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MASS SCREENING AND CONFIRMATION OF METHAQUALONE AND ITS METABOLITES IN URINE BY RADIOIMMUNOASSAY–THIN-LAYER CHROMATOGRAPHY

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SUMMARY

A sensitive, rapid, and specific procedure is described for the mass screening and confirmation of methaqualone (Quaalude) in urine specimens. The method is sensitive to 1.0 μg/ml levels of total methaqualone excretion products (free methaqualone, free hydroxylated methaqualone metabolites, and conjugated hydroxylated methaqualone metabolites). The raw urine is screened directly by radioimmunoassay, which is reactive to all the methaqualone excretion products. Specimens that are screened positive are confirmed by thin-layer chromatography using a solvent system of ethyl acetate–1,2-dichloroethane–chloroform (75:15:10) which separates methaqualone and its four major metabolites without interference from other drugs or urinary substances. The distinctive spot pattern produced by the methaqualone metabolites makes false positive results nearly impossible.

INTRODUCTION

The widespread use and abuse of methaqualone (Quaalude®) necessitates the development of a large-scale procedure for its analysis in urine specimens. A large urine drug testing laboratory must have a sensitive and specific method that can rapidly separate "true negative" from "presumptive positive" specimens. It is equally important to have a fundamentally different confirmatory method that can independently and accurately confirm the presence of methaqualone and its hydroxylated metabolites. Since the major excretion products of methaqualone are the glucuronide conjugates of the hydroxylated metabolites[1,2], the methods of analysis must be able to detect the more prevalent glucuronide conjugates as well as the free drug and free metabolite forms.

BACKGROUND

Methaqualone is very rapidly and extensively metabolized via hydroxylation. The four major metabolites are 2′-hydroxymethaqualone, 3′-hydroxymethaqualone, 4′-hydroxymethaqualone and 6-hydroxymethaqualone[1]. The major urinary excretion products of methaqualone are glucuronide conjugates of these hydroxylated metab-

olites[2]. Only small amounts of the free hydroxylated metabolites and only trace amounts of unchanged methaqualone itself are excreted[1–3].

Most laboratories use thin-layer chromatography (TLC) to screen urine specimens for various drugs including methaqualone[4,5]. The technique is relatively inexpensive, but for large scale analysis it has poor sensitivity for methaqualone due to the very limited excretion of the unchanged drug itself and due to the unextractability of the glucuronide conjugates of the hydroxylated metabolites. Although the reagents and equipment for radioimmunoassay are expensive, the consistency of results, ability to detect glucuronide forms, increased sensitivity, greater accuracy, and semi-automation of the method makes it well worth the cost involved.

Since too little unchanged methaqualone is excreted in the urine and since derivatization is required to detect the more prevalent hydroxylated metabolites[6], gas chromatography is not the most practical method for large-scale confirmation of the presumptive methaqualone positives found on radioimmunoassay. TLC, which can detect and separate the more prevalent hydroxylated forms without derivatization as well as unchanged methaqualone, is a very effective confirmation method. It is inexpensive and readily adapted to large-scale confirmation. Autoclaving to free the conjugated metabolites and thereby greatly increase the sensitivity, is readily incorporated into the procedure since fewer samples are confirmed than screened. Also since the urine specimen from a methaqualone-user will yield a very distinctive spot pattern with the ethyl acetate–1,2-dichloroethane–chloroform (75:15:10) solvent system, false positives are virtually impossible.

EXPERIMENTAL

Materials

The following materials were obtained from commercial sources: methaqualone radioimmunoassay kit (Roche Diagnostics, Nutley, N.J., U.S.A.) and ammonium sulfate, chloroform, isopropanol, hydrochloric acid, sodium hydroxyde, and methanol (Matheson, Coleman and Bell, Los Angeles, Calif., U.S.A.). All chemicals were reagent grade.

Equipment

The following equipment was used: an automatic pipetting station (Model 24004; Micromedic Systems, Philadelphia, Pa., U.S.A.), a centrifuge (Model K with 418 head; International Equipment Co., Needham Heights, Mass., U.S.A.), a γ-scintillation counter with printer (Model 5160; Packard, Los Angeles, Calif., U.S.A.), a high-speed automatic pipette (Model 25004F; Micromedic Systems, Philadelphia, Pa., U.S.A.), 50-ml round bottom centrifuge tubes with glass stoppers (Corning Glass Works, Corning, N.Y., U.S.A.), 40-ml centrifuge tubes with tabered ends (Corning), silica gel G TLC plates (VWR Scientific, Norwalk, Calif., U.S.A.), and TLC developing tanks (Brinkmann, Westbury, N.Y., U.S.A.).

Radioimmunoassay screening procedure

The urine specimens are screened for methaqualone using the Roche radioimmunoassay procedure[7] with the slight modification that automatic pipetting stations are used for all dilutions.

A specimen is recorded as presumptive positive for methaqualone if its

radioactive count is higher than the established cutoff value. The cutoff value is determined by checking all the methaqualone controls run that day and selecting a level so that all 0.5 μg/ml controls are detected. All specimens below the cutoff value are considered "negative". Specimens that give a radioactive count corresponding to 0.5 μg/ml or more of methaqualone are analyzed by TLC to confirm the presence of methaqualone metabolites.

