(6) J. B. Vaughn, *J. Pharm. Sci.*, 58, 469(1969).
(7) A. W. Archer and E. A. Haugar, *J. Pharm. Pharmacol.*, 12, 751(1960).

ACKNOWLEDGMENTS AND ADDRESSES

Received November 6, 1974, from the *Department of Pharmaceutics, Brooklyn College of Pharmacy, Long Island University, Brooklyn, NY 11216*

Accepted for publication February 4, 1975.

Presented in part at the APhA Academy of Pharmaceutical Sciences national meeting, New Orleans, November 1974.

* To whom inquiries should be directed.

High-Speed Liquid Chromatographic Analysis of Sulfasalazine (Salicylazosulfapyridine)

LYLE D. BIGHLEY *x and J. PATRICK McDONNELL ‡

Abstract □ A high-speed liquid chromatographic method for analysis of sulfasalazine (salicylazosulfapyridine) in bulk powder and tablet dosage form is presented. Analysis is accomplished with a reverse-phase partition column and 10% 2-propanol in pH 7.7 phosphate buffer as the mobile phase. The method of analysis utilizes a simple, one-step, solubilization procedure with dimethylformamide, addition of an internal standard, and chromatography. The method is specific for sulfasalazine in the presence of starting materials, degradation products, or by-products from its manufacture.

Keyphrases □ Sulfasalazine—high-speed liquid chromatographic analysis, bulk powder and tablet dosage form □ Salicylazosulfapyridine—high-speed liquid chromatographic analysis, bulk powder and tablet dosage form □ High-speed liquid chromatography—analysis, sulfasalazine bulk and tablet forms

Several analytical methods have been reported for the determination of sulfasalazine {salicylazosulfapyridine, 5-[*p*-(2-pyridylsulfamoyl)phenylazo]salicylic acid} (I). These methods include spectrophotometry (1), titration with titanium trichloride (1), polarography (2), and nonaqueous potentiometric titration (2). However, most of these methods fail to determine this compound selectively in the presence of its by-products of synthesis or degradation products. Conversely, a chromatographic method would be expected to provide simple, rapid, and specific separation and quantitation of this compound in the presence of its impurities.

Although GC has not been investigated, its use for the underivatized I probably would be unsuccessful since this compound was thermally labile when subjected to differential thermal analysis[1]. TLC, although specific, has the disadvantage of potentially long analysis times and requires a number of manipulative steps which may adversely affect precision. For example, a relative standard deviation of 2–6% resulted just from spotting and chromatography (3). (Quantitation in these experiments was effected directly on the plate with a densitometer.) Consequently, even larger errors would be expected in the overall assay if the compound is removed from the TLC plate and eluted from the adsorbent prior to quantitation.

Table I—Column Packing Materials

Column	Packing	Column Dimensions
a	Neutral alumina[a], 100–200 mesh	1 m × 0.3 cm o.d.
b	Ether stationary phase chemically bonded on a controlled porous surface[b]	1 m × 0.3 cm o.d.
c	Strong anion-exchange resin coated on a controlled porous surface[c]	0.6 m × 0.3 cm o.d.
d	Spherical siliceous particles with a controlled porous surface[d]	0.6 m × 0.3 cm o.d.
e	Diphenyldichlorosilane stationary phase chemically bonded on a controlled porous surface[e]	0.6 m × 0.3 cm o.d.
f	Octadecyltrichlorosilane stationary phase chemically bonded on a controlled porous surface[f]	1.2 m × 0.3 cm o.d.

[a] AG-7, Bio Rad. [b] Permaphase ETH, duPont. [c] Zipax SAX, duPont. [d] Corasil II, Waters Associates. [e] Corasil/phenyl, Waters Associates. [f] Corasil/C_{18}, Waters Associates.

The present study was undertaken because high-speed liquid chromatography (HSLC) appears to offer several advantages (4) for the analysis of I that other methods do not possess. For example, it can be used for thermally labile compounds, the precision is good, and the method is specific and rapid. This technique has been successfully utilized to assay I in tablet dosage forms as well as the bulk chemical.

EXPERIMENTAL

Apparatus—A liquid chromatograph[2] equipped with a UV photometric detector (254-nm radiation using a low pressure mercury source) was used. The UV detector can operate at a sensitivity of 0.02 absorbance unit full scale (aufs).

