A Liquid Chromatography Tandem Mass Spectrometry method for the Simultaneous Screening and Quantification of 10 Analgesics and Narcotics from Micro Plasma Collection Card

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Abstract

Background: Addiction and abuse of analgesics and narcotics are epidemic worldwide. It is essential to quickly identify and accurately quantify those drugs when drug poisoning is suspected. Here we present an application of micro plasma collection card for simultaneous screening and quantification of 10 typical drugs of analgesics and narcotics in plasma by liquid chromatography tandem mass spectrometry method. These drugs include Meperidine, Fentanyl, Morphine, Oxycodone, Tramadol, Acetaminophen, Heroin, Ketamine, Nimetazepam and Methamphetamine.

Methods: One drop of blood (10-20 microliter) was collected by a micro plasma collection card, and then Dried Plasma Spot (DPS) was extracted before the sample was analyzed by liquid chromatography coupled to quadrupole-time-of-flight mass spectrometry (LC-QTOF, ABI 5600+) and liquid chromatography coupled to quadrupole mass spectrometry (LC-MS/MS, ABI 5500). The drugs were identified based on retention time and exact mass acquired from molecular ions and fragment ions. After a positive identification by LC-QTOF, the sample was thereafter quantified by a LC-MS/MS method. Plasma volume factor of the 10 drugs was acquired by calculating the ratio of drug concentrations between DPS and wet plasma from a same blood sample. Hematocrit were evaluated the impact on plasma volume factor.

Results: All the drugs were well extracted from DPS with recoveries higher than 70%. For LC-QTOF screening method, the limit of detection was 10-50 ng/mL. For the LC-MS/MS quantification method, the accuracy was between 88.6-112.3% and precision was less than 10% with linearity curve ranged from 10-1000 ng/mL. Plasma volume factor of each drug was a constant value (from 0.0301 to 0.0597) when hematocrit was between 30 ~ 50% or 30 ~ 60%. The concentration conversion formula was: Wet plasma (ng·mL⁻¹) =DPS (ng·mL⁻¹) / Volume factor.

Conclusions: DPS card was a useful tool for convenient and stable biological matrix aimed for screening and quantifying the 10 analgesics and narcotics in human plasma.

Introduction

Drug addiction and abuse are prevalent worldwide, and it was critical to quickly identify the substance when drug abused was suspected. An accurately quantification is important for emergency treatment of poisoning patients. The comprehensiveness of the drug screening limits its extensive application in medical institutions.

The dried blood spot is popular in abused drug screening for it's less invasive sampling and enhanced stability while the poor quantitation limits its use. Here we present an application of micro plasma collection card for drug screening and quantification by preparing a dried plasma spot (DPS). The goal of our study is to investigate whether the DPS is a reliable tool for screening of 10 typical analgesics and narcotics and how it performs in accurate quantification.

Procedure

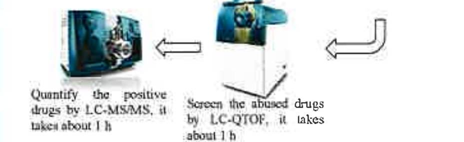

Figure 1. The workflow of DPS preparation, extraction, screening and quantification

Method

a. Preparation of DPS: A drop of blood (~ 20 μL) was dropped onto the test area of the micro plasma collection card until the control spot turned red. After three minutes, the top layer of the card was peeled off. The lower layer was dried under ambient temperature for 15 minutes and then preserved or delivered to the Lab at a room temperature.

b. Extraction and Analysis: DPS in the lower layer (usually collect a constant plasma volume of 2.52±0.03 μL) was taken out to mix with 50 μL extractant (Isopropanol: **acetonitrile**: ddH2O=3: 13: 22) including internal standard (Acetaminophen-d4 and Fentanyl-d5). After extraction, supernatant was separated by a UPLC elution procedure before screening or quantification by mass spectrometry (See Table 1).

