***N,S*-(2-Aminoethanethio)chloroaquoplatinum(II)** — 2-Aminoethanethiosulfuric acid (0.1572 g, 0.001 mole) was dissolved in 25 ml of water, and the pH was adjusted to 7.4 with 0.01 *N* NaOH. An aqueous solution (10 ml) of potassium tetrachloroplatinate(II) (0.4151 g, 0.001 mole) was added dropwise with stirring along with sufficient alkali to maintain a pH of 7.0–7.4. Stirring was continued for 1 hr after the addition; the yellow product was filtered, washed with water, and dried at 70° overnight, yielding 0.2742 g (66%), mp 340° dec.

Anal.—Calc. for $C_2H_8ClNOPtS \cdot H_2O$: C, 6.99; H, 2.93; Cl, 10.34; N, 4.07; S, 9.35. Found: C, 6.76; H, 2.46; Cl, 9.89; N, 3.80; S, 9.02.

Chloro-*N,N',S*-(cystamine)platinum(II) Chloride—Cystamine dihydrochloride (0.225 g, 0.001 mole) was dissolved in 35 ml of water, and the pH was adjusted to 7.4 with 0.01 *N* NaOH. A solution of 0.4151 g (0.001 mole) of potassium tetrachloroplatinate(II) in 10 ml of water was added dropwise with stirring, and stirring was continued for 2 hr after the addition. The yellow product was filtered, washed with water, and dried at 70° overnight. The yield was 0.42 g (52.6%) of material, which decomposed above 183°.

Anal.—Calc. for $C_4H_{12}Cl_2N_2PtS_2 \cdot 8H_2O$: C, 8.54; H, 4.98; N, 4.97; S, 11.41. Found: C, 8.70; H, 4.65; N, 4.73; S, 10.97.

REFERENCES

(1) B. Rosenberg, L. Van Camp, J. E. Trosko, and V. H. Mansour, *Nature*, **222**, 385 (1969).

(2) M. J. Cleare and J. D. Hoeschele, *Bioinorg. Chem.*, **2**, 187 (1973).

(3) B. Rosenberg, L. Van Camp, and T. Krigas, *Nature*, **205**, 698 (1965).

(4) M. J. Cleare and J. D. Hoeschele, *Platinum Met. Rev.*, **17**, 2 (1973).

(5) L. F. Lindoy, S. E. Livingstone, and T. N. Lockyer, *Aust. J. Chem.*, **20**, 471 (1967).

(6) F. Basolo, "Mechanisms of Inorganic Reactions," American Chemical Society, Washington, D.C., 1965.

(7) B. Rosenberg, *Cancer Chemother. Rep.*, **59**, 589 (1975).

(8) D. L. Klayman and R. J. Shine, *Qt. Rep. Sulfur Chem.*, **3**, 231 (1968).

(9) W. O. Foye and J. Mickles, *J. Pharm. Sci.*, **53**, 1030 (1964).

(10) S. E. Livingstone and J. D. Nolan, *Inorg. Chem.*, **7**, 1447 (1968).

(11) C. A. McAuliffe, *J. Chem. Soc.*, **1967**, 641.

(12) T. A. Connors, M. Jones, W. J. C. Ross, P. D. Braddock, A. R. Kokhar, and M. L. Tobe, *Chem.-Biol. Interact.*, **5**, 415 (1972).

(13) R. Layton, D. W. Sink, and J. R. Durig, *J. Inorg. Nucl. Chem.*, **28**, 1965 (1966).

(14) D. C. Jicha and D. H. Busch, *Inorg. Chem.*, **1**, 872 (1962).

(15) R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, **3**, 1 (1972).

(16) J. E. Huheey, "Inorganic Chemistry: Principles of Structure and Reactivity," Harper and Row, New York, N.Y., 1972.

(17) S. Mansy, B. Rosenberg, and A. J. Thomson, *J. Am. Chem. Soc.*, **95**, 1633 (1973).

