Selective GLC Determination of Epinephrine, Isoproterenol, and Phenylephrine in Pharmaceutical Dosage Forms

J. R. WATSON ˣ and R. C. LAWRENCE

Abstract □ A simple, specific GLC analytical procedure for the quantitation of epinephrine, isoproterenol, and phenylephrine in commercial tablets, powders, inhalation solutions, ophthalmic and nasal drops, and injectable preparations is presented. Samples are taken to dryness where required, the dried residue is reacted with an appropriate trimethylsilylating reagent, and the derivatives are eluted from a methyl silicone column using temperature programming. Quantitation of the flame-ionization detector signal is achieved relative to the dibenzyl succinate internal standard by an electronic integrator. The results obtained by applying the method to the analysis of each of the three drugs in several simulated decomposed mixtures were in good agreement with theoretical values, even at impurity levels of up to 80% by weight. When applied to commercial formulations, the procedure was feasible for tablets, powders, and solutions at drug concentrations of 0.2% or greater. The commonly incorporated buffering and antioxidant excipients did not interfere.

Keyphrases □ Epinephrine—GLC analysis, pharmaceutical dosage forms □ Isoproterenol—GLC analysis, pharmaceutical dosage forms □ Phenylephrine—GLC analysis, pharmaceutical dosage forms □ GLC—analyses, epinephrine, isoproterenol, and phenylephrine, pharmaceutical dosage forms □ Adrenergics—epinephrine, isoproterenol, and phenylephrine, GLC analysis, pharmaceutical dosage forms

The sympathomimetic drugs epinephrine (I), isoproterenol (II), and phenylephrine (III) comprise some of the most extensively studied pharmacological agents employed in chemotherapeutics. Their wide acceptance is reflected by the many marketed formulations and the variety of pharmaceutical dosage forms.

BACKGROUND

Because of their amphoteric properties and attendant highly unfavorable extraction characteristics into organic solvents, the analysis of epinephrine, isoproterenol, and phenylephrine in pharmaceutical dosage forms is difficult. Moreover, in an alkaline medium, the water-soluble salts formed by the catecholamines epinephrine and isoproterenol are very unstable and undergo a rapid but complex oxidative decomposition to reddish aminochromes and, subsequently, to brown melanin-type compounds (1, 2). Degradation of phenylephrine occurs less readily, but, above pH 7, cleavage of the side chain of the parent molecule results in the formation of formaldehyde, which immediately reacts with a second molecule of phenylephrine in a Pictet–Spengler-type reaction to produce a mixture of isomeric dihydroxytetrahydroisoquinoline derivatives (2).

In view of the intrinsic susceptibility of these drugs to oxidative degradation, most of their preparations are stabilized with antioxidants such as chlorobutanol, ascorbic acid, or sodium bisulfite. Several reports showed that the stability of many important epinephrine and isoproterenol pharmaceutical products may be seriously impaired by anaerobic interaction of these drugs with sodium bisulfite to give zwitterionic sulfonate derivatives. These derivatives are both optically and physiologically inactive and exhibit UV spectra identical in absorption characteristics to the parent compounds (3–8). Phenylephrine differs in this respect apparently because it lacks the 4-hydroxyl group and the required resonating quinoid structure (4).

The current USP (9) and BP (10) procedures for the assay of epinephrine in commercial formulations are based on the measurement of the specific optical rotation after conversion to the triacetyl derivative. This method is self-limiting in scope and specificity and involves tedious and time-consuming manipulations. Although limit tests for the presence of adrenalone and levarterenol (norepinephrine) are described in the monographs for the drug substance, none is specified for pharmaceutical dosage forms.

In the USP (9), both isoproterenol and phenylephrine hydrochlorides are extracted quantitatively from pharmaceutical solutions or tablet matrixes by ion-pair formation with bis(2-ethylhexyl)phosphoric acid in ether, followed by partition chromatography of the solution through a suitably buffered siliceous earth column. Isoproterenol sulfonic acid, if present, reportedly remains adsorbed on the stationary phase (8). The USP monographs include a limit test for congeneric ketone compounds in the drug substances and in the commercial isoproterenol formulations. The assay procedure may lack precision because many important variables, such as tightness of the column packing and rate of elution, are not described.

