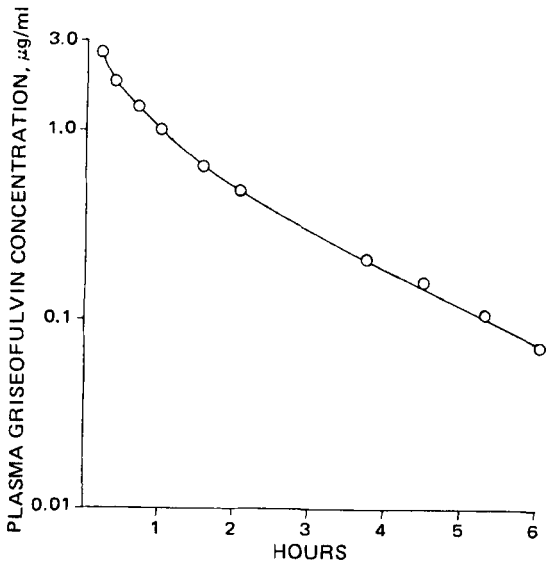


Figure 4—*Plasma griseofulvin concentration as a function of time in a 4-kg rabbit given 16 mg of griseofulvin intravenously over 1 min.*

jected into the liquid chromatograph, indicating that the peak was not due to another component or an impurity in the griseofulvin. It seems most likely that the peak was due to a metabolite of griseofulvin, although the structure of this compound is unknown. The time course of plasma griseofulvin concentrations in the rabbit is shown in Fig. 4.

The described method for quantitative determination of griseofulvin in plasma offers several advantages over the spectrofluorometric (2, 7, 8) and GLC (8) methods. The HPLC procedure is simple and rapid, with a turnaround time of 5–6 min required for each sample. The analysis uses only a small volume of plasma and is specific and reproducible; commonly used drugs do not interfere. The method is well suited for the clinical monitoring of plasma griseofulvin concentrations or for pharmacokinetic studies.

REFERENCES

(1) "The Pharmacological Basis of Therapeutics," L. S. Goodman and A. Gilman, Eds., Macmillan, New York, N.Y., 1975.

(2) M. Rowland, S. Riegelman, and W. L. Epstein, *J. Pharm. Sci.*, **57**, 984 (1968).

(3) W. L. Chiou and S. Riegelman, *ibid.*, **60**, 1376 (1971).

(4) S. Riegelman, M. Rowland, and W. L. Epstein, *J. Am. Med. Assoc.*, **213**, 426 (1970).

(5) D. Busfield, K. J. Child, R. M. Atkinson, and E. G. Tomich, *Lancet*, **2**, 1042 (1963).

(6) S. Riegelman and W. Sadee, "Clinical Pharmacokinetics: A Symposium," APhA Academy of Pharmaceutical Sciences, Washington, D.C., 1974, p. 169.

(7) V. P. Shah, S. Riegelman, and W. L. Epstein, *J. Pharm. Sci.*, **61**, 634 (1972).

(8) H. J. Schwarz, B. A. Waldman, and V. Madrid, *ibid.*, **65**, 370 (1976).

(9) W. L. Chiou, M. A. F. Gadalla, and G. W. Peng, *ibid.*, **67**, 182 (1978).

(10) J. W. Nelson, A. L. Cordry, C. G. Aron, and R. A. Bartell, *Clin. Chem.*, **23**, 124 (1977).

(11) I. Nilsson-Ehle, T. T. Yoshikawa, J. E. Edwards, M. C. Schotz, and L. B. Guze, *J. Infect. Dis.*, **135**, 414 (1977).

Stability of Furosemide in Aqueous Systems

A. G. GHANEKAR, V. DAS GUPTA ˣ, and CHARLES W. GIBBS, Jr. *

Received February 28, 1977, from the *College of Pharmacy, University of Houston, Houston, TX 77004.* Accepted for publication September 28, 1977. *Present address: Harris County Hospital District Pharmacy, Taub Loop, Houston, TX 77030.

