K53 The Direct Detection and Ultrafast Quantification of Drugs of Abuse in Serum by Probe Electrospray Ionization/Tandem Mass Spectrometry (PESI/MS/MS)

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After attending this presentation, attendees will understand the basic principles of PESI, a novel ambient ionization technique, and its use as an ultrafast and direct detection method of drugs of abuse in serum without sample preparation.

This presentation will impact the forensic science community by describing a highly sensitive and non-chromatographic analytical method capable of simultaneous sampling and ionization and ultrafast detection and quantification of drugs of abuse in serum without sample pretreatment using PESI/MS/MS.

PESI is a unique ionization technique that uses a probe needle that enables simultaneous sampling and ionization. Its high ionization efficiency is comparable to that of nano-ESI. Previous studies have reported on the effectiveness of PESI/MS/MS in the intact analysis of endogenous compounds.[1,2] The objective of this study was to evaluate the applicability of PESI/MS/MS to the direct analysis of drugs of abuse in serum without sample preparation. Quantitation was also investigated using the Internal Standard (IS) method.

PESI/MS/MS was performed using a Shimadzu LCMS-8040 equipped with PESI ion source in the Multiple Reaction Monitoring (MRM) mode using either one quantifier transition or two transitions (quantifier and qualifier) for each analyte. Serum samples were spiked with reference standards of drugs of abuse of various drug classifications. A 15µL aliquot of the spiked serum containing 50ng/mL of IS compounds were pipetted onto a sample plate. Sampling time was set at 0.30 minutes.

All target drugs selected for this study were detected by PESI/MS/MS, exhibiting that the present method can detect a wide variety of drug types. Qualitative investigation demonstrated that the majority of the drugs could be detected as low as 1ng/mL in serum, while zolpidem could be detected in serum as low as 0.1ng/mL, demonstrating the highly sensitive nature of the method. Preliminary evaluation of the present method with acetyl fentanyl, MDMA, methamphetamine, oxycodone, haloperidol, ketamine, risperidone, zolpidem, and diazepam with their respective stable-isotope-labeled analogs as IS compounds demonstrated linearity of the calibration curves over the specified range (1ng/mL-100ng/mL) with an R^2 value of 0.99. Calculated Limit of Detection (LOD) and Limit of Quantitation (LOQ) values ranged between 0.12ng/mL-0.49ng/mL and 0.33ng/mL 1.5ng/mL, respectively. Intra- and inter-day accuracy (% relative error) ranges were 0.1%-13% and 0.2%-14%, respectively. Repeatability (%RSD, *n*=5) ranged between 0.6%-14% for all target compounds. Application to real autopsy samples was tested and it was verified that the present method is suitable for practical use. Results demonstrated that PESI, having

high ionization efficiency superior to that of conventional ESI, allows for increased sensitivity with sub-nanogram level detection.

The simple, rapid, and highly sensitive direct detection method for drugs of abuse in serum by PESI-MS/MS allowed for the attainment of the ideal scenario in forensic toxicology in which no sample preparation is required for sample analysis. Samples collected at autopsy or a crime scene can be subjected to immediate and rapid drug testing. Since no chromatographic separation is required, the process of sample set-up to qualitative and quantitative MS analyses can be completed in less than one minute. Carry over between samples is negligible owing to the disposable sample plates and needles used as the ionization probe. Finally, the present method has a high potential to be applied to other biological specimens (whole blood, urine, tissue) as well as seized drugs, providing a powerful tool in the analysis of biological specimens for drug detection.

Through this research, a novel drug detection and analysis method is introduced. This method presents application possibilities in various fields such as forensic toxicology, clinical toxicology, and Therapeutic Drug Monitoring (TDM).

Reference(s):

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2. Zaitsu K., Hayashi Y., Murata T., Nakajima H., Ishikawa T., Kusano M., Tsuchihashi H., Ishii A. Intact metabolome analysis of mice biological tissues by probe electrospray ionization-tandem mass spectrometry and its preliminary application to real-time analysis. *Proceedings of the American Society for Mass Spectrometry.* 2016, San Antonio, TX.

PESI/MS/MS, Drugs of Abuse, Direct Detection