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Determination of Methaqualone in Urine by Metabolite Detection Via Gas Chromatography

Pharmaceutical preparations of the sedative and hypnotic agent methaqualone have been widely prescribed and dispensed under a variety of trade names, including Quāālude®, Sopor®, and Parest®. Cases of methaqualone abuse and overdose have been well documented [*1–3*]. The ready availability and frequent abuse of methaqualone have led to the development of methods of analysis for methaqualone and its metabolites in biologic fluids. Nowak et al used thin-layer chromatography (TLC) to study the metabolism of methaqualone in laboratory animals [*4*]. Only a small fraction of the dose was detected as the unchanged drug in the urine of the rat, while the major portion of the dose was hydroxylated and excreted as glucuronide conjugates. Preuss et al isolated twelve metabolites from urine of human subjects who had ingested methaqualone [*5*]. By comparison of these twelve metabolites with a series of synthesized monohydroxy derivatives of methaqualone they were able to identify five monohydroxy metabolites of the drug.

Gas chromatographic (GC) determination of methaqualone has been described, and quantitative data for methaqualone in blood and urine have been reported [*6–8*]. Several metabolites have been detected and identified by combined gas chromatography-mass spectrometry (GC-MS) [*3,9*]. The rapid metabolism of methaqualone and large amounts of metabolites that are excreted in the urine led to our design of a sensitive gas chromatographic method for methaqualone by detection of its principal urinary metabolites. In this study, metabolite patterns are characterized and investigated as possible indicators of methaqualone ingestion.

Experimental

A flow diagram of the extraction procedure is shown in Fig. 1. An aliquot of urine was treated with concentrated hydrochloric acid to hydrolyze glucuronide conjugates. The acid hydrolysate was adjusted to a pH of 8.0 to 8.5 with saturated potassium hydroxide, and then extracted with chloroform. The organic layer was washed with pH 8.5 phosphate buffer and filtered. An aliquot of the chloroform filtrate was evaporated to 100 μl under a stream of nitrogen and then divided into two portions. One 50-μl portion was taken to dryness, and the residue derivatized in 25 μl dimethylformamide (DMF) with 25 μl N,O-bis(trimethylsilyl)acetamide (BSA). Both the trimethylsilyl (TMS) ether-derivatized extract and the underivatized extract were subjected to gas chromatographic analysis.

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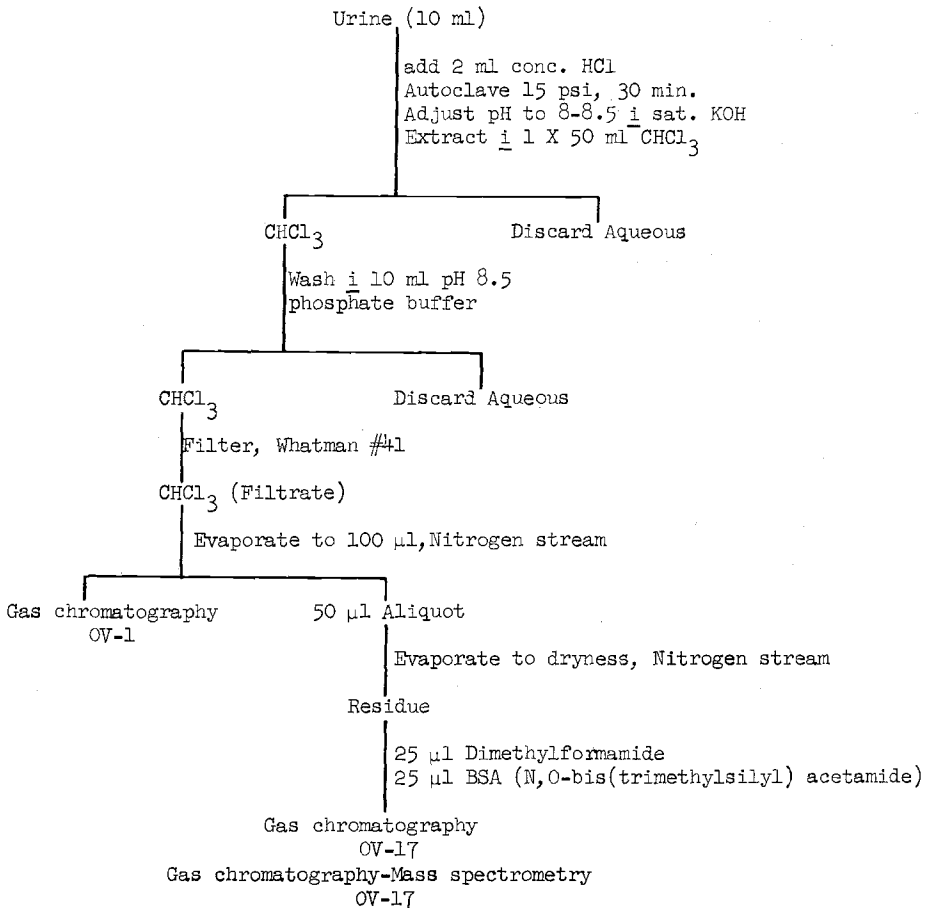

