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TECHNICAL NOTE

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Metabolite Sensitivity in the Methaqualone Radioimmunoassay

The use of radioimmunoassay (RIA) as a method capable of detecting nanogram-per-millilitre (ng/ml) quantities of methaqualone in biological specimens has been described previously [*1,2*]. Several authors have shown, by using gas chromatography or mass spectrometry, or both, that the principal products of methaqualone metabolism, as they appear in the urine, are primarily the monohydroxy derivatives [*3–5*]. These derivatives have been found to be conjugated mainly with glucuronic acid. In this study the relative sensitivity of methaqualone and its metabolites to the RIA assay was investigated.

Materials and Methods

A kit of reagents consisting of methaqualone antiserum, ^{125}I-methaqualone derivative, normal urine control, methaqualone-positive urine, and saturated ammonium sulfate was obtained from Roche Diagnostics, Hoffman-La Roche Inc., Nutley, N.J. The methaqualone, as a free base, was obtained from Wm. H. Rorer, Inc. The monohydroxylated metabolites were obtained through the National Institute on Drug Abuse and consisted of the following: 2-methyl-3-o-(2′-hydroxymethylphenyl)-4(3H)-quinazolinone (Metabolite I), 2-methyl-3-o-(3′hydroxy-2′-methylphenyl)-4(3H)-quinazolinone (Metabolite II), 2-methyl-3-o-(4′-hydroxy-2′-methylphenyl)-4(3H)-quinazolinone (Metabolite III), and 2-methyl-3-o-tolyl-6-hydroxy-4(3H)-quinazolinone (Metabolite IV).

The analytical procedure for performing the analysis was essentially the same as described by Roche Diagnostics [*6*]. In this procedure, 0.2 ml of ^{125}I-labeled methaqualone (antigen) was pipetted into an assay tube containing 0.1 ml of a urine specimen, followed by 0.2 ml of methaqualone antiserum (antibody). The resulting mixture was vortex mixed and allowed to incubate at room temperature for 1 h. During this incubation period, the labeled antigen competes with the unlabeled methaqualone for the antibody, and both bind to this moiety in proportion to their concentrations. After the incubation, 0.5 ml of saturated ammonium sulfate was added, the contents in the tube were again mixed, and

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the solution reincubated for an additional 10 min. At the end of the second incubation, the assay tubes were centrifuged for 3 min at 8000 *g* to separate the bound portion of the reaction mix, and a 0.5-ml aliquot of the supernatant was measured for radioactivity in a Beckman Biogamma counter. The quantity of radioactivity from each tube was then related directly to the amount of unlabeled methaqualone in each specimen.

All analyses were performed in triplicate and counted for 10 min in the gamma counter. To eliminate the problem of variations in reactivity of the different lots, the same RIA kit was used throughout the study [*7*].

Standard solutions of free-base methaqualone and each metabolite were prepared in methanol (1 mg/ml). The ultraviolet characteristics of the solutions were then studied by obtaining their spectra in 0.01*N* HCl [*8–10*]. Using the average molar-extinction coefficient of 1310 at 234 nm as stated in the literature, the concentrations of the methaqualone and Metabolites I, II, and III were observed to be within 0.07 mg/ml of the weighed value [*11*]. Metabolite IV, however, was noted to be 0.78 mg/ml, while the concentration by weight was 1.05 mg/ml. This metabolite also exhibited additional absorption at low wavelengths. The minor variation between these two results strongly suggests that the molar-extinction coefficient for each of these metabolites could be different. This assumption is strengthened further by the fact that the coefficients are dependent on the pH and on the location of the hydroxyl group.

Results

To compare the methaqualone equivalent of each of the metabolites, working solutions were made by diluting the methanol solutions with urine. A standard curve of the free base was used for quantitation. Table 1 indicates the free-base equivalent values and

TABLE 1—*Free-base relationship of methaqualone metabolites.*

Metabolite	Concentration *a*, ng/ml	Free-Base Equivalent *b*, ng/ml	Response Ratio *b/a*
I	103	16	0.2
II	34	27	0.8
III	20	32	1.6
IV	830	11	<0.1

the relative response ratio for each of the metabolites. The response ratio reflects the general reactivity of each metabolite in the RIA method of analysis.