Columns—The packing materials shown in Table I were used in precision-bore stainless steel columns. With the exception of Columns d, e, and f, which were purchased commercially, the columns

[1] Unpublished data.

[2] Model ALC 202, Waters Associates.

Table II—Chromatographic Behavior of Sulfasalazine

Column Packing[a]	Mobile Phase	Adjusted Retention[b] Time, min	Flow Rate, ml/min
a	Heptane	>15.0[c]	1.0
b	5% CH_3CN in water	0.6	2.0
b	7% CH_3CN in water	0.6	2.0
c	pH 9.2 borate buffer + 0.1 *M* $NaNO_3$	>28.4[c]	1.0
c	pH 7.7 phosphate buffer	>24.1[c]	1.5
c	pH 4.1 citrate buffer + 0.1 *M* $NaNO_3$	>15.3[c]	1.0
d	$CHCl_3$	>16.3[c]	1.0
d	$CHCl_3$–CH_3CN–*n*-butanol (60:15:15)	>10.3[c]	1.0
e	10% 2-propanol in pH 7.7 phosphate buffer	2.8	1.0
e	5% CH_3CN in water	0.0	1.0
e	19% 2-propanol in pH 7.7 phosphate buffer	0.0	1.0
e	15% 2-propanol in pH 7.7 phosphate buffer	1.3	1.0
f	5% CH_3CN in water	24.1	2.0
f	10% CH_3CN in water	3.4	1.5
f	5% 2-propanol in pH 7.7 phosphate buffer	31.9	1.5
f	15% 2-propanol in pH 7.7 phosphate buffer	0.6	1.5
f	10% 2-propanol in pH 6.5 phosphate buffer	3.1	1.5
f	10% 2-propanol in water	0.0	1.5
f	10% CH_3OH in pH 7.7 phosphate buffer	>16.3[c]	1.5
f	5% CH_3OH in water + 0.1 *M* ammonium carbonate	>15.6[c]	1.0
f	10% 2-propanol in pH 7.7 phosphate buffer	3.2	1.5
f	5% CH_3OH in water	>23.4[c]	1.0

[a] See Table I. [b] Times measured from the leading edge of solvent peak to the peak maximum. [c] Retention times were greater than these values, but the exact times were not determined.

were dry packed using the tap fill method of Kirkland (5). All columns were operated at ambient room temperature.

Mobile Phases—The mobile phases employed are listed in Table II. The phosphate and citrate buffers were prepared by the addition of 2.5 *N* sodium hydroxide to 0.01 *M* monobasic sodium phosphate and citric acid (monohydrate), respectively. The pH 9.2 borate buffer (0.01 *M*) was prepared by dissolving sodium tetraborate decahydrate in distilled water.

Materials—The following were used: sulfasalazine[3], salicylic acid[3], sulfapyridine[3], propylparaben[4], monobasic sodium phosphate[5], heptane[5], *n*-butyl alcohol[5], methanol[6], acetonitrile[6], sodium nitrate[6], sodium tetraborate decahydrate[6], ammonium carbonate[6], 2-propanol[6], sulfanilic acid[7], dimethylformamide[7], chloroform[7], 2-aminopyridine[8], citric acid USP (monohydrate)[9], and commercially available I tablets[10,11].

Analysis of Bulk Chemical—*Internal Standard Solution*—Weigh accurately approximately 125 mg of propylparaben and quantitatively transfer to a 25-ml volumetric flask with the aid of dimethylformamide. Dilute to volume with dimethylformamide and mix well.

Sample Solution—Accurately weigh approximately 50 mg of I and quantitatively transfer to a 50-ml volumetric flask with the aid of dimethylformamide. Add 10 ml of the internal standard solution, dilute to volume with dimethylformamide, and mix well.

Standard Solution—Prepare concurrently a standard solution, containing an amount of internal standard identical to the sample solution, and a reference lot of I in approximately the same quantity as the sample solution.

[3] Salsbury Laboratories.
[4] White Label, Eastman.
[5] Analytical reagent, Mallinckrodt.
[6] Certified ACS, Fisher.
[7] C. P., J. T. Baker.
[8] Reilly Tar and Chemical.
[9] Mallinckrodt.
[10] Rowell Laboratories.
[11] Pharmacia Laboratories.