Abstract □ A stability-indicating assay for furosemide based on high-pressure liquid chromatography was developed. The method is sensitive, accurate, and precise. The standard deviation based on six injections of the standard solution was ±1.37%. This method was used to study furosemide stability in various aqueous solutions and dosage forms. Stability tests were conducted at room temperature as well as at higher temperatures (45, 65, and 85°) at various pH values and with different vehicles. Some decomposition products were identified.

Keyphrases □ Furosemide—high-pressure liquid chromatographic analysis, stability in aqueous solutions and dosage forms □ High-pressure liquid chromatography—analysis, furosemide in aqueous solutions and dosage forms □ Stability—furosemide in aqueous solutions and dosage forms, high-pressure liquid chromatographic analysis □ Diuretics—furosemide, high-pressure liquid chromatographic analysis, stability in aqueous solutions and dosage forms

Furosemide is extensively used as a diuretic. Very little information is available concerning its stability, possibly because of the lack of a stability-indicating assay. The USP method (1) for furosemide in injections and tablets is based on UV absorption. A fluorometric method for furosemide in urine and serum also was reported (2), but any other fluorescent substances interfere with the assay. Furosemide was reported to be unstable in acidic media (3). The USP (1) indicates that the pH of the injection should be between 8.9 and 9.3.

The present investigations were undertaken to develop a stability-indicating assay for furosemide using high-pressure liquid chromatography (HPLC) and to study furosemide stability in aqueous systems.

EXPERIMENTAL

Chemicals and Reagents—All chemicals and reagents were ACS, NF, or USP quality and were used without further purification.

Preparation of Solutions and Dosage Forms—The solutions and dosage forms prepared are given in Table I.

Apparatus—A high-pressure liquid chromatograph[1] equipped with a UV detector (254 nm), a recorder[2], and an integrator[3] was used.

Column—A column[4] (30 cm × 4 mm i.d.) of a very nonpolar packing material, consisting of octadecyltrichlorosilane permanently bonded by silicon–carbon bonds, was used.

Chromatographic Conditions—The chromatographic mobile phase was 0.01 *M* $(NH_4)_2HPO_4$ in water containing 25% (v/v) methanol. The

[1] Model ALC 202, equipped with U6K universal chromatograph injector, Waters Associates, Milford, Mass.
[2] Omniscribe 5213-12, Houston Instruments, Austin, Tex.
[3] Autolab Minigrator, Spectra-Physics, Santa Clara, Calif.
[4] μBondapak C_{18}, Catalog No. 27324, Waters Associates, Milford, Mass.

Table I—Solutions and Dosage Forms Prepared

Solution	Vehicle	Furosemide Concentration, mg/ml	pH	Other Ingredients (If Any)
1	0.01 *N* NaOH	0.5[a]	—	—[b,c]
2	0.1 *N* NaOH	1.0	13	—[b,c]
3	0.1 *N* HCl	1.0	1.2	—[b,c]
4	0.04 *M* $(NH_4)_2HPO_4$	1.0	8	—[b,c]
5	Phosphate buffers (0.05 *M*)	0.5	See Table IV	—[b,c]
6	Water	0.5	4.2 (adjusted with acetic acid)	—[b,c]
7	Water	1.0[b]	See Table III	Various percentages of sugar
8	Commercial vehicle[c]	1.0 and 2.0	7.4[d] 7.5[d]	
9	50% (v/v) glycerin in water	1.0	5.6	Methylparaben, 0.005% Propylparaben, 0.002%
10	70% sorbitol in water	1.0	6.8	Methylparaben, 0.005% Propylparaben, 0.002%
11	50% sorbitol in water	1.0	8.5 (adjusted with 0.1 *N* NaOH)	Methylparaben, 0.005% Propylparaben, 0.002%
12	50% sorbitol in water with 20% (v/v) alcohol	1.0	8.5	Methylparaben, 0.005% Propylparaben, 0.002%
13	50% sorbitol in water with 10% (v/v) alcohol	1.0	8.5	Methylparaben, 0.005% Propylparaben, 0.002%
14	85% sugar in water	1.0[b]	8.2 (adjusted with 4% $NaHCO_3$)	—[b]