FIG. 1—*Flow diagram of analytical procedure.*

Gas chromatographic analysis was accomplished on a Hewlett-Packard 7600A gas chromatograph equipped with a digital integrator and flame ionization detector. The gas chromatograph was interfaced to a Hewlett-Packard 2116 computer. Separations were carried out on 1.9 m by 4 mm inside diameter glass columns packed with either 3% OV-1 or 3% OV-17 operated isothermally at 210°C and 225°C, respectively. Digital integrator output was processed in real time for peak areas, time, and retention indexes [*10*]. Concentrations of methaqualone and its monohydroxylated metabolites were calculated from standard calibration curves.

A GC-MS system consisting of an HP 5700A gas chromatograph interfaced to an HP 5930A dodecapole mass spectrometer with a single-stage permeable membrane separator and an HP 5932A data system was used for selected ion monitoring (SIM) [*11*] studies. Derivatized urine extracts were analyzed for methaqualone and the TMS ethers of monohydroxylated metabolites by monitoring ions characteristic of the electron impact spectra for each of the compounds. Separations were carried out on a 1.5 m by 2 mm inside diameter glass column packed with 3% OV-17.

A total of 737 urine specimens from the United States Air Force Europe (USAFE) Drug Abuse Detection Laboratory methaqualone screening project were screened for methaqualone by TLC before reaching our laboratory. A sampling of 65 urine specimens were

designated positive for methaqualone, and 43 doubtful positive samples were selected from this group for analysis.

Urine collected from healthy, adult, male volunteers of this laboratory who had ingested therapeutic doses of Mandrax® (250 mg) or Quāālude® (300 mg) was analyzed to determine methaqualone excretion patterns. In a controlled time study, urine specimens were collected at hourly intervals for the first ten hours to the extent possible and then casual collections were made to the end of the eleven-day study. In all studies involving volunteers, urine specimens were collected from each subject prior to administration of the dose of methaqualone and used as blanks.

The methaqualone was obtained as a gift from Wm. H. Rorer, Inc. Authentic samples of the monohydroxylated metabolites were synthesized: the 2-methyl-3-(2'-hydroxymethyl) phenyl-4(3H)-quinazolinone (Metabolite I) was prepared according to the method of Cella [*12*]; the 2-methyl-3-(2'-methyl-3'-hydroxyphenyl)-4(3H)-quinazolinone (Metabolite II), 2-methyl-3-(2'-methyl-4'-hydroxyphenyl)-4(3H)-quinazolinone (Metabolite III), and 2-methyl-6-hydroxy-3-*o*-tolyl-4(3H)-quinazolinone (Metabolite IV) were prepared according to the procedure reported by Preuss et al [*5*].

