Drug Screening of Basic Drugs in Blood Applied to Forensic Toxicology

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Abstract: Drug screening is one of the most important operations in systematic toxicological analysis. Laboratory analyses including sampling, extraction, separation and detection procedures are crucial to the diagnosis of poisoning. Determination of basic drugs from biological matrices was accomplished by using Bond Elut Certify columns and a gas chromatograph equipped with a nitrogen-phosphorus detector and a Hewlett-Packard HP-5 fused silica capillary column. The extraction procedure was found suitable for the extraction of basic drugs from plasma blood for GC-NPD analysis. The calibration curves were linear over the range of 1-17 mg l^{-1} with the confidence interval greater than 99.9%. The within and between day coefficients of variations (%CV) were less than 5% for codeine, dothiepin, mianserin, clomipramine and chlorpromazine. It is recommended for some of the drugs such as nitrazepam to be derivitised due to deterioration under heat within the column.

Key words: Drug screening · basic drugs · GC-NPD

INTRODUCTION

Drug screening is one of the most important operations in systematic toxicological analysis. Laboratory analysis is crucial to the diagnosis of poisoning [1-4]. The approach for basic drugs is initially to screen urine and/or gastric contents for the presence of compounds and their metabolites using a combination of capillary gas chromatography and thin layer chromatography [5-7]. Once identified, these compounds can be quantified in blood using alternative techniques. Analysis of urine may also be misleading, since the presence or absence of drugs and their metabolites neither confirms nor refuses recent overdosage. Drugs ingested as long as two weeks previously may be detected. An accurate drug measurement in blood is often essential to establish the correct diagnosis of poisoning [8, 9].

This paper presents an effective extraction and rapid gas chromatographic method for the accurate determination of the basic drugs in blood.

MATERIALS AND METHODS

Materials: N-butyl acetate and methanol were both HPLC grades. Analar grade 35% ammonia solution was diluted with distilled water and used as 4 molar solution. Dichloromethane, ammonia solution and isopropyl alcohol were analar grade. Phosphate buffer (100 mM, pH 6.0) was prepared by dissolving appropriate amounts of disodium hydrogen phosphate in distilled water and adjusting pH to 6 by adding phosphoric acid.

1.0 M acetic acid was prepared by diluting appropriate amounts of glacial acetic acid in distilled water. 1.0 M KOH was prepared by dissolving suitable amounts of KOH in distilled water. Pure sample of drugs and metabolites were purchased from manufacturers. The stock solutions of individual drugs were prepared by dissolving appropriate amount of each drug in methanol to make the solutions equivalent to 1 g l^{-1} for each drug. The internal standard, prazepam for the detection of benzodiazepines and butriptyline for the detection of tricyclic antidepressants and opiates, were prepared by dissolving 1g l^{-1} in methanol and then diluted to 10 mg l^{-1} in n-butyl acetate for use. Plasma samples were spiked with an appropriate amount of stock solutions. All solutions were stored in the dark at 4°C. These were stable for at least two years. Working standard solutions were prepared by dilution of the stock solutions with butyl acetate. Bond elute certify columns (130 mg of sorbent mass, 6 ml of column volume) were supplied by Varian®.

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Instrumentation: The analytical method used was a Varian model 3400 gas chromatograph equipped with a nitrogen-phosphorous detector. A Hewlett-Packard HP-5 fused silica capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness) was used. A Hamilton 701 syringe applied for injection. Operating temperatures were injection port 270°C, column and detector 300°C. Ultra pure helium CP grade was used as a carrier gas with a constant on-column pressure of 8 psi.

