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DETERMINATION OF PENTAZOCINE AND TRIPELENNAMINE IN BLOOD OF T'S AND BLUES ADDICTS BY GAS–LIQUID CHROMATOGRAPHY WITH A NITROGEN DETECTOR

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SUMMARY

A procedure for the quantitative determination of pentazocine (T's) and tripelennamine (Blues) in blood obtained from T's and Blues addicts is described. The underivatized drugs were analyzed by gas–liquid chromatography with a nitrogen detector. The retention times relative to mepivicaine (internal standard) on OV-17 at 220°C were: tripelennamine 0.69 and pentazocine 1.77. The linear ranges of blood standards were: tripelennamine, 0.10–1.00 μg/ml; pentazocine, 0.50–5.0 μg/ml. For simultaneous analysis, the within-run and between-run CVs of tripelennamine were 5.6% ($n = 23$) and 13% ($n = 12$); and for pentazocine 5.2% ($n = 23$) and 9.9% ($n = 12$). Mean recoveries over the range of standards were: tripelennamine, 103% ± 2.5% ($n = 12$); pentazocine 77.8% ± 3.6% ($n = 12$).

INTRODUCTION

During the past four years, the abuse of pentazocine and tripelennamine, known as "T's and Blues", has become epidemic among intravenous drug addicts in many of the major cities of the midwest United States[1–3]. Several procedures applicable to the detection of the drugs in urine have been reported[3–5]; however, to date, no method had addressed the simultaneous detection of the drug combination in blood. Methods described for the specific determination of pentazocine have utilized spectrofluorometry[6,7], gas–liquid chromatography (GLC)[8–11], mass spectrometry[12] and radioimmunoassay[13].

The spectrofluorometric method lacks specificity due to presence of pentazocine metabolites. The radioimmunoassay is not commercially available and the preparation of pentazocine antibodies is beyond the capabilities of most toxicology laboratories. The GLC procedures usually involve derivatization for electron-capture detection[8–10] and are designed to determine pentazocine in blood or plasma at normal therapeutic concentrations of 10–50 ng/ml. T's and Blues addicts routinely inject from 4–10 times the therapeutic dose of pentazocine, and obtain blood concentrations well above the therapeutic range. Methods for the specific detection of tripe-

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lennamine have included GLC–mass spectrometry for metabolite studies[14] or GLC and ultraviolet spectrophometry for a massive overdose[15].

This communication presents a GLC procedure using a nitrogen detector which has suitable sensitivity for simultaneous detection of tripelennamine and pentazocine in blood obtained from T's and Blues addicts. The method may be applied to clinical or post mortem blood specimens. The drugs are chromatographed underivatized.

EXPERIMENTAL

Reagents

Benzene was found satisfactory from a commercial source, distilled in glass (Burdick & Jackson Labs., Muskegon, MI, U.S.A.). Isopropanol (2-propanol) was redistilled. Carbonate buffer, pH 11, was prepared by dissolving 21.0 g of sodium carbonate and 420.0 mg of sodium bicarbonate in sufficient distilled water to make 1 l. The pH was checked and adjusted by the addition of solid carbonate or bicarbonate as necessary. All other reagents were analytical (AR) grade. All glassware was acid-washed with hydrochloric acid (1 mol/l), and then silanized with 2% dimethyldichlorosilane (Sigma, St. Louis, MO, U.S.A.) in benzene.

Standards

Stock standards, 1.00 g/l, were prepared by separately dissolving 11.42 mg of tripelennamine hydrochloride (TP) (Ciba-Geigy, Ardsley, NY, U.S.A.) and 11.29 mg of pentazocine hydrochloride (PZ) (Winthrop, New York, NY, U.S.A.) in 10.0 ml of methanol. Intermediate standards, 0.100 g/l, were prepared by dilution of 1.00 ml of each stock standard with 10.0 ml of methanol. Mixed standards in blood were prepared daily by adding the following combinations of intermediate standards to 10-ml volumetric flasks and diluting to volume with drug-free blood: 10 μl of TP and 50 μl of PZ; 20 μl of TP and 100 μl of PZ; 50 μl of TP and 250 μl of PZ; and 100 μl of TP and 500 μl of PZ. The resultant blood standards contained the following concentrations (μg/ml of blood): 0.10 of TP and 0.50 of PZ; 0.20 of TP and 1.0 of PZ; 0.5 of TP and 2.5 of PZ; and 1.0 of TP and 5.0 of PZ.

