Applications of Paired Ion High-Pressure Liquid Chromatography to Catecholamines and Phenylephrine

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Abstract □ Paired ion high-pressure liquid chromatography was useful for separating intact catecholamines (epinephrine, isoproterenol, levodopa, and methyldopa) and phenylephrine from some of their decomposition products. Furthermore, methyldopa was separated from either hydrochlorothiazide or levodopa. Under identical conditions without 1-heptanesulfonic acid (a counterion for paired ion chromatography) in the mobile phase, these separations were not possible. Paired ion chromatography also was tried successfully for the quantitative determinations of isoproterenol, levodopa, methyldopa, and phenylephrine in some dosage forms. The results were compared with the results obtained using some literature methods.

Keyphrases □ Catecholamines, various—paired ion high-pressure liquid chromatographic analyses in prepared solutions and dosage forms □ Phenylephrine hydrochloride—paired ion high-pressure liquid chromatographic analysis in prepared solutions and dosage forms □ High-pressure liquid chromatography—analyses, various catecholamines and phenylephrine hydrochloride in prepared solutions and dosage forms

Recently, paired ion high-pressure liquid chromatography (HPLC) has become popular, and the background and theory of this technique were reviewed (1). The quantitative determination of epinephrine by cation-exchange chromatography was reported (2). Watson and Lawrence reported (3–5) the quantitative determinations of catecholamines (epinephrine, isoproterenol, levodopa, and methyldopa) and phenylephrine by GLC, but this method requires derivatization and is lengthy and tedious. Recently, these authors (5) surveyed the literature on catecholamines.

This paper reports the applications of paired ion HPLC to catecholamines and phenylephrine. The catecholamines studied were epinephrine hydrochloride (I), isoproterenol hydrochloride (II), levodopa (III), and methyldopa (IV). The paper reports: (*a*) the separation of intact catecholamines from their decomposition products, (*b*) the separation of levodopa from the structurally related catecholamine methyldopa, (*c*) the separation of methyldopa from hydrochlorothiazide (a common combination product), and (*d*) quantitative determinations of II–IV and phenylephrine (V) in dosage forms using the developed method and literature methods.

EXPERIMENTAL

Chemicals and Reagents—All chemicals and reagents were USP, NF, or ACS grade and were used without further purification. Sodium 1-heptanesulfonate[1] (VI) was used as received.

Apparatus—The high-pressure liquid chromatograph[2] was capable of operating at an inlet pressure of up to 6000 psig. The multiple-wavelength detector[3] was set at 280 nm for all catecholamines except phenylephrine; for phenylephrine, it was set at 273 nm. These are wavelengths of maximum absorption. The detector was attached to a recorder[4] and an integrator[5].

The column[6] (30 cm long × 4 mm i.d.) was used as received.

Chromatographic Solvents—A 20% (v/v) solution of methanol in water containing 2% (v/v) acetic acid was used with or without 0.005 *M* sodium 1-heptanesulfonate. The pH of both solvents was 2.6 ± 0.05.

Chromatographic Conditions—The temperature was ambient. The flow rate was 1.6 ml/min (inlet pressure of approximately 1500 psig) for all catecholamines except levodopa and the levodopa–methyldopa combination for which it was 1.2 ml/min (inlet pressure of approximately 1000 psig). The absorbance unit for full-scale deflection was 0.04 except for levodopa and the levodopa–methyldopa combination for which it was 0.1. The chart speed was 30.5 cm/hr.

Stock Solutions—A 0.1% solution in water of I–V was prepared using a simple solution method. For levodopa and methyldopa, 10 and 3 ml of 0.1 *N* H_2SO_4, respectively, were added per 100 ml of the solution to obtain clear solutions. A commercial[7] 0.1% solution of epinephrine hydrochloride also contained 0.9% NaCl, 0.5% chlorobutanol, and 0.15% $NaHSO_3$. None of these compounds interfered in the assay used.

Standard Solutions—All standard solutions were prepared by diluting 10.0 ml of the stock solution to 100.0 ml with water.

