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QUALITATIVE DETECTION OF PLACIDYL (ETHCHLORVYNOL) ALONE OR IN COMBINATION WITH POLY-DRUGS IN DRUG ABUSE URINE SCREENING PROGRAMS USING ION-EXCHANGE PAPER AND/OR LIQUID–LIQUID EXTRACTION

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SUMMARY

Two procedures for the detection of ethchlorvynol are presented. Procedure I involves the use of SA-2 cation-exchange resin loaded paper. The use of multiple ion-exchange resin papers is proposed in a sequence that enables the detection of amphetamines, barbiturates, phenothiazines, propoxyphene (Darvon), phencyclidine, cocaine (benzoyl ecgonine), pentazocine (Talwin) and benzodiazepines in addition to ethchlorvynol. This procedure discusses the details of detecting ethchlorvynol in combination with the entire array of drugs of abuse depending on the needs of a clinical program. Procedure II involves the principle of liquid–liquid extraction using either raw or spent urines.

INTRODUCTION

A simple, rapid and reliable qualitative screening procedure has been developed to detect the abuse of Placidyl (ethchlorvynol) among drug dependent individuals. Ethchlorvynol, a non-barbiturate hypnotic, is a tertiary acetylenic carbinol, β-chlorovinyl ethyl ethynyl carbinol. The reports of its abuse resulting in intoxication, addiction, or death have been documented in ref. 1. Approximately 10% of the ingested drug is excreted unchanged in the urine within the first 24 h[2,3]. Ethchlorvynol can be tested qualitatively using Haux reaction[4] by treating with diphenylamine reagent or by treating with phloroglucinol and concentrated hydrochloric acid[5–6].

The proposed qualitative procedure for mass screening of urines in drug abuse prevention programs is a modification of the quantitative procedures of Haux[4] and Finkle and Bath[7]. Procedure I involves the use of cation-exchange resin loaded paper and procedure II involves the liquid–liquid extraction of raw urine.

EXPERIMENTAL

Procedure IA. Detection of ethchlorvynol only, using ion-exchange resin paper

SA-2 cation-exchange resin loaded paper is soaked in 20–50 ml of fresh

* Reprints are available at US $ 1.00 each.

undiluted urine and shaken for 20–30 min on a reciprocating shaker (Eberbach table model). The cation-exchange resin paper is then removed and transferred to a 4-oz. wide-mouthed screw-capped jar. The ion-exchange resin paper is rinsed with 10 ml of water (the rinsing being discarded) and then 5 ml of diphenylamine color reagent[4*] and 10 ml of chloroform (reagent grade) are added. The contents are shaken slowly for 10 min on a reciprocating shaker. The chloroform layer is allowed to separate and is then pipetted out and filtered through a chloroform wet non-absorbent cotton plug (using a small glass funnel) into a 15-ml non-graduated conical centrifuge tube. A teaspoon of anhydrous sodium sulfate is added to the filtrate to absorb any aqueous phase if carried over with the chloroform layer. The dried chloroform is decanted into another 15-ml non-graduated conical centrifuge tube. The chloroform extract is examined visually for pink coloration (chloroform should be rose to light pink colored depending on the concentration of the ethchlorvynol present).

For comparison of the color with that of unknown specimens it is desirable to carry standards of ethchlorvynol (0.5, 1 and 2 μg/ml) in 20–40 ml of controlled urine through the extraction procedure. The chloroform tubes which do not show pink coloration are placed in an oven having horizontal air flow maintained at 70°. The tubes are allowed to evaporate until 0.5 to 1.0 ml of chloroform is left. The tubes are then taken out, vortexed and the color is compared to the standards which have been subjected to the above extraction procedure. In case the tubes are left in the oven until completely dry, the addition of 0.5 to 1 ml of chloroform is recommended prior to vortexing and colour comparison.

Procedure IB. Detection of ethchlorvynol, poly-drugs, opiates and pentazocine using ion-exchange paper

Four procedures are presented for the detection of the various drugs of abuse depending on the needs of the clinical program:

(a) Polydrugs and ethchlorvynol. When the objective is to detect opiates (morphine, codeine and quinine), amphetamines, barbiturates, phenothiazines, phencyclidine (PCP), propoxyphene, benzoyl ecgonine in addition to ethchlorvynol, two ion papers are used in the following sequence. The first ion paper is soaked in 20–50 ml of fresh, undiluted urine and shaken for 20–30 min on a reciprocating shaker. The cation-exchange resin paper is then removed and transferred to a 4-oz. wide-mouthed screw-capped jar. The ion paper is rinsed with 10 ml of water (the rinsings discarded) and extracted at pH 10.1 using ammonium chloride–ammonium hydroxide buffer and chloroform–isopropanol (5:2) as described earlier by Kaistha and co-workers[8–12].