Both BP 1973 (10) and NF XIV (11) employ a nonspecific colorimetric method for the analysis of isoproterenol sulfate in tablets and aerosol spray preparations. In the former pharmacopeia, phenylephrine hydrochloride injection is determined by simple absorbance measurement at 273 nm after appropriate dilution with aqueous sulfuric acid. This procedure would be inadequate where the product has undergone oxidative decomposition or is contaminated with other UV-absorbing phenylalcoholamine substances.

As a consequence of the limitations, most notably in specificity, of the compendial assay procedures for epinephrine, isoproterenol, and phenylephrine in dosage forms, alternative methods that encompass various analytical techniques have appeared (12–30).

Evidence was given previously that the highly polar functional groups of levodopa (31), α-methyldopa (32), and their respective congeners can be suitably altered by trimethylsilylation to give less polar and more thermally stable derivatives. These derivatives can be easily eluted as single sharp peaks from a methyl silicone column under relatively moderate temperature conditions. Since the catecholamines and other related sympathomimetic amines readily undergo similar well-documented transformations, it seemed reasonable to apply essentially the same quantitative GLC method to the analysis of epinephrine, isoproterenol, and phenylephrine.

The present investigation was conducted to integrate the available information and to develop a rapid, accurate, and selective GLC procedure for the analysis of epinephrine, isoproterenol, and phenylephrine in both liquid and solid pharmaceutical dosage forms in the presence of excipients and high levels of decomposition products and/or contaminating congeners. The method obviates the need for extraction or prior on-column cleanup manipulations inherent in most published procedures.

EXPERIMENTAL

Materials and Solutions—The following were used: *dl*-epinephrine hydrochloride[1], *dl*-norepinephrine hydrochloride[2], adrenalone hydrochloride[1], *l*-adrenochrome free base[1], epinephrine bitartrate[3], isoproterenol hydrochloride[3], isoproterenol sulfate[4], *N*-isopropylnoradrenalone sulfate[1], phenylephrine hydrochloride[3], norphenylephrine hydrochloride[2], *N,O*-bis(trimethylsilyl)acetamide[5], and *N*-trimethylsilylimidazole[5].

Epinephrine sulfonic acid and isoproterenol sulfonic acid were synthesized by applying the general method of Schroeter and Higuchi (5). The structures of the synthesized compounds were confirmed by elemental analysis and by their IR and NMR spectral characteristics.

The following examined excipients were all analytical grade standards: glycerin, lactic acid, ascorbic acid, citric acid, lactose, chlorobutanol,

[1] ICN Laboratories Inc., Montreal, Canada.
[2] Aldrich Chemical Co., Montreal, Canada
[3] United States Pharmacopeial Convention, Rockville, Md.
[4] Abbott Laboratories, Montreal, Canada.
[5] Pierce Chemical Co., Rockford, Ill.