(18) T. A. Connors and J. J. Roberts, "Platinum Coordination Complexes in Cancer Chemotherapy," Springer Verlag, Berlin, Germany, 1974, p. 79.

(19) W. O. Foye, J. J. Lanzillo, Y. H. Lowe, and J. M. Kauffman, *J. Pharm. Sci.*, **64**, 211 (1975).

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Ion-Pair Liquid Chromatographic Assay of Decongestants and Antihistamines

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Abstract □ The quantitative determinations of combinations of antihistamine and decongestant drugs including phenylephrine, *dl*-ephedrine, ψ-ephedrine, phenylpropanolamine, pyrilamine, pheniramine, *l*-ephedrine, chlorpheniramine, brompheniramine, oxymetazoline, naphazoline, and antazoline contained in solid and liquid dosage forms are described. All active ingredients except the ephedrine optical isomers were separated from other ingredients with ion-paired high-pressure liquid chromatography. Manipulation of the mobile phase either by changing the hydroalcoholic ratio or by changing the alkyl chain length of the counterion (sulfonic acid) for achieving optimum separations is discussed. The method is simple, short, accurate, and precise.

Keyphrases □ Decongestants—analysis, ion-pair liquid chromatography, various cough and cold preparations, structure–activity relationships □ Antihistaminics—analysis, ion-pair liquid chromatography, various cough and cold preparations, structure–activity relationships □ Liquid chromatography, ion-pair—analysis, decongestants and antihistaminics in various cough and cold preparations, structure–activity relationships □ Structure–activity relationships—decongestants and antihistaminics, various cough and cold preparations

Combinations of decongestant and antihistamine pharmaceutical preparations are widely used for cough and cold treatment. Generally, such preparations contain one decongestant and one antihistamine, but several contain more than one decongestant. These combination preparations are made in various forms, *e.g.*, syrup, elixir, tablet, capsule, and timed-release tablet or capsule. Some liquid formulations may also contain preservative(s), dye(s), or flavor(s).

High-performance liquid chromatography (HPLC) has become useful for pharmaceutical preparation analysis. Decongestant and antihistamine compounds were analyzed using a strong cation-exchange column (1), and the separation of two decongestants and one antihistamine was demonstrated using a nonpolar reversed-phase column and heptanesulfonic acid as an ion-pairing agent (2, 3). This report describes a comprehensive analytical procedure applicable to numerous decongestants and antihistamines and discusses ways to achieve a desired separation by changing the alkyl chain length of sulfonic acid or by altering the hydroalcoholic composition of the mobile phase.

EXPERIMENTAL

Chemicals and Reagents—The following drugs and preservatives were used: phenylephrine (I), *dl*-ephedrine (II), ψ-ephedrine (III), phenylpropanolamine (IV), pyrilamine (V), pheniramine (VI), *l*-ephedrine (VII), chlorpheniramine (VIII), brompheniramine (IX), diphenhydra-

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Table I—Detection Limit and Linear Detection Range at 254 nm of Various Drugs Tested

Drug	Minimum[a] Detection Limit, ng	Linear[a] Detection Range	Correlation Coefficient
Phenylephrine hydrochloride	40	40 ng–100 μg	0.9759
Ephedrine	60	60 ng–60 μg	0.9990
Phenylpropanolamine hydrochloride	140	140 ng–8 μg	0.9976
Pyrilamine maleate	18	18 ng–40 μg	0.9849
Pheniramine maleate	20	20 ng–50 μg	0.9991
Guaifenesin	40	40 ng–100 μg	0.9990
Pseudoephedrine hydrochloride	80	80 ng–60 μg	0.9972
Chlorpheniramine maleate	14	14 ng–20 μg	0.9996
Brompheniramine maleate	20	20 ng–20 μg	0.9949
Antazoline phosphate	18	18 ng–10 μg	0.9910
Naphazoline hydrochloride	24	24 ng–20 μg	0.9964
Oxymetazoline hydrochloride	280	280 ng–30 μg	0.9930

[a] Amount injected.

mine (X), guaifenesin (XI), caffeine (XII), aspirin (XIII), oxymetazoline (XIV), naphazoline (XV), antazoline (XVI), methylparaben (XVII), and propylparaben (XVIII). All were USP or NF quality and were used without further purification.