Decomposition of Catecholamines and Phenylephrine—A 5.0-ml quantity of the stock solution was mixed with 5.0 ml of an appropriate solution of sodium hydroxide (0.01, 0.1, or 1 *N*). A sodium hydroxide solution of lower concentration was preferred as long as the mixture became discolored within a few minutes, so the 0.1 *N* solution generally was used. However, for II and V, the 0.01 and 1 *N* solutions were used, re-

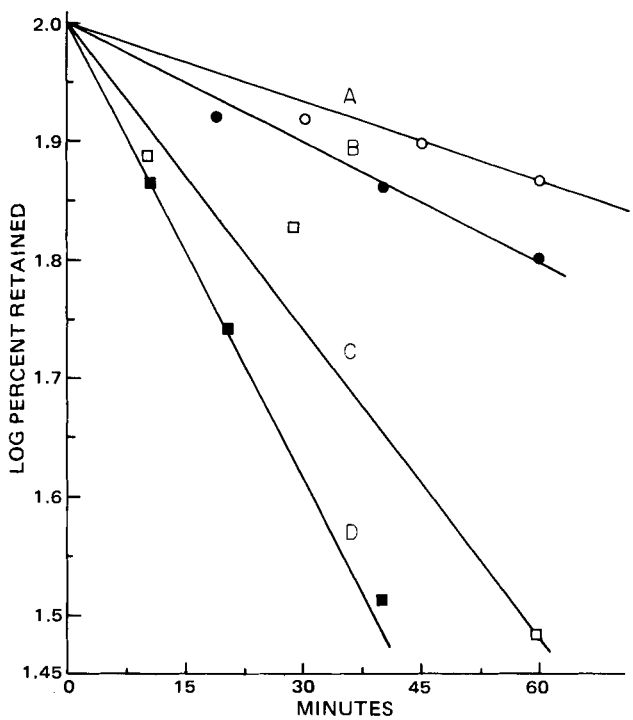

Figure 1—*Plots of log percent retained* versus *time. Key: A, II; B, IV; C, I; and D, III.*

[1] Eastman Kodak Co., Rochester, NY 14650.
[2] Model ALC 202 equipped with a U6K Universal injector, Waters Associates, Milford, Mass.
[3] Spectroflow monitor 770, Schoeffel Instrument Corp., Westwood, N.J.
[4] Omniscribe 5213-12. Houston Instruments, Austin, Tex.
[5] Autolab Minigrator, Spectra-Physics, Santa Clara, Calif.
[6] μBondapak C_{18}, Waters Associates, Milford, Mass.
[7] Parke, Davis & Co., Detroit, Mich.

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Table I—Assay Results on the Dosage Forms and Standard Deviations on I–V

Compound	SD^a, %	Dosage Form Assayed	Results[b], % of Claim
I	0.53	None	—[c]
II	0.84	Injection	100.0[d]
III	0.76	Capsules	100.7[e]
IV	0.83	Tablets	98.4[f]
V	0.98	Nose drops	99.9[g]
IV with hydrochlorothiazide		Tablets	100.2[d]

[a] Based on five injections of the standard solution. [b] Average of two. [c] No dosage form was tested. Only standard deviation was determined. [d] The result with the Kaistha method (6) was 99.6%. [e] The result with the USP method (7) was 100.3%. [f] The result with the USP method (8) was 98.4%. [g] The result with the Koshy and Mitchner method (9) was 99.8%.

spectively. After an appropriate period, the reaction mixture was quenched by adding 5.0 ml of sulfuric acid of an appropriate concentration. The mixture was brought to volume (50.0 ml) with water and assayed as discussed later.

Extraction of Levodopa and Methyldopa from Solid Dosage Forms—Twenty tablets or the contents of 20 capsules were ground to a fine powder, and powder representing 100.0 mg of catecholamine was weighed accurately and transferred to a mortar. Then 10 ml (20 ml for levodopa) of 1:350 sulfuric acid in water was added and mixed thoroughly for 2–3 min. The mixture was brought to volume (100.0 ml) with water and filtered if necessary. The first 10–15 ml of the filtrate was rejected, and then 10.0 ml was diluted to 100.0 ml with water and assayed as discussed later.