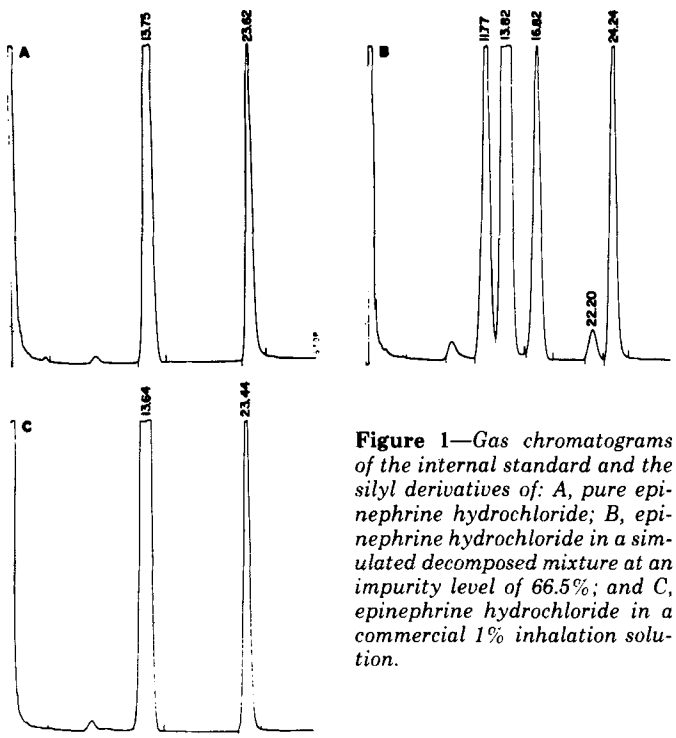

Figure 1—*Gas chromatograms of the internal standard and the silyl derivatives of: A, pure epinephrine hydrochloride; B, epinephrine hydrochloride in a simulated decomposed mixture at an impurity level of 66.5%; and C, epinephrine hydrochloride in a commercial 1% inhalation solution.*

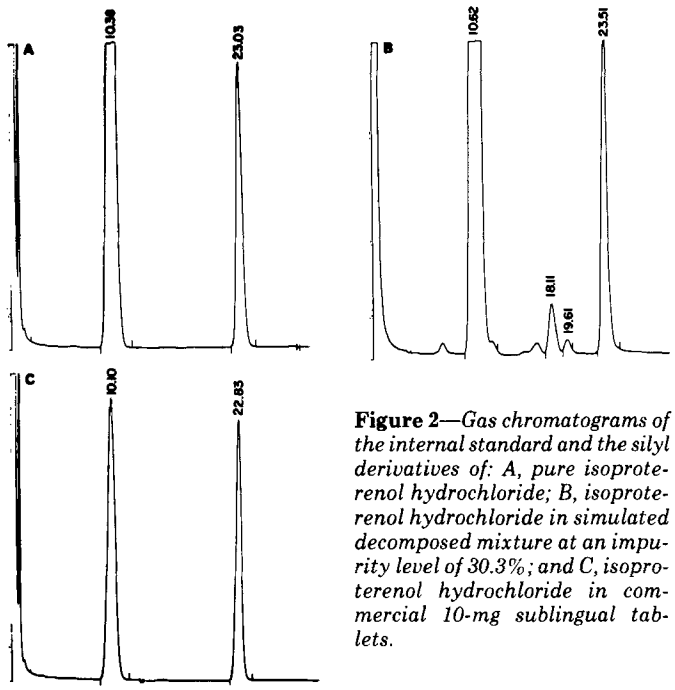

Figure 2—*Gas chromatograms of the internal standard and the silyl derivatives of: A, pure isoproterenol hydrochloride; B, isoproterenol hydrochloride in simulated decomposed mixture at an impurity level of 30.3%; and C, isoproterenol hydrochloride in commercial 10-mg sublingual tablets.*

saccharin sodium, sodium bisulfite, sodium citrate, edetate sodium, phenol, and benzalkonium chloride.

The internal standard solution was dibenzyl succinate[6] in spectrograde dimethylformamide[6], accurately weighed to contain about 2.5 mg/ml.

Sample Preparation—*Response Factor Calibration Mixtures*—For I, II, or III, varying quantities of the pure substance (as the salt), ranging from about 2 to 18 mg, were accurately weighed into five separate 5-ml septum-sealed vials. Then 2.00 ml of the internal standard solution was dispensed into each vial from a 5-ml microburet graduated in 0.01 ml.

Simulated Decomposed Mixtures—For each drug, five separate simulated decomposed mixtures of widely differing impurity levels were prepared by accurately weighing known quantities of the following compounds into 5-ml septum-sealed vials to obtain about 20–30 mg of material in each mixture.

For epinephrine mixtures (Series 1), epinephrine hydrochloride, epinephrine sulfonic acid, norepinephrine hydrochloride, adrenalone hydrochloride, and adrenochrome free base were used. The impurity range varied from about 20 to 80%.

For isoproterenol mixtures (Series 2), isoproterenol hydrochloride, isoproterenol sulfonic acid, norepinephrine hydrochloride, and *N*-isopropylnoradrenalone sulfate were used. The impurity range was from about 16 to 77%.

For phenylephrine mixtures (Series 3), phenylephrine hydrochloride and norphenylephrine hydrochloride were used. The impurity range was from about 7 to 26%.