Sample preparation: Plasma was spiked with the appropriate drugs. Internal standard was added to the plasma. The plasma was then diluted with 1ml of phosphate buffer (pH 6.0). The tube was vortex mixed. The Bone elute Certify were preconditioned with 3 ml methanol. Addition of 3ml deionised water was followed by 1 ml of 100 mM phosphate buffer (pH 6.0). A low vacuum (=3 inches Hg) was used in the preparation step to prevent drying of sorbent. The pre-treated samples were passed through the preconditioned columns (1-2 ml min⁻¹). The columns were rinsed with 3 ml deionised water, 1 ml of 1 M acetic acid and 3 ml methanol. The columns were dried by vacuum for 5 minutes at = 10 inches Hg. The drugs were eluted by 3 ml dichloromethane/isopropyl alcohol/ ammonia solution (78:20:2). This solution was passed through the columns and collected at 1-2 ml/min. Finally, the eluate was evaporated in a gentle stream of nitrogen at = 40°C.

Calibration and Method Validation: Identification of drugs was made by their retention time and comparing these to the listed values shown in Table 1.

Calibration curves of the drugs were generated by least-square linear regression. They were constructed by plotting the peak area ratios of the drugs to internal standards. Construction of graphs of peak area ratio to the internal standard of known amounts of drug standard versus concentration enabled quantification of sample concentrations. Duplicate analyses were made and the mean results taken.

Precision was expressed as the relative standard deviation of the concentration values. The precision was calculated after analysis of the three replicates on the same day (within-day precision) and after repeated analysis over several days (between-day precision) [10, 11].

RESULTS

The retention times of more than 25 basic drugs are listed in Table 1. Using codeine, dothiepin, mianserin,

Table 1: Retention times of the basic drugs

Compound	RT(Min)	Compound	RT(Min)
Chlordiazepoxide	36.0	Thioridazine	43.0
Methamphetamine	10.2	Bupivacaine	32.1
D-propoxyphene	31.3	Imipramine	29.9
Dihydrocodeine	33.1	Dothiepin	33.5
Chlorpromazine	35.5	Mianserin	30.8
Trifluoperazine	36.6	Midazolam	35.0
Trimeprazine	32.5	Prazepam	35.6
Nortriptyline	31.0	Methadone	34.8
Clomipramine	34.0	Oxazepam	33.0
Trimipramine	31.1	Haloperidol	40.5
Flunitrazepam	36.7	Codeine	33.8
Desipramine	31.6	Cocaine	29.5
Pethidine	28.0	Diazepam	34.4
Butriptyline	37.7	Heroin	36.4

clomipramine and chlorpromazine as examples, calibration curves were linear over the range of 1-17mg l⁻¹ with the confidence interval greater than 99.9%. The within and between day coefficients of variation (%CV) were less than 5% for codeine, dothiepin, mianserin, clomipramine and chlorpromazine. Representative gas chromatograms from 2 cases which illustrate the value of the method in clinical and forensic toxicology are shown if Figures 1 and 2.