Dilutions of Other Dosage Forms—The contents of an isoproterenol hydrochloride injection (contained 0.02% solution of II) were diluted two times with water and assayed as described later. Then 2.0 ml of 0.5% phenylephrine hydrochloride nose drops was diluted to 100.0 ml with water and assayed as described later.

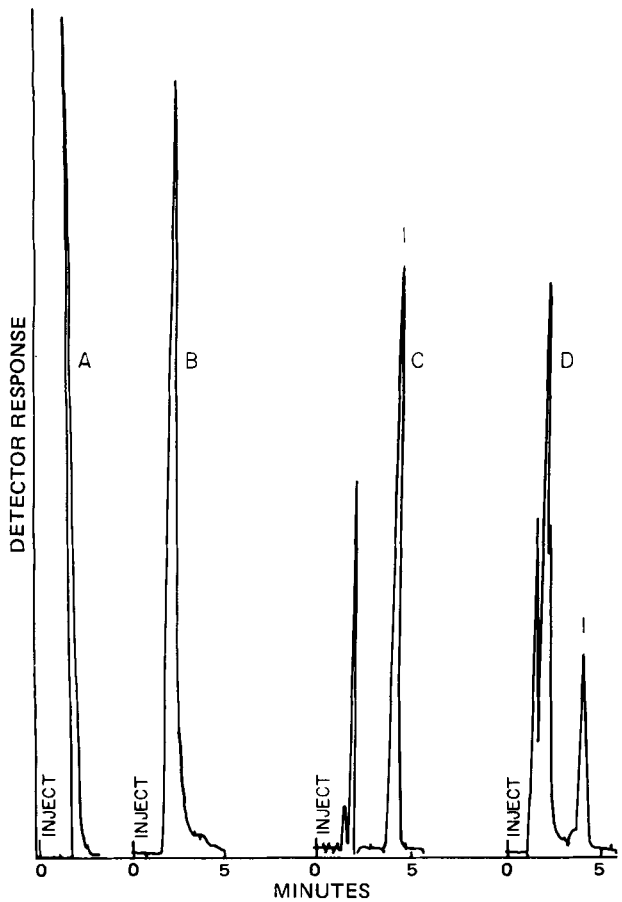

Figure 2—*Sample chromatograms of I (peak 1 is from I). Key: A, from a standard solution using mobile phase without VI; B, from a 1-hr decomposed sample using mobile phase without VI; and C and D, same as A and B, respectively, except that the mobile phase contained VI.*