Results and Discussion

Monohydroxy derivatives are the principal products of methaqualone metabolism, and the major metabolic pathway appears to be hydroxylation in the liver at one of ten possible sites (Fig. 2) [*2,13*]. We have found that hydroxylation is actually semi-specific in that Metabolites I, II, III, IV, and V predominate. In addition to these metabolites, other products of metabolism have been detected in the urine. Minor metabolic pathways involving dihydroxy and hydroxymethoxy biotransformation of methaqualone exist. Quantities of the disubstituted metabolites in the urine are low, however, when compared with concentrations of the monohydroxylated metabolites.

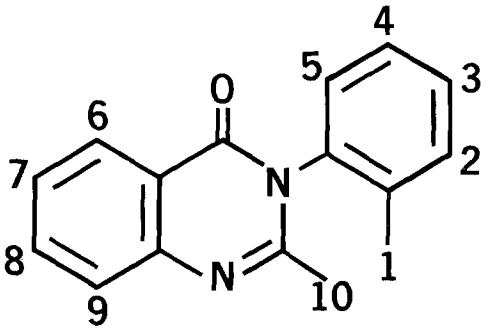

FIG. 2—*Structure of methaqualone labeled to indicate ten possible positions where monohydroxylation can occur: 1, Metabolite I; 2, Metabolite II; 3, Metabolite III; 7, Metabolite IV; and 10, Metabolite V.*

Retention indexes for methaqualone and its principal metabolites are listed in Table 1. The molecular weight of the monohydroxy metabolites is only 16 AMU or about 6% greater than methaqualone, and an increase in retention index can be predicted for this increase in molecular weight. One would expect the retention indexes of the metabolites on an OV-1 column to be only slightly higher than methaqualone if molecular weight was the only factor influencing chromatographic behavior of the compounds. Note, however, that the retention indexes of the metabolites are almost 200 to 300 retention index units higher than methaqualone. A major contributing factor responsible for these increases in retention indexes is the highly polar nature of the monohydroxylated metabolites. Since

TABLE 1—*Retention indexes.*

	OV-1		OV-17	
	Underivatized TMS	Derivative	Underivatized TMS	Derivative
Methaqualone	2115	...	2575	...
2-Hydroxymethyl methaqualone (Metabolite V)	2285	2335	2775	2720
2′-Hydroxymethyl methaqualone (Metabolite I)	2340	2320	2880	2705
3′-Hydroxy methaqualone (Metabolite II)	2435	2435	2995	2830
4′-Hydroxy methaqualone (Metabolite III)	2465	2465	3025	2850
6-Hydroxy methaqualone (Metabolite IV)	2465	2470	3040	2860

the five metabolites share the same empirical formula, one can relate the polarity of this series of compounds directly with retention index. It is therefore apparent that the hydroxymethyl metabolites are less polar than the tolyl and quinazolinone ring-substituted metabolites. Because of the wide range of polarity of monohydroxylated methaqualone metabolites, urine extracts were first analyzed for methaqualone and the free hydroxy metabolites, and the results confirmed by detection of the respective TMS derivatives.

Controlled time studies were conducted to determine the qualitative and quantitative relationships between methaqualone and methaqualone metabolites. Quantitative data is given for methaqualone and its principal monohydroxylated metabolites to demonstrate the reliability of metabolite detection as a means of identifying methaqualone usage (Table 2). Gas chromatographic separations of underivatized extracts of urine on an OV-1 column are shown in Fig. 3. The chromatograms represent typical urinary excretion patterns obtained from a study in which a volunteer ingested a 300-mg dose of Quāālude®. The lower chromatogram was obtained from urine collected 4 hours, and the upper from an extract of urine collected 24 hours, after dose. Note that the peak heights and corresponding peak areas of the metabolites are considerably greater in magnitude than that of methaqualone. Methaqualone and components 2 and 3, the 2-hydroxymethyl and 2′-hydroxymethyl metabolites, respectively, have been completely resolved. While these can be chromatographed with separation adequate for quantitative measurement, the three remaining monohydroxy metabolites cannot. The 4′-hydroxy and 6-hydroxy metabolites, labeled components 5 and 6 respectively, although not completely separated from the 3′-hydroxy metabolite (II), labeled component 4, contribute to the characterization of the methaqualone excretion. Quantitative data reported for these metabolites (Table 2) were ob-

