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Rapid and quantifiable screening method for 64 drugs in human blood by direct probe ionization/ tandem mass spectrometry (DPiMS)

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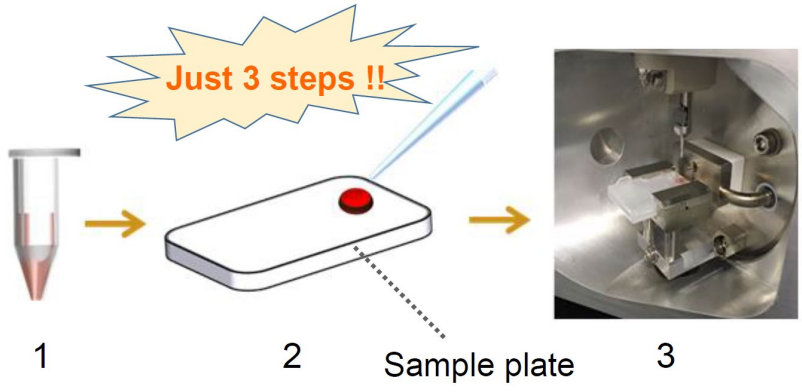
Introduction

LC-MS/MS-based drug screening analysis is a gold standard in forensic toxicology because LC-MS/MS allows for the simultaneous determination of drugs in a single run. LC-MS/MS has gradually achieved high-throughput analysis, though more rapid and higher user-friendly method are preferable for drug screening. In particular, sample preparation such as extraction is mandatory for instrumental analysis, though it is a time-consuming process for analysts. Therefore, innovative analytical method without tedious sample preparation step is strongly required for next-generation drug screening analysis. Recently, ambient ionization techniques (AITs) have been improved and they are able to directly analyze target compounds in biological specimens. Probe electrospray ionization (PESI) is an ambient ionization technique invented by Prof. Kenzo Hiraoka in 20071, and it enables us to analyze drugs directly in biological specimens including tissue samples. We have first combined PESI with tandem mass spectrometry and have succeeded in analyzing intact endogenous metabolites not only in mouse liver but also in brain2-4. Here, we present a novel ultra-rapid drug screening analysis by direct probe ionization-tandem mass spectrometry (DPiMSTM-8060) and demonstrate its usability.

Material and Methods

Sample Preparation



1. Whole blood (10 µl) is diluted 10-fold with an IS* (50 ng/ml) aq.
   Then the diluted solution is further diluted 2-fold with ethanol.
2. 10 µl of the final diluted sample is pipetted onto a sample plate.
3. Start direct analysis!

*50ng/mL Diazepam-d5 aq.

⇒ Total 5 min

Fig.1 Schematic of analytical protocol

Analytical Condition

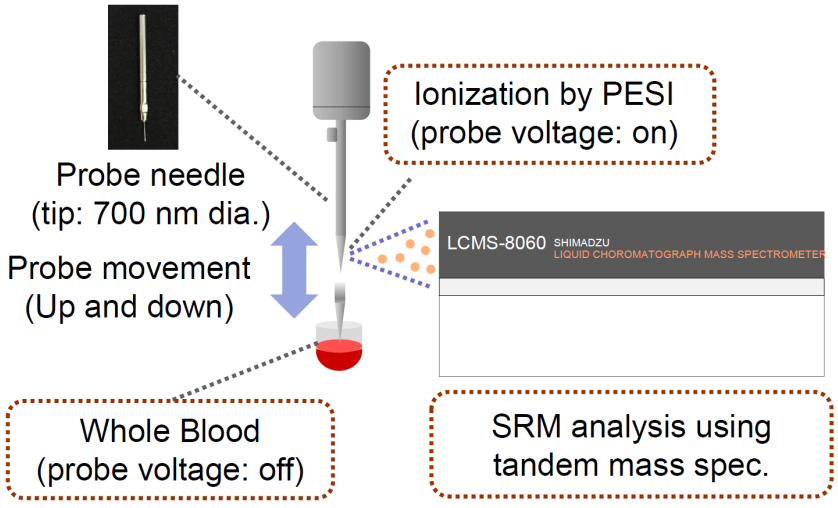


Fig. 2 Schematic of DPiMS-8060 system

Instruments: LCMS-8060 tandem mass spec. with DPiMS-8060 (Shimadzu Corporation)
Total number of the target analytes: 64 compounds (Table 2)
Data acquisition mode: SRM mode for each compound with scheduled SRM (Fig. 3).
Ionization mode: PESI positive/negative
Dwell time: 1 msec for each transition
Applied voltage: ±3.0 kV