Stock internal standard, 10.0 g/l, of the internal standard was prepared by dissolving 115.0 mg of mepivicaine hydrochloride (Breon, New York, NY, U.S.A.) in 10.0 ml of methanol. An intermediate standard, 0.025 g/l, was prepared by dilution of 50 μl of the stock standard with 10.0 ml of methanol. The extracting solvent containing the internal standard was prepared by diluting 1.00 ml of the intermediate standard with 500 ml of benzene–2-propanol (9:1).

Gas chromatography

All analyses were performed on a Perkin-Elmer Sigma 2 Gas Chromatograph equipped with a nitrogen detector (Perkin-Elmer, Norwalk, CT, U.S.A.). The detector response was recorded and integrated with a Perkin-Elmer Sigma 10 Data Station. The detector bead adjustment was 5.0 (2.26 A). Chromatography was performed on a glass column (1.8 m × 4 mm I.D.) packed with 3% OV-17 on Chromosorb W HP, 80–100 mesh (Alltech Assoc., Arlington Heights, IL, U.S.A.). The column was conditioned by temperature programming from 50°C at 2°C/min to 350°C,

which was held for 12 h before being connected to the detector. The temperatures were: injection port, 250°C; column, 220°C; and detector, 275°C. Gas flow-rates and/or pressures were: nitrogen carrier gas, 30 ml/min, 100 p.s.i.g.; air, 26 p.s.i.g.; and hydrogen, 12 p.s.i.g.

Under these conditions, the retention times were: tripelennamine, 2.7 min; mepivicaine (internal standard), 3.9 min; and pentazocine, 6.9 min. The relative retention times (RRT) of tripelennamine and pentazocine to the internal standards were 0.69 and 1.77, respectively.

Procedure

Pipet 2.0 ml of blood (samples, bood standards, and drug-free blood as a blank) into 15-ml glass culture tubes with PTFE-lined screw caps. Adjust the pH to *ca.* 11 by addition of 2.0 ml of carbonate buffer. Add 10 ml of benzene–2-propanol (9:1) *containing the internal standard*, and extract for 5 min. Centrifuge (1000 *g* for 3 min) and transfer the solvent (upper) layer to a second set of 15-ml screw-capped tubes. Add 5.0 ml of HCl (0.5 *N*), extract for 5 min, and after centrifugation, aspirate and discard the solvent (upper) phase. Add 0.5 ml of NaOH (5.0 *N*) to the acid extract to adjust the pH to 10–11 (check pH, and add extra NaOH dropwise if necessary), then add 5.0 ml of benzene–2-propanol (9:1) *without internal standard, extract* for 5 min and centrifuge. Transfer the solvent (upper) layer to a 10-ml test tube and dehydrate over anhydrous Na_2SO_4. Transfer the dried solvent to a 10-ml centrifuge tube add one drop of 1% ethanolic HCl and evaporate under dry nitrogen at 60°C to *ca.* 1.0-ml volume. Remove heat, and allow the remaining solvent to evaporate at room temperature under dry nitrogen. Dissolve the residue in 50 μl of methanol and inject 2–4 μl into the gas chromatograph. Rinse the syringe thoroughly between injections with HCl (1 *N*) mol/l followed by methanol to prevent carry-over. Routinely chromatograph methanol between every fourth analysis to check for carry-over or "ghosting".

Calculate the peak-area ratio (peak area of tripelennamine and/or pentazocine to that of mepivicaine internal standard) for each sample and determine the blood concentration by comparison to the peak-area ratios (relative peak areas) of the extracted standards.