TABLE 2—*Methaqualone*[a] *and metabolite*[b] *levels in urine, μg/ml.*

Sample	Methaqualone	I	II	III	IV	V
4 hour	0.20	1.48	3.67	2.52	0.80	1.00
24 hour	0.25	11.6	11.6	31.5	3.58	0.78
4508	0.29	7.95	9.88	16.5	0.70	0.56
4526	1.24	5.89	22.0	29.4	6.90	10.6

[a] 1.9 m by 4 mm inside diameter 3% OV-1 column used for quantification.
[b] 60 m by 0.6 mm inside diameter SE-30 on Silanox 101 SCOT capillary column used for quantification.

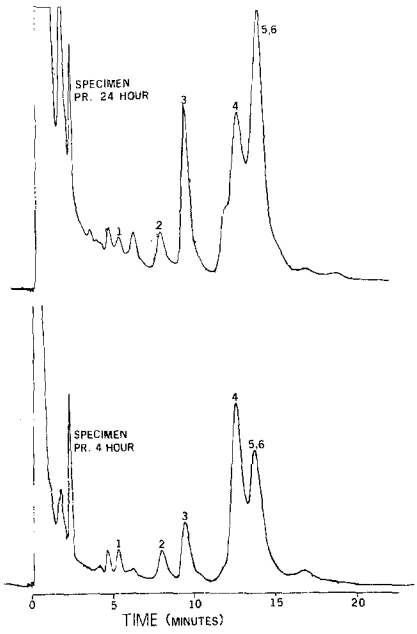

FIG. 3—*Gas chromatographic separation of urine extracts from a controlled time study on a 1.9 m by 4 mm inside diameter column packed with 3% OV-1 on 80/100 mesh Supelcoport. Temperatures of column, flash heater, and detector were 210°C, 250°C, and 275°C, respectively. Peaks are identified as 1, methaqualone; 2, Metabolite V; 3, Metabolite I; 4, Metabolite II; 5, Metabolite III; and 6, Metabolite IV.*

tained by analyzing TMS derivatized urine extracts on high resolution, support coated, open tubular (SCOT) capillary columns [*14*], similar to columns developed by German et al [*15*]. The significance of these metabolites is noted in a controlled time study and in urine samples collected from the methaqualone abuser. In this time study, levels of methaqualone and the monohydroxylated metabolites were monitored for a period of eleven days after ingestion of the therapeutic dose. Metabolites II, III, and IV were detected in the extracts of hydrolyzed urine from one hour after the dose to the end of the study. Methaqualone was first detected in the urine at a concentration of 0.20 μg/ml two hours after dose. The concentrations of Metabolites I through V were 3 to 14 times the concentration of methaqualone in this sample. Similarly, high metabolite to methaqualone ratios are recognized in the chromatograms in Fig. 3. For example, the ratio of Metabolite I to methaqualone in the urine collected at 4 hours is 7:1, and 47:1 in the 24-hour sample. A peak concentration of 0.46 μg methaqualone per ml of urine was reached within 7.2 hours after dose. A total of 350 μg or 0.1% of the dose was excreted as the unchanged drug in the urine during the first 24 hours of the study. These values are low when compared with the concentrations of metabolites, but are consistent with concentrations that can be predicted when a therapeutic dose is ingested [*6*]. These data from experimentally controlled subjects can be correlated with data obtained from urine specimens collected in drug screening programs.

All 65 specimens from the USAFE methaqualone screening project found positive for methaqualone ingestion by TLC were confirmed positive by GC. Of the 43 samples determined doubtful positive by TLC, GC analyses were positive for methaqualone and metabolites for 12. Ten of the remaining 31 samples had peaks with retention indexes corresponding to that of methaqualone, but did not have peaks that could be attributed to metabolites. The peaks with retention indexes corresponding to methaqualone were

negative for the drug by MS. Twelve of the 65 specimens found positive by both TLC and GC did not have peaks associated with methaqualone but were positive for monohydroxy metabolites. Methaqualone ingestion was therefore established by the presence of characteristic patterns of metabolites.