[a] For a standard solution, it was further diluted with water to a concentration of 50 μg/ml. [b] Prepared from furosemide injection. [c] Syrpalta syrup, Emerson Laboratories, Dallas, Tex. The syrup also contained dye(s), flavor(s), and sodium benzoate (as confirmed during these investigations). An 8.0-ml quantity of 4% $NaHCO_3$/50 ml of the syrup was added to raise the pH to keep furosemide in solution. [d] The pH values are of the recompounded samples; pH values of the original samples were not determined.

temperature was ambient, and the flow rate was 2.0 ml/min. The detector was set at a sensitivity of 0.16 absorbance unit for full-scale deflection, and the chart speed was 30.5 cm/hr.

Procedure for Furosemide—*In Solution and Liquid Dosage Forms*—An appropriate quantity of the solution or dosage form was diluted with water to a 50-μg/ml concentration, and 40 μl was injected. For purposes of comparison, an identical volume of the standard solution was injected after the assay solution was eluted.

In Tablets—One tablet was ground and mixed with 4 ml of methanol. Then enough 0.05 *M* sodium bicarbonate was added to bring the solution to 100.0 ml. The solution was filtered (if necessary), the clear filtrate was diluted with water to a concentration of 50 μg of furosemide/ml, and 40 μl was injected as already described.

Calculations—Since preliminary investigations indicated that the peak area of furosemide was directly related to the concentration, the results were calculated using the following equation:

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

where A_a is the peak area of the assay sample and A_s is the peak area of the standard sample. The results on commercial tablets and injection were 101.8 and 99.6% of the label claim, respectively. The standard deviation based on six injections of the standard solution was ±1.37%.

A sample chromatogram is presented in Fig. 1A.

The investigations indicated (Fig. 1B) that the developed method was stability indicating *versus* the USP method (1), which was not. For example, a 42-month-old sample in an 85% solution of sugar in water whose pH was adjusted to 8.2 with sodium bicarbonate gave 26.1% by the developed method (Fig. 2C) *versus* 82.1% by the USP method. Therefore, stability studies on the dosage forms and solutions were conducted using the developed method.

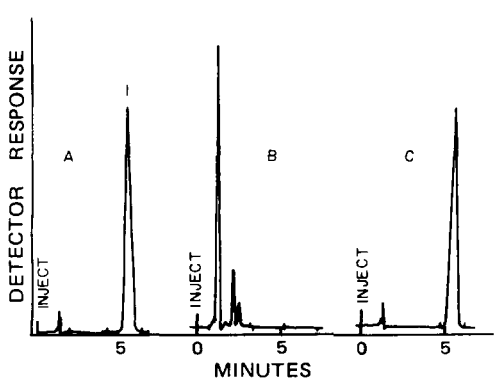


Figure 1—*Chromatograms of furosemide from solutions. Peak 1 is from furosemide. Key: A, from a standard solution; B, from a solution in 0.1 N HCl after 30 days of storage at 65°; and C, from a solution in 0.1 N NaOH after 30 days of storage at 65°.*

Stability Studies—*Solution in Commercial Vehicle Containing 2.0 mg of Furosemide/ml (Table I)*—This solution was divided into various portions and stored at 45, 65, and 85° as well as at room temperature. The portions were assayed frequently (Table II). A solution of furosemide in the commercial vehicle (1.0 mg/ml) when stored at room temperature showed more decomposition than the 2.0-mg/ml solution (Table II).

Solutions Containing Sugar—The solutions of furosemide (1.0 mg/ml) in water containing various percentages of sucrose were stored at 65° and assayed after 3 weeks (Table III).