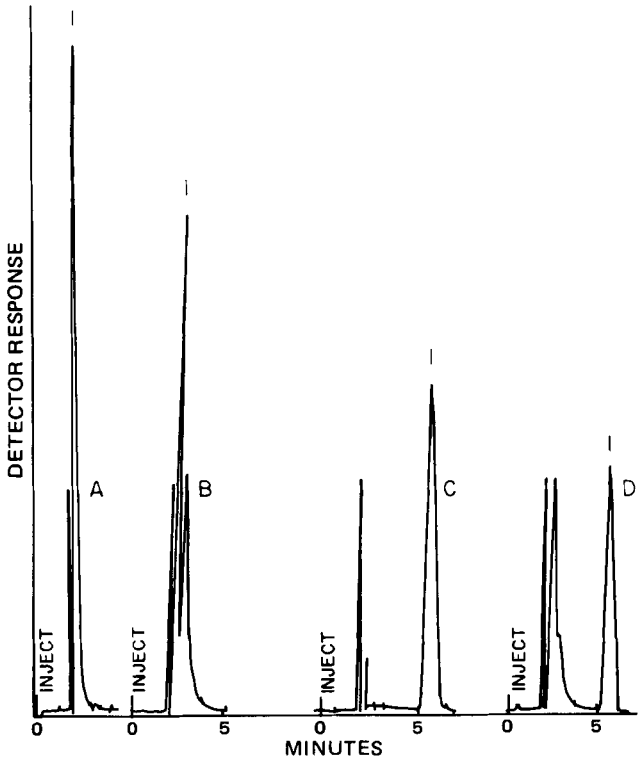

Figure 3—*Sample chromatograms of II (peak 1 is from II). Key: A, from a standard solution using mobile phase without VI; B, from a 1-hr decomposed sample using mobile phase without VI; and C and D, same as A and B, respectively, except that the mobile phase contained VI.*

Assay for All Catecholamines and Phenylephrine—The standard solution (10.0 μl) was injected using the described conditions and solvent containing VI. After the standard eluted out, 10.0 μl of the assay solution was injected. For comparison, this procedure was then repeated using solvent without VI. In another experiment, standard solutions of III and IV were injected together to determine if these two structurally related catecholamines would separate.

Calculations—Since preliminary investigations indicated that concentration (0.5–1.5 μg) was directly related to the peak area, the results were calculated by comparing the peak areas of the standard (A_s) and assay sample (A_a) as follows:

$$\frac{A_a}{A_s} \times 100 = \text{percent of claim} \qquad \text{(Eq. 1)}$$

The results are presented in Table I and Fig. 1. The sample chromato-

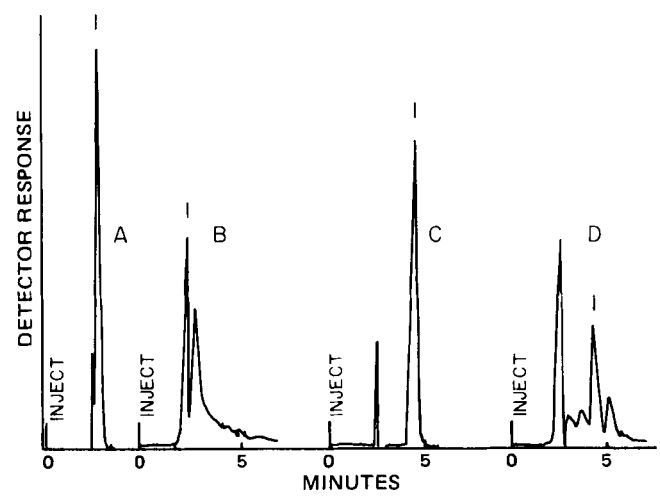

Figure 4—*Sample chromatograms of III (peak 1 is from III). Key: A, from a standard solution using mobile phase without VI; B, from a 1-hr decomposed sample using mobile phase without VI; and C and D, same as A and B, respectively, except that the mobile phase contained VI.*

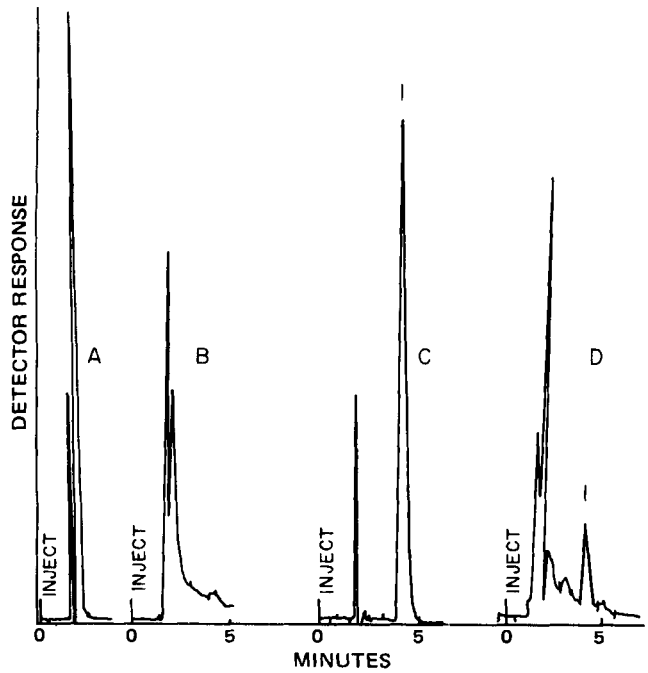

Figure 5—*Sample chromatograms of IV (peak 1 is from IV). Key: A, from a standard solution using mobile phase without VI; B, from a 2-hr decomposed sample using mobile phase without VI; and C and D, same as A and B, respectively, except that the mobile phase contained VI.*