Chromatograms in Fig. 4 represent typical urinary methaqualone excretion patterns for the casual drug abuser. The time intervals between dose and urine collection are not known for Samples 4508 and 4526 that were obtained in the USAFE methaqualone screening project. Lack of documentation, however, does not affect recognition of similarities between these samples and the chromatograms of urine extracts obtained from controlled studies. The assignment of components 1 through 6 for methaqualone and metabolites is consistent with the labeling of peaks in the chromatograms of the controlled time study. The peak areas and corresponding concentrations of methaqualone are again low when compared with the respective concentrations of monohydroxylated metabolites. Sample 4526 contained 1.24 μg methaqualone per ml of urine. This is more than two times the maximum concentration of methaqualone detected in studies involving volunteer ingestion of therapeutic doses of methaqualone. Since it is common practice for the drug abuser to consume multiple doses of methaqualone, it is not unusual to detect methaqualone at a concentration of 1 μg/ml of urine [*16,17*]. This, however, is still only a small percentage of methaqualone-related compounds detected in the acid hydrolyzed urine. The bulk of the dose was detected as monohydroxylated metabolites. In this case, each of the five monohydroxy metabolites is at least five times the concentration of methaqualone, with 24:1 calculated for the Metabolite III to methaqualone ratio. A concentration of 0.29 μg methaqualone per ml of urine was detected in sample 4508. The concentration of Metab-

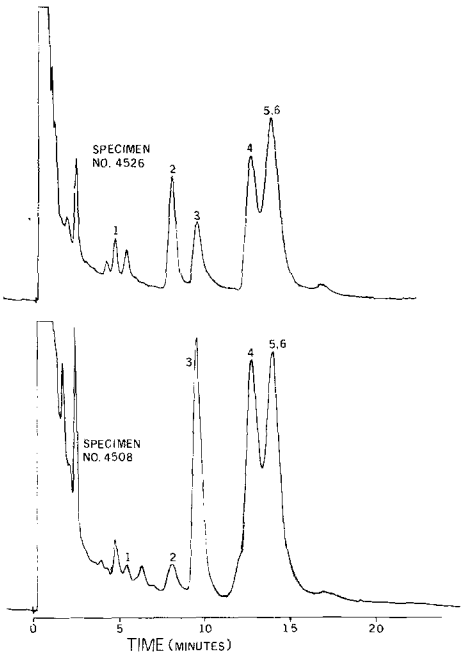

FIG. 4—*Gas chromatographic separation of urine extracts of specimens from methaqualone abuse screening project on a 1.9 m by 4 mm inside diameter column packed with 3% OV-1 on 80/100 mesh Supelcoport. Temperatures of column, flash heater, and detector were 210°C, 250°C, and 275°C, respectively. Peaks are identified as 1, methaqualone; 2, Metabolite V; 3, Metabolite I; 4, Metabolite II; 5, Metabolite III; and 6, Metabolite IV.*

olite I is 7.95 μg/ml urine or 27 times that of methaqualone. Both Metabolites II and III are prominent components of the chromatogram, with concentrations even higher than Metabolite I. A ratio of over 90:1 is calculated when the concentrations of these metabolites are compared with that of the unchanged drug.

An OV-17 column was used to confirm identification of methaqualone metabolites. A chromatogram of hydroxy metabolites as TMS derivatives is shown in Fig. 5. Note that thirteen components of the chromatogram of this reaction mixture are associated with methaqualone ingestion and subsequent metabolism. Many of these are highly polar disubstituted hydroxy and hydroxy-methoxy metabolites that are difficult to chromatograph as the underivatized components. The TMS derivatization decreases interaction of the hydroxy metabolites with column material and enhances overall sensitivity. The difference in polarity between the free hydroxy Metabolites I and V is greater than the respective TMS derivatives. This is reflected in retention index data and decreased resolution between these compounds. A change in the order of elution from the column is also noted. The TMS ether of Metabolite I now elutes before the Metabolite V analog. Separation of the Metabolite II and III TMS ether derivatives, components 6 and 7, is adequate for qualitative identification.

