Determination of Sodium *o*-Iodohippurate in Pharmaceutical Dosage Forms by High-Speed Liquid Chromatography

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Abstract □ A method is presented for the quantitative analysis of sodium *o*-iodohippurate by high-speed liquid chromatography. Following a preliminary extraction to remove benzyl alcohol, the samples are acidified and extracted with ethyl acetate. After evaporation of the solvent, the residue is dissolved in an aliquot of ethyl acetate containing an internal standard and assayed by comparison of peak heights after liquid chromatography. This method is independent of the amount of radioactively tagged material present, thus permitting analysis of the total sodium *o*-iodohippurate present.

Keyphrases □ Sodium *o*-iodohippurate injection—analysis, high-speed liquid chromatography □ High-speed liquid chromatography—analysis, sodium *o*-iodohippurate injection

Sodium *o*-iodohippurate, radioactively tagged with ^{131}I, is referred to in USP XVIII (1) as a diagnostic aid for renal function determinations. Recent interest in such determinations has been stimulated by its use for early postoperative evaluation of the status of transplant patients (2) and for the prediction of function in renal failure (3).

Previous analytical methods have relied on paper chromatography (1), or paper chromatography in combination with electrophoresis (4), followed by detection with autoradiography, β-ray scanning, or scintillation measurements. However, these methods of detection are limited to radioactive material and will not measure the total amount of sodium *o*-iodohippurate present. In addition, Høye (5) indicated that nonradioactive and ^{131}I-tagged sodium *o*-iodohippurate behave differently when chromatographed on paper.

An alternative approach was sought for the quantitation of sodium *o*-iodohippurate exclusive of its radioactivity. GLC and TLC techniques were tried, but difficulty was encountered in the preparation of stable volatile derivatives in the former and in recovery in the latter, which resulted in poor precision. Recently, high-speed liquid chromatography has been applied successfully to pharmaceutical analysis (6–8). This article describes the application of high-speed liquid chromatography to the estimation of sodium *o*-iodohippurate in sterile solution for injection.

EXPERIMENTAL

Apparatus—A high-pressure liquid chromatograph[1], equipped with a UV detector (254 nm) capable of operating at inlet pressures up to 1200 psig, was used.

Column—The column was purchased from the instrument manufacturer and was a 1-m × 2.1-mm i.d. × 0.6-cm o.d., precision bore stainless steel tube. The column packing was an adsorption material[2].

Reagents—Ethyl acetate, chloroform, hydrochloric acid, sodium hydroxide, acetic acid, dibasic sodium phosphate, and anhydrous sodium sulfate were reagent grade and required no further purification. Methyl 2,5-dihydroxybenzoate was prepared by methylation of 3,5-dihydroxybenzoic acid[3] in the usual manner (9). Sodium *o*-iodohippurate[4], *o*-iodobenzoic acid[5], and water for injection, bacteriostatic, with 1% benzyl alcohol[6], were used without further purification.

A simulated solution for injection[7] was prepared in this laboratory. It contained 2 mg/ml sodium *o*-iodohippurate and 0.5 mg/ml dibasic sodium phosphate in water for injection, bacteriostatic, with 1% benzyl alcohol at pH 7.5.

Acetic Acid in Chloroform (1% v/v)—Dilute 5.0 ml of acetic acid to 500 ml with chloroform.

Internal Standard Solution—Dissolve approximately 200 mg of methyl 3,5-dihydroxybenzoate in 200 ml of ethyl acetate to yield a final concentration of approximately 1 mg/ml.

Standard Reference Solution—Accurately weigh 30 mg of sodium *o*-iodohippurate into a screw-cap vial large enough to hold 20 ml. Pipet 10.0 ml of the internal standard solution into the vial, followed by approximately 0.5 ml of acetic acid, and shake to dissolve all solids.

Sample Preparation—Pipet 15.0 ml of the sample solution (*i.e.*, 30 mg of sodium *o*-iodohippurate) into a 125-ml separator

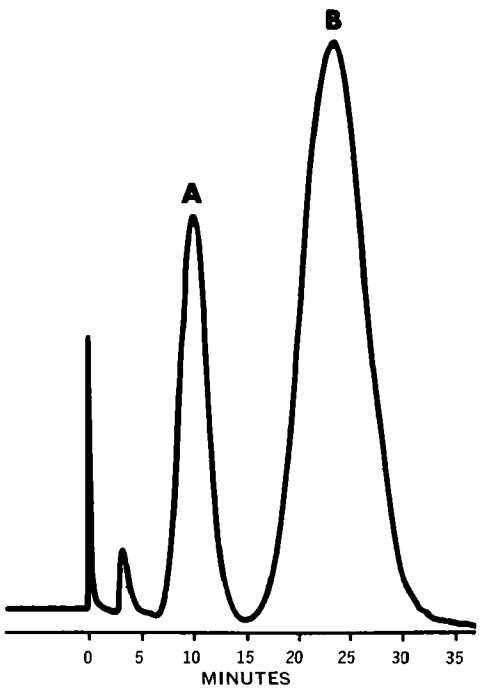

Figure 1—*High-speed liquid chromatogram of a sample extract with internal standard. Key: A, o-iodohippuric acid; and B, methyl 3,5-dihydroxybenzoate (internal standard). The two earlier peaks are artifacts caused by injection and a solvent peak, respectively.*

[1] DuPont model 820, duPont de Nemours and Co., Wilmington, Del.
[2] Sil-X, Nester/Faust Mfg. Corp., Newark, Del.
[3] Naftone, Inc., New York, N.Y.
[4] Mallinckrodt Chemical Works, St. Louis, Mo.
[5] Eastman Kodak Co., Rochester, N.Y.
[6] Abbott Laboratories, North Chicago, Ill.
[7] Hippuran, Abbott Laboratories, was prepared for this experiment with nonradioactive materials.