RESULTS

Standard curves constructed from analyses of blood containing known concentrations of tripelennamine and pentazocine are presented in Fig. 1. The least-squares linear regression equations for tripelennamine and pentazocine were: y (peak area ratio, tripelennamine/mepivicaine) = $5.870x$ (tripelennamine, μg/ml) + 0.10, (r = 0.998); and y (peak area ratio, pentazocine/mepivicaine) = $0.9135x$ (pentazocine, μg/ml) − 0.22, (r = 0.999). The within-run coefficient of variation (c.v.) for tripelennamine (target concentration, 0.20 μg/ml) was 5.6% (n = 23) and that for pentazocine (target concentration, 1.0 μg/ml) was 5.2% (n = 23). Chromatograms of an extracted blood standard and blood obtained from a T's and Blues addict are presented in Figs. 2 and 3, respectively. The between-run C.V. calculated by three determinations on a single day for four successive weeks were: tripelennamine (target concentration, 0.20 μg/ml) 13% (n = 12); and pentazocine (target concentration,

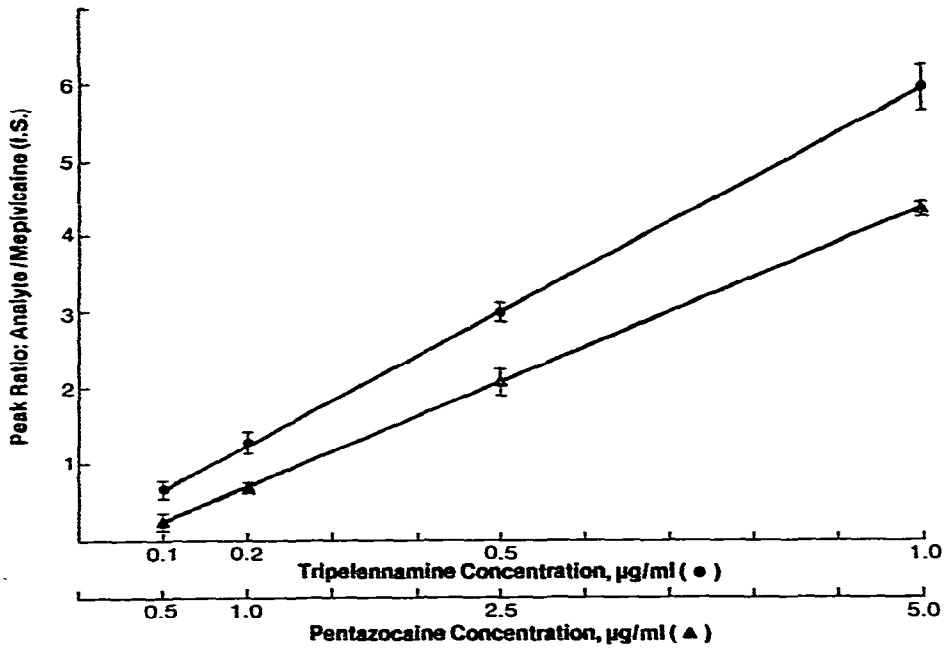
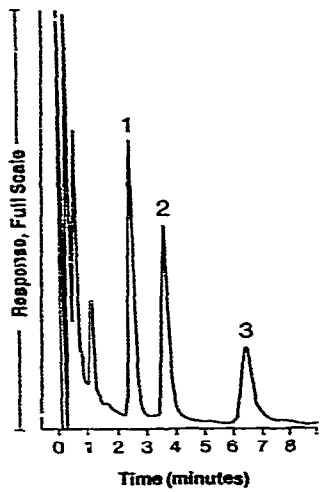


Fig. 1. Calibration curve for blood extracted standards of tripelennamine (●) and pentazocine (▲). Points represent the mean of three determinations, 2 S.D. in brackets.

