

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

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## 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

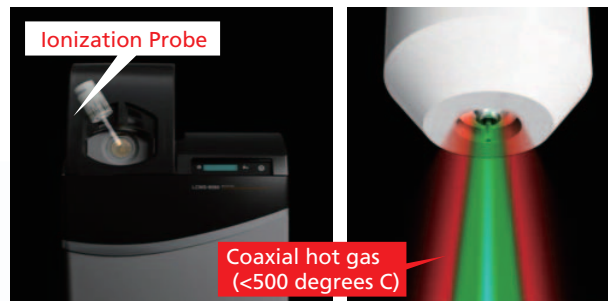
desolvation devices coupled to a highly sensitive triple quadrupole mass spectrometer utilizing a coaxial hot gas ion source and the heated multi-orthogonal interface (Hot Source Induced Desolvation: HSID) mounted at the inlet of mass spectrometer. The combination of the coaxial hot gas and the HSID 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

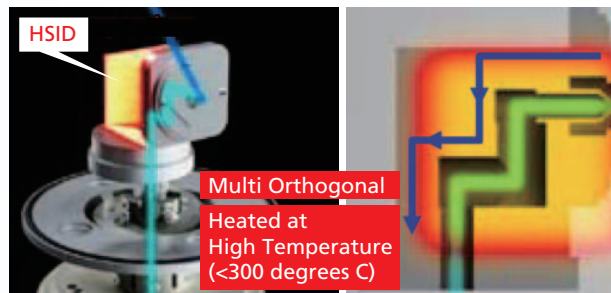
coaxial hot gas and HSID were optimized for each sample. Chromatographic separations were carried out on a Nexera MP system (Shimadzu Corporation, Japan) using a Shim-pack XR-ODSIII (50 mmL. x 2.0 mmI.D., 1.6 mm).

### 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.

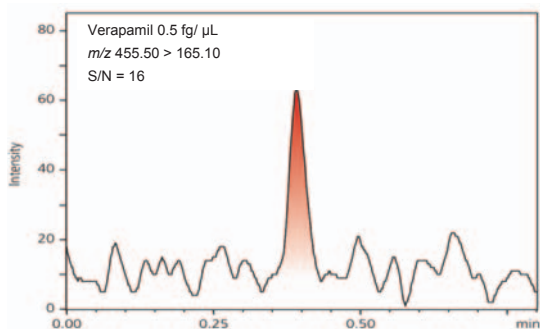


Fig. 1 LCMS-8080 triple quadrupole mass spectrometer.

Fig. 2 Coaxial hot gas and HSID.

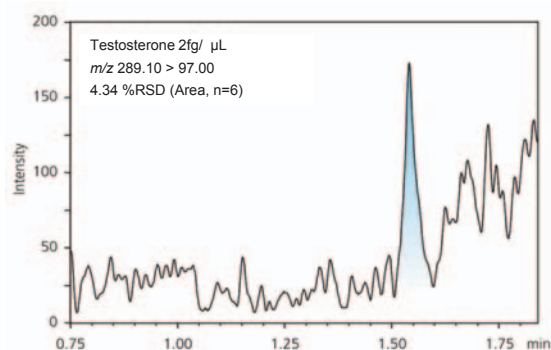
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## Results



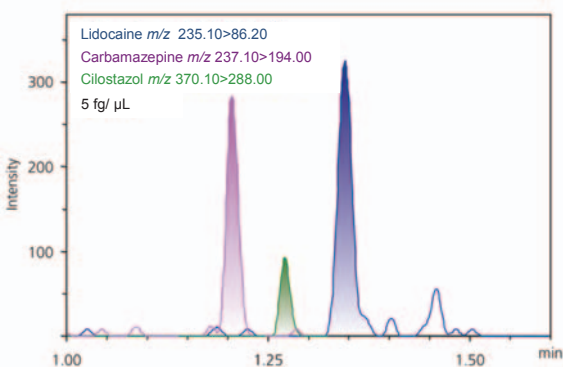
MRM chromatogram for verapamil at 0.5 fg/µL. Extremely high sensitivity has been achieved with the lower limit of detection in the region of below femto-gram level.

Coaxial Hot Gas : 500 degrees C  
HSID : 280 degrees 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 degrees C  
HSID : 240 degrees 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 degrees C  
HSID : 280 degrees C  
Mobile phase A : 5mM 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 (R2) and Area %RSD for each compound

Compounds	Dynamic Range (fg/µL)	R <sup>2</sup>	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

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With cilostazol calibration curves, an extremely good linearity value of  $R^2 = 0.9999$  was obtained across a dynamic range from 2 fg/  $\mu\text{L}$  to 50,000 fg/  $\mu\text{L}$ . In addition,

even at low concentrations, a relative error under 10 % and RSD under 15 % were achieved.

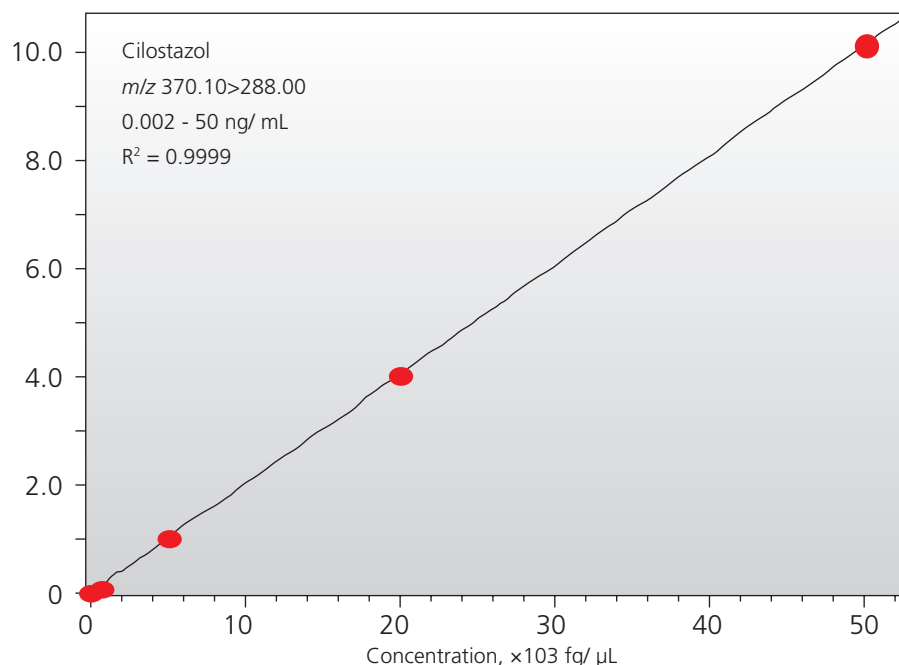


Fig. 4 Calibration curves for Cilostazol.

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

Perpared Concentration $\times 10^3$ fg/ $\mu\text{L}$	Measured Concentration $\times 10^3$ fg/ $\mu\text{L}$	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.



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