 2.4. Hence, the depressing effect of citric acid on the dissolution rate of ergotamine in a pH 5 buffer is consistent with predictions based on the Noyes–Whitney model.

CONCLUSIONS

1. Chloride ions, a common constituent of the contents of the stomach and duodenum, significantly reduce the concentration of ergotamine in solution by forming ergotamine chloride, a substance with a solubility product of $\sim 5 \times 10^{-6}\ M^2$.

2. The dissolution rate of ergotamine tartrate in water is not depressed appreciably by chloride ions because the precipitation rate of ergotamine chloride apparently is slow compared to the dissolution rate.

3. Protonation of the diionized tartrate anion of ergotamine tartrate by the addition of small amounts of citric acid or tartaric acid increases the solubility of ergotamine. Large amounts of these acids result in the precipitation of 1:1 salts of ergotamine and the acid monoanions. Sodium citrate depresses the solubility of ergotamine.

4. The Noyes–Whitney model for dissolution accounts for the rate-enhancing effect of caffeine and citric acid on ergotamine dissolution at pH 1.0 and the rate-depressing effect of citric acid at pH 5.0.

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Wall-Coated Open Tubular Column Coupled with Nitrogen-Selective Detector for Routine GLC Determination of Diazepam, Meprobamate, Phenylbutazone, and Thioridazine in Serum

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Received March 28, 1979, from the *Laboratory of Pharmacology, Medical School, Laboratory of Functional Explorations B, University Hospital Center of Caen, 14000 Caen, France.* Accepted for publication February 26, 1980.

Abstract □ The selectivity and sensitivity provided by a wall-coated open tubular column coupled with a nitrogen-selective detector allowed rapid, accurate determination of diazepam, meprobamate, phenylbutazone, and thioridazine in serum in the same chromatographic system using 100–200 μl of sample.

Keyphrases □ Diazepam—GLC determination in serum, wall-coated open tubular column coupled with nitrogen-selective detector □ Meprobamate—GLC determination in serum, wall-coated open tubular column coupled with nitrogen-selective detector □ Phenylbutazone—GLC determination in serum, wall-coated open tubular column coupled with nitrogen-selective detector □ Thioridazine—GLC determination in serum, wall-coated open tubular column coupled with nitrogen-selective detector □ GLC—analysis, diazepam, meprobamate, phenylbutazone, and thioridazine in serum, wall-coated open tubular column coupled with nitrogen-selective detector

Various columns and detectors have been used in the GLC determination of diazepam (I) (1–7), meprobamate (II) (8, 9), phenylbutazone (III) (10–14), and thioridazine (IV) (15–17) in serum. Packed columns were used in all of the methods and were coupled to a ^{63}Ni-electron-capture detector (1–5, 7, 14), a flame-ionization detector (6, 8–13,

Table I—Extraction Conditions

Serum Sample Extracted, μl	Drug (Solvent)	Concentrations Tested, μg/ml	Amount of Internal Standard (Solvent)	Volume of Acid or Base	Volume of Extraction Solvent	Agitation Time, min	Recovery of Drug, %	Recovery of Internal Standard, %
100	Diazepam (methanol)	0.1, 0.2, 0.5, 1, 1.5, 2	0.125 μg of flunitrazepam (chloroform)	30 μl of 1 *N* NaOH	6.0 ml of ether–chloroform (70:30 v/v)	6	89	69
100	Meprobamate (methanol)	5, 10, 15, 20, 25, 30	0.250 μg of thiopental (water)	200 μl of 0.05 *N* HCl	0.3 ml of chloroform	5	67	62
100	Phenylbutazone (water)	25, 50, 75, 100, 125, 150	5.0 μg of diazepam (methanol)	500 μl of pH 2 phosphate buffer	0.5 ml of chloroform	6	70	77
200	Thioridazine (methanol)	1, 1.5, 2, 2.5, 3, 3.5	1.0 μg of haloperidol (chloroform)	100 μl of 1 *N* NaOH	5.0 ml of hexane–chloroform (70:30 v/v)	12	88	60

Table II—Chromatographic Conditions and Results

Drug	Volume of Sample Injected, μl	Sensitivity and Attenuation	Column Temperature	Linear Carrier Gas Velocity[a], cm/sec	Drug Retention Time	Internal Standard Retention Time	Minimum Detectable Quantity, ng
Diazepam	3–5	1 × 8	235°	46	2 min, 6 sec	3 min, 6 sec	0.5
Meprobamate	10	1 × 2	170°	29	3 min, 28 sec	4 min, 26 sec	5
Phenylbutazone	1–2	1 × 16	245°	36	2 min	2 min, 18 sec	2
Thioridazine	6–8	1 × 2	260°	50	4 min, 36 sec	3 min, 12 sec	6

[a] Nitrogen U.