Aqueous Solutions in Phosphate Buffers, Acetic Acid, Hydrochloric Acid, and Sodium Hydroxide—These solutions were stored at 65° for 30 days and assayed (Table III).

Dibasic Ammonium Phosphate—An aqueous solution in dibasic ammonium phosphate was stored for 24 hr at room temperature and

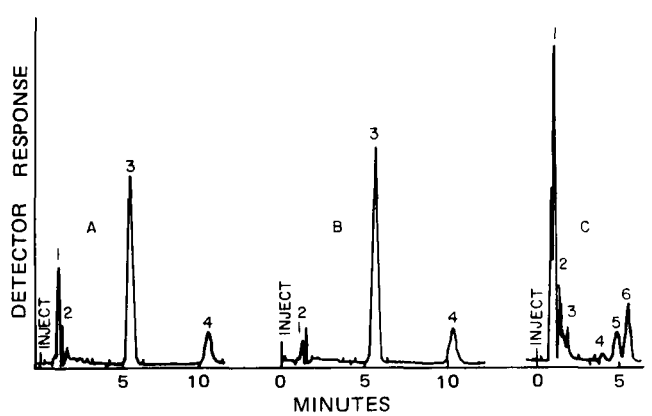


Figure 2—*Chromatograms of furosemide from dosage forms. A: in 50% aqueous solution of sorbitol after 3 weeks of storage at 65°. Peak 3 is from furosemide, peak 4 is from methylparaben, and peaks 1 and 2 are from the solvent and decomposition products of furosemide. B: same as A except that 20% (v/v) alcohol was substituted for 20% water. C: in simple syrup after 3.5 years of storage at room temperature. Peak 6 is from furosemide; peaks 1 and 2 are from sugar, solvent, levulinic acid, and 4-chloro-5-sulfamoylanthranilic acid; peaks 3 and 5 are similar to the peaks obtained from a solution of furfuryl alcohol in water after 3 weeks of storage at room temperature; and peak 4 is from furfuryl alcohol.*

Table II—Assay Results on Furosemide [a] (with 8.0 ml of 4% Sodium Bicarbonate) in Conmercial Vehicle at Various Temperatures

Storage Temperature (±1°)	Original Concentration, mg/ml	Percent of Furosemide Retained after 7 Days	14 Days	21 Days	28 Days	30 Days	42 Days	49 Days	56 Days	60 Days	87 Days	90 Days
45°	2	—	94.1[b]	—	92.9	—	91.2	—	—	—	84.9	—
65°	2	—	91.4[b]	—	91.7	—	—	87.1	84.7	—	70.9[b]	—
85°	2	92.5	87.5	81.9	68.9[b]	—	—	—	—	—	—	—
24°	2	—	—	—	—	93.8	—	—	—	88.0[c]	—	87.5[c]
24°	1	—	—	—	—	95.7	—	—	—	89.6	—	86.2

[a] Lasix injection was used to make these solutions. [b] These results may not be reliable due to minor mechanical problems in the integrator, but they do not affect the conclusions of this study. [c] These results may not be reliable due to the growth of fungus, but they do not affect the conclusions of this study.

Table III—Percentages of Furosemide Retained in Various Aqueous Solutions after 30 Days (21 Days for Sugar Solutions) of Storage at 65°

Solution Containing	Initial pH	Final pH	Percent Retained
10% sugar	8.5	4.9	82.3
20% sugar	8.1	4.3	60.5
30% sugar	8.2[b]	4.3	55.5
50% sugar	7.9	4.0	43.9
60% sugar	7.8	4.0	44.1
70% sugar	7.7	3.9	40.3
85%[b] sugar	7.6	3.9	40.3
Phosphate buffer	5.0	—	88.6
Phosphate buffer	4.8	—	76.8
Phosphate buffer	4.65	—	72.1
Phosphate buffer	4.55	—	67.1
Acetate	4.2	—	11.5
Hydrochloric acid	1.2	—	0.0
Sodium hydroxide	13	—	99.5