Table III—Individual Retention Times for Several Possible Impurities of Sulfasalazine

Compound	Adjusted Retention Time[a], min
Impurity 1[b]	0.0
Impurity 2[b]	0.0
2-Aminopyridine	1.8
Sulfapyridine	0.0
Impurity 3[b]	8.9
Sulfanilic acid	0.0
Impurity 4[b]	4.7
Salicylic acid	0.0

[a] Time measured from the leading edge of solvent peak to the peak maximum. [b] Isolated but unidentified compounds.

Liquid Chromatography—Inject alternately 5 μl of the sample and standard solutions with a 10-μl syringe[12], using the stop-flow technique. Operational parameters for the liquid chromatographic separations are listed with the appropriate figures.

Quantitation—Quantitation was accomplished either by measurement of peak heights or of peak areas with a planimeter. The

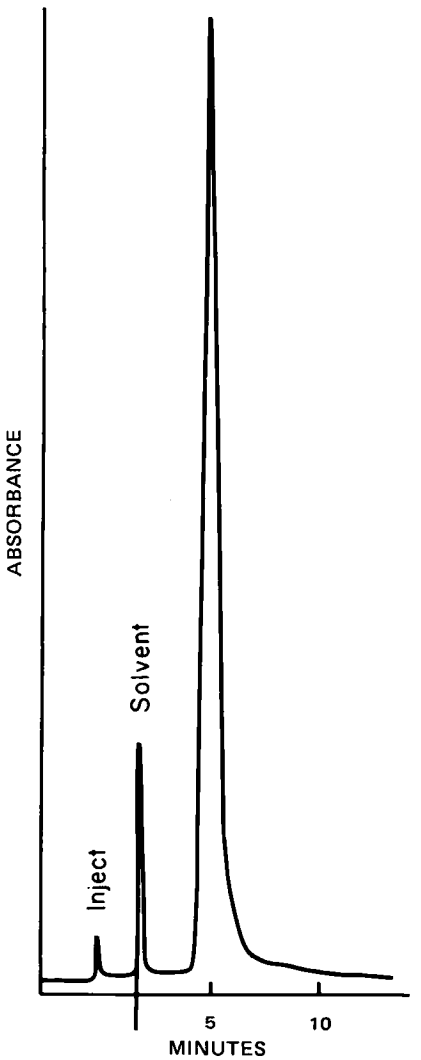

Figure 1—*Typical chromatogram of sulfasalazine. Chromatography conditions were: Column f; solvent, 10% 2-propanol in pH 7.7 phosphate buffer; chart speed, 30.48 cm (12 in.)/hr; flow, 1.5 ml/min; detector sensitivity, 0.16 aufs; and sample size, 5 μl.*

[12] Hamilton 701 NWG.

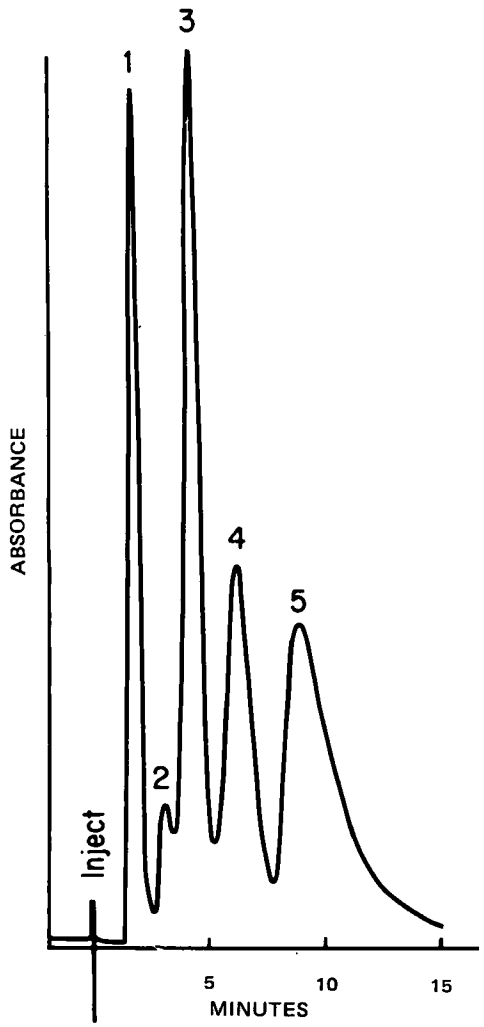

Figure 2—*Separation of a synthetic mixture containing sulfasalazine and some possible impurities. Key: peak 1, impurities 1 and 2, sulfapyridine, sulfanilic acid, and salicylic acid; peak 2, 2-aminopyridine; peak 3, sulfasalazine; peak 4, impurity 4; and peak 5, impurity 3. Chromatography conditions were the same as in Fig. 1.*