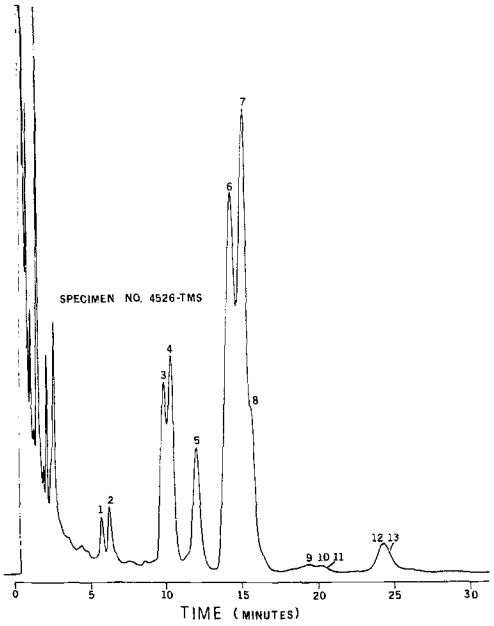

FIG. 5—*Gas chromatographic separation of a TMS derivatized urine extract of a specimen from methaqualone abuse screening project on a 1.9 m by 4 mm inside diameter column packed with 3% OV-17 on 80/100 mesh Supelcoport. Temperatures of column, flash heater, and detector, 225°C, 250°C, and 275°C, respectively. Peaks identified as 1, desmethyl derivative; 2, methaqualone; 3, Metabolite I; 4, Metabolite V; 5, Bonnichsen's metabolite X_t; 6, Metabolite II; 7, Metabolite III; 8, Metabolite IV; 9, dihydroxy metabolite; 10, dihydroxy metabolite; 11, hydroxy-methoxy metabolite; 12, dihydroxy metabolite; and 13, hydroxy-methoxy metabolite.*

Derivatized urine extracts were analyzed by SIM. The total ion plots of Sample 4526 (Fig. 6) indicate that some resolution was lost by passing the effluent from the OV-17 column through the single stage membrane separator, but the resolution was adequate for SIM. The TMS derivatives of all monohydroxylated metabolites studied have charac-