Table 1. LC condition and gradient elution program

Time(min)	A%	B%	Flow rate
0	90	10	
8	0	100	
12	0	100	0.2 ml/min
12.1	90	10	
16	90	10	

Column: ACQUITY-UPLC HSS PFP (2.1×150mm, 1.8μm) from WATERS
Mobile phase A contains 0.2% folic acid, 20 mM ammonium acetate, 1.87% isopropanol, 8.13% acetonitrile and 90% ddH2O.
Mobile phase B contains 0.2% folic acid, 20 mM ammonium acetate, 16% isopropanol, 69% acetonitrile and 15% ddH2O.

c. Identification and Quantification: The abused drugs were screened by TOF-MS IDA product ion scan (ABI QTOF5600+) based on retention time and exact mass acquired from molecular ions and fragment ions. After a positive identification, the supernatant was reanalyzed to quantify the concentration of the target drugs by a multiple reaction monitoring mode (ABI 5500).

d. Plasma volume factor: Plasma Volume Factor was used to convert the concentration of the drug on DPS to plasma. It was acquired by calculating the ratio of drug concentrations in DPS and in wet plasma (See test program below). Here, the influence of hematocrit to plasma volume factor was considered.

Figure 2. Test program of Plasma Volume Factor

Results

A. The chromatographic behaviour of 10 analgesics and narcotics in DPS were performed well. No obvious interference was observed.

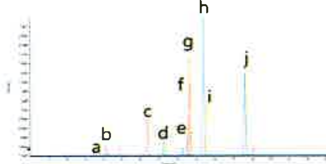

Figure 3. The typical chromatograms of 10 analgesics and narcotics in DPS. a. Acetaminophen, b. Morphine, c. Oxycodone, d. Methamphetamine, e. Heroin, f. Nimetazepam, g. Tramadol, h. Ketamine, i. Meperidine, j. Fentanyl.

B. All the drugs were well extracted from DPS with recoveries higher than 70%. For LC-QTOF screening method, the limit of detection was 10-50 ng/mL.

C. For the LC-MS/MS quantification method of the 10 drugs, the accuracy was between 88.6-112.3% and precision was between 2.46-9.66% with linearity curve ranged from 10-1000 ng/mL (Table 2).

D. Plasma Volume Factor was an independent constant value for each drug (from 0.0301 to 0.0597) when hematocrit was between 30 ~ 50% or 30 ~ 60%.

Table 2. Summary of retention time, Q1 to Q3 transition, LOD, accuracy, precision, recovery, plasma volume factor and permitted Hematocrit (%) of the DPS detection in our method

No.	Analytes	Retention time	Q1>Q3	LOD (ng/mL)	Accuracy (%)	Precision (%)	Recovery (%)	Plasma Volume Factor	Hematocrit (%)
a	Acetaminophen	4.1	152.3>110.2	50	93.00-103.2	9.66	78.84	0.0343	30~50
b	Morphine	4.2	286.1>201.2	50	95.20-111.3	6.34	86.39	0.0482	30~60
c	Oxycodone	6.3	316.2>298.1	10	95.40-105.8	4.72	85.91	0.0589	30~60
d	Methamphetamine	7.1	150.1>119.1	50	97.42-102.5	6.58	80.09	0.0597	30~60
e	Heroin	8.3	370.2>268.2	20	94.52-105.0	3.48	71.59	0.0401	30~50
f	Nimetazepam	8.6	296.1>250.2	20	95.39-104.6	6.09	78.63	0.0329	30~60
g	Tramadol	8.5	264.2>58.0	10	97.38-112.3	5.42	81.57	0.0365	30~60
h	Ketamine	9.4	238.1>179.1	15	100.2-105.6	5.88	74.29	0.0476	30~50
i	Meperidine	9.5	248.3>220.3	20	88.60-111.3	2.46	81.43	0.0465	30~60
j	Fentanyl	11.5	337.2>188.3	10	90.62-108.7	7.86	84.68	0.0301	30~60

Hematocrit: Red blood cell volume, Reference Range(s): >18 Years 38.5-50.0 % for male, 35.0-45.0 % for female

Conclusions

- Micro Plasma Collection Card is a convenient microsampling technique for screening 10 analgesics and narcotics in human plasma in our study.
- In our study, the plasma volume factor of DPS is drug dependent which is constant when hematocrit is within reported Reference Ranges.
- The described Micro Plasma Collection Card provides an alternative to wet plasma or DBS during screening and quantitation for 10 analgesics and narcotics.

References

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3. [illegible]

Acknowledgements

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