The aqueous buffer phase is retained and used for the extraction of benzoyl ecgonine as reported by Kaistha and Tadrus[11]. Spent urine is used for the detection of ethchlorvynol by soaking the second ion paper, shaking for 20–30 min and processing as described under Procedure IA for ethchlorvynol.

(b) Opiates, pentazocine, propoxyphene and phencyclidine and poly-drugs. When the aim is to detect mini poly-drugs (opiates, pentazocine, phencyclidine, propoxyphene) and ethchlorvynol, two ion-exchange papers are used in the same sequence

* A 200-mg amount of diphenylamine reagent grade is dissolved in 50 ml of concentrated sulfuric acid and this solution is added slowly to 50 ml of water, cooled to room temperature and stored in the dark at room temperature. This solution was found satisfactory for use over a 2-month period.

as explained above under procedure IB (a). The first ion paper is extracted as described above. The extracted residue is chromatographed using two-phase solvent systems. The plate is first developed in solvent D^{8-12}, where drugs such as pentazocine, methadone and its metabolite, propoxyphene and phencyclidine are separated and detected[13]. The plate is then dried and developed in solvent C^{8-12} for the separation and detection of opiates as described elsewhere by Kaistha and Tadrus[13]. The second ion paper is used for the detection of ethchlorvynol as described under Procedure IA.

(c) Ethchlorvynol, poly-drugs and pentazocine. Three ion-exchange papers are used when the aim is to test the entire array of drugs of abuse plus pentazocine and ethchlorvynol. The first ion paper is soaked alone in 20–50 ml of urine to absorb a wide variety of abuse drugs and processed as described under Procedure IB (a). The second and third ion papers are soaked together in the spent urine and shaken for 30 min. Both ion papers are removed and one is used for pentazocine detection as described by Kaistha and Tadrus[13]. The other ion-exchange paper is used for the detection of ethchlorvynol as described under Procedure IA.

(d) Ethchlorvynol, diazepam, chloridazepoxide and other benzodiazepines using spent urine. A 5-ml volume of the spent urine is used for the detection of ethchlorvynol using the liquid–liquid extraction procedure as described below (Procedure II).

The remaining spent urine 20–40 ml is used for the detection of benzodiazepines and morphine conjugates. The procedure involves acid hydrolysis as described by Kaistha and Tadrus[12].

Procedure II. Detection of ethchlorvynol using liquid–liquid extraction

To a 50-ml round-bottomed non-graduated centrifuge tube are added 5 ml of fresh or spent urine and 5 ml of diphenylamine color reagent. The contents are mixed and 5 min later, 10 ml of chloroform (reagent grade) are added. The tubes are shaken on a reciprocating shaker (Eberback tube model) at a low speed, and the method is continued as described under Procedure IA.

RESULTS

Confirmation of ethchlorvynol color product using thin-layer chromatography

Thin-layer chromatography is recommended when the color of the concentrated chloroform extract obtained under Procedures IA and IB is doubtful (reddish brown unstead of rose to pink). Those doubtful tubes are then vortexed and the entire extract of the unknown specimens is spotted on 20 × 20 cm Gelman precoated silica-gel glass-microfiber sheets (ITLC Type SA) with a layer thickness of 250 μm. The standard of ethchlorvynol (0.5 μg/ml of urine) carried through the assay procedure is spotted along with the unknown specimen. The plate is heated for 5 min in an oven maintained at 70° and is then placed in a developing tank containing 100 ml of chloroform. The plate is taken out after the spot has travelled a distance of about 11–12 cm, air dried for 10 min and then dried in oven at 70° for 5 min. A 100-ml volume of acetone–methanol (90:10) is placed in a developing tank and the plate is again redeveloped up to 11–12 cm in the same direction in this second solvent. The plate is taken out, air dried and is examined for pink color spots. The pink color spots of the unknown specimens and the standard should be at the same level (R_F value of major spot about 0.80 and minor spot R_F about 0.50). The purpose of developing the

plate in two separate solvents is to allow the colors due to impurities to travel first, and the color due to ethchlorvynol moves only in the second solvent.

DISCUSSION

The proposed procedures for the detection of ethchlorvynol are precise and reliable. Drugs such as chlorpromazine, trifluoperazine, thioridazine (mellaril), imipramine, amitriptyline and nor-triptyline were carried through assay procedure and no interference with ethchlorvynol detection procedure was observed up to 500 μg of the above drugs per ml of urine. The use of ion-exchange papers in the sequence discussed in procedures IB, a, b and c is very essential if the goal is to detect poly-drugs along with ethchlorvynol. Soaking two ion papers simultaneously in the same urine will cause uneven distribution of adsorption of minute concentrations of drugs. Thus the use of one of these papers for poly-drug detection will result in an unsatisfactory analysis. However, two ion papers can be soaked simultaneously if the aim is to detect pentazocine and ethchlorvynol only. Studies conducted by these laboratories revealed that pentazocine and ethchlorvynol are present in sufficient amounts if second and third ion papers were soaked in the same urine containing 1 μg/ml of each.

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