K43 Determination of Alprazolam in Oral Fluid

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After attending this presentation, attendees will learn the disposition of alprazolam in oral fluid and understand the degree of sensitivity necessary for its detection.

This presentation will impact the forensic community and/or humanity by demonstrating how, as oral fluid gains acceptance as a suitable specimen type for roadside collection, procedures for the detection of low level benzodiazepines are very important.

Methods: Oral fluid is increasingly being studied as a suitable matrix for roadside collection and determination of driving under the influence of drugs. A low dosage, potent benzodiazepine with anxiolytic properties, alprazolam, was selected for this experiment, due to its potential contribution to impaired driving. Benzodiazepines have not been widely detected in oral fluid since the saliva:plasma (S:P) ratio is less than 0.5 for most of the drug class. The newer benzodiazepines are also given in low dosage regimens making their detection in oral fluid even more difficult.

Extraction: Calibrators were prepared in Quantisal™ transportation buffer at concentrations of 0.1, 0.2, 0.5, 1, 2 and 5 ng/mL of alprazolam. Deuterated (d5) alprazolam was added at a concentration of 5 ng/mL. Mixed mode (cation exchange:hydrophobic) solid phase extraction columns were conditioned with methanol (3 mL), deionized water (3 mL) and 0.1M phosphate buffer (pH 6.0; 2 mL). The specimens were loaded onto the column and allowed to run through. The columns were washed with deionized water (3 mL) and 0.1M phosphate buffer:acetonitrile (80:20, v,v: 2 mL). The columns were dried for 5 minutes, then hexane (1 mL) was added. The alprazolam was eluted with ethyl acetate:ammonium hydroxide (98:2 v,v; 2 mL) and evaporated to dryness. BSTFA + TMCS (50 DL) was added and the samples were heated at 70°C for 45 min.

Analysis: In order to achieve the sensitivity necessary for the detection of low level anxiolytic benzodiazepines in oral fluid, a two- dimensional gas chromatographic system was employed, with negative chemical ionization mass spectral detection. The system functioned optimally when the phases of the two gas chromatographic columns were as different as possible. The primary gas chromatographic column was a DB-35 MS column (30m x 0.25mm ID x 0.25 µM film thickness), the inlet pressure was 54.3 psi, and the average linear velocity was 81 cm/sec. The length of the restrictor column was calculated by software, and was dependant on the dimensions of the column and the pressure. The restrictor tubing was connected to the Deans switch and the other end was attached to a secondary detector.

In a Deans switch mode, the flow from the primary column plus a switching flow are passed onto the secondary column. The secondary column was a DB-1 stationary phase (15 m x 0.25 mm i.d. x 0.25 Dm film thickness). The Deans Switch (Auxiliary Port #3) was programmed to operate at a pressure of 31.2 psi. It allowed all the flow from the primary column to vent through the flame ionization detector for 11.2 min. For 1.1 min the flow was then switched to allow the carrier gas to enter the secondary analytical column. At 12.3 min, the flow was returned to the secondary vent.

In order to "trap" the analyte using the cryo-focusing unit, the focuser was cooled from the oven temperature of 280°C to 100°C beginning at a run time of 10.5 min. The ramp rate for cooling was as high as it was possible to set the software and was set at 777°C/ minute. It was held at 100°C for 3 min, thereby allowing the alprazolam to trap in the cryofocuser. At a retention time of 13.5 min, the focuser was heated at a rate of 777°C/minute to a final temperature of 280°C.

Injection and Oven Parameters: The front inlet was operated in pulsed splitless mode at an initial temperature of 280°C. The pressure was 54.3 psi and the pulse time was 1 minute. The purge flow was 20 mL/min and the purge time was 1 minute. The injection volume was 2 µL.

The oven was programmed from 190°C for 1 min; ramped at 30°C/min to 320°C where it was held for 10.67 min.

Mass Spectrometer Parameters: The instrument was tuned in negative chemical ionization mode, using ammonia. The flow of the ammonia collision gas into the source was maintained between 8.0 x 10^{-5} and 1.0 x 10^{-4} Torr. The MS source was held at 150°C, the quadrupole at 106°C, the transfer line at 280°C, and was operated at 800eV over tune. The MSD was operated in selected ion monitoring mode with four ions in a single group. Ions 313 and 315 were monitored for D_5-alprazolam; 308 and 310 for alprazolam with a dwell time of 50 ms for each ion. The retention time of alprazolam was 14.4 min. The method was linear over the range tested.

Results: The procedure was applied to specimens collected using

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the Quantisal™ oral fluid collection device, from a subject who was a prescription user of alprazolam. The profile of alprazolam detection over a time course of 16 hours after ingestion will be presented.

Summary: A method for the extraction and highly sensitive detection of alprazolam in oral fluid is described. The method was applied to oral fluid specimens taken from a prescription user of alprazolam.

Alprazolam, Driving Under the Influence of Drugs, Oral Fluid

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