15, 17), or a ^{90}Sr-ionization detector (16). The columns were packed with Carbowax 20M (1), 3% OV-17 (2–4), 3% OV-225 (5), and 3% SE-30 (6) for I; 3% SE-30 (8) and 3.8% OV-17 (9) for II; 3% OV-210 (10), 3% Apiezon L (11), 3% OV-7 (12), 10% DC 200 (13), and 5% OV-17 (14) for III; and 3% XE-60 (15) and 3% OV-17 (16, 17) for IV.

To overcome the disadvantages of using many materials, one polyvalent chromatographic system, a high-performance wall-coated open tubular column coupled with a nitrogen-specific detector, was used for the determination in serum of these four drugs of various molecular weights and polarities.

The great efficiency (18, 19) and short elution time of the wall-coated open tubular column and the sensitivity and selectivity (20) of the nitrogen detector allow rapid, accurate determination of drugs at therapeutic levels with small samples (100–200 μl) using a simple extraction and no derivatization steps.

EXPERIMENTAL

Standard Solutions—Meprobamate[1] (50 μg/ml), thiopental[2] (5 μg/ml), phenylbutazone[3] (100 μg/ml), diazepam[4] (1, 5, and 100 μg/ml), thioridazine[5] (10 μg/ml), haloperidol[6] (10 μg/ml), and flunitrazepam[4] (5 μg/ml) solutions were prepared by dissolving the pure products in appropriate solvents as indicated in Table I. The solutions were stored at 4°.

Solvents—Chloroform, *n*-hexane, ether[7], and methanol[8] were of high grade purity.

Instrumentation—The gas–liquid chromatograph[9] was equipped with a nitrogen-selective detector[10] and a solid injector[10] and was connected to a recorder[11] with a scale range of 1 mv.

[1] Clin-Comar-Byla, Paris, France.
[2] Abbott, St-Remy sur Avre, France.
[3] Geigy, Rueil-Malmaison, France.
[4] Roche, Neuilly, France.
[5] Sandoz, Rueil-Malmaison, France.
[6] Lebrun, Paris, France.
[7] Merck, Darmstadt, West Germany.
[8] Prolabo, Paris, France.
[9] Model 3000 FC 1 ERPT, Girdel, Suresnes, France.
[10] Girdel, Suresnes, France.
[11] Servotrace PU, Sefram, Paris, France.

Table III—Assay Accuracy

	Serum Concentration, μg/ml		
	Theoretical	Experimental	Percent Difference
Diazepam	0.1	0.095	5
	0.2	0.185	7.5
	0.5	0.515	3
	1	0.985	1.5
	1.5	1.510	0.7
	2	1.995	0.25
Meprobamate	2.5	2.3	8
	5	4.8	4
	10	9.7	3
	15	15.3	2
	20	20.1	0.5
	25	25.5	2
Phenylbutazone	25	24.5	2
	50	50	0
	75	74.5	0.7
	100	99.5	0.5
	125	126.5	1.2
	150	152	1.3
Thioridazine	1	0.98	2
	1.5	1.5	0
	2	1.95	2.5
	2.5	2.37	5.2
	3	2.98	0.7
	3.5	3.54	1.7

The glass capillary column (11.5 m × 0.39 mm) with a phase of SE-30 0.4 μm thick was pretreated and tested by the manufacturer[12]. According to the spot test, it presents an effective number of theoretical plates (n = 16,600) for heptabarbital at 200° for a velocity of 18 cm/sec of the carrier gas (nitrogen U) and a capacity factor of three.

The chromatographic conditions were: injector temperature, 290°; detector temperature, 290°; make-up gas (nitrogen U) flow rate, 40 ml/min; hydrogen flow rate, 24 ml/min; air flow rate, 460 ml/min; and chart speed, 2.5 or 5 mm/min.

The other specific conditions for each compound are summarized in Table II.

Procedure—To 100 or 200 μl of human serum[13] in polytef-lined screw-capped glass test tubes (enveloped in a sheet of aluminum foil to ensure darkness for the thioridazine) were added the drug, the internal standard, acid or base, and the extracting solvent (Table I). The mixture was shaken and centrifuged for 5 min at 15° and 3500 rpm.

