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Direct analysis of 10 antipsychotics in serum by the online system integrating solid-phase extraction with UHPLC-MS/MS

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Overview

A fully-automatic system integrating solid-phase extraction with ultra-high performance liquid chromatography-tandem mass spectrometry was successfully applied to the direct analysis of ten antipsychotics in serum.

Introduction

Antipsychotic (AP) drugs are widely prescribed for the treatment of schizophrenia and psychosis. However, it has been shown that these drugs can increase the risk of sudden cardiac death with studies showing that the risk of sudden cardiac death is increased threefold among patients treated with APs. Detection of these drugs is necessary to establish their use and possible contribution to the death. Increasingly, LC-MS/MS methods are being commonly utilized for the detection of antipsychotic drugs in a wide range of tissues including blood. Usually the blood sample need to be manually pretreated by liquid–liquid extraction (LLE) or solid-phase extraction (SPE) to eliminate the matrix effects and the non-target protein. This offline pretreatment is time-consuming and easy to produce error.
In this paper, a fully-automatic system integrating SPE with UHPLC-MS/MS was successfully applied to the direct analysis of ten APs in serum

Methods

LC-MS/MS Analysis

SPE conditions	
SPE column	: Shim-pack MAYI-C8(G) 4.6 mmi.d.×10 mmL, 50 µm
Extraction solvent	: FA/H_2O/ACN=0.2/95/5, v/v
Extraction flow rate	: 2 mL/min
Injection volume	: 50 µL
Extraction time	: 2 min
Desorption solvent	: ACN/MeOH=80/20, v/v
Desorption flow rate	: 0.2 mL/min
LC conditions	
Separation column	: Shim-pack XR-ODS III, 2.0 mmi.d.×50 mmL, 1.6 µm
Mobile phase A	: 0.1%FA+5mM ammonium acetate in H_2O
Mobile phase B	: ACN
Flow rate	: 0.5 mL/min
Column oven	: 40 °C
Time program	: see Table 1

All analytes were determined by multiple reaction monitoring (MRM) in positive ionization mode. The desolvation temperature, heat block temperature, nebulizing gas and drying gas were optimized, respectively. The dwell time and pause time for each ion channel were set at 12 ms and 3 ms, respectively.
The whole analysis procedure was automatically controlled by Shimadzu workstation LabSolutions (Version 5.60).

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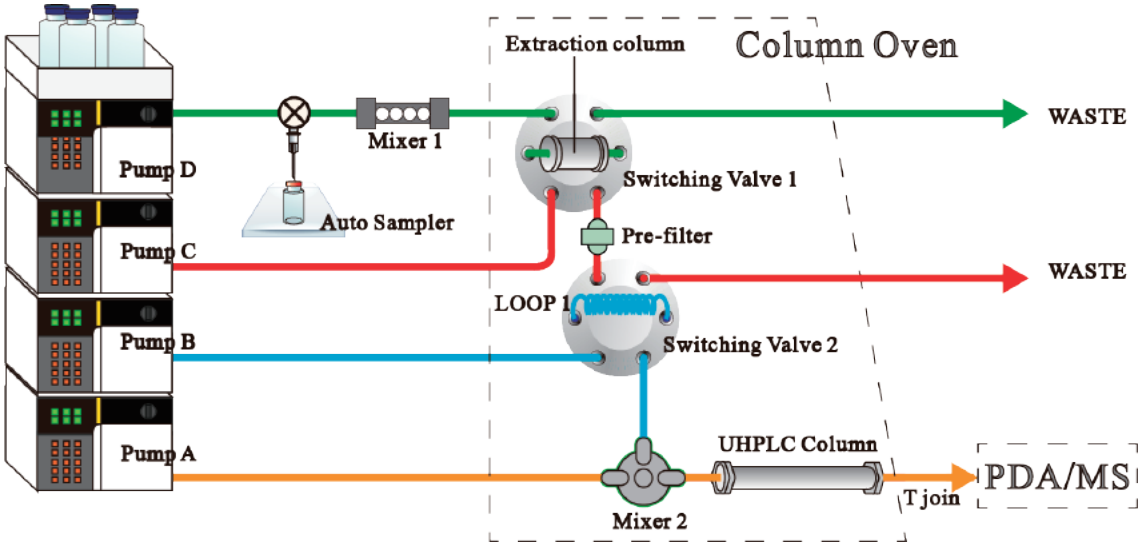

Figure 1 Schematic diagram of the online SPE-UHPLC-MS/MS system

Table 1 LC time program

Time (min)	Module	Command	Value
2.00	Column Oven	CTO.RVL	0
3.40	Pumps	SV(Pump C)	B
3.50	Column Oven	CTO.RVR	0
3.60	Column Oven	CTO.RVL	1
4.50	Pumps	Pump B Conc.	8
8.50	Column Oven	CTO.RVR	1
8.50	Pumps	Pump B Conc.	60
8.50	Pumps	SV(Pump C)	A
8.60	Pumps	Pump B Conc.	100
9.98	Pumps	Pump B Conc.	100
9.99	Pumps	Pump B Conc.	8
10.00	Controller	Stop	

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Results

The online system was realized by a dual-dilution strategy. The first dilution allowed the direct injection of complex samples with minimal pretreatment, and the second dilution enabled large volume of strong eluent to be directly introduced into the UHPLC column without causing peak broadening or distortion. A restricted access media column with C8 modified inside was used as the extraction column to selectively concentrate the APs in the diluted serum. Afterwards, the APs were desorbed and online diluted into weak mobile phase and then stacked onto the column head. This compression strategy enables the online system to tolerate as much as 500 µL injection volume of strong solvents without peak broadening and distortion.

