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Modification of Metoclopramide GLC Assay: Application to Human Biological Specimens

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Received January 4, 1979, from the **Faculty of Pharmaceutical Sciences and the* ‡*Faculty of Medicine, University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5.* Accepted for publication April 11, 1979.

Abstract □ A modified electron-capture GLC assay for metoclopramide in human biological specimens is reported. This assay involves the incorporation of a back-extraction method to remove endogenous contaminants. Its applicability was demonstrated by studying the time course of metoclopramide in plasma and urine from a human subject. The lowest quantifiable metoclopramide concentration in plasma was 7 ng/ml, provided 0.5 ml of plasma was used.

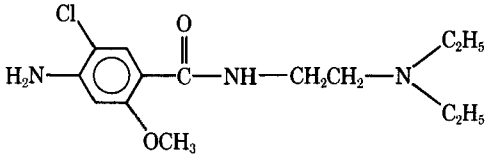
Keyphrases □ Metoclopramide—analysis, electron-capture GLC, human plasma and urine □ Antiemetic agents—metoclopramide, electron-capture GLC analysis, human plasma and urine □ GLC, electron capture—analysis, metoclopramide, human plasma and urine

A highly sensitive electron-capture GLC assay was developed recently for the determination of metoclopramide (I), mol. wt. 299.81, in biological specimens from the rat (1). However, when the same procedures were used to analyze human plasma, quantitation of I was impossible due to interferences from endogenous substances (Fig. 1*a*). A slight modification of this method, involving a "cleanup step," successfully removed endogenous substances from the human plasma samples. The purpose of this report is to describe this improved assay.

EXPERIMENTAL

Materials—Metoclopramide monohydrochloride monohydrate[1] (II) (mol. wt. 336.31), benzene[2], 4% ammonium hydroxide[3], 1 *N* NaOH[4], 5 *N* NaOH[4], 1 *N* HCl[5], heptafluorobutyric anhydride[6] (III), and diazepam[7] were used.

GLC—A reporting gas–liquid chromatograph[8] equipped with a ^{63}Ni-electron-capture detector (^{63}Ni-15 mCi) and a 1.2-m × 2-mm i.d. glass column, containing 3% OV-225 coated onto 80–100-mesh Chromosorb W, was used to analyze plasma samples. The operating temperatures for routine analysis were: injection port, 250°; oven, 245°; and detector, 350°. The carrier gas (95% argon–5% methane) flow rate was 40 ml/min. For urine analyses, all GLC conditions were identical except that a 0.6-m × 2 mm-i.d. 3% OV-225 column was used and the oven temperature was 230°.



I

Extraction Procedure—*Plasma*—A 0.5-ml sample of human plasma (blank) was spiked with serial concentrations of II (concentration equivalent to 7–93 ng of I/ml of water) (Table I). To each spiked sample, 0.5 ml of 1 *N* NaOH was added. The final volume was made up to 2 ml with water (pH ≃ 12). The aqueous phase was extracted with 8 ml of benzene by shaking with a wrist-action shaker[9] for 15 min. After centrifugation at 3000×*g* for 10 min, 7 ml of the organic phase was removed and back-extracted (extraction time, 15 min) with 2 ml of 1 *N* HCl.

The benzene layer was aspirated, and the sample was washed two times with benzene (4 ml each). The remaining aqueous layer was alkalinized with 0.5 ml of 5 *N* NaOH (pH ≃ 12). Finally, this layer was extracted with 6 ml of benzene (extraction time, 15 min). A 5-ml aliquot of the organic phase was removed and dried under a gentle nitrogen stream at ambient temperature. The residues were reconstituted with 0.5 ml of internal standard solution (50 ng of diazepam/ml of benzene).

Urine—A 0.1–0.5-ml volume of urine containing I was extracted in the same manner as described by Tam and Axelson (1).

