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High Sensitivity Identification of Drug Metabolites with Increasing Ionization Efficiency using A Novel LC-ESI Interface and Q-TOF

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1. Introduction

Identification and quantitation of drug metabolites is an essential part of the drug discovery process. High sensitivity and high mass accuracy analysis is required to detect and identify low levels of potentially undesirable metabolites. High levels of chemical background and matrix effects interfere with the ability of mass spectrometers to detect and characterize metabolites. Conventional LC flow rate instruments may be unable to detect minor metabolites due to the lack of sensitivity. Although nanoflow ESI helps to enhance the signal, the analytical time becomes longer and robustness is challenging. In this work, we demonstrate the ability of a micro-flow LC-ESI system with a high performance Q-TOF to detect low levels of drug metabolites.

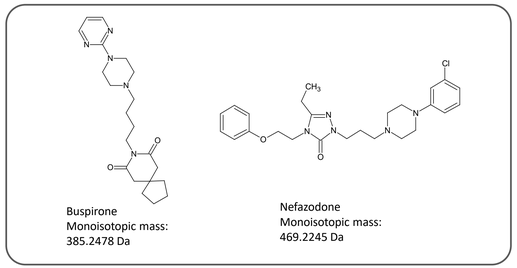

Figure 1 Structure of parent drugs used for drug metabolites analysis

2. Methods

Authentic standards of several well-studied drug targets were subjected to in-vitro metabolic processing with human liver microsomes. The reaction was pre-incubated by adding 10 μL of target compound(500 μM) to 80 μL of microsomes from human male liver at 0.25 mg/mL in potassium phosphate buffer (pH 7.4) and waiting 5 min at 37 ˚C. Then after 5 min, NADPH was added to start the reaction. After 60 min, 20 μL of stop solution (92:5:3 water: acetonitrile: formic acid) was added. Samples were analyzed after centrifugation to remove particulates.

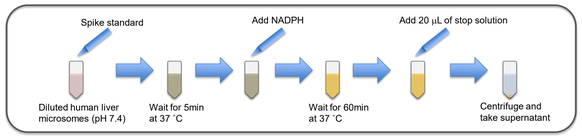

Figure 2 Sample preparation procedure

Figure 3 LCMS-9030 quadrupole time of flight(Q-TOF) mass spectrometer with Nexera Mikros microflow liquid chromatograph

3. Results

3-1. Method development

The authentic standards of several well-studied drug targets shown in figure 1 were subjected to in-vitro metabolic processing with human liver microsomes. Reactions were analyzed by LC with a microflow interface coupled to a high performance Q-TOF mass spectrometer. LC, ESI and MS conditions were optimized to maximize performance. MS1 Scan and data-dependent tandem MS analysis was carried out to characterize drug metabolites. A microflow C18 column was used for all targets and metabolites to improve the signal of some minor metabolites without loss of throughput. To confirm the improvement of signal by using microflow, conventional flow analysis was also carried out. Data analysis was performed to detect and tentatively identify metabolites using accurate mass analysis.

UHPLC conditions (Conventional flow)

Column: Shim-pack GISS C18 50mm × 2.1mm, 1.9 μm
Mobile phase A: 0.1% Formic acid
B: 0.1% Formic acid in acetonitrile
Flow rate: 300 μL/min
Time program: B conc.10%(0.5 min) -50%(6.5min) - 98%(6.5-8.5min)
Column temperature: 40 ˚C

UHPLC conditions (Microflow)

Column: Shim-pack MC C18 50mm × 0.175mm, 1.9 μm
InertSustainSwift C18 150mm × 0.175mm, 1.9 μm (for buspirone)
Mobile phase A: 0.1% Formic acid
B: 0.1% Formic acid in acetonitrile
Flow rate: 2 μL/min
Time program: B conc.10%(3 min) -50%(9min) - 98%(9-11min)
B conc.10%(3 min) -61%(10min) - 98%(10-12.5min) (for buspirone)
Column temperature: 40 ˚C

MS conditions (Conventional flow)

Nebulizing gas: 3.0 L/min
Heating gas: 10.0 L/min
Drying gas: 10 L/min
Interface Temperature: 300 ˚C
DL temperature: 250 ˚C
Heat block temperature: 400 ˚C

MS conditions (Microflow)

Nebulizing gas: 0.5 L/min
Heating gas: 3.0 L/min
Drying gas: OFF
Interface Temperature: 100 ˚C
DL temperature: 100 ˚C
Heat block temperature: 400 ˚C

3-2. Comparison of Chromatogram between Conventional and Microflow

Figure 4 and Figure 5 show scan chromatograms of tentatively identified metabolites of buspirone and nefazodone detected after 60 min in-vitro metabolic processing with human liver microsomes. Analysis took less than 15 minutes per sample, including column rinsing, and excellent separation and high sensitive detection with microflow were obtained. Table 1 and Table 2 show the signal improvement realized by using microflow. Some tentatively identified metabolite peaks were detected only by microflow analysis and all peak signals were improved. The data stability of microflow was equivalent to conventional flow and both methods showed good stability even with weak signal peaks, because the high resolution mass analysis gave low background levels.