To simulate the analysis of aqueous commercial formulations, each of the 15 mixtures was dissolved in water (1 ml) and the water was then removed at 60° under a stream of pure dry nitrogen on a heating block module[5]. To ensure complete desiccation, the vial was placed in a drying pistol assembly under vacuum over phosphorus pentoxide at 80° for 30 min under subdued light conditions. The dried residues were then treated with 2.00 ml of the internal standard solution.

Commercial Products—Ten tablets were selected at random, weighed, and finely powdered. An amount of powdered tablet (or powder) equivalent to about 10–15 mg of isoproterenol hydrochloride (or sulfate) was weighed into a 5-ml vial, and 2.00 ml of the internal standard solution was dispensed into the vial.

Aqueous formulations included inhalation solutions, ophthalmic and nasal drops, and injectable preparations. A volume of solution theoretically equivalent to about 5–15 mg of active drug was pipetted into a 5-ml vial, and the water was removed as indicated previously. Then 2.00 ml of the internal standard solution was added to the dried residue in the vial.

[6] J. T. Baker Chemical Co., Phillipsburg, N.J.

Derivatization Procedure—Each isoproterenol sample solution containing the internal standard was treated with *N*-trimethylsilylimidazole (1.0 ml) and allowed to stand for 30 min in the dark at ambient temperature with occasional shaking.

Each epinephrine and phenylephrine sample solution containing the internal standard was treated with *N,O*-bis(trimethylsilyl)acetamide (1.0 ml) and allowed to stand for 30 min in the dark at ambient temperature with occasional shaking.

GLC—Two microliters of silylated solution was injected into a gas chromatograph[7] (flame-ionization detector) fitted with a 5% OV-101[5] on Chromosorb 750[8] (100–120-mesh) U-shaped glass column [1.82 m (6 ft) × 6 mm o.d.] preconditioned at 265° for 18 hr. The support was coated using a fluidizer[9] maintained at 150° for 2 hr with a suitable nitrogen flow to ensure gentle, yet thorough, drying of the packing material.

Temperature conditions were: column, 170° (10 min) and then programmed to 245° at 2°/min; injection port, 225°; and detector, 225°. Gas flows were: nitrogen, 70 ml/min; hydrogen, 40 ml/min; and air, 380 ml/min.

The detector signal was fed to an electronic integrator[10] with an input signal range capacity of 0–1 v.

RESULTS AND DISCUSSION

Although the catecholamine acids are readily silylated in acetonitrile, the catecholamine salts are not, probably because of poor solubility in this solvent. For these drugs, dimethylformamide is a superior medium because it possesses enhanced solubilizing properties and accelerates the trimethylsilylation 10–20 times by comparison with pyridine and other solvents (23).

The criteria adopted for choosing the appropriate silylating reagent for each of the three drugs were based on the following considerations: (*a*) the derivatization of the drug should take place quantitatively in a minimum amount of time and at ambient temperature if possible, (*b*) the silyl derivative of the pure drug should elute as a single sharp peak, and (*c*) this peak should be clearly resolved from that of the internal standard and from the peak or peaks generated by potential contaminating by-products or decomposition products. In the present study, *N,O*-bis(trimethylsilyl)acetamide was employed for the silylation of epinephrine hydrochloride (and bitartrate) and phenylephrine hydrochloride but was less suitable for isoproterenol sulfate, yielding two peaks in the approximate area ratio of 4:1. Consequently, *N*-trimethylsilylimidazole was selected as the preferred silyl donor for the analysis of isoproterenol hydrochloride and isoproterenol sulfate formulations.

[7] Bendix series 2500.
[8] Applied Science Laboratories Inc., State College, Pa.
[9] Hi-Eff, Applied Science Laboratories.
[10] Hewlett-Packard series 3380A reporting integrator.