All sulfonic acids were received as their sodium salts except methanesulfonic acid, which was received as a free acid. All reagents were used as received[1]. The chromatographic solvents were distilled-in-glass quality[2].

Chromatography—The modular liquid chromatograph consisted of a constant flow pump of 6000 psi maximum operating pressure[3], a variable wavelength detector set at 254 nm[4], and a constant volume loop injector[5]. The chromatograms were recorded using a strip-chart recorder[6]. The column (moderately polar phenyl bonded) was purchased commercially[7].

Mobile Phase—The mobile phase was water–methanol–glacial acetic acid (55:44:1 v/v) containing enough heptanesulfonic acid sodium salt to yield a 0.005 *M* solution. The flow rate was 2.0 ml/min.

Sample Preparation—*Diluent*—The diluent was methanol–water (1:1 v/v).

Elixirs and Syrups—Three elixirs and two syrups were analyzed. Ten milliliters of elixir or syrup were diluted with the diluent to 50 ml in a volumetric flask.

Table II—Retention Data of Various Antihistamine and Sympathomimetic Drugs and Related Compounds

Drug	k'[a]	Tailing Factor[b]
Phenylephrine	1.2	100
dl-Ephedrine	2.2	100
Phenylpropanolamine	1.9	96
Pyrilamine	8.8	86
Pheniramine	4.4	91
l-Ephedrine	2.2	100
Guaifenesin	1.4	100
ψ-Ephedrine	2.2	100
Chlorpheniramine	8.8	86
Brompheniramine	10.5	86
Oxymetazoline	9.8	63
Naphazoline	4.6	90
Antazoline	11.0	73
Diphenhydramine	8.6	43
Methylparaben	2.2	100
Propylparaben	5.0	91
Caffeine	1.4	100
Aspirin	2.2	100

[a] k' is the capacity factor calculated as $(V_R - V_0)/V_0$, where V_R is the apparent retention volume and V_0 is the dead volume of the column. [b] Calculated by drawing a perpendicular line from the baseline to the peak maximum and then measuring the base width of the second half (y) of the chromatographic peak relative to the first half (x). Measurements were made at 10% of the peak maximum. Tailing factor = $x/y \times 100$.

[1] Eastman Kodak Co., Rochester, N.Y.
[2] Burdick & Jackson, Muskegon, Mich.
[3] Waters Associates, Milford, Mass.
[4] Schoeffel Instruments Corp., Westwood, N.J.
[5] Valco Instruments, Houston, Tex.
[6] Model 56, Perkin-Elmer.
[7] μBondapak phenyl (4 mm i.d. × 30 cm), Waters Associates, Milford, Mass.

Tablets—Four tablet preparations were assayed. Ten tablets were ground to a fine powder, and the powder equivalent to one tablet weight was transferred into a 50-ml volumetric flask. Approximately 30 ml of the diluent was added, and the flask was shaken for 15 min. The solution was diluted to volume with the diluent, and ~10 ml of this solution was filtered through a 0.45-μm filter for chromatographic analysis.

Timed-Release Capsules or Tablets—Three dosage forms were analyzed. The contents equivalent to one capsule or tablet were transferred to a 100-ml volumetric flask, and 10 ml of 1.0 *N* HCl was added. The flask was shaken for 15 min. Approximately 50 ml of diluent was added, and the flask was shaken for another 15 min. The solution was diluted to volume with the diluent, and ~10 ml was filtered through a 0.45-μm filter.