Derivative Formation and Quantitative Analysis—A 20-μl volume of III was added to the reconstituted solution. The reaction mixtures were incubated at 55° for 10 min. Excess III was removed by hydrolysis with 0.5 ml of water and neutralization with 0.5 ml of 4% NH_4OH (1). A 5-μl volume of the derivative solution was injected into the reporting gas–liquid chromatograph by an automatic sampler. Quantitative estimation of I in human plasma and urine samples was accomplished by plotting the area ratios of the derivative and the internal standard against a range of solutions of known I concentration.

Human Study—A 20-mg metoclopramide monohydrochloride[10] (IV) dose was administered orally to a fasted normal healthy volunteer during a drug interaction study with griseofulvin. Five-milliliter blood samples were withdrawn at 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, and 24 hr after drug administration. The blood samples were immediately centrifuged, and the plasma was separated. Cumulative urine samples were collected up to 96 hr after drug consumption. Both the plasma and urine samples were stored at −20° until analysis.

Table I—Quantitative Metoclopramide Estimation in Human Plasma

Metoclopramide Concentration, ng/ml	n^a	Area Ratio	*SD*
7	3	0.409	±0.03
15	3	0.812	±0.05
37	3	2.140	±0.25
56	3	2.935	±0.20
93	3	4.768	±0.36

[a] Each *n* is the average of three injections.

[1] Analysis No. 9207, A. H. Robins, Montreal, Canada.
[2] Distilled in glass, Caledon, Georgetown, Ontario, Canada.
[3] Reagent ACS code 1293, Allied Chemical Canada Ltd., Pointe Claire, Quebec, Canada.
[4] Mallinckrodt Chemical Works, St. Louis, Mo.
[5] Reagent ACS, Allied Chemical Canada Ltd., Pointe Claire, Quebec, Canada.
[6] Pierce Chemical Co., Rockford, Ill.
[7] Lot R-6685, Hoffmann-La Roche, Montreal, Canada.
[8] Hewlett-Packard model 5840.
[9] Patent pending, Burrell Corp., Pittsburgh, Pa.
[10] Maxeran tablets (10 mg), Nordic, Laval, Quebec, Canada.

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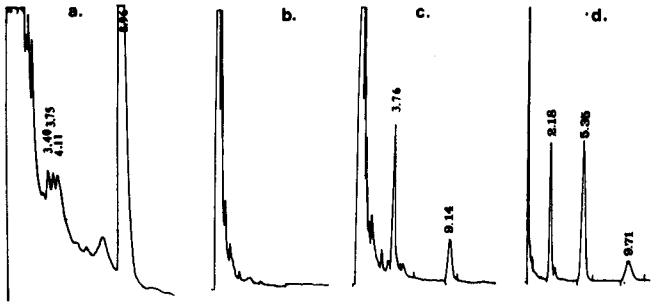

Figure 1—*Representative chromatograms of: (a) a blank plasma extract after a single extraction; (b) blank plasma after back-extraction; (c) a plasma sample containing 60 ng of metoclopramide/ml (3.76 min) and 50 ng of diazepam/ml (9.1 min); and (d) a urine sample using a 0.6-m 3% OV-225 column instead of a 1.2-m 3% OV-225 column. Peaks at 2.18, 5.35, and 9.71 min are the derivative of metoclopramide, diazepam, and an unidentified metabolite, respectively. Chart speed was 0.5 cm/min.*

RESULTS AND DISCUSSION

Extraction and GLC—With the single-extraction method (1), it was observed that not only I but also other endogenous substances that contributed to the extraneous peaks were effectively removed from human plasma samples (Fig. 1*a*). These interfering peaks prevented the quantitation of I in human plasma. Those contaminants responsible for the interfering peaks were successfully removed by back-extracting I into an acidic medium (Fig. 1*b*). A representative chromatogram of the plasma extract after the plasma was spiked with II is shown in Fig. 1*c*. The retention times of the derivatives of I and diazepam were 3.75 and 9.14 min, respectively. Back-extraction of human urine samples was unnecessary since no interference was observed in the chromatogram (Fig. 1*d*).