Table I—Standard Addition

Sodium *o*-Iodohippurate Added[a], mg	Sodium *o*-Iodohippurate Found, mg	Percent Recovered of Amount Added
0.00	19.62	
5.65	25.32	100.9
11.30	30.40	95.6
16.95	36.40	99.0

[a] Milligrams added to 10.0 ml of a solution for injection containing 2.0 mg/ml of sodium *o*-iodohippurate.

and extract twice with 50-ml portions of ethyl acetate, saving both layers. Combine the organic layers and extract with 15 ml of 0.001 *N* sodium hydroxide, discarding the organic phase containing the benzyl alcohol. Combine the remaining aqueous phases; adjust the pH of the solution, using a pH meter, to 2.0 with 1.2 *N* hydrochloric acid; and extract the aqueous phase three times with 50-ml portions of ethyl acetate, discarding the aqueous layer after the final extraction. Filter the combined ethyl acetate layers over approximately 40 g of anhydrous sodium sulfate, and rinse the filter with approximately 20 ml of ethyl acetate, being careful that no water remains in the organic phase after filtration. Evaporate the ethyl acetate to dryness in a current of air (no heat). Pipet 10.0 ml of the internal standard solution into the evaporating dish, followed by approximately 0.5 ml acetic acid, and swirl to dissolve all of the residue. The solution may now be chromatographed or transferred to a screw-cap vial for storage.

Chromatographic Procedure—The analytical column was already described. The mobile phase was 1% acetic acid in chloroform, the temperature was ambient, and the solvent flow was approximately 0.8 ml/min (at an inlet pressure of 1200 psig). The precision photometer detector (254 nm) was set at a sensitivity of 0.04 absorbance unit full-scale.

Samples and standards of 3 μl (approximately 9 μg of sodium *o*-iodohippurate) were injected with the flow stopped. A standard was injected for every four sample injections, and the peak heights of *o*-iodohippuric acid and the internal standard were measured.

Calculations—The response factor, *F*, is the ratio of the internal standard peak height to the *o*-iodohippuric acid peak height in the standard reference solution, multiplied by the weight of sodium *o*-iodohippurate in the standard. The concentration of sodium *o*-iodohippurate in milligrams per milliliter in the sample is then calculated:

$$\text{mg/ml sodium } o\text{-iodohippurate} = (F)\left(\frac{H_1}{H_2}\right)\left(\frac{1}{15}\right) \quad \text{(Eq. 1)}$$

where H_1 and H_2 are the peak heights of *o*-iodohippuric acid and the internal standard in the sample, respectively.

RESULTS AND DISCUSSION

Figure 1 shows the chromatogram of a typical sample extract containing the internal standard. If present, *o*-iodobenzoic acid, the major degradation product, elutes with the solvent and does not interfere with the analysis. Ethyl acetate was chosen as the solvent for extraction because of the success previously shown for the extraction of hippuric acid (10). In addition, the first extraction from a basic aqueous phase serves to remove benzyl alcohol which would interfere with the assay.

The linearity of response (Table I) has been shown for sample solutions containing 1.0–4.0 mg/ml of sodium *o*-iodohippurate.

The precision of the method (Table II) was determined from 10 replicate determinations of sodium *o*-iodohippurate in a solution for injection performed by three analysts over 4 days.

Table II—Precision and Accuracy

Trial	Sodium *o*-Iodohippurate Found[a], mg/ml	Percent Label Claim
1	1.97	98.0
2	1.94	96.4
3	1.98	98.5
4	2.00	99.6
5	1.96	97.7
6	1.97	98.0
7	1.96	97.7
8	1.99	99.2
9	2.02	100.6
10	2.05	102.0
	Mean	98.8%
	Relative standard deviation	1.6%

[a] Theory: 2.008 mg/ml sodium *o*-iodohippurate.

Peak height rather than peak area was chosen as the method of measurement because, as observed in this laboratory and as previously reported (6), peak height varies less with fluctuations in inlet pressure (flow rate) than does peak area.

CONCLUSION

This is a stability-indicating assay because the excipients are separated by the formation of *o*-iodohippuric acid and its subsequent extraction, and the major degradation product, *o*-iodobenzoic acid, is separated chromatographically.

The method, as written, is applicable to nonradioactive material. Therefore, it may be used to determine the concentration of sodium *o*-hippurate in solutions regardless of activity. However, for use with highly radioactive material, the sample preparation procedure may be scaled down accordingly to avoid exposure to radiation.

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