

QUANTIFICATION OF BUPRENORPHINE IN POST-MORTEM BLOOD BY TWO-DIMENSIONAL GAS CHROMATOGRAPHY – MASS SPECTROMETRY

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INTRODUCTION

Buprenorphine is a semi-synthetic opioid. It is chemically similar to morphine but its potency is up to 40 times greater (1). It has been used successfully for the management of moderate to chronic pain and for opioid dependency treatment. Some analysts classify less than 5 ng/mL in plasma as therapeutic while others suggest 2 – 20 ng/mL (2,3).

There has been a substantial rise in illicit availability of buprenorphine on the black market (4). It has been documented that even at therapeutic concentrations buprenorphine can be fatal (5).

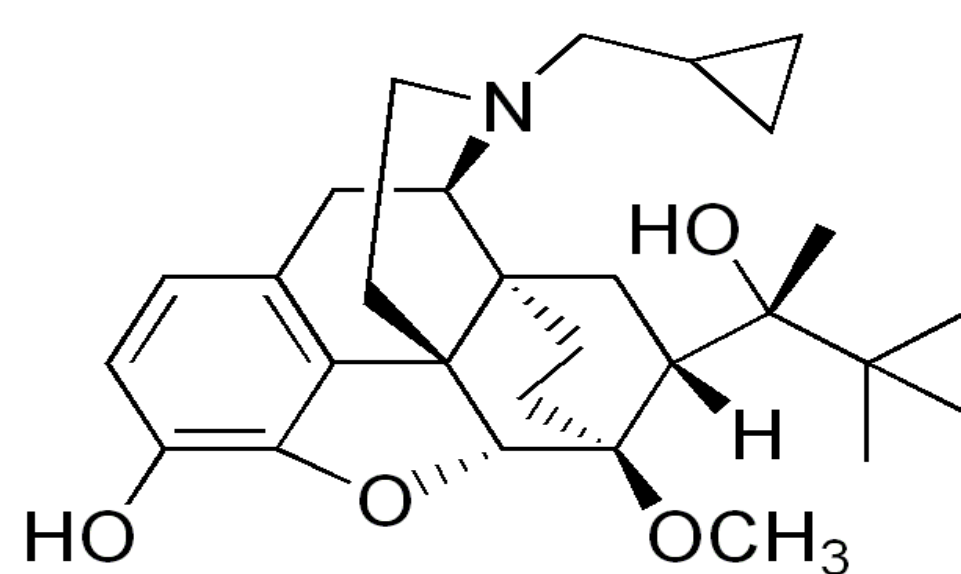


FIGURE 1: Buprenorphine

AIM

To validate a method for the quantification of buprenorphine in post-mortem blood using solid-phase extraction and two-dimensional gas chromatography-mass spectrometry (2D GC-MS) and to demonstrate method applicability to authentic post-mortem blood samples.

METHOD

Six point calibration curve in whole blood: Blank, Zero, 1.0 – 50.0 ng/mL
Internal standard: Buprenorphine-d₄

Sample preparation consisted of a two-step protein precipitation process using acetonitrile and adjusting blood pH to 5 using a phosphate buffer. Buprenorphine was purified using mixed mode (C8/SO₃⁻) solid-phase extraction cartridges. Endogenous water-soluble compounds and lipids were removed from the cartridges using firstly a water wash and then a hexane wash. Samples were eluted using chloroform:isopropanol: ammonia (80:20:2) mix. The samples were then concentrated and derivatised using a trimethylsilyl reagent. The samples were analysed using a 2D GC-MS (6).