The peaks at 2.18, 5.35, and 9.71 min were the derivatives of I, diazepam, and an unidentified metabolite, respectively. The identification of this metabolite by mass spectrometry is planned. Since simultaneous quantitation of this metabolite and I is desirable, and the elution time of the peaks would be relatively long if a 1.2-m column (Fig. 1*c*) were used, a shorter column might be preferable in the urine sample analyses. A 0.6-m column provided baseline separation between the peaks, and a short elution time for all three compounds also was observed (Fig. 1*d*). Alternatively, a 1.2-m column was required to separate the derivative peak from the solvent front in plasma analyses due to interfering substances not observed in urine.

Chromatographic response was linear in the range studied (concentration equivalent to 7–93 ng of I/ml of plasma and 0.04–2.2 μg of I/ml of urine). The calibration curves were obtained by analyzing blank urine or plasma samples spiked with various drug concentrations (Tables I and II). From the linear regression analyses, the best-fit line through the data points was described by $y = 0.051x - 0.062$ for plasma and $y = 1.345x - 0.446$ for urine, with $r^2 = 0.99$ in both cases. The recovery of I after

Table II—Quantitative Metoclopramide Estimation in Human Urine

Metoclopramide Concentration, μg/ml	n^a	Area Ratio	*SD*
0.04	3	0.075	±0.001
0.37	3	0.569	±0.002
0.7	3	1.058	±0.017
1.1	3	1.531	±0.024
1.5	3	2.030	±0.054
1.9	3	2.562	±0.113
2.2	3	3.044	±0.053

[a] Each *n* is the average of three injections.

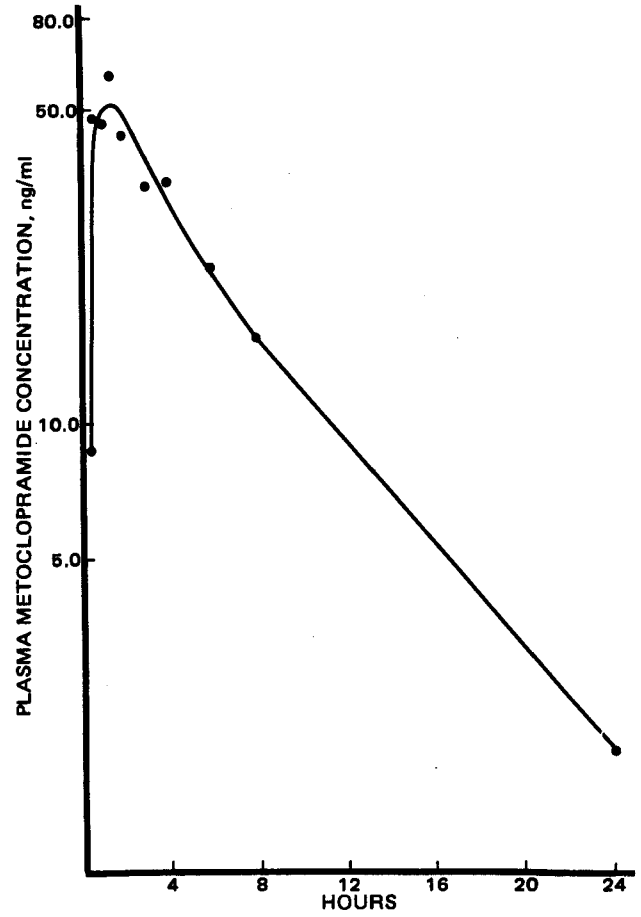

Figure 2—*Plasma metoclopramide profile after a dose of 20 mg of metoclopramide monohydrochloride po to a normal healthy volunteer.*