To observe the effects that various amounts of metabolites would produce in this analysis, dilutions ranging from zero to 100 ng/ml were made of the free base and Metabolites I, II, and III. These dilutions produced a family of curves when the counts obtained for each concentration were graphed. Figure 1 shows that all the curves are similar in shape but of varying reactivity. Since Metabolite IV has such a low free-base equivalent value, it was not feasible to plot it in this range of concentrations.

Discussion

The wide range of reactivity of the various metabolites to the free base presents a problem in the use of the RIA method of analysis for total methaqualone quantitation in physiological specimens. This is of particular importance when using an RIA kit in which the reference standard is the free methaqualone base in urine.

Methaqualone is almost completely metabolized in the body and is excreted in the

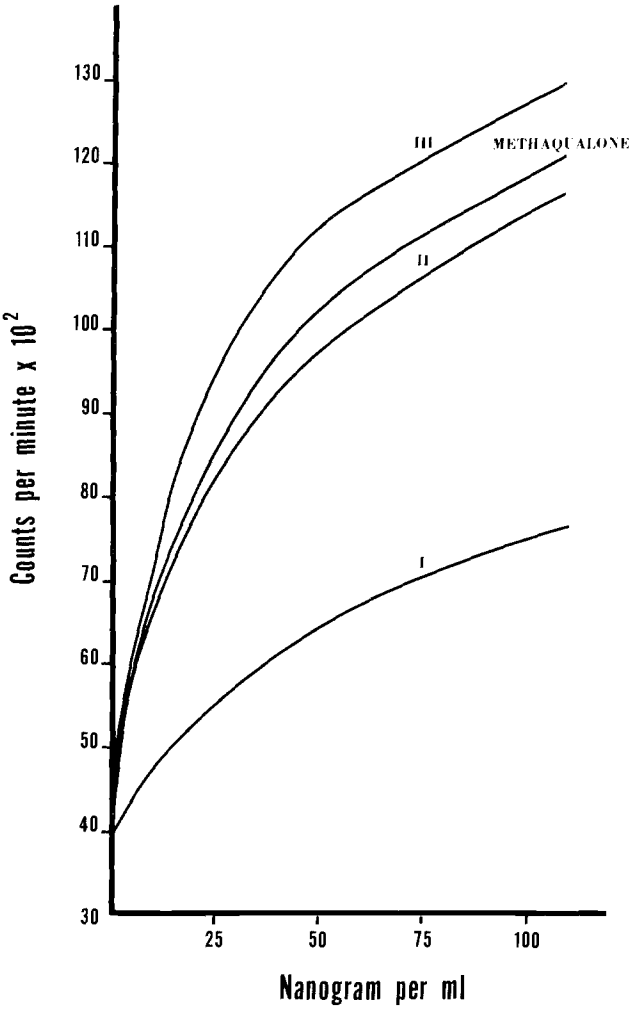

FIG. 1—*Graph showing curves for the quantitation of methaqualone and its metabolites.*

urine, mainly conjugated with glucuronic acid [*3*]. As many as twelve metabolites have been isolated from urine, but the monohydroxy derivatives predominate [*12*]. The methaqualone metabolism in the liver varies with time and from person to person, thus causing a wide variation in the concentration of the individual metabolites in physiological specimens.

Summary

Recent literature reflects the increased use of RIA as a qualitative and quantitative tool [*13*]. This study exemplifies the problems that may arise when this technique is used for quantitation. Caution must be exercised, and a physiological and toxicological understanding of each particular drug is essential to provide credible results. It is evident that the sensitivity and ease of the RIA method for methaqualone makes it invaluable as a screening test, but confirmation and quantitation of this drug must remain with those methods that can quantitate each metabolite.

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