To 0.5 mL of oral fluid specimens were added 50 μL of ethyl acetate in tubes. The tubes were vortex mixed for 5 min and centrifuged (3000 rpm) for 3 min. The ethyl acetate was transferred to a 5-mL glass tubes and the extracted samples were dissolved in 50 μL of ethyl acetate and 2 μL of amphetamine-d5 (IS) were added. Samples were capped mixed and incubated for 30 min at 65 °C.

HFBA was added. Samples were capped mixed and incubated for 30 min at 65 °C. Ethyl acetate and 2 μL of amphetamine-d5 (IS) were used as quantifier and qualifier ions. The analytes and internal standard (amphetamine-d5) were extracted from oral fluid with ethyl acetate and derivatized with heptafluorobutyric anhydride (HFBA) at 60 °C for 30 min.

RESULTS: The calibration curves were linear (p=0.98) in the concentration range 20–2000 ng/mL, for all analytes. The intra- and inter assay imprecisions were within (1.6–12.5%) and (1.5–9.5%), respectively for all analytes. Intra-assay accuracies were between -5.9 and 6.7% for all analytes. The method was successfully applied to detect and quantify the target analytes from oral fluid specimens collected from khat and methcathinone users.

CONCLUSIONS: The method was well validated and successfully applied to saliva specimens collected from khat and methcathinone users.

Introduction

Herbal cathinones are phenylalkylamine alkaloids naturally found in the leaves of the khat plant (Catha edulis) [1]. This plant is commonly found in East Africa and Arabian Peninsula; people chew the leaves of this plant for their stimulant effect [2]. The main natural cathinones present in the khat are cathinone [S-(+)-norpseudoephedrine] and cathine [S,S-(+)-norpseudoephedrine]. Cathinone is the main psychoactive constituent of this plant, and it produces an effect similar to amphetamines.

While blood and urine routinely used for drugs of abuse testing, oral fluid has become a popular choice as an alternative matrix for drug testing. In contrast to blood or urine samples, oral fluid is easy to collect, difficulty of adulteration and noninvasive [3-5].

To our knowledge, information on natural cathinone and methcathinone related ephedrine detectability in oral fluid using GC-MS is not reported. Here, a GC-MS method was developed and validated to detect and quantify cathine, cathinone, methcathinone and ephedrine in oral fluid samples. Human subject samples were analyzed using the developed method to demonstrate the applicability of the method.

GC-MS condition

A Varian 450-GC gas chromatograph with a Varian 240-MS ion trap mass spectrometer was used for the detection of the analytes. Separation of analytes was performed with VF-5ms (30 m × 0.25 mm) column (Varian, Inc., Palo Alto, USA).

Sample preparation

To 0.5 mL of oral fluid specimens were added 50 μL of amphetamine-d5 (IS), 100 μL of saturated sodium bicarbonate adjusted to pH 9.0 with 1.0 N of sodium carbonate and 4.0 mL of ethyl acetate in tubes. The tubes were vortex mixed for 5 min and centrifuged (3000 rpm) for 3 min. The ethyl acetate was transferred to a 5-mL glass tubes and evaporated to dryness using a stream of nitrogen at room temperature.

Derivatization

The extracted samples were dissolved in 50 μL of ethyl acetate, and then 50 μL of HFBA was added. Samples were capped mixed and incubated for 30 min at 65–70°C in heat block. Samples were allowed to cool at room temperature, and then evaporated to dryness under a stream of nitrogen. Samples were then reconstituted with 50 μL of ethyl acetate and 2 μL was injected in GC-MS system.

Results

Figure 1: Mass spectra of HFBA derivatives of cathine, cathinone, methcathinone and amphetamine-d5.

Table I: Retention Times and Ions Monitored for GC-MS Analysis of HFBA Derivatives.

Table II: Accuracy and Imprecision for Cathine, Cathinone, Methcathinone and Ephedrine in Oral Fluid.

Table III: Concentration Levels of Cathine, Cathinone, Methcathinone and Ephedrine (ng/mL) in Oral Fluid cases.

Figure 2. The total ion chromatograms of HFBA derivatives of cathine (2), cathinone (4), methcathinone (5), and ephedrine (6) and (1) for the analysis of splited oral fluid at concentration of 2000 ng/mL, (a) authentic samples received from khat (b) and methcathinone (c) users.

RCMS method was successfully developed and validated to quantify cathine, cathinone, methcathinone and ephedrine in oral fluids. The method was successfully applied to detect and quantify all analytes in human subject samples, which can be employed by clinical and forensic laboratories. Oral fluids is acceptable as alternative biological samples to use to detect and quantify the target cathinones and related ephedrines.

Conclusions

A GC-MS method was successfully developed and validated to quantify cathine, cathinone, methcathinone and ephedrine in oral fluids. The method was successfully applied to detect and quantify target analytes in human subject samples, which can be employed by clinical and forensic laboratories. Oral fluids is acceptable as alternative biological samples to use to detect and quantify the target cathinones and related ephedrines.

References