[a] Initial pH values were higher since the samples were made using furosemide injection whose pH was 8.9. [b] The pH of simple syrup was approximately 6.5.

stability. The pH is apparently indirectly responsible for furosemide decomposition in sugar solutions (Table III) since they changed considerably and were acidic (Table III). The pH changes in sorbitol solutions were not as wide as in sugar or glycerin (Tables III and IV), so sorbitol solutions were studied further. Alcohol appears to have a stabilizing effect (Table IV). Since more than 10% (v/v) alcohol is a reasonable upper limit in pediatric dosage forms, alcoholic concentrations were kept to a minimum. Only two concentrations (10 and 20% v/v) were studied.

The samples prepared in the commercial vehicle (2.0 mg/ml) that contained sodium benzoate as the preservative showed fungus growth after about 30 days. This growth was expected since sodium benzoate is not a good preservative in a basic medium. It is the undissociated benzoic acid that prevents fungus growth. No such problem was noticed with parabens as the preservatives (0.005% methylparaben and 0.002% propylparaben). These preservatives did not interfere with the assay (Figs. 2A and 2B).

The decomposition of furosemide (I) appears to follow Scheme I as reported by Kovar *et al.* (3). The presence of decomposition products in a 3.5-year-old sample (Fig. 2C) was confirmed by injecting solutions of furfuryl alcohol (II) (0.1 ml/250 ml of water), levulinic acid (III) (1 ml/250 ml of water), and 4-chloro-5-sulfamoylanthranilic acid (IV) (20 mg/100 ml of methanol). The solution of furfuryl alcohol in water decomposed quite fast, even at room temperature. The decomposition of furfuryl alcohol appears to be complex (Fig. 2C) and not a simple decomposition to levulinic acid as reported by Kovar *et al.* (3).

Table IV—Percentages of Furosemide Retained when Solution Contained Either Glycerin, Sorbitol, or Sorbitol with Alcohol

Solution in	pH	Stored at (±1°)	Percent Retained after 21 Days	30 Days	61 Days	120 Days	155 Days	182 Days
50% glycerin	5.7	65°	78.9 (4.6)[a]	—	—	—	—	—
70% sorbitol	6.8	65°	88.4 (5.7)	—	—	—	—	—
50% sorbitol	8.5[b]	65°	81.7 (4.9)	—	—	—	—	—
50% sorbitol with 20% alcohol	8.5[b]	65°	93.3 (4.7)	—	77.4 (4.7)	—	—	—
50% sorbitol with 20% alcohol	8.5[b]	24°	99.5 (7.1)	—	99.0 (6.7)	—	—	—
50% sorbitol with 10% alcohol	8.5[b]	65°	90.8	—	81.1 (4.8)	21.7 (4.0)	10.4 (3.9)	0 (3.9)
50% sorbitol with 10% alcohol	8.5[b]	24°	99.3	99.2 (6.1)	—	97.5[c] (5.8)	99.2 (5.6)	99.3 (5.5)

[a] Observed pH. [b] The pH was adjusted with 0.1 *N* NaOH solution in water. [c] It appears that there is an error in this reading.

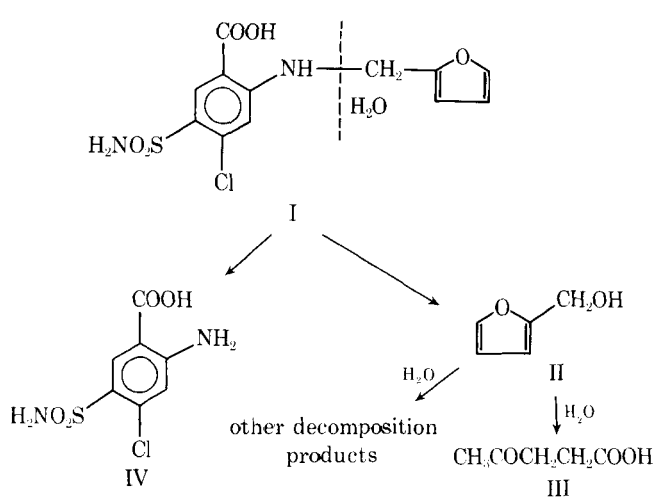
assayed to determine if furosemide, like ethacrynic acid[5], undergoes fast decomposition in the presence of ammonium ions. No decomposition of furosemide was noted.