[12] Catalog No. 2101-201, LKB, Bromma, Sweden.
[13] Biotrol OOA, Biotrol, Paris, France.

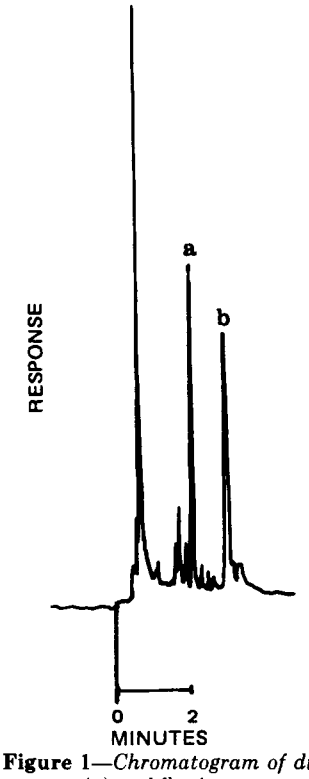

Figure 1—*Chromatogram of diazepam (a) and flunitrazepam as the internal standard (b).*

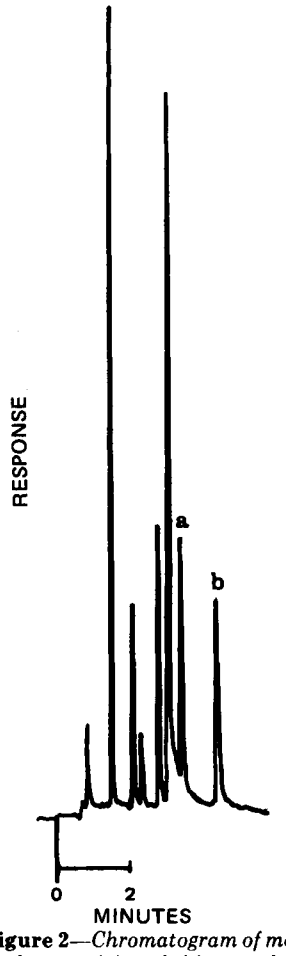

Figure 2—*Chromatogram of meprobamate (a) and thiopental as the internal standard (b).*

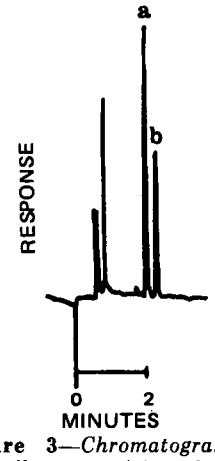

Figure 3—*Chromatogram of phenylbutazone (a) and diazepam as the internal standard (b).*

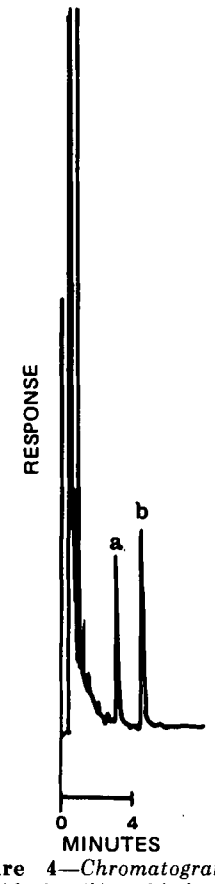

Figure 4—*Chromatogram of thioridazine (b) and haloperidol as the internal standard (a).*

An aliquot (2–10 μl) of the organic phase was injected directly into the chromatograph for II and III. The entire organic phase was evaporated to dryness for I (50° under an air stream) and IV (20° under a nitrogen stream), and the residue was dissolved in 50 μl of chloroform before injection.

RESULTS AND DISCUSSION

Figures 1–4 illustrate the chromatographic responses of the drugs and standards. The chromatographic conditions, retention time, and minimum detectable concentration of each compound are summarized in Table II.

The range of each drug was chosen according to the therapeutic concentrations: 0.7–1.5 (21) or 0.5–2.5 μg/ml (22) for I, 3.4–26.6 (21) or 10 μg/ml (22) for II, 60–150 (21) or 100 μg/ml (22) for III, and 1.1–6.2 (21) or 1–1.5 μg/ml (22) for IV.

The slopes of the regression lines were 1.312, 0.058, 0.015, and 0.527 for I, II, III, and IV, respectively. The correlation coefficient was 0.999 in all cases. The standard deviations ranged from 5 to 3% for 0.5–2 μg of I/ml, from 4 to 3% for 5–30 μg of II/ml, from 4 to 2.5% for 25–150 μg of III/ml, and from 6 to 3% for 1–35 μg of IV/ml over 3 months. Accuracy (Table III) was tested by analyzing spiked samples. Accuracy was improved by avoiding the evaporation step for drugs having sufficiently high therapeutic levels (II and III) and by evaporating the extraction mixture (IV) under nitrogen and in darkness.

