K24 A Fast and Sensitive LC/MS/MS Method for the Quantitation and Confirmation of 30 Benzodiazepines and Non-Benzodiazepine Hypnotics in Forensic Urine Samples

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After attending this presentation, attendees will learn about using LC/MS/MS for analysis of benzodiazepines and non-benzodiazepine hypnotics in urine. These drugs are of interest because of increased use and abuse.

This presentation will impact the forensic science community by presenting a fast, simple, and sensitive technique to detect and quantify ben- zodiazepines and other hypnotic drugs in urine. The method presented has several advantages over the other techniques that are used.

Introduction: Benzodiazepines and other nonbenzodiazepine hypnotics, such as Zaleplon, Zolpidem, and Zopiclone are widely prescribed psychoactive drugs for the treatment of anxiety and sleep disorders. These substances frequently lead to dependence and abuse and some of them can affect judgment and behavior. As a result, these compounds are of great interest in forensic, toxicological and clinical research laboratories. The screening for benzodiazepines with immunoassay tests does not provide enough sensitivity and specificity. Analysis using gas chromatography with different detectors is difficult or impossible because of thermal instability and requires time consuming derivatization and clean-up steps. Liquid chromatography (LC) with UV detection cannot detect benzodiazepines at required concentration levels and lacks in selectivity.

LC with tandem mass spectrometric detection (MS/MS) with electrospray ionization (ESI) is the ideal technology for the analysis of polar and thermally labile drugs and their metabolites, yielding high sensitivity and specificity. Sample preparation is also fast and simple. The developed LC/MS/MS method detects 30 analytes in a single chromatographic run using two Multiple Reaction Monitoring (MRM) transitions to allow quantitation and confirmation.

Experimental and Results: Urine samples of forensic cases were diluted after addition of internal standards. LC separation was carried out using a Shimadzu Prominence LC using mobile phases of: (A) water with 0.2% formic acid and 2mM ammonium formate, and (B) acetonitrile with 0.2% formic acid and 2mM ammonium formate. A 3200 Q TRAP® LC/MS/MS system equipped with an ESI source operated in the MRM mode was used for detection. Two transitions were monitored. The first MRM was used to quantify the analyte and a ratio of the quantifier to the second qualifier MRM was used for confirmation.

Limits of quantitation in urine samples, accuracy and reproducibility of all analytes were studied. All targeted compounds could be quantified in urine samples after a dilution step at a concentration of at least 10 ng/mL. The high selectivity of MRM detection allows compound specific detection without interference of urine matrix or other drugs or metabolites being present in the sample. The dilution step of the urine sample preparation additionally ensures elimination or reduction of ion suppression which could be caused because of co-eluting matrix components.

Benzodiazepines, LC/MS/MS, Toxicology

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