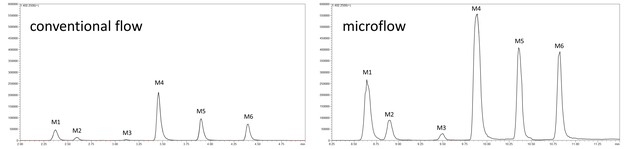

Figure 4 Chromatograms of tentatively identified metabolites of buspirone

Table 1 Comparison of nefazodone metabolite signals between conventional and microflow LC

Peak Height (signal)			
#	Conventional	Micro	Ratio
M1	46,338	266,339	5.7
M2	12,663	83,413	6.6
M3	4,475	29,391	6.6
M4	211,525	550,084	2.6
M5	95,727	399,358	4.2
M6	71,713	383,743	5.4

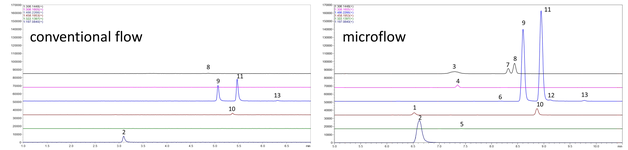

Figure 5 Chromatograms of tentatively identified metabolites of nefazodone

Table 2 Comparison of nefazodone metabolite signals between conventional and microflow LC

Peak Height (signal)					
#	Conventional	Micro	Ratio	CV% (conventional)	CV% (micro)
1	ND	2,931	-	-	5.7
2	7,512	27,218	3.6	6.7	1.8
3	ND	2,595	-	-	7.0
4	ND	3,218	-	-	3.7
5	ND	356	-	-	9.4
6	ND	244	-	-	5.7
7	ND	6,484	-	-	1.8
8	443	12,930	29.2	6.3	2.5
9	19,283	88,963	4.6	3.0	2.0
10	1,953	8,023	4.1	3.3	4.6
11	27,012	111,528	4.1	5.6	5.4
12	ND	1,361	-	-	3.5
13	662	991	1.5	7.6	5.7

*CV% were calculated by area (n = 3)

3-3. Qualitative Analysis of Metabolites of Small drugs

Metabolites were tentatively identified using high mass accuracy Q-TOF analysis. The information from the high resolution mass spectrum and better sensitivity helps to characterize exactly what kind of metabolites were detected. Figure 6 shows spectra of two buspirone metabolites collected by microflow Q-TOF. Microflow improved the sensitivity of buspirone metabolites more than four fold compared with conventional flow and could detect well known hydroxylated metabolites accurately. From this result, it was found that microflow Q-TOF has the capability to detect and characterize minor compounds much better than conventional flow. Figure 7 shows the structural assignment of nefazodone metabolites. Peak number 4 was only detected by microflow Q-TOF (shown on Figure 5) was identified with the specific spectrum.

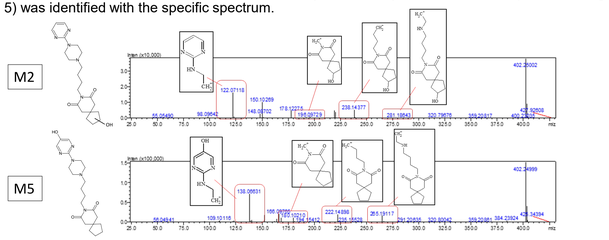

Figure 6 Structural estimation of metabolites derived from buspirone

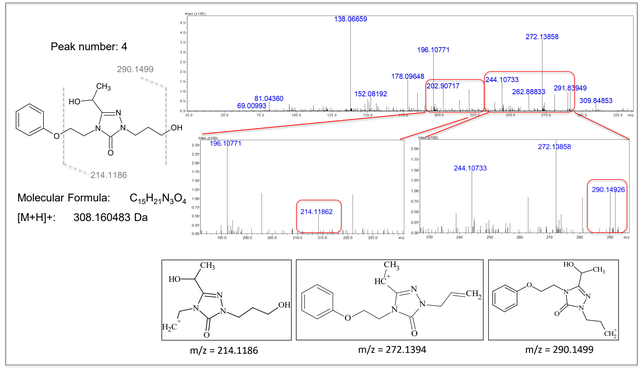

Figure 7 Identification of minor nefazodone metabolites detected by microflow Q-TOF

4. Conclusions

- By using microflow, all metabolite peaks had improved signal and the better sensitivity would help to discover potentially toxic metabolites of drugs not detectable with conventional flow.
- The better results were achieved without sacrificing analytical run time and the data stability was also equivalent to conventional flow.
- Analytical data showed that the stability was maintained even at low flow rate.