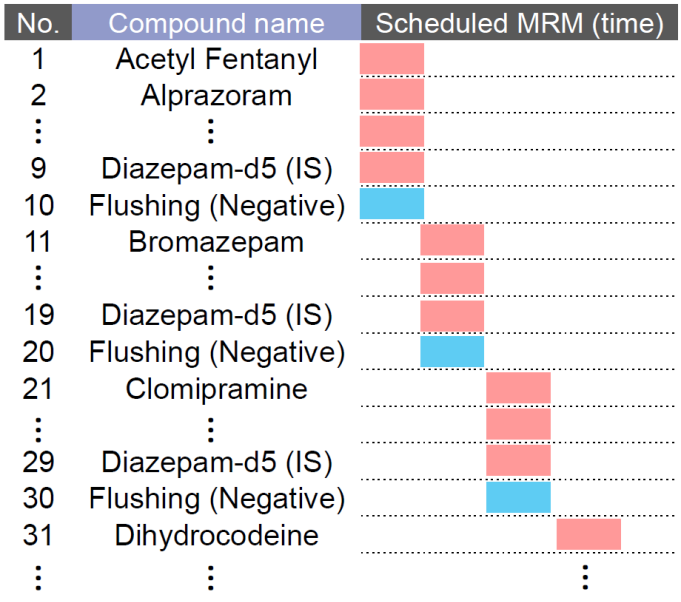


Fig. 3 Schematic of scheduled SRM method

Table 1 Comparison of data acquisition time between DPiMS-8060 and LC-MS/MS

	DPiMS/MS	LC-MS/MS
Data Acquisition time (64 compounds screening)	**3.2** mins ⇒ ultra-rapid!!	> 30 mins

Results

The method was optimized to contain 64 MRM transitions for drugs compounds to be monitored simultaneously, and their quantitative performance was evaluated in control blood samples (Table 2). The limits of quantitation were found to be 1 ng/mL for 25 compounds (Figure 3), demonstrating more than sufficient sensitivity for the screening purpose. Further validation experiment is in progress, evaluating intra-day and inter-day accuracy and precision at spike levels of 40 ng/mL and 80 ng/mL (data not shown).

Table 2. Target drugs in the panel and their calibration ranges after optimization

Compound Name	Calibration Range (ng/mL)	Linearity (R^2)	Compound Name	Calibration Range (ng/mL)	Linearity (R^2)
Acetyl Fentanyl	1 – 100	0.995	Cloxazolam	5 – 100	0.980
Alprazolam	10 – 100	0.965	Clozapine	1 – 100	0.996
Amitriptyline	5 – 100	0.993	Cocaine	1 – 100	0.992
Amoxapine	5 – 100	0.998	Colchicine	5 – 100	0.936
Atropine	1 – 100	0.999	Desipramine	1 – 100	0.991
Blonanserin	1 – 100	0.999	Diazepam	5 – 100	0.973
Bromazepam	10 – 100	0.973	Dihydrocodeine	1 – 100	0.993
Brotizolam	5 – 100	0.976	Diphenhydramine	5 – 100	0.989
Bupivacaine	1 – 100	0.998	Diphenidine	10 – 100	0.933
Carbamazepine	1 – 100	0.991	Dosulepin	5 – 100	0.981
Carpipramine	1 – 100	0.972	Duloxetine	5 – 100	0.979
Chlorpromazine	5 – 100	0.960	Escitalopram	1 – 100	0.991
Clobazam	10 – 100	0.969	Estazolam	5 – 100	0.969
Clocapramine	5 – 100	0.958	Etizolam	5 – 100	0.976
Clomipramine	25 – 100	0.983	Fludiazepam	10 – 100	0.97
Clotiazepam	1 – 100	0.988	Flunitrazepam	50 – 100	0.99

Table 2. (continued)