INSTRUMENTATION: 2D GC-MS

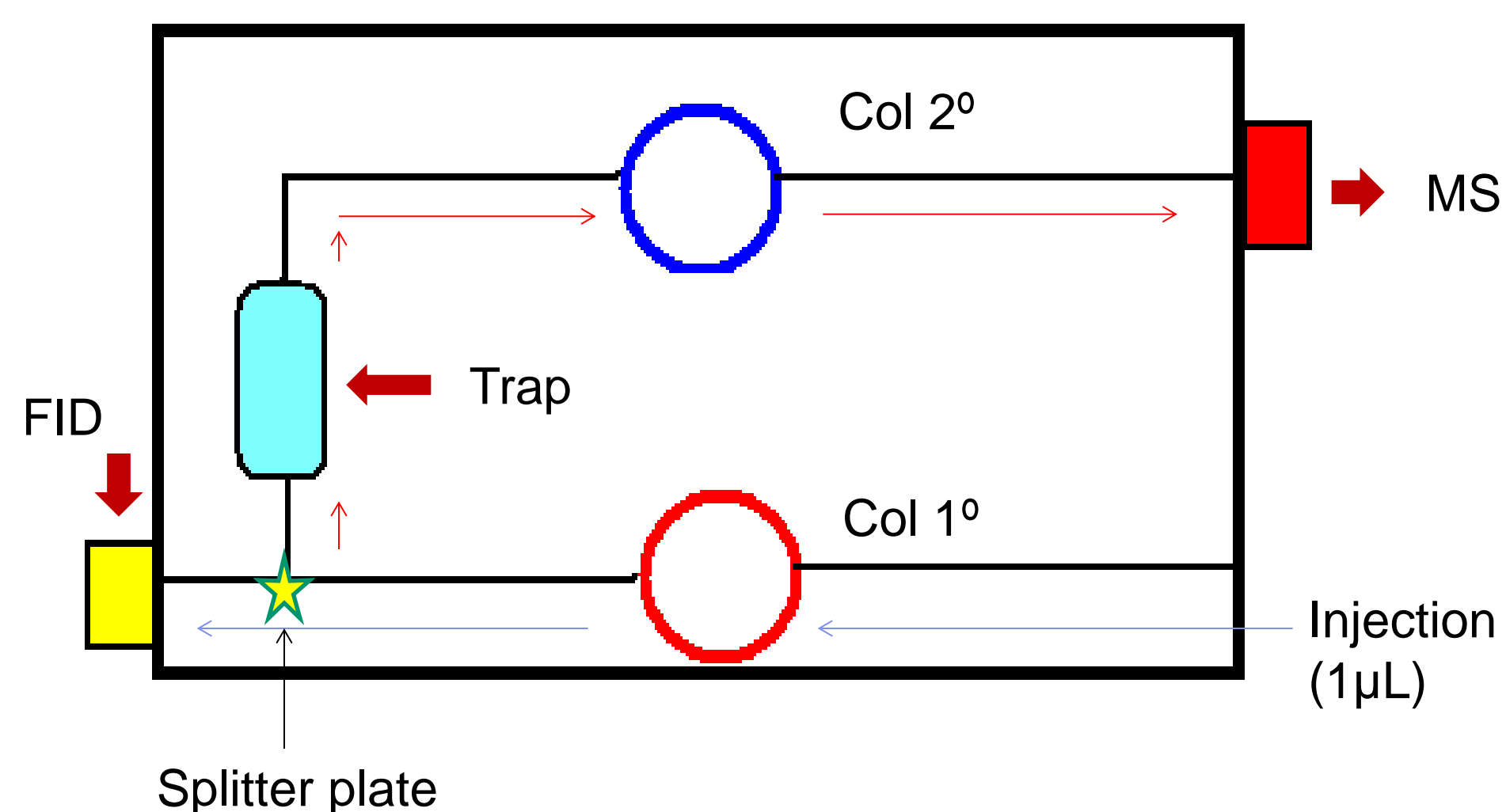


FIGURE 2: 2D GC-MS (Agilent Technologies)

The GC oven contains two columns of different polarity. Compounds that do not separate on the 1^o column may do so in the 2^o column. Only the analytes of interest are “cut” from the 1^o column on to the 2^o column and enter the MS. Endogenous unwanted matrix is removed through the FID and does not enter the MS. Sensitivity and selectivity for the analytes are therefore enhanced.

RESULTS AND DISCUSSION

The assay was linear from 1.0 - 50.0 ng/mL ($r^2 > 0.99$). Intra-day and inter-day imprecisions (%RSD) were less than 5% and the average recovery was 60%. The limit of detection was 0.5 ng/mL and limit of quantification was 1.0 ng/mL.