Table I—GLC Data on Epinephrine, Isoproterenol, Phenylephrine, and Associated Compounds

Compound	Silylation Method[a]	Number of Peaks	Retention Time, min	Relative[b] Response Factor, %
Epinephrine hydrochloride	A	1	13.7	0.528 ± 2.52
Epinephrine bitartrate	A	2	3.1 (tartaric acid)	—
			13.7	0.849 ± 1.52
Norepinephrine hydrochloride	A	1	11.5	—
Adrenalone hydrochloride	A	1	16.3	—
Epinephrine sulfonic acid	A	—	—	—
Adrenochrome	A	1	22.6 (broad and small)	—
Isoproterenol hydrochloride	B	1	10.4	0.608 ± 2.67
Isoproterenol sulfate	B	1	10.4	0.686 ± 1.84
Norepinephrine hydrochloride	B	2	18.1 (91%)	—
			19.5 (9%)	—
N-Isopropylnoradrenalone sulfate	B	3	9.2 (12%)	—
			14.7 (11%)	—
			16.1 (77%)	—
Isoproterenol sulfonic acid	B	—	—	—
Phenylephrine hydrochloride	A	1	6.3	0.604 ± 2.38
Norphenylephrine hydrochloride	A	1	5.2	—
Dibenzyl succinate	—	1	23.4	1.0000

[a] A = *N,O*-bis(trimethylsilyl)acetamide in dimethylformamide, and B = *N*-trimethylsilylimidazole in dimethylformamide. [b] Relative to dibenzyl succinate, expressed as weight/area, and representing the average of five determinations.

Table II—GLC Analysis of Simulated Mixtures[a]

Mixture Series	Components	1		2		3		4		5	
		Added	Rec[b]	Added	Rec[b]	Added	Rec[b]	Added	Rec[b]	Added	Rec[b]
1	Epinephrine hydrochloride	17.99	17.78	14.15	14.32	11.84	11.93	8.47	8.23	5.44	5.28
	Norepinephrine hydrochloride	1.03	—	3.60	—	2.15	—	4.12	—	5.31	—
	Adrenalone hydrochloride	1.33	—	3.09	—	2.87	—	4.37	—	5.36	—
	Epinephrine sulfonic acid	1.04	—	2.82	—	2.02	—	4.18	—	7.65	—
	Adrenochrome	1.01	—	2.86	—	2.02	—	4.17	—	5.51	—
	Percent impurity in mixture	19.7		46.6		43.4		66.5		81.4	
2	Isoproterenol hydrochloride	17.30	17.89	14.38	14.76	11.44	11.54	8.62	8.48	5.22	4.95
	Norepinephrine hydrochloride	1.02	—	1.77	—	3.27	—	4.10	—	5.33	—
	Isoproterenone sulfate	1.10	—	1.73	—	3.10	—	4.17	—	6.24	—
	Isoproterenol sulfonic acid	1.07	—	2.76	—	3.06	—	4.23	—	5.50	—
	Percent impurity in mixture	15.6		30.3		45.2		59.3		76.6	
3	Phenylephrine hydrochloride	19.57	19.28	18.54	18.62	17.45	17.58	16.50	16.49	15.48	15.31
	Norphenylephrine hydrochloride	1.35	—	2.13	—	3.17	—	4.20	—	5.50	—
	Percent impurity in mixture	6.5		10.3		15.4		20.3		26.2	

[a] All values are in milligrams. [b] The recovery values listed represent the average of duplicate injections of the mixture.

Table III—GLC Analysis of Commercial Formulations

Drug	Dosage Form	Manufacturer	Label Claim[a]	Percent Recovery of Label Claim
Epinephrine hydrochloride	Inhalation solution	M	3.5%	97.9
	Inhalation solution	D	2.25%	102.9
	Ophthalmic solution	C	2.0%	106.8
	Inhalation solution	H	2.25%	87.8
	Inhalation solution	H	2.25%	94.0
	Ophthalmic solution	B	2.0%	102.5
	Inhalation solution	K	2.25%	114.5
	Inhalation solution	I	1.0%	114.3
Epinephrine bitartrate	Ophthalmic solution	F	2.0%	95.9
Isoproterenol hydrochloride	Tablets	O	10 mg/tablet	104.8
	Tablets	O	10 mg/tablet	102.5
	Inhalation solution	O	0.5%	107.9
	Inhalation solution	L	0.5%	112.9
Isoproterenol sulfate	Powder mix	A	25%	106.0
Phenylephrine hydrochloride	Nasal drops	O	1.0%	94.0
	Nasal drops	E	0.25%	91.7
	Ophthalmic solution	G	0.2%	90.1
	Ophthalmic solution	O	10%	99.5
	Ophthalmic solution	J	10%	100.6
	Ophthalmic solution	N	10%	106.4
	Injection	O	1%	91.7

[a] The percent values are defined in grams per 100 ml of solution.