TLC confirmation procedure

To 15 ml of each urine specimen screened positive by radioimmunoassay add 2 ml of concentrated hydrochloric acid. Autoclave the specimens for 15 min (15 p.s.i. and 121°) then cool them to room temperature in an ice-water bath. Add 4–5 pellets of sodium hydroxide and vortex the specimens until the pellets are dissolved. Adjust the pH of each specimen to 9.0 ± 0.2 by the dropwise addition of 6 *N* HCl or 6 *N* NaOH as necessary. Add 20 ml of 5% isopropanol–chloroform, then cap and shake each specimen for 15 min at slow speed on a horizontal platform shaker. After extraction, aspirate the urine (top) layer and filter the organic layer into a dry 40-ml conical centrifuge tube. Evaporate the organic layer to dryness in a 70° water-bath under nitrogen. Reconstitute the extraction residue with 0.1 ml of methanol and spot the solution on a silica gel G TLC plate. Develop the plate in an unsaturated TLC tank containing a solvent system of ethyl acetate–1,2-dichloroethane–chloroform (75:15:10). After development air dry the plate then spray it with acidified iodoplatinate to visualize the methaqualone metabolite spots. A positive specimen will produce spots for at least 2′-hydroxymethaqualone, 3′-hydroxymethaqualone, and 4′-hydroxymethaqualone. Larger positives will produce a spot for 6-hydroxymethaqualone and possibly even methaqualone itself.

RESULTS AND DISCUSSION

The radioimmunoassay–TLC system is suitable for screening and confirming methaqualone excretion products in urine specimens on a large scale. The procedure can consistently detect 1.0 μg/ml levels of methaqualone excretion products (unchanged, hydroxylated, and conjugated).

The radioimmunoassay screening method is considered superior to other screening techniques such as TLC. Methaqualone, 3′-hydroxymethaqualone, and 4′-hydroxymethaqualone are very reactive with the radioimmunoassay methaqualone antibody; 2′-hydroxymethaqualone is somewhat reactive; and 6-hydroxymethaqualone is weakly reactive[8]. Thus a 0.5 μg/ml radioimmunoassay cutoff easily allows detection of 1.0 μg/ml levels of methaqualone excretion products.

Each specimen found positive by radioimmunoassay is confirmed by TLC. Acid hydrolysis liberates the conjugated hydroxylated metabolites, substantially increasing the sensitivity obtained. Excellent separation of the four major metabolites and methaqualone itself are obtained with the ethyl acetate–1,2-dichloroethane–chloroform (75:15:10) solvent system without interference from other drugs, metabolites, or other urinary substances (Table I). The distinctive spot pattern produced by the methaqualone metabolites is such that it makes reporting false positives virtually impossible.

Two other solvent systems were tested and found to be usable for metha-

TABLE I

R_F VALUES OF METHAQUALONE METABOLITES AND OTHER DRUGS

Solvent systems: 1 = ethyl acetate–1,2-dichloroethane–chloroform (75:15:10); 2 = ethyl acetate–methanol–conc. NH_4OH (86:10:4); 3 = ethyl acetate–benzene–chloroform (40:40:20).

Substance	*Solvent systems*		
	1	*2*	*3*
Methaqualone	0.75	0.91	0.64
2′-Hydroxymethaqualone	0.35	0.87	0.15
3′-Hydroxymethaqualone	0.63	0.82	0.41
4′-Hydroxymethaqualone	0.56	0.76	0.30
6-Hydroxymethaqualone	0.48	0.71	0.28
Benzoylecgonine	0	0	0
Cocaine	0.13	0.91	0.12
Codeine	0.09	0.53	0
Methadone	0.07	0.93	0.05
Morphine	0.01	0.23	0
Nicotine	0.07	0.83	0.05
Phencyclidine	0.08	0.98	0.07
Propoxyphene	0.19	0.93	0.18

qualone confirmation, but not as good as the ethyl acetate–1,2-dichloroethane–chloroform (75:15:10) solvent system (Table I). The ethyl acetate–methanol–conc. NH_4OH (86:10:4) solvent system gives excellent separation of methaqualone and its metabolites, but methaqualone itself is interfered with by cocaine, propoxyphene, and methadone (Table I). The ethyl acetate–benzene–chloroform (40:40:20) solvent system eliminates interferences with methaqualone and gives fair separation of methaqualone and its metabolites, but the separation is not as good as with the other two solvent systems (Table I) and the solvent system contains benzene which is being seriously considered as a possible carcinogen.

By using the radioimmunoassay–TLC system a series of spiked urine specimens containing varying concentrations of methaqualone were analyzed. The data obtained showed above 99% accuracy at the 0.7 μg/ml level. Urine specimens spiked with various concentrations of other drugs (Table I) were tested and found to be negative with both radioimmunoassay and TLC.

This radioimmunoassay–TLC method offers a sensitive and reliable analysis procedure for ascertaining the presence or absence of methaqualone excretion products in urine on a large scale.

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