0.50 µg/ml) 9.9% (n = 12). The absolute, uncorrected analytical recovery both for tripelennamine and pentazocine, calculated by comparison of peak areas obtained from supplemented blood samples with those of non-extracted standards, is pre-



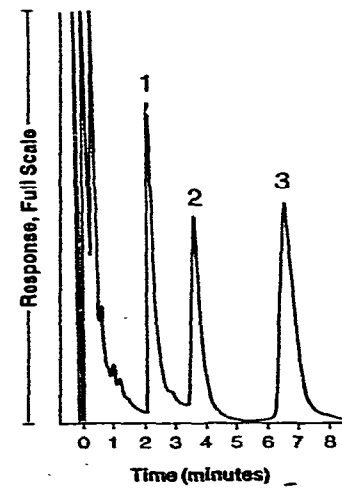


Fig. 2. Chromatogram of an extracted blood standard. Peaks: 1 = tripelennamine, 0.02 µg/ml; 2 = mepivicaine (I.S.); 3 = pentazocine, 0.50 µg/ml.

Fig. 3. Chromatogram of an extract of post mortem blood obtained from a T's and Blues addict. Peaks: 1 = tripelennamine, 0.20 µg/ml; 2 = mepivicaine (I.S.); 3 = pentazocine, 3.3 µg/ml.

sented in Table I. The mean recoveries over the concentration range of the standards for tripelennamine and pentazocine were: 103% + 2.5% and 77.8% + 3.6%, respectively. This incomplete recovery of pentazocine, as well as variations in aliquoting, were corrected by use of extracted blood standards and internal standard (mepivicaine).

TABLE I

RECOVERY OF TRIPELENNAMINE AND PENTAZOCINE ADDED TO BLOOD

Tripelennamine (μg/ml)			*Pentazocine (μg/ml)*		
Added	*Recovered**	*Recovery (%)*	*Added*	*Recovered**	*Recovery (%)*
0.10	0.105 ± 0.006	105%	0.50	0.405 ± 0.01	81%
0.20	0.21 ± 0.011	105%	1.0	0.780 ± 0.03	78%
0.50	0.50 ± 0.030	100%	2.0	1.59 ± 0.07	79.5%
1.0	1.02 ± 0.041	102%	5.0	3.64 ± 0.17	72.8%

* Mean ± S.D. ($n = 3$).

Thirty-two basic drugs which are commonly encountered in clinical or post mortem blood analyses (Table II) were screened for interference in the proposed assay by comparing the retention times of their non-extracted methanol solutions with that of tripelennamine, pentazocine and mepivicaine. Acidic drugs, as well as morphine and hydromorphone, were not screened because they would not be extracted under the alkaline conditions of the assay.

TABLE II

RELATIVE RETENTION TIMES (RRT) TO MEPIVICAINE OF 32 COMMON BASIC DRUGS SCREENED FOR INTERFERENCE

Drug	*RRT*	*Drug*	*RRT*
Amitriptyline	1.31	Medazepam	1.60
Cholordiazepoxide	4.48	Meperidine	0.30
Chlorpromazine	2.48	Methadone**	1.03
Cocaine*	1.70	Methaqualone*	1.69
Codeine	1.32	Nordiazepam	4.25
Chlorpheniramine***	0.70	Normeperidine	0.46
Desalkyl flurzazepam	3.50	Nortriptyline	1.55
Desipramine*	1.79	Oxazepam	2.24
Diazepam	3.06	Phencyclidine	0.44
Doxepin	1.56	Phendimetrazine	0.18
Flurazepam	6.30	Phenmetrazine	0.19
Haloperidol	>6.00	Propoxyphene	1.15
Imipramine	1.48	Procaine	0.90
Ketamine	0.53	Quinine	>6.00
Lidocaine	0.47	Thioridazine	>6.00
Methapyrilene***	0.68	Tranylcypromine	0.18

* Chromatographs as pentazocine.
** Chromatographs as mepivicaine.
*** Chromatographs as tripelennamine.

Cocaine, desipramine and methaqualone, when injected simultaneously with pentazocine, produced a single peak. While these compounds are not resolved under the conditions of the assay, in our experience they have not represented a significant problem. Under the conditions of the assay cocaine is seldom detected in blood except in rare instances of drug overdose and methaqualone is not co-administered with T's and Blues by local addicts. To date, no case of T's and Blues abuse has also involved these drugs. Methadone elutes with mepivicaine internal standard. While methadone is commonly used in the treatment of heroin addiction, its use in the treatment of pentazocine dependency is controversial, since methadone's addiction potential is greater than that of pentazocine[16]. While performing post mortem toxicological analyses in over 40 T's and Blues cases we have not encountered methadone in any case. Chlorpheniramine and methapyrilene elute as tripelennamine. However, chlorpheniramine is seldom present in blood in detectable concentrations, and methapyrilene has been removed from the pharmaceutical market in the U.S.A. by the Food and Drug Administration due to its potential as a carcinogen. The other basic drugs in Table I did not interfere with the assay.