Quantitation—Standard curves for the appropriate compounds were established. A 20-μl sample was injected, and the peak response was measured (as peak height). The drug concentration was measured either directly from the standard curve or by regression analysis. The amount of drug present in the dosage form was calculated by:

$$\% \text{ label claim} = (C)(D)\left(\frac{100}{L}\right) \qquad \text{(Eq. 1)}$$

where C is the concentration of the sample (milligrams per milliliter) read from the standard curve, D is the total dilution (milliliters), and L is the label amount of drug in the dosage form (milligrams).

RESULTS AND DISCUSSION

All compounds previously mentioned were tested for linear detection range and minimum detection limit. The data were obtained using six to 10 calibration points (Table I).

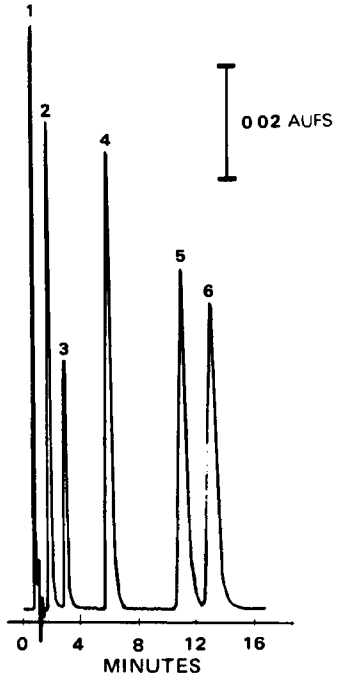

Figure 1—*Chromatogram of five common cough and cold drugs. Key: 1, maleic acid; 2, phenylephrine; 3, pseudoephedrine; 4, naphazoline; 5, chlorpheniramine; and 6, brompheniramine.*

Table III—Regression Analysis of the Relationship between Retention Volume and the Hydroalcoholic Composition of the Mobile Phase

Compound	Correlation Coefficient	Slope	Intercept
Chlorpheniramine	0.974	-6.7×10^{-3}	4.3×10^{-1}
Pheniramine	0.995	-7.1×10^{-3}	4.9×10^{-1}
ψ-Ephedrine	0.998	-6.7×10^{-3}	5.3×10^{-1}
Phenylephrine	0.997	-5.4×10^{-3}	5.3×10^{-1}

Table IV—Liquid Pharmaceutical Preparations Analyzed

Active Ingredient	Label, mg/5 ml	Assay, mg/5 ml
Elixir A[a]		
Pseudoephedrine hydrochloride	30.0	30.1
Chlorpheniramine maleate	2.0	2.0
Syrup B[b]		
Phenylpropanolamine hydrochloride	20.0	18.7
Phenylephrine hydrochloride	5.0	4.72
Phenyltoloxamine citrate[c]	7.5	—
Chlorpheniramine maleate	2.5	2.48
Syrup C		
Phenylephrine hydrochloride	2.5	1.9
Chlorpheniramine maleate	1.0	0.96
Elixir D[d]		
Brompheniramine maleate	4.0	4.2
Phenylephrine hydrochloride	5.0	5.3
Phenylpropanolamine hydrochloride	5.0	4.85
Elixir E[e]		
Phenylephrine hydrochloride	5.0	5.0
Chlorpheniramine maleate	1.0	1.0
Chloroform	13.5	—

[a] Deconamine elixir, Cooper Laboratories. [b] Naldecon syrup, Bristol Laboratories. [c] Could not be analyzed due to lack of reference standard. [d] Dimetapp elixir, A. H. Robins. [e] Novahistine elixir, Dow.