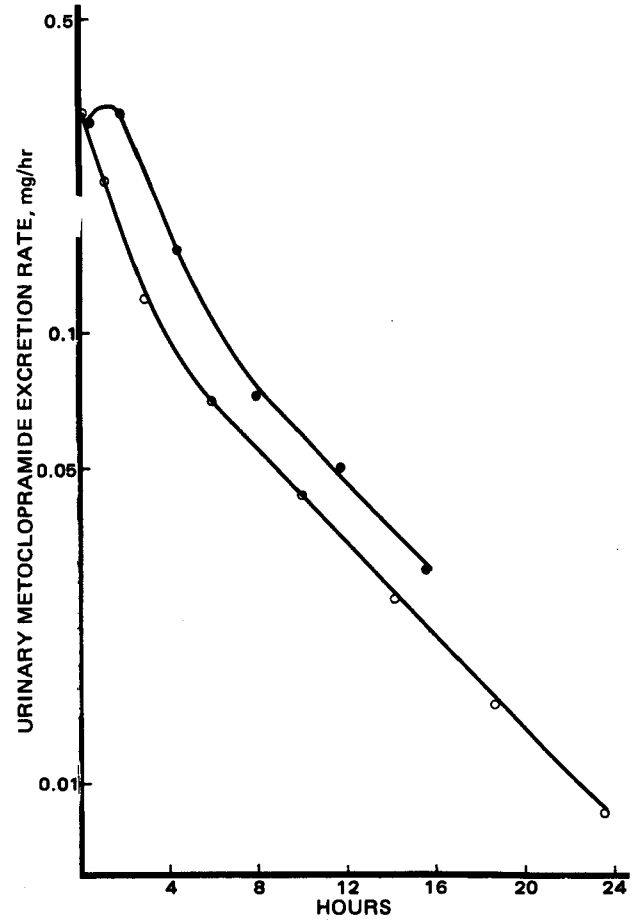

Figure 3—*Midpoint urinary excretion rate plots. Key: ●, urine samples collected along with blood samples; and ○, urine samples collected in a separate study using the same dose (20 mg of metoclopramide monohydrochloride) and subject.*

extraction from the plasma samples was 84 ± 9%. The minimum quantifiable amount of I was ~25 pg/injection.

Human Data—To demonstrate the application of the assay modification, urine and plasma I concentrations were measured in a human subject receiving IV[10] during a drug interaction study.

The peak plasma I concentration was achieved at ~1.5 hr after an oral dose of IV. Despite the somewhat scattered plasma data, the elimination half-life could be estimated using truncated plasma data, as reported previously (2, 3). When the truncated 8-hr plasma data were evaluated, an apparent half-life of 3.9 hr was noted; other investigators noted a half-life of 2.8 hr (3) and 4.0 hr (2) using similar data. However, when a 24-hr plasma sample was taken into account (Fig. 2), the elimination half-life was 5.4 hr. It becomes apparent that the use of 8-hr plasma data results in an underestimation of the terminal elimination half-life. Since no blood samples were taken between 8 and 24 hr after drug administration, an accurate estimation of the true elimination half-life was hampered using plasma data.

It was possible, however, to assess accurately the I elimination half-life using the urinary excretion rate data. When the urinary excretion rate of I was determined, the peak excretion rate was at 1.5 hr, in agreement with the time required for peak plasma concentrations to occur in this subject (Fig. 3), and the elimination half-life was 6 hr. In the postabsorptive phase of the plasma concentration and urinary excretion rate data, a biexponential decline was observed (Figs. 2 and 3), suggesting that I may follow multicompartment kinetics in humans. Following intravenous administration, Bateman *et al.* (3) reported biexponential kinetics for I, but these investigators may have missed the true terminal elimination phase noted in this subject (Fig. 3). This finding was confirmed by the fact that measurement of the half-life for I using truncated urinary excretion data (0–8 hr only, Fig. 3) yielded a half-life of 3 hr, in agreement with the reported value (3).

When the urinary excretion rate for I was determined in another experiment, the time for the maximal excretion rate was attained more rapidly. Slopes observed in the postabsorptive phase in both experiments (Fig. 3) were virtually identical, yielding a half-life of 6 hr. Thirteen percent of the dose was recovered as the intact drug after 96-hr cumulative urinary excretion. Acid hydrolysis of urine samples yielded a threefold increase in intact drug recovered.

Although urinary excretion rate data strongly suggest a longer half-life for I, the precise determination of the I elimination half-life in human plasma requires more blood samples. Additional studies are planned to confirm this observation.