Relevant information on the average retention times of epinephrine, isoproterenol, and phenylephrine salts and their respective congeners and/or interaction and degradation products are presented in Table I. In the epinephrine series, the bitartrate salt of the drug afforded two peaks. The first (at 3.1 min) was due to the tartaric acid moiety while the adrenochrome gave only one small broad noninterfering peak at around 22 min. For the two compounds in the isoproterenol series for which more than one peak was generated, the individual peak values, expressed as percentages of total area, have been included in parentheses. *N*-Isopropylnoradrenalone sulfate appears to be particularly prone to multiple peak formation, due perhaps to *in situ* enolization, but none of the peaks would interfere with the quantitation of isoproterenol even in the presence of unusually large amounts of *N*-isopropylnoradrenalone.

No data are given with respect to the aminochrome analog of isoproterenol since it was not commercially available and is not readily prepared in the laboratory. However, it appears safe to assume a flame-ionization detector response for this compound quite similar to that of adrenochrome. The sulfonic acids of epinephrine and isoproterenol were not converted to silyl derivatives of sufficient volatility or thermal stability to be detectable under the GLC conditions. However, attempts to investigate further the nonelutive properties of these two compounds were not made.

In Table I, the listed response factors of the pure drugs were computed automatically by the digital processor and are expressed in weight/area relative to dibenzyl succinate. Their respective coefficients of variation were well within acceptable limits of precision. The chromatograms of the silyl derivatives of pure epinephrine hydrochloride, isoproterenol hydrochloride, and phenylephrine hydrochloride are reproduced in Figs. 1A, 2A, and 3A, respectively.

The results obtained by applying the GLC method to the analysis of several simulated decomposed mixtures of each drug are given in Table II and support the conclusion that the procedure is feasible and selective even at unrealistically high levels of contamination. The term "decomposed" is employed here in the broader sense to embrace other chemical entities that would not arise from the normal degradation but rather are artifacts incompletely removed during drug synthesis. The mixtures were dissolved in water prior to sample treatment and analysis to duplicate as closely as possible the conditions existing in the assay of commercial aqueous formulations and to demonstrate that the correct amount of chemically intact parent drug could be recovered.

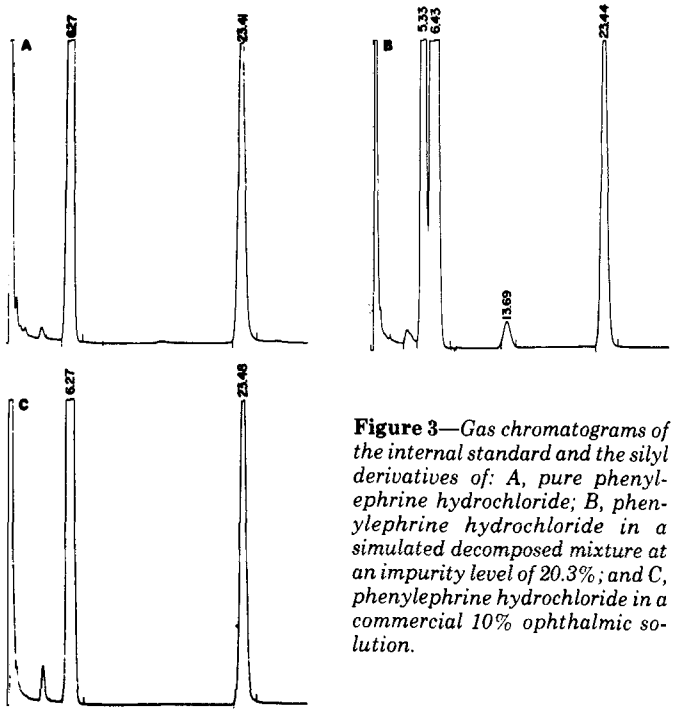

Figure 3—*Gas chromatograms of the internal standard and the silyl derivatives of: A, pure phenylephrine hydrochloride; B, phenylephrine hydrochloride in a simulated decomposed mixture at an impurity level of 20.3%; and C, phenylephrine hydrochloride in a commercial 10% ophthalmic solution.*

The percent recovery values varied from 97.2 to 101.2% for epinephrine, from 94.9 to 103.4% for isoproterenol, and from 98.5 to 100.7% for phenylephrine. The value of 94.9% for isoproterenol Mixture 5 is admittedly somewhat of an outlier, but the high level of impurity in this case (76.6%) is really not one that would normally be encountered in the day-to-day analysis of commercial formulations. Sample chromatograms of each of the three drugs in simulated decomposed mixtures are presented in Figs. 1B, 2B, and 3B.