For 100-μl serum samples, the minimum detectable concentrations were 0.05, 2, and 5 μg/ml for I, II, and III, respectively. For a 200-μl serum sample of IV, it was 0.3 μg/ml. The twofold determination of the patient serum levels gave the accuracy of the method (Tables III–V). No endogenous interfering substances were found. Exogenous substances such as phenobarbital and lidocaine did not interfere with II; nor did vincamine, quinidine, or clonazepam interfere with I, III, and IV.

The great sensitivity of the wall-coated open tubular column coupled with a nitrogen-specific detector allowed small samples to be used

Table IV—Serum Concentrations of Diazepam in Five Epileptic Patients

Age of Patient, years	Daily Diazepam Dose, mg	Comedication (Daily Dose)	Serum Concentration, μg/ml
8	10	Valproate sodium, 400 mg	0.30 ± 0.02
8	10	Valproate sodium, 800 mg	0.17 ± 0.02
13	8	Phenobarbital, 50 mg; valproate sodium, 600 mg	0.70 ± 0.01
50	15	Phenobarbital, 300 mg	0.42 ± 0.02
56	10	Phenobarbital, 100 mg	0.23 ± 0.01

Table V—Serum Concentrations of Meprobamate in Four Intoxication Cases

Age of Patient, years	Serum Concentration, μg/ml
16	87 ± 2
17	190 ± 5
18	70 ± 1
33	138 ± 2

Table VI—Serum Concentrations of Phenylbutazone in a Nursing Mother after Administration of a 250-mg Suppository Twice Daily for 10 Days

Days of Treatment	Serum Concentration, μg/ml
3	52.0 ± 2.0
6	79.0 ± 2.5
11	66.0 ± 2.0

(100–200 μl), instead of the previous requirements of 1–3 ml for II and III (8, 10–13) and 4–5 ml for IV (15, 17), without increasing the lowest detectable serum concentration. The high resolution of the capillary column (23) reduced the extraction stage to a single step instead of the three steps often suggested to eliminate the interfering substances (12, 13, 15, 17). Furthermore, derivatization steps such as silylation (10) and methylation (12) were unnecessary. The rapid elution time (24) shortened the retention times. These conditions led to shorter extraction and chromatographic stages. Nevertheless, the accuracy and sensitivity were usually improvements over previous assays (3–5, 9, 11, 12, 15, 17).

The determination in serum of four drugs useful in toxicology (25–29) or therapeutic monitoring (30–32) demonstrated the versatility and potential of this chromatographic system and its possible routine use in view of the growing importance of serum level determinations of an increasing number of diverse drugs in clinical practice.

The method was demonstrated by the determination of serum concentrations of diazepam in five epileptic patients (Table IV), of meprobamate in four cases of voluntary overdose (Table V), and of phenylbutazone in a nursing mother given a 250-mg suppository twice daily for 10 days (Table VI).

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Immobilization of Proteins in Microspheres of Biodegradable Polyacryldextran

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Abstract □ Macromolecules were immobilized by an emulsion polymerization technique in biodegradable microspheres of polyacryldextran, prepared by copolymerizing bisacrylamide with acryldextran. Such particles can be characterized by D–T–C expressions, where D denotes the concentration of derivatized dextran, T is the total concentration of acrylic compounds in the monomeric solution, and C denotes the fraction of cross-linker. In microparticles based on dextran T40 with a D–T–C of 11–1–75, the yield of immobilized protein was greater than in polyacrylamide particles. The properties of the immobilized proteins, *e.g.*, K_m and V_{max}, were retained. The heat stability of the proteins was improved so that 5–10% of carbonic anhydrase still was active after 30 min at 100°. However, the leakage of proteins from the polyacryldextran particles was greater than from polyacrylamide particles.

Keyphrases □ Microspheres—biodegradable polyacryldextran particles, immobilization of proteins □ Polyacryldextran—microspheres, immobilization of proteins □ Proteins—immobilization in polyacryldextran microspheres

The therapeutic use of proteins *in vivo* usually is hampered by rapid protein inactivation or by immunological reactions when the proteins are immunogenic. For instance, asparaginase is not active for long periods in the treatment of acute leukemias (1). However, the noncovalent immobilization of the proteins in microspheres of

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