SHIMADZU
Excellence in Science

PO-CON1205E

Evaluation of the higher sensitive LC/MS/MS incorporates novel desolvation technologies to achieve low femto-gram LOQ

IMSC 2012 PMo-006

Shinjiro Fujita, Natsuyo Asano, Kazuo Mukaibatake
SHIMADZU CORPORATION, Kyoto, JAPAN

SHIMADZU
Excellence in Science

Evaluation of the higher sensitive LC/MS/MS incorporates novel desolvation technologies to achieve low femto-gram LOQ

Introduction

The triple quadrupole mass spectrometer is widely used in various application fields to quantify the trace amount of compounds because of excellent sensitivity and selectivity. In order to exceed the low femto-gram barrier, many researchers have been developing new desolvation technologies at either ESI sprayer or MS inlet.

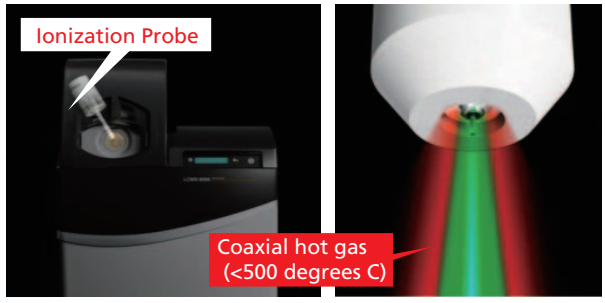
In this paper we present the development novel desolvation devices coupled to a highly sensitive triple quadrupole mass spectrometer utilizing a coaxial hot gas ion source and the heated multi-orthogonal interface (Hot Source Induced Desolvation: HSID) mounted at the inlet of mass spectrometer. The combination of the coaxial hot gas and the HSID enhanced desolvation efficiency which resulted in low femto-gram limit of quantitation.

Methods

7 commercially available drug samples (Verapamil, Alprazolam, Carbamazepine, Cilostazol, Lidocaine, Fluticasone and Testosterone) were prepared for the sensitivity evaluation of LCMS-8080 triple quadrupole mass spectrometer (Shimadzu Corporation, Japan) equipped with coaxial hot gas and HSID interface. All samples were analyzed by the Multiple Reaction Monitoring (MRM). MRM parameters including MRM transitions and collision energy as well as compound dependent ion transfer voltages were optimized through automatic MRM optimization functionality incorporated in LabSolution software (Shimadzu Corporation, Japan). The temperatures of coaxial hot gas and HSID were optimized for each sample. Chromatographic separations were carried out on a Nexera MP system (Shimadzu Corporation, Japan) using a Shim-pack XR-ODSIII (50 mmL.× 2.0 mmI.D., 1.6 mm).

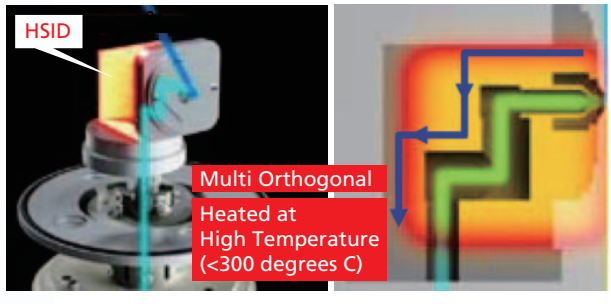
Fig. 1 LCMS-8080 triple quadrupole mass spectrometer.

Coaxial Hot Gas



High temperature gas blowing around the electro spray coaxially achieves highly efficient desolvation and accelerates ionization, resulting in larger volumes of ions introduced into the mass spectrometer.

HSID (Hot Source Induced Desolvation)

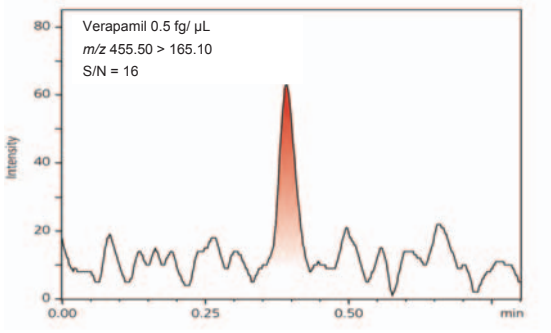


Noise derived from neutral species or unwanted ions are strongly reduced in the multi orthogonal region which is heated to a high temperature and achieves excellent signal to noise ratio.

Fig. 2 Coaxial hot gas and HSID.

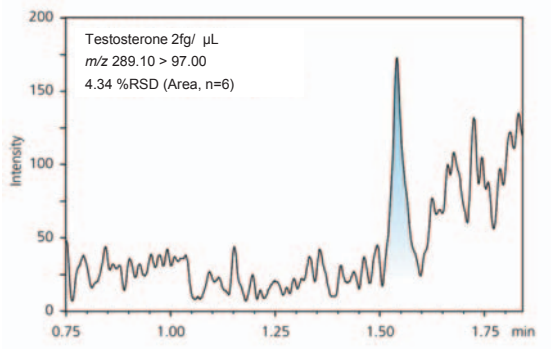
Evaluation of the higher sensitive LC/MS/MS incorporates novel desolvation technologies to achieve low femto-gram LOQ

Results



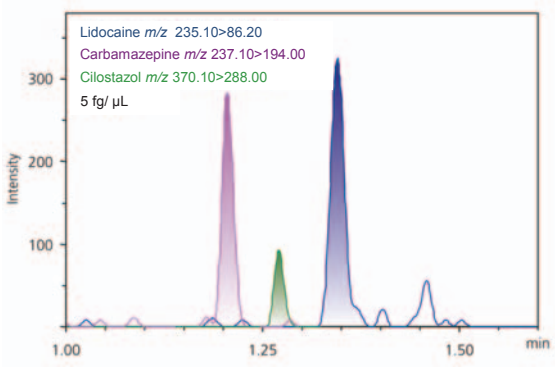
MRM chromatogram for verapamil at 0.5 fg/ µL. Extremely high sensitivity has been achieved with the lower limit of detection in the region of below femto-gram level.