DISCUSSION

The drugs covered include tricyclic antidepressants, benzodiazepines, local anesthetics and opiates. In compare with liquid-liquid extraction, the lack of hazard solvents for the extraction procedure is an advantage of the method [12]. The sensitivity of the method is more than adequate for the purposes of clinical and forensic toxicology. In most cases a 500 μL sample gives a good response for medium to high therapeutic concentrations. The reproducibility of the retention data over time was excellent. The chromatograms of blank sample are very clean. There was no chromatogram for a few numbers of the drugs such as lorazepam, benzoyl ecgonine and nitrazepam. Partial decomposition of nitrazepam in the column is probably caused nitrazepam not to be detected. Since, the deterioration happens within the column, it causes poor sensitivity by gas chromatograph and high detection limits for the drugs. Ketazolam in converted to diazepam due to high temperature of the column [13, 14]. Derivitisation of these compounds can solve the problem. It increases
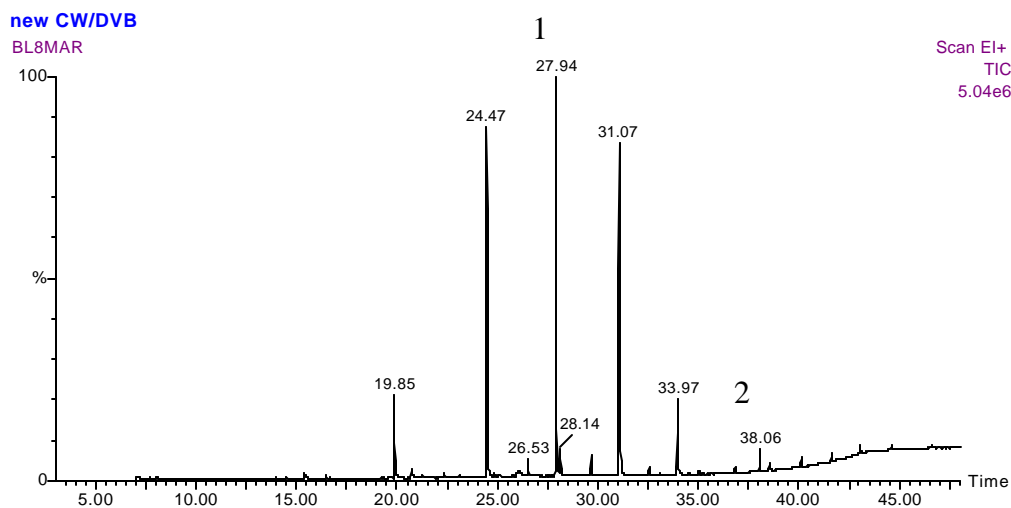
Fig. 1: Chromatogram from blood extract of a patient who admitted to hospital with a suspicious of pethidine overdose. Peaks are (1) pethidine and (2) prazepam (Internal standard). Pethidine 5.2 mg l^{-1} was present in the blood. The excessive amount of pethidine in the blood was probably the cause of death
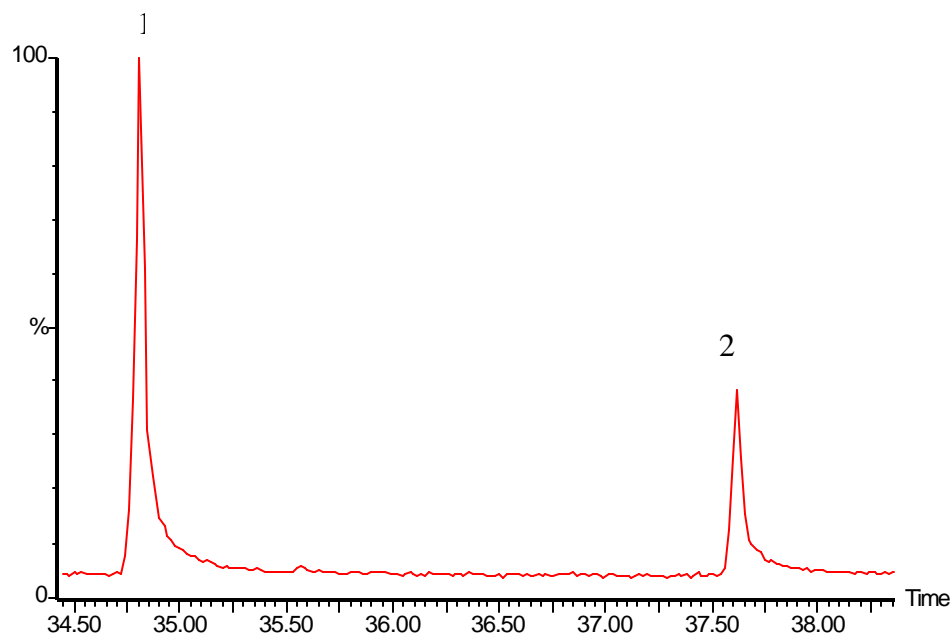

Fig. 2: Chromatogram from blood extract of an addicted man who took an overdose of methadone. Peaks are (1) methadone and (2) butriptyline (Internal Standard). Methadone 7.3 mg l^{-1} was present in the blood. The excessive amount of methadone in the blood was probably the cause of death

thermal stability and improve separation and detector response [15, 16].

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