

An automated SPE procedure for the simultaneous extraction of the majority of medicinal drugs that affect the mind

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INTRODUCTION: This study investigated the solid phase extraction (SPE) of antidepressant, antipsychotic and hypnotic type drugs at blood concentrations ranging from sub-therapeutic to fatal.

METHOD: A SPE method was developed for blood using Phenomenex Strata X™ polymeric sorbent cartridges coupled with the Zymark Rapid Trace™ SPE Workstation. 0.1M (pH 6) Phosphate buffer (5 mL) was added to whole blood (0.4 mL) and the solution sonicated for 15 minutes and then centrifuged for 10 minutes. The sample was then loaded onto a pre-conditioned Strata X cartridge which was rinsed with water (1 mL), 0.01M aqueous acetic acid (2 mL) and finally 5% aqueous acetonitrile (2 mL). The cartridge was dried for 3 minutes and the drugs were eluted using 0.01M acetic acid in acetonitrile (2 mL). The extracted fraction was divided into two parts. One part was analysed for benzodiazepine type drugs using LC/MS/MS in positive ion mode using a 10 mM ammonium formate/acetonitrile gradient with a Phenomenex Luna 3µ Phenyl Hexyl, 50 × 2 mm column. The remaining portion was analysed by GC/MS/NPD using a HP-5MS, 0.25µm column 30m × 0.32mm.

Recoveries of drugs representative of various drug types were determined at three concentration levels ranging from sub-therapeutic to above therapeutic by comparison with an external calibration.

RESULTS:

Drug	mg/L Actual	mg/L measured mean (n=5)	%CV
alprazolam	0.015 - 0.20	0.013 - 0.15	9 - 4
clobazam	0.20 - 2.6	0.23 - 1.9	10 - 3
clonazepam	0.017 - 0.23	0.019 - 0.15	38 - 7
diazepam	0.16 - 2.14	0.15 - 1.63	19 - 4
lorazepam	0.007 - 0.090	0.009 - 0.054	24 - 10
lormetazepam	0.003 - 0.046	0.002 - 0.034	16 - 11
midazolam	0.019 - 0.25	0.02 - 0.18	24 - 7
nitrazepam	0.019 - 0.26	0.014 - 0.22	20 - 4
nordiazepam	0.16 - 2.1	0.21 - 1.8	5 - 4
oxazepam	0.16 - 2.1	0.13 - 1.6	8 - 8
temazepam	0.12 - 1.6	0.12 - 1.1	7 - 3
triazolam	0.002 - 0.020	0.002 - 0.014	25 - 8
zopiclone	0.03 - 0.42	0.23 - 0.40	13 - 3

amitriptyline	0.17 - 2.3	0.14 - 2.0	5 - 3
citalopram	0.13 - 1.8	0.10 - 1.5	5 - 2
codeine	0.12 - 1.9	0.11 - 1.2	11 - 9
cyclizine	0.02 - 0.32	0.02 - 0.29	18 - 3
diphenhydramine	0.35 - 4.7	0.31 - 4.5	6 - 3
dothiepin	0.12 - 1.9	0.08 - 1.8	15 - 2
fluoxetine	0.13 - 2.0	0.10 - 1.9	12 - 7
methadone	0.16 - 2.4	0.14 - 2.2	7 - 2
nortriptyline	0.27 - 3.6	0.22 - 2.7	8 - 2
paroxetine	0.13 - 2.0	0.11 - 1.8	15 - 5
pheniramine	0.11 - 1.4	0.1 - 1.3	6 - 4
quetiapine	0.16 - 2.2	0.13 - 1.7	32 - 4
thioridazine	0.27 - 4.2	0.24 - 3.7	8 - 2
venlafaxine	0.23 - 3.1	0.21 - 3.1	3 - 1
pentobarbitone	2.0 - 27	1.8 - 21	11 - 13
phenobarbitone	1.7 - 22	1.6 - 11	18 - 11
thiopentone	1.2 - 15.6	1.0 - 6.7	18 - 14

The method was then used to determine semi-quantitative levels of drugs by comparison of the response of each drug to a common internal standard. Eleven drugs (pethidine, ketamine, tramadol, methadone, nortriptyline, lamotrigine, dothiepin, diazepam, paroxetine, thioridazine and quetiapine) were analysed at 2 levels (therapeutic and above therapeutic) using this approach. The CV ranged from 3% to 23% (n=5). The method was applied to forensic cases and results compared to those obtained using existing methodology. These results will be discussed.

DISCUSSION: The results obtained showed that the method efficiently extracted a wide variety of drugs in blood over more than a ten-fold concentration ranging from sub therapeutic levels. The method enabled the semi-quantitative analysis of drugs that currently require three extraction methods.

CONCLUSIONS: This single extraction method proved to be a useful tool for screening a wide range of drugs for forensic cases ranging from drug-facilitated sexual assaults to drug-related fatalities.

KEYWORDS: *SPE, Medicinal drugs that affect the mind, Blood*

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