percentage of I in the sample was determined by:

$$\% \text{ I in sample} = \frac{(P_1W_2)/(P_2W_1)}{(P_3W_4)/(P_4W_3)} \times 100 \qquad \text{(Eq. 1)}$$

where P = peak height or peak area, W = weight, subscript 1 = I in sample, subscript 2 = internal standard in sample, subscript 3 = I in standard, and subscript 4 = internal standard in standard.

Since the weight of the internal standard is identical in the sample and standard solutions, this term can be deleted from Eq. 1. In addition, this equation can be simplified by using the ratio of the peak area (or peak height) of I/peak area (or peak height) of propylparaben for both standard and sample, $R_{sample} = P_1/P_2$ and $R_{std} = P_3/P_4$. Then it can be shown that:

$$\% \text{ I in sample} = \frac{R_{sample}W_3}{R_{std}W_1} \times 100 \qquad \text{(Eq. 2)}$$

Analysis of Tablets—Place one tablet (label claim 500 mg of I) in a beaker, add approximately 20 ml of dimethylformamide, and crush the tablet with a stirring rod. Quantitatively transfer the contents of the beaker to a 100-ml volumetric flask and rinse the beaker with three additional portions of dimethylformamide. Dilute to volume with dimethylformamide and mix well.

Pipet 10 ml of this solution into a 50-ml volumetric flask, add 10 ml of the internal standard solution previously described, and dilute to volume with dimethylformamide. Alternate 5-μl injections of the standard solution with the tablet formulation solution. The standard solution is prepared as described under *Analysis of Bulk Chemical.*

Quantitation is done as described under *Analysis of Bulk Chemical.* Since the weight of internal standard is identical for the tablet formulation and the standard solution, the equation is:

$$\text{I in tablet} = \frac{R_{sample}W_3D}{R_{std}} \qquad \text{(Eq. 3)}$$

where D = dilution factor, *i.e.*, 10 with this dilution; subscript 1 = I in tablet; subscript 2 = internal standard in tablet solution; subscript 3 = I in standard; subscript 4 = internal standard in standard; $R_{sample} = P_1/P_2$; $R_{std} = P_3/P_4$; and the other symbols have the same meaning as before.

RESULTS AND DISCUSSION

Initially, the chromatographic behavior of I was studied as a function of several column packing and mobile phase combinations; the adjusted retention times relative to the dimethylformamide peak for these systems are presented in Table II. Studies were not continued with those packing and mobile phase combinations giving long retention times. With the other systems, it became evident that an adjusted retention time for I of approximate-