grams (with and without VI in the mobile phase) are presented in Figs. 2–7. The standard deviations on all catecholamines and phenylephrine based on five areas from the identical volumes of the standard solutions are presented in Table I.

RESULTS AND DISCUSSION

Paired ion HPLC was very useful for separating intact catecholamines from some of their decomposition product(s) (Figs. 2–5). However, under identical conditions without VI in the mobile phase, there were no such separations (Figs. 2–5). All catecholamines allowed to react overnight with sodium hydroxide decomposed almost completely except V. The phenylephrine hydrochloride solution did not decompose much (less than 3%) even on reaction with 1 *N* NaOH (Fig. 6) *versus* all catecholamines where the concentrations of sodium hydroxide were lower (0.1 *N* except that for II it was 0.01 *N*).

Decomposition for all catecholamines in the presence of sodium hydroxide appeared to be pseudo first order (Fig. 1). The *K* values at room temperature were estimated to be 0.0197, 0.00506, 0.0301, and 0.00755 min^{-1} for I, II, III, and IV, respectively. Since the kinetic analysis was done for comparison purposes, only readings up to 60 min (40 min for levodopa because of faster decomposition) were treated kinetically. The strength of the sodium hydroxide solution used for isoproterenol was 0.01 *N versus* 0.1 *N* for all other catecholamines. Moreover, the solution of epinephrine (see *Stock Solutions*) contained preservative, which may explain the wider deviations from linearity in Fig. 1. It was not possible to determine the *K* value for V since it did not decompose more than 3% when left overnight in the presence of 1 *N* NaOH or on boiling for 2 hr with 0.1 *N* NaOH.

The results on 10 and 60 min and overnight decomposed samples of I were compared with the results obtained by a cation-exchange[8] method (2). The results were similar, and no new peak was recorded. The other catecholamines did not separate from their decomposition products using the cation-exchange method (2). An overnight decomposed sample of V gave a 97.6% result by the Koshy and Mitchner method (9) *versus* 97.4% by the developed method. The result on an overnight decomposed sample of III was almost 0% by the developed method *versus* 174.5% by the USP method (7) based on UV absorption. On methyldopa, the results on an overnight decomposed sample were 56.4% by the USP colorimetric method (8) and 0% by the developed method.

[8] Zipax SCX (100 cm × 2.1 mm i.d.), DuPont Instruments, Wilmington, Del.

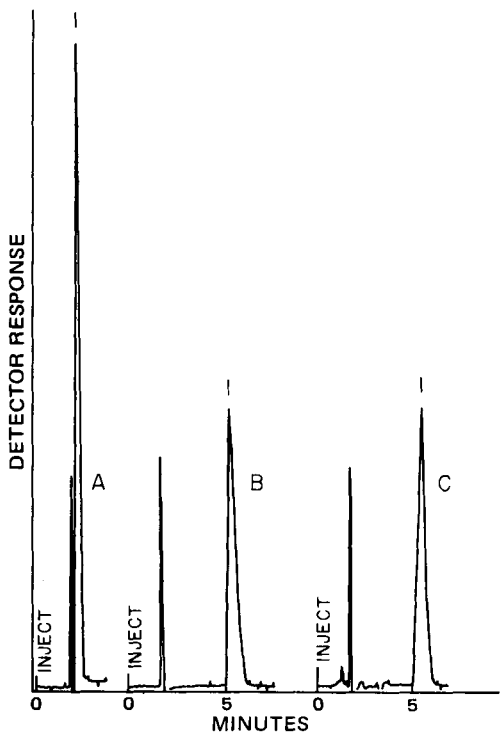

Figure 6—*Sample chromatograms of V (peak 1 is from V). Key: A, from a standard solution using mobile phase without VI; B, from a standard solution using mobile phase with VI; and C, from an overnight decomposed (with 0.1 N NaOH) sample using mobile phase with VI.*