The chromatographic separation of five common drugs used for cough and cold treatment is shown in Fig. 1, and the chromatographic retention data are listed in Table II. The phenyl column, of moderate polarity, is capable of resolving almost all of the compounds listed except the stereoisomers such as *l*-ephedrine and *d*-ephedrine. A nonpolar hydrocarbon-bonded column[8] (C_{18} bonded) did not produce satisfactory results. For example, chlorpheniramine showed unacceptable peak tailing, and pyrilamine and pheniramine could not be separated (2).

The retention of compounds can be varied to obtain a desired separation. Four compounds of decreasing polarity (phenylephrine, ψ-ephedrine, pheniramine, and chlorpheniramine) were studied to show how a slight modification of the mobile phase can allow almost any desired separation. Two relationships were studied: (*a*) change in the hydroalcoholic composition, and (*b*) change in the alkyl chain length of the counterion, *i.e.*, alkylsulfonic acid.

Figure 2 shows the effect of changing the water–methanol ratio on the retention volume. The retention volumes of all compounds increased with the increased water ratio in the mobile phase. A linear relationship for each drug was obtained by plotting percent water in the mobile phase *versus* V_R^{-1}. This result is due to a decrease in the solubility (hydrophobicity) of the solute molecule in the mobile phase. The regression analyses of the data points are given in Table III.

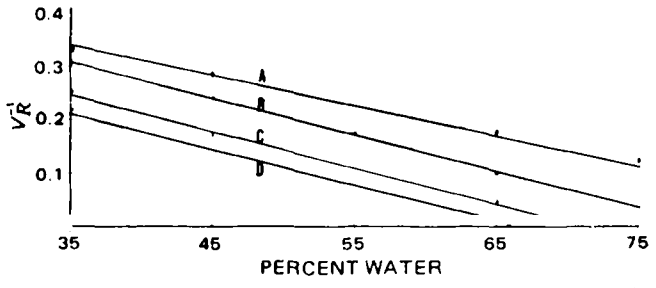

Figure 2—*Relationship between hydroalcoholic composition of mobile phase and drug retention volume. Key: A, θ, chlorpheniramine; B, O, pheniramine; C, ×, pseudoephedrine; and D, ■, phenylephrine.*

[8] μBondapak-C_{18}, Waters Associates, Milford, Mass.

Table V—Solid Dosage Forms Analyzed

Active Ingredient	Label, mg	Assay, mg
Tablet A[a]		
Pseudoephedrine hydrochloride	60.0	60.5
Chlorpheniramine maleate	4.0	4.02
Tablet B[b]		
Dexbrompheniramine maleate	2.0	2.01
d-Isoephedrine maleate	60.0	60.3
Tablet C[c]		
Phenylephrine hydrochloride	10.0	9.24
Chlorpheniramine maleate	2.0	1.85
Tablet D[d]		
Phenylpropanolamine hydrochloride	50.0	50.2
Phenindamine tartrate[e]	24.0	—
Chlorpheniramine maleate	4.0	4.15

[a] Deconamine tablets, Cooper Laboratories. [b] Disophrol tablets, Schering. [c] Novahistine Fortis, Dow. [d] Nolamine tablets, Carnrick. [e] Could not be analyzed due to lack of reference standard.

The effect on drug retention of changing the alkyl chain length of the counterion (sulfonic acid) in the mobile phase is shown in Fig. 3. The plot of the carbon number of the alkyl chain *versus* $\log_e$ retention volume shows a linear relationship for pheniramine and chlorpheniramine, but the plot for ψ-ephedrine and phenylephrine is dual sloped. This result is due to the fact that with pheniramine and chlorpheniramine, the increase in the carbon chain length of the counterion decreases the polarity of the "ion-paired" molecule as a whole, causing a linear increase in the retention volume in the reversed-phase system. However, there is very little change in polarity for ψ-ephedrine and phenylephrine when the counterion is methane through butanesulfonic acid. A linear increase in the retention is noticed ($\log_e V_R$ *versus* carbon number of the alkyl chain of the counterion) from pentane to octanesulfonic acid.