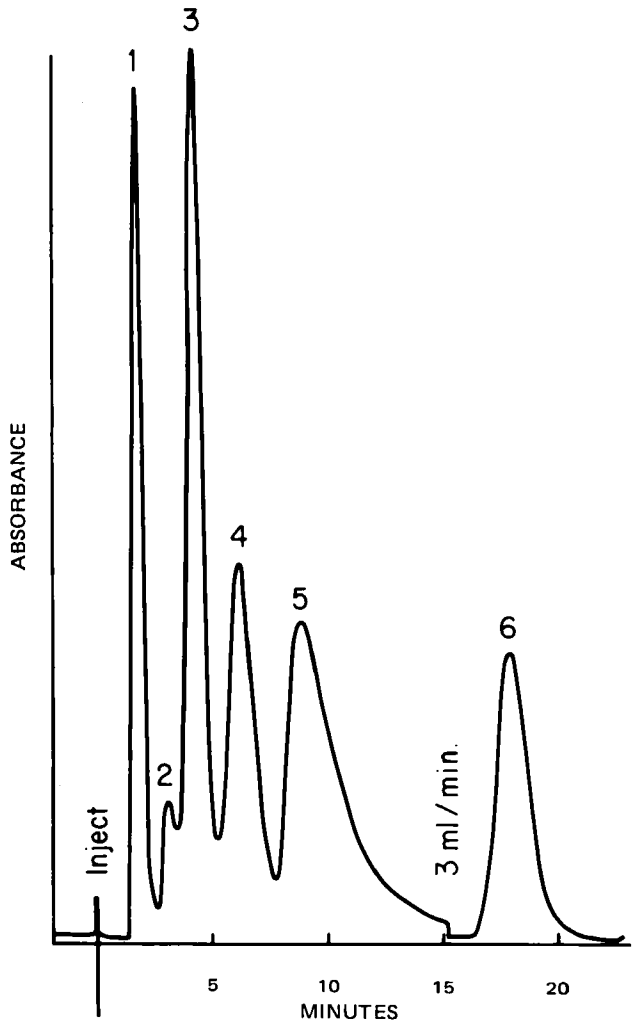

Figure 3—*Separation of a synthetic mixture containing sulfasalazine, some possible impurities, and an internal standard. Key: peak 1, impurities 1 and 2, sulfapyridine, sulfanilic acid, and salicylic acid; peak 2, 2-aminopyridine; peak 3, sulfasalazine; peak 4, impurity 4; peak 5, impurity 3; and peak 6, propylparaben. Chromatography conditions were the same as in Fig. 1, except the flow rate was increased to 3 ml/min after 15 min.*

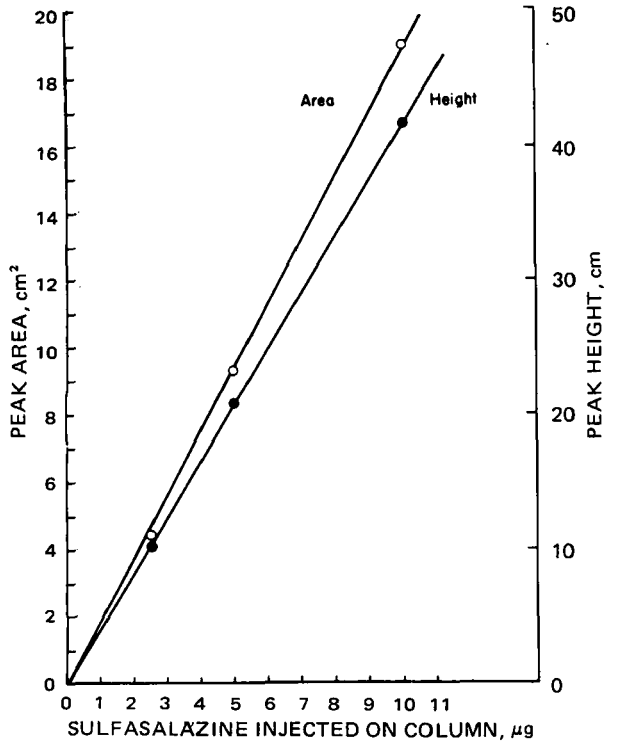

Figure 4—*Linearity of detector response to sulfasalazine.*

ly 3 min was necessary, since certain known impurities which may be present in I elute at, or immediately after, the solvent front. Consequently, only four column packing and mobile phase combinations met this requirement: Column e or f with 10% 2-propanol in pH 7.7 phosphate buffer, and Column f with either 10% acetonitrile in water or 10% 2-propanol in pH 6.5 phosphate buffer (Table I).

Of these four systems, only Column f with 10% 2-propanol in pH 7.7 phosphate buffer was satisfactory. When this system was used with the conditions shown in Fig. 1, I has an adjusted retention time of approximately 3.2 min. The other three systems were undesirable for various reasons. For example, Column f with 10% acetonitrile in water was unacceptable because certain by-products from synthesis had the same retention time as I or were not eluted. When 10% 2-propanol in pH 6.5 phosphate buffer was used with this packing, the peak width broadened slightly. Similarly, Column e in combination with 10% 2-propanol in pH 7.7 phosphate buffer resulted in a broadening of the I peak.

