B110 LC/MS Analysis of Flunitrazepam (Rohypnol®) Solid Dosage Tablets

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After attending this presentation, attendees will understand the qualitative and quantitative analysis of flunitrazepam by Liquid Chromatography Mass Spectrometry (LC/MS). This poster will convey the process of extracting and quantitating this drug by LC/MS. It includes quantitation and confirmation by LC/MS.

This presentation will demonstrate to the forensic community that LC/MS will provide an improved methodology over GC/MS methods related to the analysis of this drug. This chromatograpy and sensitivity is a significant improvement over current GC/MS techniques.

The quantitative analysis of seized flunitrazepam tablets can be performed using liquid chromatography-mass spectrometry (LC/MS). LC/MS is ideal because of the physical properties of flunitrazepam and the faster analysis times relative to gas chromatograph-mass spectrometry (GC/MS). Gas chromatography-mass spectrometry (GC/MS) techniques are not ideal for molecules that are polar and have low volatility such as flunitrazepam. Liquid-liquid extraction (LLE) was used to isolate flunitrazepam from the inert water-soluble constituents of the tablet. The technique is rapid, sensitive and confirmatory for flunitrazepam in solid dosage tablets.

Flunitrazepam is a class of potent hypnotic agents available on prescription in most European and Latin American countries. In the United States flunitrazepam is a scheduled drug of abuse. Many date-rape cases have been linked to the incapacitating effects of flunitrazepam. These effects are exacerbated by the presence of alcohol typically found in environments where flunitrazepam is illegally utilized. The availability of these pills originates from the illegal smuggling from countries that manufacture this drug for therapeutic purposes.

The tablets are dissolved in water to break up the tablet matrix. The tablets form an aqueous suspension that is mixed with an equal volume of chloroform. The sample is vortexed and sonicated for a few minutes. The chloroform layer is allowed to separate by centrifugation for a few minutes. The denser chloroform layer is aspirated with a pipette to avoid contamination of the organic phase with the aqueous phase. The procedure should be repeated five times to be able to achieve quantitative recovery of flunitrazepam. It should be noted that basic extractions should be avoided because of the instability of flunitrazepam in alkaline medium. The higher the pH the faster the degradation kinetics of flunitrazepam. We used distilled water instead of an alkaline aqueous solution to perform the liquid-liquid extraction.

The assay used a deuterated internal standard, flunitrazepam-d7, to correct for changes in running conditions. A C-18 reverse phase column was used to 4.6 x 150mm, 1 mL/min, methanol:water (70:30) mobile phase with isocratic elution. A plot of the ratio of the areas of [Flunitrazepam]/[Flunitrazepam-d7] versus the respective concentration gave us an excellent linear regression fit for the standard curve. The linearity range of the assay was 0 to 500 ng/mL. The standard linear regression line had a coefficient of correlation, r = 0.999. The limit of detection (LOD: S/N =3) was 12 ng/mL (injection volume 20uL) and the limit of quantitation (LOQ: S/N = 10) was 50 ng/mL. The assay was run in selected ion monitoring mode (SIM) choosing several ions that can be used for qualifying and quantitation. The need to produce fragmentation can be achieve by increasing the cone voltage of the Finnigan aQa Navigator™ LC/MS system. This is important for forensic work where a minimum of three ions, ion ratios within +/-20%, and retention times are part of the confirmatory requirements for selected ion monitoring.

The ions monitored for flunitrazepam were m/z 314, 300, 272, 239 and for the deuterated internal standard, flunitrazepam-d7, m/z 321, 307, 279, 246. The highest sensitivity for this assay can be obtained with a lower cone voltage, but the fragmentation pattern would not be achieved. The analysis of solid dosage tablets is not usually dependent on sensitivity of the assay because of the high concentrations of drug found in the tablets.

The analysis performed on a confiscated pill presumably manufactured by Roche had a label concentration that read 1 mg. The tablet appears to be legitimate based on its identifying marks. The quantitative analysis by SIM mode LC/MS gave a recovery of approximately 1.08 mg flunitrazepam. The label number seems to indicate that this was a 1 mg flunitrazepam tablet. The assay showed quantitative recovery of flunitrazepam, recovery of tri-level controls were within 10% of target value and our negative controls were negative. This indicates that our deuterated standards did not contain detectable quantities of nondeuterated standard. In addition, no carryover effects were present at the highest concentration of the linear range. The assay developed is a rapid, sensitive and confirmatory for the presence of flunitrazepam in solid dosage tablets. The extraction technique is simple, efficient and quantitative. It allows for a robust confirmatory technique for flunitrazepam by LC/MS.

Flunitrazepam, Rohypnol®, Roofies

 ** Presenting Author*