Aqueous Solutions in Glycerin and Sorbitol—These solutions were stored at 65° and at room temperature for varying periods and reassayed (Table IV). Since the investigations indicated that sorbitol was better than glycerin (from a stability point of view) and that alcohol had a stabilizing effect, dosage forms in sorbitol in which part of the water was replaced with alcohol were also investigated (Table IV).

DISCUSSION

The method developed for the quantitative determination of furosemide appears to be stability indicating (Figs. 1A, 1B, and 2A–2C). It is accurate, precise (standard deviation based on six injections of the standard solution was ±1.37%), and sensitive. The peak area was directly related to concentration (range tested was 0.5–3 μg of furosemide) with a correlation coefficient of 0.99996. The USP method (1), based on UV absorption, is not stability indicating since a 3.5-year-old sample in simple syrup gave 82.1% *versus* only 26.1% by the developed method. In the two commercial dosage forms (injection and tablets), no decomposition was noted in samples that were at least 3 years old. Furosemide was very unstable in acidic media and very stable in basic media (Table III).

Sugar appears to have an adverse effect (Table III) on furosemide



Scheme I

[5] The study on ethacrynic acid will be presented in a separate report.

Considering this discussion and the results, it is possible to formulate a liquid dosage form with limited stability containing sorbitol, alcohol (10–20% v/v), and preservatives (methylparaben and propylparaben). The pH should be adjusted to at least 8.5 with sodium hydroxide in water. The shelflife of the dosage form may be extended for more than 1 year by adding an overage of about 5% of furosemide.

The data were not treated mathematically because the pH values were not constant (Table III) because of the hydrolysis of sugar. The commercial vehicle also contained high concentration of sugar. No attempt was made to buffer the dosage forms heavily to prevent pH changes. If a biologically safe buffer can be used to maintain the pH value at around 8.5, it would certainly stabilize the dosage form further.

REFERENCES

(1) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, pp. 213, 214.

(2) A. W. Forrey, B. Kimpel, A. D. Blair, and R. E. Cutler, *Clin. Chem.*, **20,** 152 (1974).

(3) K. A. Kovar, G. P. Wojtovicz, and H. Auterhoff, *Arch. Pharm.*, **307,** 657 (1974).

ACKNOWLEDGMENTS

The authors thank Hoechst-Roussel Pharmaceuticals Inc. for a generous supply of furosemide powder and injections.

Quantitation of Cocaine and Its Principal Metabolite, Benzoylecgonine, by GLC–Mass Spectrometry Using Stable Isotope Labeled Analogs as Internal Standards

SATYA P. JINDAL ˣ and PER VESTERGAARD

Received June 27, 1977, from the *Rockland Research Institute, Orangeburg, NY 10962.* Accepted for publication October 12, 1977.

Abstract □ A quantitative GLC–mass spectrometric assay was developed for the determination of cocaine and its principal metabolite, benzoylecgonine, in human urine. The assay utilizes selective ion focusing to monitor in a GLC effluent the molecular ions of cocaine and benzoylecgonine generated by electron-impact ionization. Cocaine-d_3 and benzoylecgonine-d_3 were the internal standards. The assay can measure 2 ng of cocaine/ml and 5 ng of benzoylecgonine/ml with about 5% precision. The curves relating the amounts of cocaine and benzoylecgonine added *versus* the amounts found over a large range of cocaine and benzoylecgonine concentrations were straight lines with nearly zero intercepts and slopes of 0.98 ± 0.01 and 0.97 ± 0.01, respectively. The method was used for the analysis of urinary cocaine and benzoylecgonine in cocaine addicts. Assay specificity was confirmed by complete identity of the mass spectra of cocaine and benzoylecgonine with those of authentic materials.