Compound Name	Calibration Range (ng/mL)	Linearity (R^2)	Compound Name	Calibration Range (ng/mL)	Linearity (R^2)
Flurazepam	1 – 100	0.999	Perphenazine	25 – 100	0.998
Fluvoxamine	5 – 100	0.986	Pimozide	10 – 100	0.964
Levomepromazine	5 – 100	0.985	Prazepam	5 – 100	0.995
Lidocaine	1 – 100	0.993	Promethazine	5 – 100	0.996
Maprotiline	1 – 100	0.996	Propericiazine	1 – 100	0.997
MDA	5 – 100	0.995	Quetiapine	1 – 100	0.993
MDMA	5 – 100	0.999	Quazepam	5 – 100	0.980
Medazepam	25 – 100	0.975	Risperidone	1 – 100	0.988
Methamphetamine	5 – 100	0.984	Sildenafil	25 – 100	0.968
Mianserin	5 – 100	0.972	Sulpiride	1 – 100	0.989
Midazolam	1 – 100	0.985	Tandospirone	1 – 100	0.999
Mirtazapine	1 – 100	0.992	Tofisopam	1 – 100	0.999
Morphine	25 – 100	0.940	Trazodone	1 – 100	0.995
Nortriptyline	5 – 100	0.986	Triazolam	5 – 100	0.991
Nitrazepam	25 – 100	0.970	Zolpidem	1 – 100	0.992
Pemoline	50 – 100	0.986	Zotepine	5 – 100	0.986

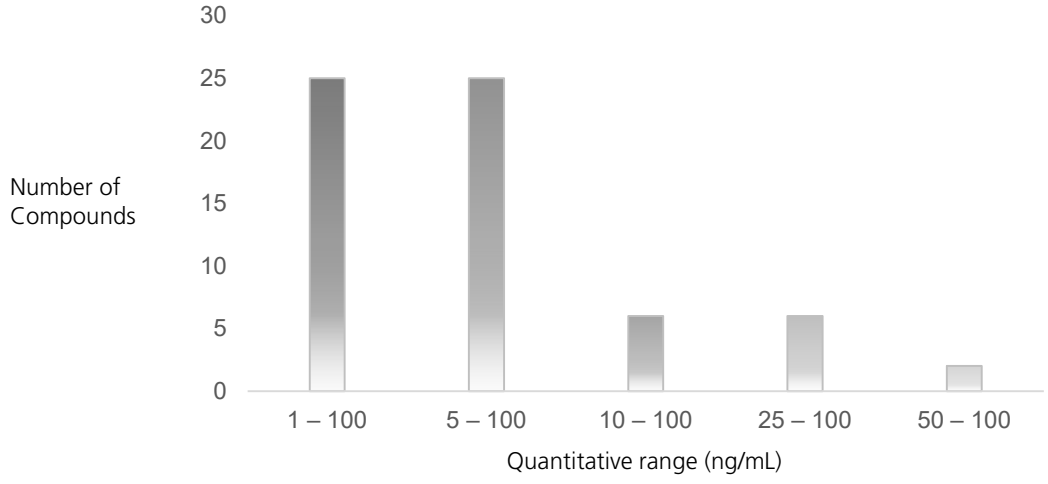


Figure 3. Quantitative ranges of 64 drugs in blood measured by DPiMS

Comparison of screening results for samples between DPiMS-8060 and LC-MS/MS

Table 3 Comparison of screening results

Sample No. (Real postmortem whole bloods)	DPiMS-8060	LC-MS/MS With in-house library
	Detected Compounds	
1	Alprazolam, Amitriptyline, Flunitrazepam, Nortriptyline, Fluvoxamine	Alprazolam, Amitriptyline, Flunitrazepam, Nortriptyline
2	Atropine, Lidocaine, Clomipramine, Fluvoxamine	Atropine, Lidocaine
3	Estazolam, Risperidone, Trazodone, Diphenhydramine, Diazepam, Fludiazepam, Flunitrazepam	Estazolam, Risperidone, Trazodone
4	Methamphetamine, Amitriptyline	Methamphetamine
5	Methamphetamine	Methamphetamine

Screening result by DPiMS-8060 correlated with the results by the established LC-MS/MS, demonstrating its applicability to real postmortem whole bloods.

Conclusion

Ultra-rapid and highly user friendly drug screening without cumbersome sample preparation was achieved by DPiMS-8060, and quantitative performance of the method was fully validated for 64 various drugs, demonstrating the practicality of the method.

References

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