Another advantage of paired ion HPLC appears to be its ability to separate catecholamines from other active ingredient(s). In this study, methyldopa was successfully separated from hydrochlorothiazide (Fig. 7B). The separation without the presence of VI was not as sharp (Fig. 7A) as with VI in the mobile phase (Fig. 7B). Furthermore, only with paired ion HPLC could methyldopa be separated from a structurally related catecholamine, levodopa (Fig. 7D). Without VI, no such separation was possible (Fig. 7C).

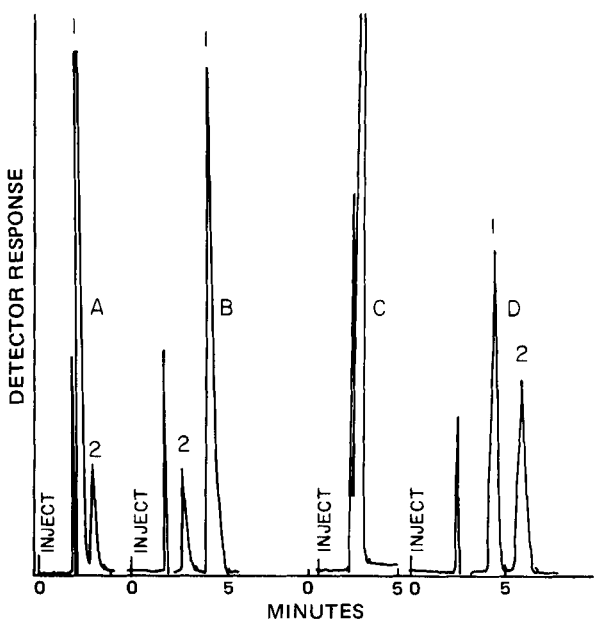

Figure 7—*Sample chromatograms of separation of IV from hydrochlorothiazide and levodopa. Key: A, separation of IV from hydrochlorothiazide using mobile phase without VI (peaks 1 and 2 are from IV and hydrochlorothiazide, respectively); B, same as A except that the mobile phase contained VI; C, separation of IV from III using mobile phase without VI; and D, same as C except that mobile phase contained VI (peaks 1 and 2 are from III and IV, respectively).*

In addition, the new method requires only one solvent–column system for all catecholamines studied and phenylephrine.

The developed method was used for the quantitative determinations of II–V in dosage forms, and the results were in agreement with the results obtained using literature methods (Table I). The presence of methylparaben and propylparaben in the commercial dosage (phenylephrine hydrochloride nose drops) did not interfere (Table I). In all catecholamines studied and phenylephrine, the areas of the peaks were directly related to concentration (range 0.5–1.5 μg). The sensitivity of the method can be further improved by reducing the absorbance unit for full-scale deflection. The standard deviations based on five injections of the standard solution were estimated to be 0.53, 0.84, 0.76, 0.83, and 0.98% for I, II, III, IV, and V, respectively.

REFERENCES

(1) "Paired Ion Chromatography, An Alternative to Ion Exchange," Brochure D61, Waters Associates, Milford, Mass., 1975, p. 3.

(2) C. Fu and M. J. Sibley, *J. Pharm. Sci.*, **66,** 425 (1977).

(3) J. R. Watson, *ibid.*, **63,** 96 (1974).

(4) J. R. Watson and C. R. Lawrence, *J. Chromatogr.*, **103,** 63 (1975).

(5) J. R. Watson and C. R. Lawrence, *J. Pharm. Sci.*, **66,** 560 (1977).