Keyphrases □ Cocaine—GLC–mass spectrometric analysis in human urine □ Benzoylecgonine—GLC–mass spectrometric analysis in human urine □ GLC–mass spectrometry—analyses, cocaine and benzoylecgonine in human urine □ Narcotics—cocaine and benzoylecgonine, GLC–mass spectrometric analyses in human urine

Cocaine abuse (1–5) has prompted considerable interest in methods for the detection and quantitation of the parent drug and its metabolites in biological fluids and tissues. TLC and the enzyme multiplied immunoassay technique (EMIT) are the most frequently employed screening methods for the detection of cocaine in human urine. However, these methods are inherently only semiquantitative at best. Although cocaine may be determined by GLC, the amount of unchanged cocaine excreted in human urine (2, 6) is generally below the method's limits of detection (7, 8).

BACKGROUND

A rather sensitive GLC procedure (9) for cocaine is based on the reduction of cocaine with lithium aluminum hydride, derivatization of the reduced product with pentafluoropropionic anhydride, and subsequent detection by electron capture. This method is sensitive but unspecific, and it is not applicable to cocaine in biological specimens. Besides cocaine, benzoylecgonine, ecgonine, and possibly other metabolites (10) of cocaine are converted by the procedure (9) to the same derivative. Therefore, the GLC-measured cocaine concentration cannot be the true concentration of cocaine alone.

Recently, a GLC procedure for cocaine and benzoylecgonine in the urine of cocaine users was reported (11). The method involves methylation of urinary benzoylecgonine to cocaine by treatment with diazomethane, thereby measuring combined benzoylecgonine and cocaine. Original cocaine was determined in a separate extract without the methylation step. Thus, benzoylecgonine could be determined by difference. Cocaine is metabolized in the human body rather rapidly (12), benzoylecgonine being the major metabolite (13, 14). Consequently, most urine samples of cocaine users contain trace amounts of unchanged drug and large concentrations of benzoylecgonine. Furthermore, cocaine and benzoylecgonine have rather widely different and variable extraction efficiencies, and one ends up dealing with differences of rather large and very small numbers. As a result, this assay is of limited usefulness.

This paper describes a GLC–mass spectrometric assay of cocaine and benzoylecgonine in human urine. Selective ion monitoring, the technique built on combined GLC–mass spectrometry with selective focusing on suitable fragments of the molecular ion (mass fragmentography) or the molecular ion itself, is a well-established technique, used widely in pharmacology (15, 16). This technique was used to develop a sensitive and specific assay for cocaine and benzoylecgonine in human urine with site-specific deuterium-labeled cocaine and benzoylecgonine as internal standards.

EXPERIMENTAL

Materials—Analytical grade cocaine hydrochloride[1], benzoylecgonine[2], norcocaine hydrochloride[3], methyl iodide-d_3[4], and *N*-ethyl-*N*-nitro-*N*-nitrosoguanidine[5] were used without further purification. All solvents were analytical grade[6]. Silanized tubes[7] (10 ml) with screw caps[8] were used for urine extraction; final solvent evaporation was performed in 5-ml glass-stoppered centrifuge tubes[9]. Pasteur pipets with hand-

[1] Mallinckrodt Chemical Works, New York, N.Y.
[2] Techman Inc., Park Forest South, Ill.
[3] Norcocaine hydrochloride was a gift from Dr. Sally, Chemistry Department, University of Wisconsin.
[4] International Chemical & Nuclear Corp., Irvine, Calif.
[5] Pfaltz & Bauer, Flushing, N.Y.
[6] Fisher Scientific Co., Pittsburgh, Pa.
[7] Kimble, Owens-Illinois, Toledo, Ohio.
[8] Lined with Teflon (DuPont).
[9] Pyrex 8084.