Further studies were undertaken with Column f and 10% 2-propanol in pH 7.7 phosphate buffer to determine if this system would resolve I from various structurally related compounds which could be present as impurities under normal circumstances. Adjusted individual retention times for several possible degradation products, starting materials, and by-products of synthesis were found to be sufficiently different from the retention time of I (Table III). Consequently, a synthetic mixture was prepared by dissolving approximately equal amounts of I and these compounds

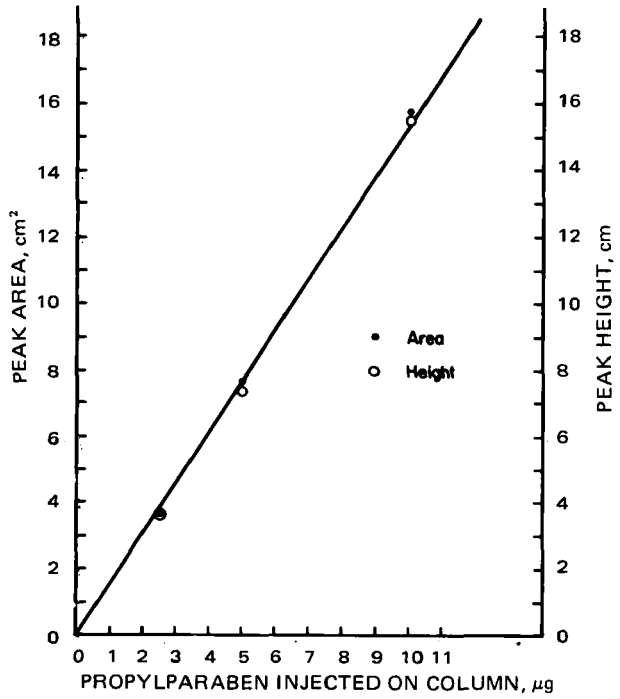

Figure 5—*Linearity of detector response to propylparaben.*

Table IV—Precision Study for Sulfasalazine Using Peak Height Measurements

Sample	Individual Assays, %			Average of Duplicate Assays, %
1	A	93.3		
	B	94.9		
	Average			94.1
2	A	99.1		
	B	97.4		
	Average			98.3
3	A	92.8		
	B	98.7		
	Average			95.8
4	A	95.1		
	B	95.9		
	Average			95.5
5	A	97.6		
	B	96.5		
	Average			97.0
6	A	92.1		
	B	92.5		
	Average			92.3
Mean individual assay, %		95.49	Mean duplicate assays, %	95.49
SD, %		2.44	*SD*, %	2.11
RSD, %		2.55	*RSD*, %	2.21

Table V—Precision Study for Sulfasalazine Using Peak Area Measurements

Sample	Individual Assays, %			Average of Duplicate Assays, %
1	A	93.2		
	B	94.7		
	Average			94.0
2	A	95.6		
	B	89.3[a]		
	Average			92.5
3	A	93.9		
	B	92.7		
	Average			93.3
4	A	95.1		
	B	97.1		
	Average			96.1
5	A	96.6		
	B	93.9		
	Average			95.3
6	A	96.8		
	B	97.8		
	Average			97.3
Mean individual assay, %		94.7	Mean duplicate assays, %	94.8
SD, %		2.36	*SD*, %	1.81
RSD, %		2.49	*RSD*, %	1.91

[a]The mean individual assay, standard deviation, and relative standard deviation calculated with Sample 2B removed (Z score = −2.29, $p < 0.02$) are 95.2, 1.70, and 1.79%, respectively.

Table VI—Analysis of Commercial Tablet Formulations of Sulfasalazine[a]

Using Peak Areas	Found, mg
Product A	498.5
Product B	496

[a] Label claim: 500 mg of sulfasalazine.

in dimethylformamide. A chromatogram of this mixture is shown in Fig. 2. Although baseline separation was not achieved, separation of I was adequate to permit quantitation even in the presence of these large amounts of impurities.

The specificity of this HSLC system was further confirmed by repeatedly injecting a sample of approximately 85% pure I and collecting the effluent when the peak with an adjusted retention time of 3.2 min appeared. When this effluent was subjected to TLC, using the solvent system of Kiger and Kiger (6), only I was found in the trapped samples. Plates that were sprayed with a solution containing 4-dimethylaminocinnamaldehyde and titanium trichloride (7) also only showed one spot.

