B138 Identification of Principal Components in Khat Leaves (Catha edulis) Using Liquid Chromatography Atmospheric Pressure Electrospray Ionization Mass Spectrometry

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After attending this presentation, attendees will understand the methodology for the identification of the two principal components of khat: cathinone and cathine using LC/MS

This presentation will impact the forensic community and/or humanity by demonstrating a new LC/MS application that will assist the forensic drug chemist in identifying controlled compounds in khat leaves. Present methodologies make conclusive identification especially GC/MS difficult, requiring derivatization to confirm cathinone and cathine.

A liquid chromatography mass spectrometric (LC-MS) method using atmospheric pressure electrospray ionization (API-ES) was developed for the identification of the two principal alkaloids in seized khat, cathinone (2-amino-1-phenyl-1-propanone) and cathine ((+)-norpseudoephedrine).

The leaves and young shoots of *Catha edulis* Forsk are usually referred to as khat. Khat is an evergreen shrub or tree that grows wild pri- marily in eastern Africa and the southern Arabian Peninsula. The chewing of khat is endemic in certain parts of Africa and the Arabian Peninsula. Users chew the fresh plant material for its stimulant and euphoric effects, similar to the effects of amphetamine. Cathinone (Schedule 1) has been determined to be the principal alkaloid responsible for the pharmacological and stimulant effects, similar to amphetamine in potency. Cathine (Schedule IV) had been originally identified as the main naturally occurring alkaloid present in khat responsible for its stimulating effects, together with its diastereomer norephedrine. Cathine occurs mainly in older plants and is also formed by reduction of cathinone during drying and storage. In fresh or well preserved khat material, cathine exists as only a minor component in comparison to cathinone.

Present confirmatory techniques for the identification of cathinone and cathine have relied on gas chromatography-mass spectrometry (GC- MS) and gas chromatography-infrared detection (GC-IRD). However, GC-MS of cathinone and cathine gives weak spectral information and mole- cular ion confirmation is difficult, requiring the need for derivatization. GC-IRD offers more specificity in the identification of cathinone and cathine. The use of liquid chromatography-mass spectrometry (LC-MS) and in-source collision induced dissociation fragmentation using a single quadrupole mass spectrometer provides another analytical technique pos- sessing good discriminating power for the identification of the principal khat alkaloids.

Sample preparation involved soaking the freeze dried khat leaves (approximately 10 g) in 0.1N H_2SO_4 followed by extraction with chloroform to remove neutral organic compounds. The acidic solution is basified with 5% sodium carbonate and the cathinone and cathine are extracted into methylene chloride. The methylene chloride extract layers are evaporated to dryness under a stream of air and reconstituted in methanol for LC-MS analysis. Separation was performed on a 15 cm x 3.0 mm Phenomenex Polar-RP column using 10mM ammonium formate pH 3.7 (93%): 7% acetonitrile as the mobile phase along with diode array detection at 250nm and 210nm. Baseline selectivity was achieved for norephedrine (4.4min), cathine (4.7min), cathinone (5.4min), ephedrine (5.9min) and amphetamine (7.6min). Electrospray parameters were optimized via flow injection analysis and in-source collision induced dissociation experiments were performed to optimize fragmentation of the compounds of interest. Ionization is effected via electrospray in positive mode resulting in a proto- nated pseudomolecular ion (M+H) for the compounds of interest. The method utilizes dynamic fragmentor voltage ramping (*m/z* 152 (80V), *m/z* 150 (90V), *m/z* 134(140V), *m/z* 132 (150V), *m/z* 117 (200V), *m/z* 91 (240V), *m/z* 50 (300V)), resulting in collision induced spectra, with cathinone exhibiting a prominent pseudomolecular ion of m/z 150 and cathine exhibiting a pseudomolecular ion of m/z 152. The method is robust and allows for the rapid screening of multiple exemplars of seized khat submis- sions for the identification of cathinone and cathine.

LC/MS, Cathinone, Cathine