Cutting-edge mass spectrometric screening methods for new psychoactive substances

Kei Zaitsu
Nagoya University Graduate School of Medicine
Japan

Scope of the lecture:
Cutting-edge mass spectrometric screening for drugs of abuse, especially new psychoactive substances (NPSs), using our newly-developed probe electrospray ionization/tandem mass spectrometry (PESI/MS/MS) will be outlined. In addition, advantages and disadvantages of drug screening methods including PESI/MS/MS, LC-Q-TOFMS and high-end LC-MS/MS will be discussed with our postmortem cases.

Learning objectives:
1. To comprehend the principles of PESI/MS/MS and its features.
2. Be acquainted with the details of "rapid-fire" screening by PESI/MS/MS.
3. To understand the analytical pros and cons from cutting-edge to general mass spectrometric screening methods.

Extended abstract:

1. Introduction

New psychoactive substances (NPSs) are new derivatives of the already-known drugs of abuse and/or newly-emerged psychotropic drugs. NPSs are one of the most troublesome targets in forensic or clinical drug analyses because general target screening methods such as triggered-selected reaction monitoring (SRM) analysis are almost ineffective for them. In addition, sample preparation such as extraction is mandatory for conventional mass spectrometry, but "God only knows" what the appropriate sample preparation methods for those newly-encountered drugs are. The most preferable way for comprehensive NPS analysis then is, paradoxically, "no sample preparation" – but is it possible? The answer is "Yes": ambient ionization techniques have been recently improving and most of them do not require sample preparation, even for biospecimens.
In this lecture, I will introduce a cutting-edge screening method using our newly-developed technique, probe electrospray ionization/tandem mass spectrometry (PESI/MS/MS). Pros and cons of non-target or target screening methods including PESI/MS/MS, LC-Q-TOFMS and high-end LC-MS/MS will be also discussed with postmortem cases encountered in our lab.

2. Rapid-fire screening by PESI/MS/MS

Probe electrospray ionization (PESI) is an ambient ionization technique invented by Prof. Kenzo Hiraoka in 2007[1], and it enables us to analyze drugs directly in biological specimens including tissue samples. We have first combined PESI with tandem mass spectrometry (PESI/MS/MS) and have succeeded in analyzing intact endogenous metabolites not only in mouse liver but also in brain[2,3]. The schematics of PESI/MS/MS is shown in Fig. 1.
PESI uses a thin solid needle (tip diameter: 700 nm) both as sampling and ionization units, and the needle probe moves up and down speedily, leading to high-speed data acquisition. At the bottom position, the probe needle penetrates into samples and target analytes are quickly adsorbed onto the probe surface. At the top position, high voltage is applied to the probe for ionization of the adsorbed analytes. Ionization efficiency of PESI is quite high comparable to that of nano-electrospray ionization[4], enabling direct analysis of target compounds in biospecimens without tedious sample preparation.

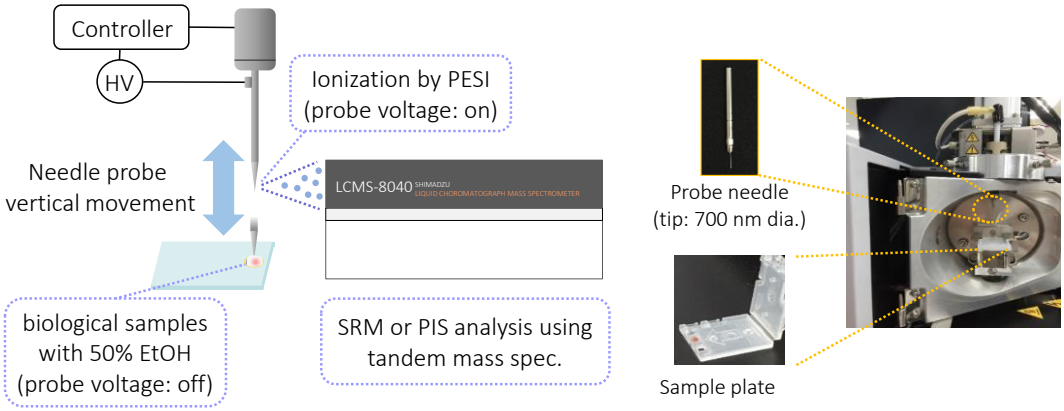

Fig. 1 Schematics of PESI/MS/MS

We applied PESI/MS/MS to the screening of drugs in serum and whole blood, succeeding in direct detection of 160 drugs within ca. 6 min. Although some drugs were only detectable above 10 ng/ml, almost all drugs could be detected at 10 ng/ml or lower concentration levels, demonstrating the practicality of PESI/MS/MS. Since sensitivity of drugs changes with its solvent for enhancing ionization efficiency, appropriate selection of solvent is important in accordance with the drug's physical property. Quantitative analysis by PESI/MS/MS is also feasible.

This technique can be applied to the screening for NPSs by their "all ions MS/MS" analysis like SWATH[5]: all precursor ions existing in the target *m/z* range are selected, and product ion scan for each precursor ion is executed. Method optimization for all ions MS/MS for NPS screening is now underway.

3. Pros and cons from cutting-edge to general mass spectrometric screening methods.

High-resolution mass spectrometry such as Q-TOFMS is known to be useful for screening of unknown drugs, especially NPSs, though the detection limits are generally higher than those in target analysis using triggered-SRM method by high-end tandem mass spectrometry. In other words, lower criteria settings for non-target screening generally decreases the quality of product ion spectra. In a postmortem case tested in our lab, extremely low concentration of the unchanged synthetic cannabinoid was detectable only in blood by high-spec LC/MS/MS, while Q-TOFMS was unable to detect it. In this case, however, its tentative urinary metabolites were easily detected by non-target screening by Q-TOFMS, which can be a critical clue to reinvestigate for the ingested drugs themselves. It suggests that expansion and/or frequent updates of in-house and/or commercially-available database are highly important for screening of continuously emerging NPSs.

In a strict sense of "screening test", however, it is most preferable for anyone to do the test with ease and rapidity; in reality, sample preparation and operation of a mass spectrometer is too difficult for laypersons without specialized training. From such a point of view, PESI/MS/MS is most likely to match the definition of user-friendly "drug screening" if its database is further improved and updated.

4. **Conclusions**

Rapid-fire screening by PESI/MS/MS is valuable not only for NPSs and drugs of abuse, but also for pharmaceuticals. Therefore, we will apply PESI/MS/MS to clinical toxicology/ therapeutic drug monitoring and/or to other biospecimens such as saliva to verify its availability for on-site drug analysis. Likewise to PESI/MS/MS, other ambient ionization techniques such as paper spray ionization[6] and desorption corona beam ionization (DCBI)[7]

combined with MS/MS or Q-TOFMS will be generally used for drug screening in near future.

References

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