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(3) D. N. Bateman, C. Kahn, K. Mashiter, and D. S. Davis, *Br. J. Clin. Pharmacol.*, **6**, 401 (1978).

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Kinetic Studies of Hydralazine Reaction with Acetaldehyde

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Received February 21, 1979, from the *School of Pharmacy, West Virginia University, Morgantown, WV 26506.* Accepted for publication April 12, 1979.

Abstract □ *In vitro* kinetic studies of the reaction of hydralazine with acetaldehyde at physiological concentrations and pH were conducted. This reaction, which leads to the formation of 3-methyl-*S*-triazolo[3,4-*a*]phthalazine, may occur in the plasma and may represent an alternative pathway for hydralazine metabolism. The reaction of hydralazine with acetaldehyde followed second-order kinetics with an activation energy of 16.9 kcal/mole. At 37°, the half-life of the reaction for a solution containing 2.3 μg of acetaldehyde/ml and 1 μg of hydralazine/ml was 4.5 hr. The rate increased with increasing acetaldehyde concentrations.

Keyphrases □ Hydralazine—reaction with acetaldehyde, kinetics, half-life, therapeutic implications □ Antihypertensive agents—hydralazine, reaction with acetaldehyde, kinetics, half-life, therapeutic implications □ Kinetics—hydralazine reaction with acetaldehyde □ Acetaldehyde—reaction with hydralazine, kinetics, half-life, therapeutic implications

Since the early 1950's, hydralazine has been used to treat hypertension by direct peripheral vasodilation. More recently, the drug has been used in the treatment of severe congestive heart failure (1, 2). Its use, however, has been limited due to inadequate pharmacokinetic data on the drug and its metabolites.

BACKGROUND

The complete metabolism of hydralazine remains undefined. Studies (3, 4) showed that hydralazine undergoes polymorphic *N*-acetylation to form 3-methyl-*S*-triazolo[3,4-*a*]phthalazine as the major metabolic pathway, with phenotypically slow acetylators having higher steady-state plasma concentrations than fast acetylators. However, it was pointed out that the thermal half-life of hydralazine among slow acetylators is only slightly longer than or is not significantly different from that of fast acetylators (5). Thus, it seems doubtful that the enzymatic *N*-acetylation can represent the major metabolic pathway of hydralazine in humans.

Hydralazine metabolism is also more complicated because hydralazine is chemically reactive. It undergoes condensation with biogenic aldehydes and ketones to form hydrazones. Studies (6) in this laboratory showed that the reaction of hydralazine with formaldehyde and acetaldehyde forms *S*-triazolo[3,4-*a*]phthalazine and 3-methyl-*S*-triazolo[3,4-*a*]-phthalazine, respectively. Both products are known hydralazine metabolites (3, 4).

The formation of 3-methyl-*S*-triazolo[3,4-*a*]phthalazine from acetaldehyde and hydralazine was mentioned previously (7). These findings suggest that the formation of the hydralazine metabolites in humans may be at least partially due to the reaction of hydralazine with acetaldehyde. The acetaldehyde concentration in biological fluids is subject to significant increase by alcohol consumption and certain disease states (8). Thus, metabolite formation *via* this chemical reaction is significant with respect to proper drug use, especially in alcoholism, diabetes, and fasting states and on prolonged hydralazine administration.

To examine the effect of this chemical reaction on hydralazine metabolism and pharmacokinetics, the *in vitro* kinetics of 3-methyl-*S*-triazolo[3,4-*a*]phthalazine formation from hydralazine and acetaldehyde were studied. The importance of this reaction is discussed.

EXPERIMENTAL

Materials—Hydralazine (I), hydrochloride salt[1], and acetaldehyde[2] (II) were used as obtained. 3-Methyl-*S*-triazolo[3,4-*a*]phthalazine (III)

[1] Courtesy of Dr. M. Wilhem, Ciba-Geigy, Summit, N.J.
[2] Aldrich Chemical Co., Milwaukee, Wis.

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