Thus, the point from which a change in polarity is noticed could be called the "polarity threshold" of each compound. Varying the carbon chain length of the counterion below the polarity threshold causes no significant variation of retention volumes. The concept of polarity threshold can be exploited to manipulate drug analyses, as shown by the following example.

A dosage form containing ψ-ephedrine and chlorpheniramine was analyzed using the solvent system described in the *Experimental* section. When heptanesulfonic acid was replaced by pentanesulfonic acid as the counterion, the retention volumes of both ψ-ephedrine and chlorpheniramine were reduced from 6.0 and 20.4 ml to 3.9 and 11.9 ml, respectively. Further replacement of pentanesulfonic acid by butanesulfonic acid selectively reduced the chlorpheniramine retention to 9.6 ml without affecting the ψ-ephedrine retention (3.8 ml) because of the polarity threshold.

Change of the alkyl chain of sulfonic acid also can be of advantage when the dosage form contains acidic molecules such as parabens or aspirin, which do not ion-pair with sulfonic acid. Naphazoline and propylparaben had a very similar k' (Table II) and could not be separated from each

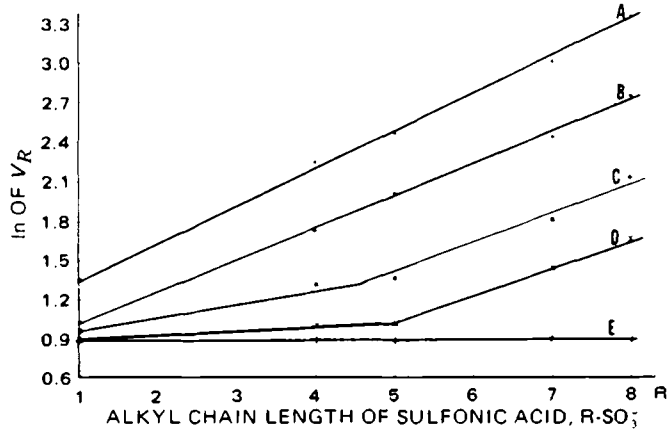

Figure 3—*Relationship between alkyl chain length of the counterion and drug retention volume. Key: A, ■, chlorpheniramine; B, O, pheniramine; C, *, pseudoephedrine; D, ×, phenylephrine; and E, +, maleate.*

Table VI—Timed-Release Dosage Forms Analyzed

Active Ingredient	Label, mg	Assay, mg
Capsule A[a]		
Pseudoephedrine hydrochloride	120.0	118.8
Chlorpheniramine maleate	8.0	7.8
Timed-Release Tablet B[b]		
Phenylpropanolamine hydrochloride	40.0	44.0
Phenylephrine hydrochloride	10.0	10.7
Chlorpheniramine maleate	5.0	4.8
Phenyltoloxamine citrate[c]	15.0	—
Capsule C[d]		
Chlorpheniramine maleate	8.0	7.43
Phenylephrine hydrochloride	15.0	20.4
Methscopolamine[c]	2.5	—

[a] Deconamine capsules, Cooper Laboratories. [b] Naldecon timed-release tablets, Bristol. [c] Could not be analyzed due to lack of reference standard. [d] Cosea-D capsules, Center.

other. By changing the counterion from heptanesulfonic acid to pentanesulfonic acid, the naphazoline retention was reduced without affecting the propylparaben retention, and a complete resolution of these compounds was possible.

To ensure the specificity of the proposed method, all drugs were artificially degraded. All drugs were well separated from their degradation products. Twelve pharmaceutical preparations were analyzed by this method. The versatility of the method was demonstrated by the analysis of various combination preparations (Tables IV–VI). The reproducibility of the method was excellent. Five replicate analyses of Liquid Preparation I (Table IV) had a coefficient of variation of 0.9%. The variation coefficients of five replicate analyses of two solid dosage forms (Preparation I, Table V; and Preparation I, Table VI) were 1.4 and 1.9%, respectively.

