Determination of the “legal high” 4-methylethcathinone (4-MEC) in blood by LC-MS/MS and report of a 4-MEC related death

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Introduction

4-Methylethcathinone (4-MEC) is a synthetic derivative of the pharmacologically active alkaloid cathinone (Figure 1) and is structurally similar to mephedrone (4-methylmethcathinone), which has been linked to a number of fatalities in the U.K.1,2 This has led to cathinone derivatives being controlled as Class B substances under the Misuse of Drugs Act in April 2010. Although there is limited clinical data on 4-MEC, information on related cathinones would suggest that 4-MEC acts via inhibition of the monoamine transporters for dopamine, serotonin and noradrenaline. The reported common clinical signs and symptoms associated with cathinone toxicity include agitation, vomiting, sweating, tachycardia, palpitations, seizures and cardiopulmonary arrest. The aim was to develop a quantitative method for 4-MEC in blood by LC-MS/MS. This has not previously been reported in the literature.

![Figure 1: Structures of cathinone, mephedrone and 4-MEC](image1)

Sample Preparation

- Samples were spiked with internal standard (d3-mephedrone), buffered to pH 6 followed by vortexing, sonication and centrifugation
- Samples underwent solid phase extraction (Strata Screen-C) with elution by chloroform / isopropanol / ammonia at low speed (1 - 2 mL/min)
- Samples were concentrated under nitrogen at 60°C
- Samples were reconstituted with 10% methanol and transferred to LC-MS/MS

LC-MS/MS Conditions

Chromatographic separation was carried out on an Agilent Technologies Zorbax SB-C18 column with gradient elution (glacial acetic acid/water/acetic acid/methanol) at a flow rate of 0.6 mL/min. Analytes were detected on an Agilent Technologies 6140 Triple Quadrupole operated in multiple reaction monitoring (MRM) mode with ESI positive ionisation. Parameters for MRM transitions were designated as follows (Figure 2). Fragmentation voltages were 100V and 90V for 4-MEC and internal standard respectively. Collision energy was 20V and dwell times were 50ms.

![Figure 2: MRM transitions used in 4-MEC quantitative method.](image2)

Case Report

A 22 year old male was thought to have consumed alcohol and cannabis and have insufflated an unknown quantity of 4-MEC at a friend’s house. A short time later he suddenly collapsed, started to fit and coughed up blood. Resuscitation was attempted by trained staff both at the scene and in hospital. He was pronounced dead approximately 4.5 hours after taking 4-MEC.

Toxicological analysis of femoral venous blood revealed ethanol (30 mg/dL), 4-MEC (167 ng/mL) and a sub-therapeutic level of paracetamol (5 mg/L). For comparative purposes the UK driving limit for ethanol in blood is 80 mg/dL. Ethanol, 4-MEC and paracetamol were also detected in urine. Following a complete autopsy, no natural disease or injury which could have caused, contributed to or accelerated death were identified.

Conclusions

A quantitative LC-MS/MS method for the detection of 4-MEC in blood is described. 4-MEC was quantitated in a fatal case, where death was attributed to 4-MEC toxicity due to the presence of the drug and the absence of an alternative cause of death. To our knowledge there have been no fatalities due to 4-MEC previously reported. The measured 4-MEC concentration in the above case may assist in the interpretation of blood 4-MEC levels in future cases of suspected 4-MEC related fatalities.

References