Coaxial Hot Gas : 500°C
HSID : 280°C
Mobile phase A : 5mM Annmonium acetate-water
Mobile phase B : Acetonitrile
Flow rate : 0.5 mL/min



MRM chromatogram for testosterone, a type of steroid. At 2 fg/ µL, at the LOQ region, the RSD is 4.34% for 6 repeated analysis.

Coaxial Hot Gas : 450°C
HSID : 240°C
Mobile phase A : 0.1 % formic acid - water
Mobile phase B : Acetonitrile
Flow rate : 0.4 mL/min



MRM chromatogram for 3 drugs at 5 fg/ µL. In each case, the area repeatability (RSD) was less than 6%, a low noise level has been achieved, and with excellent selectivity, the LOQ is several femto-gram or less.

Coaxial Hot Gas : 500°C
HSID : 280°C
Mobile phase A : 5 mM Annmonium acetate-water
Mobile phase B : Acetonitrile
Flow rate : 0.4 mL/min

Fig. 3 MRM chromatograms at lower concentration.

Table 2 ynamic range, linearity (R^2) and Area %RSD for each compound

Compounds	Dynamic Range (fg/ µL)	R^2	Area %RSD
Verapamil	0.5-200,000	0.9995	0.08-10.70
Alprazolam	2-100,000	0.9998	1.78-7.92
Carbamazepine	2-20,000	0.9999	0.93-6.54
Cilostazol	2-50,000	0.9999	0.42-12.38
Lidocaine	5-50,000	0.9996	0.36-8.10
Fluticasone	5-200,000	0.9999	0.39-18.63
Testosterone	2-20,000	0.9997	0.40-4.34

Evaluation of the higher sensitive LC/MS/MS incorporates novel desolvation technologies to achieve low femto-gram LOQ

With cilostazol calibration curves, an extremely good linearity value of R^2 = 0.9999 was obtained across a dynamic range from 2 fg/ µL to 50,000 fg/ µL. In addition, even at low concentrations, a relative error under 10 % and RSD under 15 % were achieved.

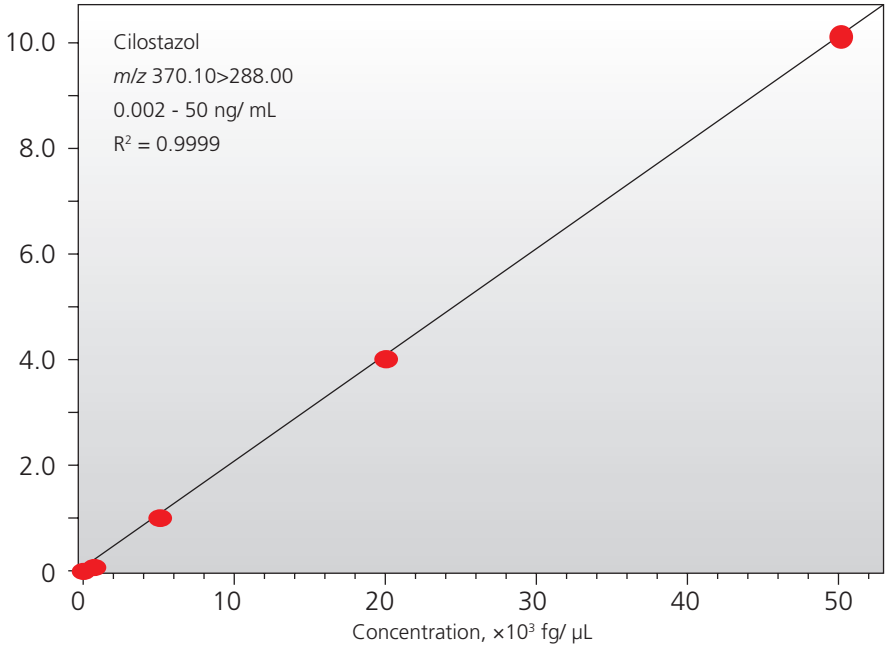


Fig. 4 Calibration curves for Cilostazol.

Table 3 Relative Error (RE) and Area %RSD for each concentration of Cilostazol.

Perared Concentration x10³ fg/ µL	Measured Concentration x10³ fg/ µL	Rerative Error (RE) %	Area %RSD (N=5)
0.002	0.0021	5.89	12.38
0.005	0.0048	-3.76	5.03
0.05	0.049	-1.62	2.78
0.2	0.20	-1.35	1.47
0.5	0.50	-0.20	1.46
5	4.99	-0.24	0.42
20	20.09	0.43	0.93
50	49.93	-0.14	0.57

Conclusions

Using LCMS-8080 equipped with coaxial hot gas and HSID, low femto-gram LOQs were achieved for all 7 drugs (Verapamil, Alprazolam, Carbamazepine, Cilostazol, Lidocaine, Fluticasone and Testosterone) delivering excellent linearity of R^2 > 0.999 across wide range from low to high concentrations.

First Edition: September, 2012

Shimadzu Corporation
www.shimadzu.com/an/