(6) K. K. Kaistha, *ibid.*, **59,** 241 (1970).

(7) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, p. 281.

(8) *Ibid.*, p. 320.

(9) K. T. Koshy and H. Mitchner, *J. Pharm. Sci.*, **52,** 802 (1963).

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High-Performance Liquid Chromatographic Assay of Sulfapyridine and Acetylsulfapyridine in Biological Fluids

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Received October 28, 1977, from the *Clinical Pharmacokinetics Laboratory, Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Amherst, NY 14260.* Accepted for publication December 28, 1977. *Present address: Clinical Pharmacokinetics Laboratory, Millard Fillmore Hospital, Buffalo, NY 14209.

Abstract □ A high-pressure liquid chromatographic method for the sensitive, rapid, and specific determination of sulfapyridine and its *N*-acetyl derivative in plasma and saliva was developed. A cyano-bonded, reversed-phase, high efficiency column was used. The system detected these sulfonamides in serum to 0.25 mg/liter and within only 6 min. Sulfapyridine was separated from its acetyl derivative with little interference from other drugs. The assay reproducibility was within 3%. The assay was highly useful for routine monitoring of patients receiving sulfasalazine for inflammatory bowel disease.

Keyphrases □ Sulfapyridine and acetyl metabolite—high-performance liquid chromatographic analyses in biological fluids □ High-performance liquid chromatography—analyses, sulfapyridine and acetyl metabolite in biological fluids □ Dermatitis suppressants—sulfapyridine and acetyl metabolite, high-performance liquid chromatographic analyses in biological fluids

The measurement of sulfasalazine[1] metabolites in serum is useful for monitoring patients with inflammatory bowel disease (1–4). As a combination drug, sulfasalazine (I) is composed of sulfapyridine (II) azo linked to 5-aminosalicylic acid (IV). The azo linkage is cleaved by bacteria in the colon, releasing II and IV. Compound II metabolizes to acetylsulfapyridine (III), sulfapyridine *O*-glucuronide (VI), and acetylsulfapyridine *O*-glucuronide (VII).

[1] Azulfidine, Pharmacia; SAS-500, Rowell Laboratories; and Rorasul, Rorer.

BACKGROUND

Das and coworkers (1–3) established the relationship among the sulfasalazine metabolites (II, III, VI, and VII) in serum, acetylation phenotype, and sulfasalazine therapeutic efficacy and side effects. Total serum concentrations of II, III, VI, and VII between 20 and 50 mg/liter were associated with disease remission (1). Side effects were more likely to occur in slow acetylators when total sulfapyridine metabolite concentrations were greater than 50 mg/liter (2). Among the several metabolites, only II and III are present in sufficient concentrations in serum (3) and saliva (5) to be important in acetylator phenotyping and to be clinically useful as a guide to dosage adjustment.

Compound I and its metabolites were identified previously by TLC and by colorimetric methods. Schroder and Campbell (6) used TLC to help establish the metabolic pathway of sulfasalazine in humans. Subsequent work by Das and coworkers (1, 2, 4) made use of a modified Bratton–Marshall colorimetric assay (7) that measures only free II. Therefore, all metabolites of II must be hydrolyzed with acid or with enzymes to release free II for quantitation. These hydrolyses require temperature control. It is then necessary to diazotize the free II to produce the color for colorimetric analyses. Two of the several reagent solutions must be made fresh weekly because of their instability. Although the Bratton–Marshall method is reliable for investigative study, the procedural complexity limits its utility for routine clinical monitoring of patient samples on a daily basis and, of course, it is relatively nonspecific.

Current work to identify and quantitate sulfonamides utilizes high-pressure liquid chromatography (HPLC). HPLC was used in measuring I in tablets and powders (8). Sharma *et al.* (9) measured various sulfas and acetyl metabolites in animal urine by HPLC. Cobb and Hill (10) determined the relative retention times of various sulfonamides eluted from an HPLC column. The measurement of II and III from the serum

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