The proposed method is useful for assaying of cough and cold drugs. The method is simple, requires no elaborate sample preparation techniques, and is specific, accurate, and reproducible. Many separations can be achieved by proper manipulation of the mobile phase.

REFERENCES

(1) T. L. Sprieck, *J. Pharm. Sci.*, **63**, 591 (1974).
(2) V. D. Gupta and A. G. Ghanekar, *ibid.*, **66**, 895 (1977).
(3) A. Yacobi, Z. M. Look, and C.-M. Lai, *ibid.*, **67**, 1668 (1978).

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Specific and Sensitive High-Performance Liquid Chromatographic Determination of Glyburide

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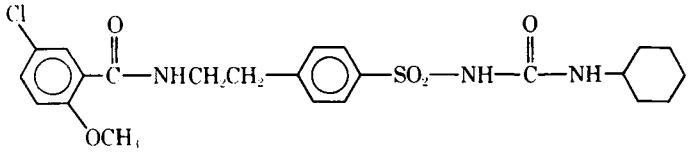
Received December 18, 1978, from the *Pharmaceutical Research and Development and Control Research and Development Laboratories, The Upjohn Company, Kalamazoo, MI 49001.* Accepted for publication March 21, 1979.

Abstract □ A specific and sensitive high-performance liquid chromatographic method has been developed for the rapid determination of intact glyburide in dog serum. With butylparaben as an internal standard, 1 ml of acid-buffered serum was extracted with toluene and an aliquot of the toluene was evaporated to dryness. The redissolved residue was chromatographed on a microparticulate reversed-phase column, and quantitation was achieved by monitoring the UV absorbance of the eluate at 228 nm. The response was linear, and the lower detection limit was ~20 ng/ml. Assay precision, as estimated by analyzing replicate samples of a laboratory standard, was better than 6% (*CV*). The utility of the analytical methodology for the determination of this highly potent sulfonylurea in pharmacokinetic studies in the dog was demonstrated.

Keyphrases □ Glyburide—analysis, high-performance liquid chromatography, dog serum □ Antidiabetic agents—glyburide, high-performance liquid chromatographic analysis, dog serum □ High-performance liquid chromatography—analysis, glyburide in dog serum

Glyburide[1] (I), 1-[[4-[2-(5-chloro-2-methoxybenzamido)ethyl]phenyl]sulfonyl]-3-cyclohexylurea, is a potent, orally active sulfonylurea for the management of maturity-onset diabetes mellitus (1). A number of analytical methods for the determination of glyburide in serum have been reported. These methods include procedures based on UV spectrophotometry, colorimetry following reaction with 2,4-dinitrofluorobenzene, fluorometry (2), and competitive protein binding (3, 4). However, only a recently developed radioimmunoassay (4) is sufficiently sensitive for monitoring blood levels following administration of



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therapeutic doses (2.5–10 mg). Although this radioimmunoassay is highly sensitive, with the lower limit of detection being ~4 ng/ml of serum, the method lacks specificity because of cross-reactivity of the hydroxy metabolites of glyburide. In addition, it is necessary to prepare standard curves using pretreatment serum from each subject because the assay response is subject dependent (4).

GLC has been used extensively for the analysis of sulfonylureas in biological fluids (5–14). However, this technique requires a time-consuming derivatization step to form volatile, thermally stable derivatives unless on-column derivatization is employed with concomitant loss in reproducibility[2] (12). Furthermore, the technique frequently lacks specificity since the intact compound and its metabolites may form identical derivatives (14, 15). Finally, the high molecular weight and inherently low volatility of glyburide or its derivatives make GLC analysis difficult if not impossible.

[1] Micronase, The Upjohn Co., Kalamazoo, MI 49001.

[2] D. G. Kaiser, The Upjohn Co., Kalamazoo, MI 49001, personal communication.

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