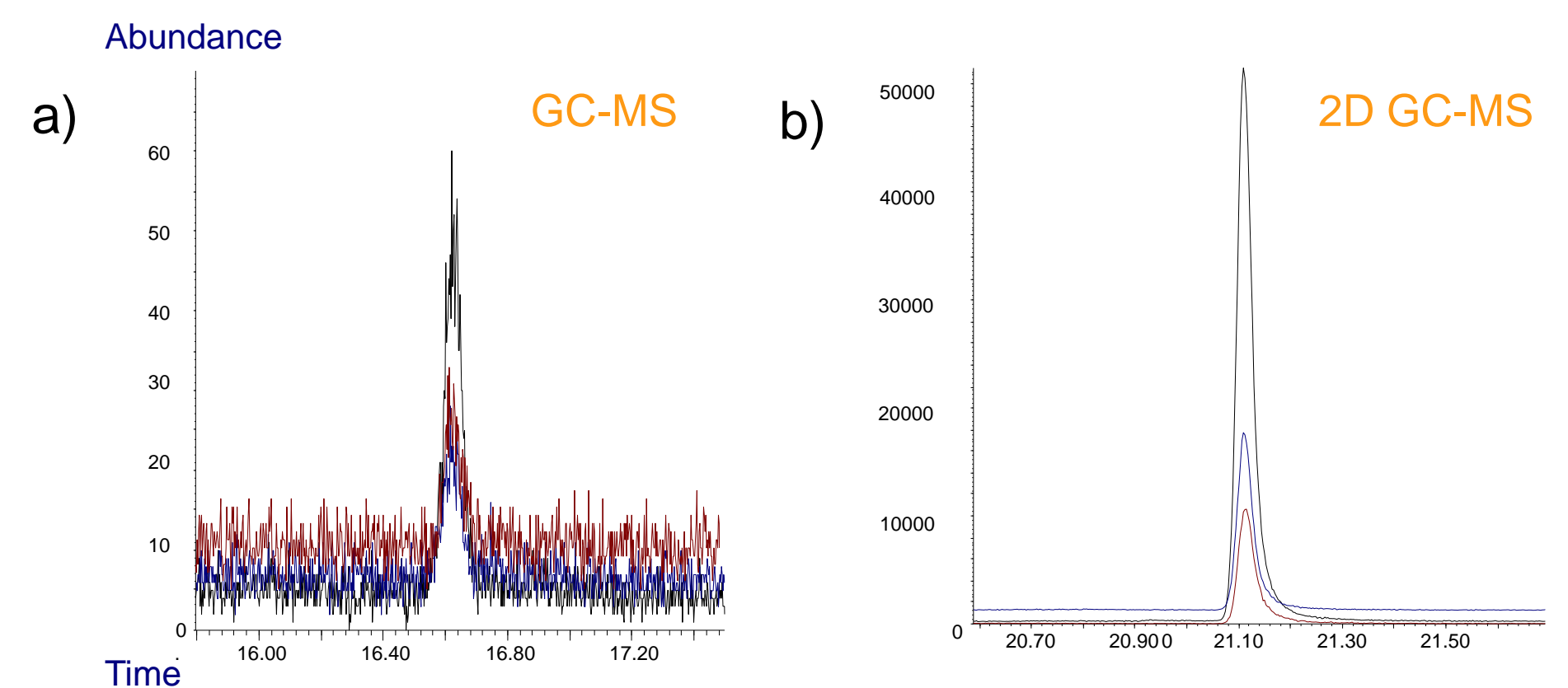


FIGURE 3: Extracted ion chromatogram of derivatised buprenorphine at 10 ng/mL (extracted from blood) analysed on a) GC-MS and b) 2D GC-MS

2D GC-MS improved the limit of detection for buprenorphine by 20-fold compared to analysis on a conventional GC-MS

TABLE 1: Buprenorphine concentration in post-mortem case bloods

	History	Buprenorphine concentration (ng/mL)	Other drugs concentration (µg/mL)	Ethanol (mg%)
1	Hanging	43.0	Morphine: 0.05	<10
2	Mental health issues	16.0	Sertraline: 1.35 Prochlorperazine and Chlorpheniramine: Therapeutic	<10
3	Possible overdose	11.0	Dothiepin: 6.71, Fluoxetine: 0.40	159
4	Illicit drug user	8.3	Cocaine: recreational use Quetiapine: Therapeutic	67
5	Illicit drug user	7.8	Morphine: 0.48, Codeine: 0.03 6-acetylmorphine: 0.02 Diazepam and Nordiazepam: low therapeutic	142
6	Sudden death	5.0	Carbamazepine: therapeutic	13
7	Illicit drug user	4.2	Morphine: 0.87, Codeine: 0.05 Cocaine: recreational use Amitriptyline: therapeutic	13
8	Mental health issues	4.1	Promethazine: 5.60, Paracetamol: 284 Dothiepin: high therapeutic	114
9	Illicit drug user	4.0	Cocaine: recreational use, Lignocaine: positive Amitriptyline, Citalopram and Olanzapine: High therapeutic	35
10	Illicit drug user	3.8	Dihydrocodeine: 5.40, Morphine: 0.03 Quetiapine, Diazepam and Nordiazepam: therapeutic	<10
11	Illicit drug user On buprenorphine tablets	3.0	Morphine: 0.02 Diazepam and Nordiazepam: therapeutic Cocaine: recreational use	<10
12	Illicit drug user	2.3	Morphine: 0.39, Codeine: 0.05	127
13	Found collapsed	2.0	None	<10
14	Illicit drug user	1.8	Cocaine: recreational use, Paroxetine: therapeutic	<10

Concentrations of buprenorphine in 14 post-mortem blood samples ranged from 1.8 – 43.0 ng/mL.

CONCLUSION

2D GC-MS demonstrated increased sensitivity for buprenorphine analysis compared to conventional GC-MS. We report a simple, efficient and cost-effective solid-phase extraction 2D GC-MS method for the quantification of buprenorphine in post-mortem blood. 2D GC-MS is a complementary method to both conventional GC-MS and LC-MS/MS.

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