DISCUSSION

When 2 μl of the extracted standards are injected, this method gives a linear response for tripelennamine and pentazocine over the range of concentrations presented, 0.10–1.0 mg/ml and 0.5–5.0 mg/ml, respectively. If the concentration of either drug in a test sample fell below the standards the chromatogram was repeated using 4 μl of extract.

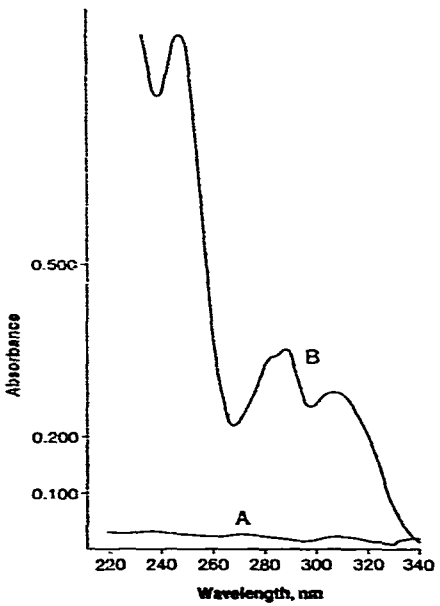


Fig. 4. Ultraviolet absorption spectrum of an extract (0.5 *N* H_2SO_4) of liver obtained from T's and Blues addict. (A) Blank of 0.5 *N* H_2SO_4 extract of drug-free liver; (B) characteristic mixed spectra of tripelennamine, 308 nm and 243 nm, and pentazocine, 288 nm.

T's and Blues addicts dissolve various ratios of tripelennamine and pentazocine tablets in water to produce an injectable solution[3]. Therefore there is a wide variation in the amount of each drug administered and the resultant blood concentrations. Concentrations of tripelennamine and pentazocine in blood obtained from T's and Blues addicts generally range from 0.1–0.5 μg/ml and 0.25–3.5 μg/ml, respectively. No constant ratio of tripelennamine to pentazocine has been observed in blood samples analyzed in our laboratory. In a few instances, extraction of a second blood sample with appropriate adjustment of the internal standard and concentration into 25 μl of methanol has been necessary to determine low blood concentrations of either drug. While the method is less sensitive than previous GLC pentazocine methods, it does permit simultaneous quantitative determination of both drugs at blood concentrations observed in T,s and Blues addicts.

The method is not presented as a general blood drug screening procedure. When available, urine should be analyzed prior to the blood analysis in order to determine the presence of other drugs[3–5]. For post mortem analysis when urine is not available, we recommend that drug screening be performed on liver specimens. Both tripelennamine and pentazocine may be readily determined in liver by direct extraction into diethylether[17]. Tripelennamine and pentazocine produce a characteristic mixed ultraviolet absorption spectrum in 0.5 *N* H_2SO_4 extract of liver (Fig. 4). The maxima at 306–310 nm and 238–243 nm are typical of tripelennamine, and the maximum at 286–289 nm is a slightly distorted pentazocine spectrum. The acid extract is back-extracted into organic solvent and subjected to thin-layer chromatographic analysis to resolve the mixture and/or confirm the presence of other basic drugs. If blood is the only specimen available, we routinely perform temperature-programmed GLC drug screening analysis on SE-30 liquid phase as described by Peel and Perrigo[18,19]. Those drugs which may interfere with the presented T,s and Blues procedure (chlorpheniramine, cocaine, desipramine, methadone, methapyrilene and methaqualone), if present, are readily detected and resolved from tripelennamine and pentazocine by any one of these screening methods.

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