Under optimized conditions, 50 µL of serum were directly injected into the online system. Under the optimized conditions, the whole analysis procedure took only 10 min. The linearity in the range of 0.008000~1250 µg/L were build based on spiked blood samples. All the correlation coefficients were greater than 0.9972, the limits of detection and the limits of quantitation were in the range of 0.000801~0.688 µg/L and 0.00321~2.75 µg/L, respectively. Respectabilities of the R.T. and peak area of ten APs were in the range of 0.01~0.06% and 1.12~4.54%, respectively. The recoveries and the RSD of the real samples ranged from 58.75~136.75% and 0.87~7.69%, respectively. According to the results, this online analysis system was sensitive, reliable and applicable to trace analysis in complex samples.

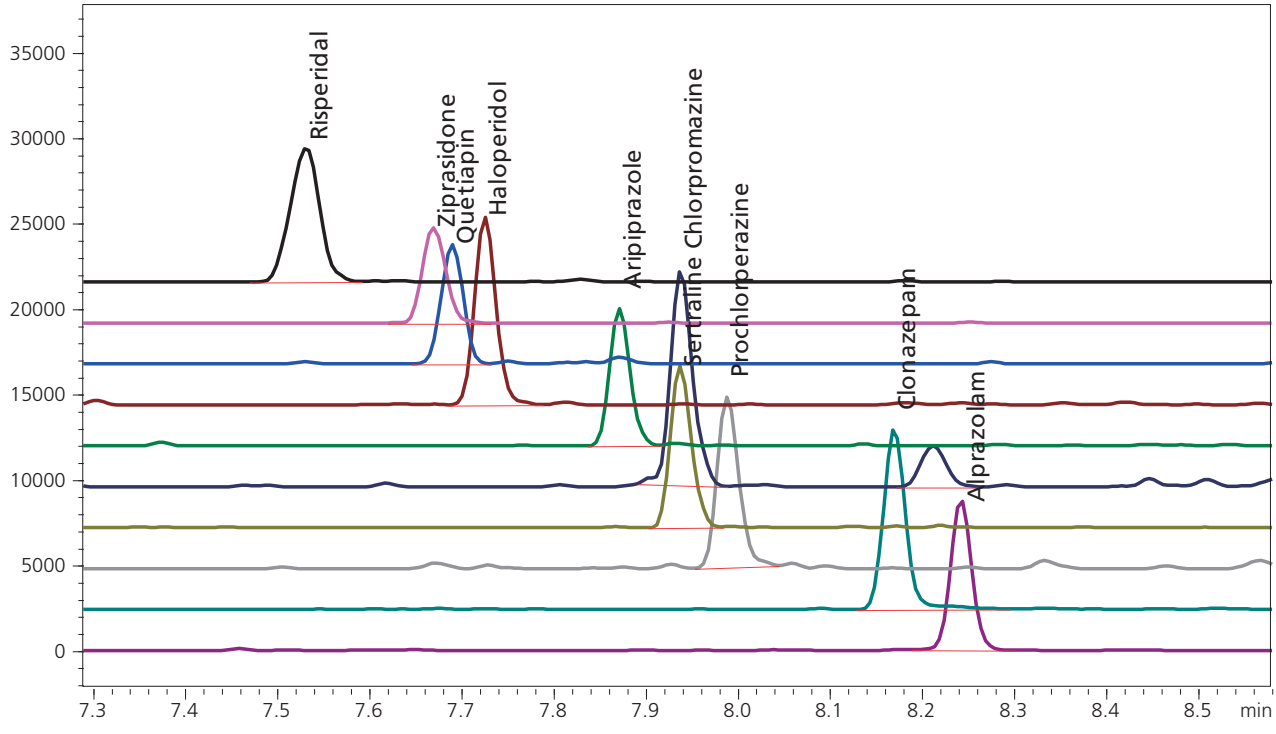

Figure 2 Representative MRM chromatograms of ten AP (0.00800~5.00 µg/L)

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Table 2 Accuracy and precision for the analysis of amlodipine in human plasma (in pre-study validation, n=3 days, six replicates per day)

Compound	Spiked concentration (0.040-25 µg/L)		Spiked concentration (0.800-500 µg/L)	
	RSD (%)	Average Recovery (%)	RSD (%)	Average Recovery (%)
Risperidal	4.04	103.04	1.56	136.75
Ziprasidone	3.78	58.75	1.56	83.17
Quetiapin	7.45	108.00	1.17	128.00
Haloperidol	1.93	93.50	0.87	132.67
Aripiprazole	3.33	98.00	3.85	112.50
Chlorpromazine	7.48	125.04	6.39	110.42
Sertraline	2.64	101.33	1.24	116.83
Prochlorperazine	3.14	112.25	7.69	109.67
Clonazepam	1.78	75.93	1.24	98.67
Alprazolam	6.54	82.00	1.43	112.33

Conclusions

A fully-automatic versatile SPE-UHPLC-MS/MS system was successfully applied to the direct analysis of ten Aps in serum. Excellent repeatability, low LODs and satisfactory recoveries were obtained under the optimized conditions. In conclusion, the online SPE-UHPLC-MS/MS system is a simple and effective technique for the automatically analysis of trace compounds in complex matrix.

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