To document further the validity of the present GLC method for the analysis of commercial formulations, 12 commonly incorporated buffering and antioxidant excipient compounds were chromatographed after dissolution or suspension in the silylation medium at ambient temperature for 30 min. No potentially interfering signals were observed for any substance examined. Most adjuvants listed under *Experimental* either gave peaks that were eluted close to or under the solvent or produced no peak that could be detected under the temperature conditions used. Single sharp peaks, well resolved from those of the particular drug with which they were formulated, were observed for citric acid (7.6 min) and ascorbic acid (12.6 min). Benzalkonium chloride gave four small noninterfering peaks at about 17, 26, 36, and 44 min.

The results of the analysis of commercial tablets, powder mixes, nasal drops, inhalation and ophthalmic solutions, and an injectable preparation are delineated in Table III. Chromatograms for epinephrine, isoproterenol, and phenylephrine in commercial formulations are given in Figs. 1C, 2C, and 3C.

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High-Performance Liquid Chromatographic Analysis of Isoniazid and Its Dosage Forms

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Abstract □ A high-performance liquid chromatographic analysis is described for isoniazid as a drug entity and in its tablet and injectable dosage forms. After incorporation of the drug or dosage form in a solvent mixture and addition of an internal standard, tribenzylamine, an aliquot is chromatographed using a pellicular silica gel medium followed by UV spectrophotometric detection at 254 nm. The response of the chromatographic system was linear over a concentration range corresponding to 20–200% of the labeled amount of isoniazid. Comparison of the results with those obtained by the official USP XIX method indicates similar accuracy and precision. The advantages of the proposed method are its simplicity and rapidity, its potential for automation, and its specificity. The specificity was demonstrated in the presence of potential degradation products of isoniazid, other drugs used with isoniazid in combination dosage forms, and an adduct formed by the reaction of isoniazid with lactose in the tablet.

Keyphrases □ Isoniazid—high-performance liquid chromatographic analysis, tablets and injectables □ High-performance liquid chromatography—analysis, isoniazid, tablets and injectables □ Tuberculostatic antibacterials—isoniazid, high-performance liquid chromatographic analysis, tablets and injectables

Because isoniazid (isonicotinic acid hydrazide) is the drug of choice for the treatment of tuberculosis, many attempts have been made to develop methods for its quantitation in dosage formulations. The procedures rely primarily on the redox reactivity of the hydrazide group or upon color formation with an appropriate chromogen.

One analysis is based on the addition of excess standard iodine followed by back-titration with standard thiosulfate (1). This procedure was official in four USP revisions but was supplanted in the current USP XIX (2) by a nitrous acid titration similar to that used for the determination of sulfa drugs. The method official in the BP (3) is similar to the earlier USP method, except that excess bromine is used instead of iodine. These oxidimetric methods and a nonaqueous titration procedure were reviewed and compared previously (4).

Another method (5) involves the determination of isoniazid and other hydrazine-derived drugs by potentiometric titration in an acid solution, using chloramine-T as the titrant. However, its applicability to dosage forms was not explored. A colorimetric method was proposed based on the reaction of isoniazid with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (6). A colorimetric procedure uses 9-chloracridine as the chromogenic reagent (7).

The purpose of this study was to investigate the utility of high-performance liquid chromatography (HPLC) in the determination of isoniazid in its dosage forms. The speed and accuracy of this technique and the availability of relatively inexpensive modular instrumentation should make such analysis particularly applicable in quality assurance situations.

The proposed method is simple, involving only the dissolution of the sample, addition of the internal standard, and introduction of an aliquot of the resulting mixture onto a liquid chromatograph with an adsorption column and a