HPLC-MS-MS Methods for the Detection of the Khat Constituents: Cathinone and Norephedrine/Norpseudoephedrine (Total) in Blood and Urine

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BACKGROUND

Khat (Catha edulis Forsk), also known as Quat, Qat and Chat, is a psychotropic herbal drug, cultivated in Eastern Africa and the Arabian Peninsula. Khat is a leafy evergreen shrub that can grow to a height of up to 20 feet. Its leaves contain cathinone and cathine (norpseudoephedrine), which produce amphetamine-like stimulant effects. The more pharmacologically active component, cathinone, which has half the potency of amphetamine, is unstable and breaks down to the less active cathine and norephedrine. The effects of khat are therefore greatest when the leaves are chewed fresh, within 48 hours of picking.

To preserve its potency khat is usually sprinkled with water and packaged in plastic bags or wrapped in banana leaves [Figure 1]. The leaves and bark of the plant are sometimes smoked or brewed into tea and are also sold as a chewable paste, dried or crushed or in powdered form. A small bunch of leaves usually costs around £4. Khat chewing is usually a social affair and in a typical session between 50 and 200 grams of leaves are chewed over a number of hours.

Chewing leads to feelings of euphoria and makes the user feel more alert and talkative. Khat suppresses appetite and can lead to a state of calm when chewed over several hours. Recent research conducted at King's College in London suggests that the phenylpropylamine alkaloids present in khat can boost mens' fertility. However, prolonged use may lead to impotence, insomnia, anxiety, aggression and worsening of any pre-existing mental health problems. Inflammation of the mouth and oral cancer may also result from overuse. Regular use can lead to some psychological dependence.

As khat loses its potency fairly rapidly after being harvested its use has largely been confined to those countries where it is grown. However khat chewing is becoming more common in the UK due to migration and because of air transportation. Whilst khat is a controlled substance in countries such as America, Canada, Norway, Sweden and Switzerland it is not yet controlled by law in the UK. However, its active ingredients cathinone and cathine are classified as class C drugs under the Misuse of Drugs Act 1971. For these reasons it is important for forensic toxicology laboratories to be aware of its use and have methodology in place for its detection.

Figure 1. Khat is usually sold wrapped in banana leaves to keep the leaves moist and fresh.

EXPERIMENTAL

In April 2004 Cardiff Bioanalytical Services Ltd, circulated a UKNEQAS blood sample containing cathinone, cathine and norephedrine for quantitative toxicology. Only one participant returned a result for each analyte, suggesting that most laboratories either do not routinely screen for khat or do not have methodology in place for its detection. We describe an HPLC-MS-MS method for the detection of the khat constituents in cathinone and norephedrine/norpseudoephedrine (total) in blood and urine. The UKNEQAS blood sample was analysed for comparison against the spike values and previously reported results. A urine sample was donated by a volunteer who chewed 15g of previously frozen khat leaves for 10 minutes and ingested the juice, but not the residue. Urine samples were collected at 0, 3.5, 6.5, 8.5, 28 and 43 hours post dose.

Materials

Cathinone and norephedrine were kindly provided by Cardiff Bioanalytical Services Ltd. Bupivicaine was obtained from Sigma-Aldrich. HPLC grade acetonitrile and methyl-tert-butyl-ether (MTBE) were purchased from Rathburns Chemicals Limited (Walkerburn, Scotland). Analar grade formic acid and sodium hydroxide (40% solution) were obtained from BDH (Poole, Dorset, England). De-ionised water was prepared on site (ELGA Limited).

Extraction

The simultaneous extraction of the khat constituents: cathinone and norephedrine/norpseudoephedrine (total) involves liquid-liquid extraction of 100µL of sample into MTBE (1.5mL) after the addition of 100mmol sodium hydroxide (250µL). 250µL of Bupivicaine (1:0.25, cathinone:bupivicaine) is used as internal standard. The samples are mixed (approx. 5 minutes) and then centrifuged (3500rpm, 5 minutes). Cathinone and norephedrine/norpseudoephedrine are then back-extracted into 0.1% formic acid (250µL). The extracts are transferred to autosampler vials and left to stand for 5 minutes to allow any residual MTBE to evaporate, prior to analysis. 50µL of extract is injected onto the HPLC-MS-MS.

HPLC Conditions and MS Parameters

The HPLC equipment consisted of a Perkin Elmer PE200 series autosampler and pump and a Schimadzu CTO-10A column oven. Detection was by tandem mass spectrometry (HPLC-MS-MS), using a Sciex API2000 triple quadrupole mass spectrometer equipped with a turbo-ion spray interface (Applied Biosystems). The analytical column used was a Supelcosil LC-Si, 10cm x 4.6, 5µm and the mobile phase used was acetonitrile:de-ionised water:formic acid (50:50:0.1, v/v/v). The method was run in positive ionisation mode and set to detect the precursor and product ion transitions of cathinone (m/z: 149.9/131.9) norephedrine/ norpseudoephedrine (m/z: 151.9/134.1) and bupivicaine (m/z: 289.1/140.1). Cathinone, norephedrine/norpseudoephedrine and bupivicaine eluted after 4.6, 4.4 and 4.7 minutes, respectively.

Concentrations of both analytes could be detected at 20ng/mL.

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RESULTS

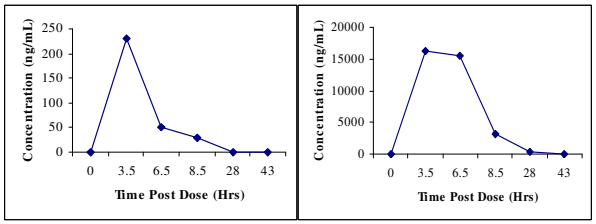

Figure 2. The measured urinary concentrations of cathinone (left) and norephedrine/norpseudoephedrine (total) (right).

Urinary cathinone and norephedrine/norpseudoephedrine peaked between 3.5 and about 6 hours. The concentration of norephedrine/norpseudoephedrine greatly exceeded that of cathinone and is representative of both that present in the khat leaves and that produced via metabolism of the ingested cathinone.

The circulated UKNEQAS blood sample was spiked with 130.5, 70.4 and 90.8ng/mL of cathinone, cathine and norephedrine respectively. The only centre to return results reported 42.2, 121.0 and 140.0ng/mL. Using the methodology described here we obtained results of 52ng/mL for cathinone and 208ng/mL for norephedrine/norpseudoephedrine (total). The differences in the spike value and measured concentrations is likely to be attributable to enzymatic breakdown of cathinone to its metabolites.

CONCLUSION

The weight of khat and the duration of consumption investigated here is significantly less that that which would be consumed in a typical khat session. The khat leaves had also been frozen previously, which is likely to have lead to further degradation of the active ingredient. Despite this, cathinone and its metabolites were detectable in urine over a number of hours post dose. The methodology presented is sensitive and sufficient for the detection of the constituents of khat in both urine and blood, and has also since been effectively applied to post mortem blood samples.

Khat chewing is becoming more popular in the UK due to migration. There are a number of concerns about the long term health risks of using this currently legal drug. A recent Home Office survey of 600 Somalis living in London, Birmingham, Bristol and Sheffield, showed that 49% would support moves to make khat illegal. Given the rise in usage and the abuse potential of this herbal drug the investigators feel that toxicology laboratories should have in place methodology for its detection.

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