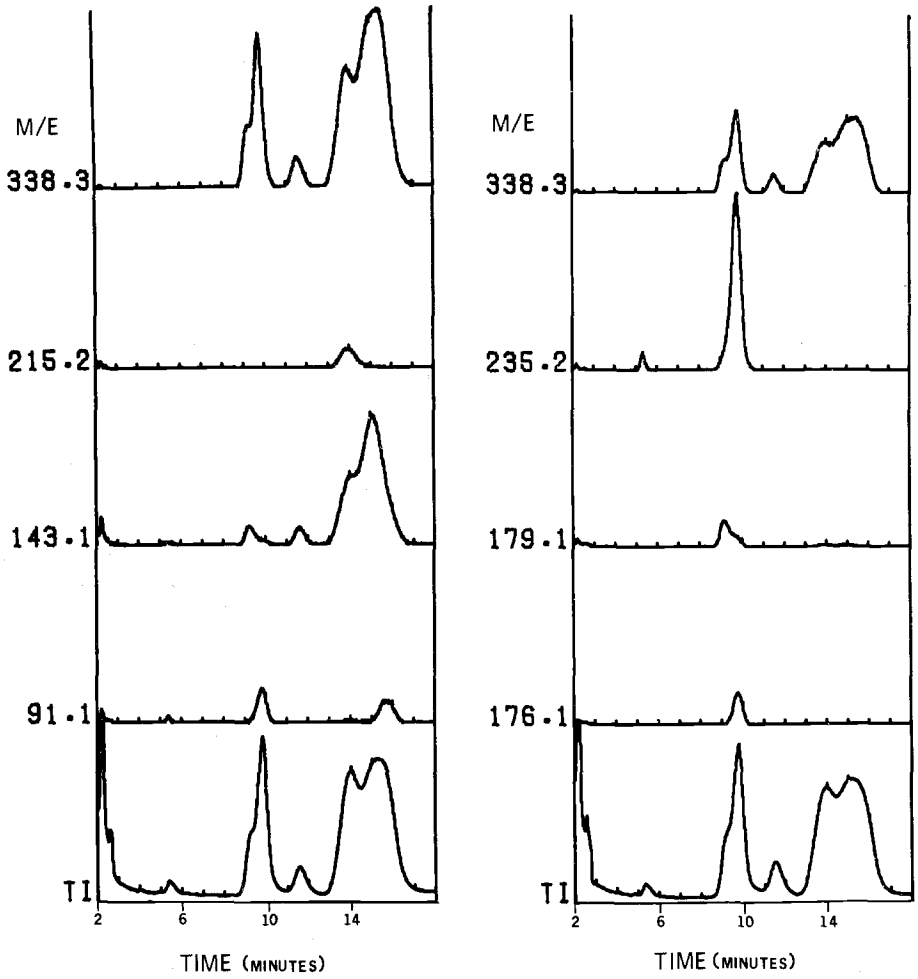

FIG. 6—*Selected ion monitoring of a TMS derivitized urine extract of specimen 4526 obtained from a methaqualone screening project, subsequent to gas chromatographic separation on a 1.5 m by 2 mm inside diameter column packed with 3% OV-17 on 100/120 mesh Gas Chrom® Q. Operating conditions: column temperature, 200°C, flash heater, 250°C; transfer line, 250°C; separator, 240°C; ion source, 180°C; mass filter, 80°C; ionization current, 300 μA; electron energy, 70eV.* Left panels: *total ion chromatogram; ions of* m/e *91.1, 143.1, 215.2, and 338.3.* Right panels: *total ion chromatogram; ions of* m/e *176.1, 179.1, 235.2, and 338.3.*

teristic molecular ions at *m/e* 338 with relative abundances greater than 50%. Ions of *m/e* 338, 235, 179, and 176 were selected for the identification of Metabolites I and V. The single peak at *m/e* 235 at 5.3 minutes suggests the presence of methaqualone. A peak of *m/e* 235 is also present in each of the electron impact fragmentation patterns of the hydroxymethyl metabolite TMS ethers (Metabolites I, V); detection of this peak, coupled with retention time data, is indicative of their presence in the sample. The specificity of SIM is demonstrated and these metabolites clearly identified by studying ions that are unique in the fragmentation of each compound. Metabolite V may be distinguished from Metabolite I by monitoring ions of *m/e* 176 and 179 that are prominent in their respective mass spectra. The magnitude of these ions is consistent with what would be expected from the quantitative data of the two metabolites.

Ions of *m/e* 91, 143, 215, and 338 were selected for identification of the TMS ethers of Metabolites II, III, and IV. The ion of *m/e* 143 is present in the spectra of Metabolites II and III but is insignificant in Metabolite IV; *m/e* 91 is a major fragment of the TMS ether of Metabolite IV, and although its concentration is much lower than its analog, Metabolite III, it is detected by mass spectrometry. The ion of *m/e* 215 is characteristic of the Metabolite II TMS derivative and is used to distinguish Metabolite II from III. The mass spectra of the TMS ethers of Metabolites II and III are very similar except for this ion and minor differences in the relative intensities of other ions.

Summary and Conclusions

It has been shown that methaqualone rapidly undergoes biotransformation to monohydroxylated metabolites, and concentrations of metabolites in urine are many times greater than the unchanged drug. Drugs at submicrogram concentrations are often difficult to discern from extraneous materials, and interferences from normal urinary constituents complicate the analysis. Retention indexes of blank urine constituents and methaqualone are so close that interpretation of quantitative and even qualitative data at these low concentrations is subject to considerable error. Gas chromatographic separation of monohydroxylated metabolites of methaqualone from urinary interferences has been demonstrated; metabolite patterns have been characterized and recognized as important for the identification of methaqualone ingestion.

Acknowledgments

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