Quantitative analysis of I samples was accomplished by the internal standard technique, using propylparaben as the internal standard. This compound was selected because it could be separated from the impurities shown in Fig. 2. Since these impurities elute immediately before and after the I peak, the compound selected as the internal standard had to have a relatively long retention time; and propylparaben was the only compound tested that met this requirement. Figure 3 shows the synthetic mixture containing propylparaben. The flow rate was increased from 1.5 to 3 ml/min after 15 min to hasten the elution of the propylparaben. The change in flow rate at this time permits elution of the impurities without overlapping either the I or propylparaben.

The UV detector response to I and propylparaben is linear over the concentration ranges used in this study. Plots of absorbance peak heights or areas *versus* concentration are shown in Figs. 4 and 5.

The precision of the HSLC method was determined by comparing the results obtained on six subsamples of I taken from one lot of bulk chemical. Peak heights are shown in Table IV, and peak areas are shown in Table V. Values for each injection and the average of duplicate injections are shown for each analysis. The standard deviation and relative standard deviation were calculated for individual and duplicate injections and are consistent with values reported for the liquid chromatographic analysis of other compounds (8–11).

The precision of measurement of peak areas probably could have been further improved in this study if integration were done with either a mechanical or electronic integrator. However, since a planimeter was used, precision of area measurements was maximized by increasing the recorder chart speed so that the peak area could be determined more precisely.

It is evident from the precision studies that either peak height or area can be used for quantitative analysis. However, if peak heights are used, the time at which the flow rate is increased from 1.5 to 3 ml/min must be closely regulated to prevent band spreading, which results from increased retention time. Use of peak areas would not require such close attention with respect to the time at which the flow rate was changed because the area of the propylparaben should remain the same even if some band spreading does occur, provided resolution remains adequate for accurate quantitation. Since the propylparaben is well resolved from I and other possible impurities, slight band spreading would not be a problem.

Two commercially available tablet formulations were examined for their I content to determine the feasibility of this method for tablet dosage forms. Preliminary results (Table VI) indicate that this procedure is applicable. Tablet excipients did not interfere with the analysis and need not be separated from I prior to liquid chromatography.

Since dimethylformamide was used as the solvent for I in the assay procedure and since solutions were occasionally prepared several hours prior to injection, it was desirable to determine whether this solvent degrades I. Therefore, a sample of I was dissolved in dimethylformamide and subjected to thermal stress at 80° for 196 hr. No degradation was observed when this solution was analyzed by TLC and HSLC.

REFERENCES

(1) "Tests and Standards for New and Nonofficial Remedies," J. B. Lippincott, Philadelphia, Pa., 1953, p. 256.

(2) B. Nygard, J. Olofsson, and M. Sandberg, *Acta Pharm. Suecica*, **3**, 343(1966).

(3) V. M. Novacek, *Amer. Lab.*, **5**, 85(1973).

(4) A. F. Michaelis, D. W. Cornish, and R. Vivilecchia, *J. Pharm. Sci.*, **62**, 1399(1973).

(5) J. J. Kirkland, *J. Chromatogr. Sci.*, **10**, 129(1972).

(6) J. L. Kiger and J. G. Kiger, *Ann. Pharm. Fr.*, **24**, 593(1966).

(7) D. R. Powell and B. A. Burton, *J. Pharm. Sci.*, **63**, 1290(1974).

(8) J. A. Mollica and R. F. Strusz, *ibid.*, **61**, 444(1972).

(9) J. A. Mollica, G. R. Padmanabhan, and R. Strusz, *Anal. Chem.*, **45**, 1859(1973).

(10) R. L. Stevenson and C. A. Burtis, *J. Chromatogr.*, **61**, 253(1971).

(11) W. F. Beyer, *Anal. Chem.*, **44**, 1312(1972).

ACKNOWLEDGMENTS AND ADDRESSES

Received September 23, 1974, from the *College of Pharmacy, University of Iowa, Iowa City, IA 52242, and ‡Salsbury Laboratories, Charles City, IA 50616

Accepted for publication February 4, 1975.

Presented in part at the Pharmaceutical Analysis and Control Section, APhA Academy of Pharmaceutical Sciences, San Diego meeting, November 1973.

Supported by a grant from Salsbury Laboratories, Charles City, IA 50616

ˣ To whom inquiries should be directed.