SHIMADZU

LC-MS Application Data Sheet No. 51

Analysis of N-nitroso-fenfluramine using **LC-MS**

A provisional method for analyzing N-nitroso-fenfluramine has been indicated by the Japanese National Institute of Health Sciences, where LC-MS or LC-PDA, or alternatively GC-MS is utilized. The data shown here was generated by obtaining 50 mg from a capsule of a diet food product, adding 5 mL of methanol, and analyzing the extract with a LCMS-2010A system (extraction ratio and recovery rate were not studied). Figure 1 shows the TIC (total ion chromatogram) and mass chromatogram for the sample and Figure 2 shows the mass spectra. Fenfluramine was eluted with a retention time of six minutes and a MH^+ m/z 232 peak was detected. In addition, after an N-nitroso-fenfluramine retention time of 16.5 - 17.5 min, two peaks indicating isomers were eluted, where MH^+ m/z 261 and $[M+H+CH_3CN]^+$ m/z 302 peaks were detected.

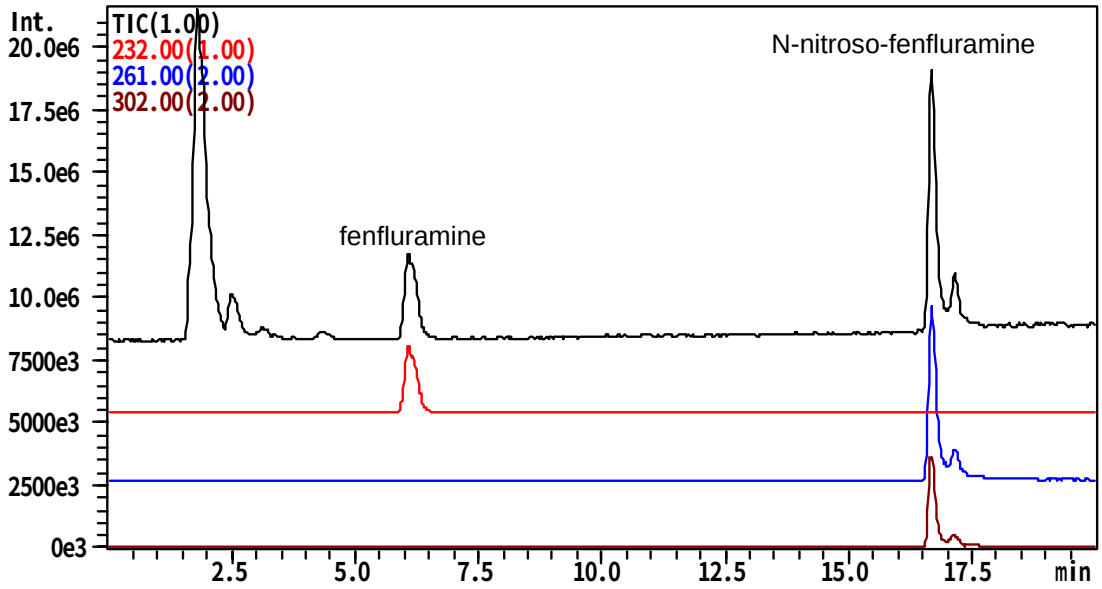

Fig. 1 Total ion chromatogram and mass chromatograms

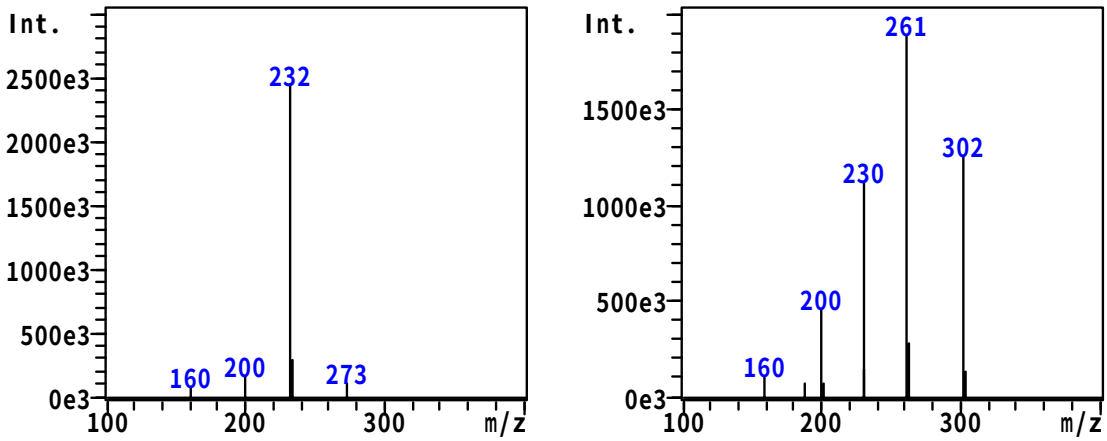

Fig. 2 Mass spectra of fenfluramine (left) and N-nitroso-fenfluramine (right)

Since a standard fefluramine product is available in market, SIM (selected ion monitoring) was used for measurement and generating calibration curves. Detection at 1 ng/mL (2 pg) was possible and the correlation coefficient at 1 - 100 ng/mL (n=2) was over 0.999

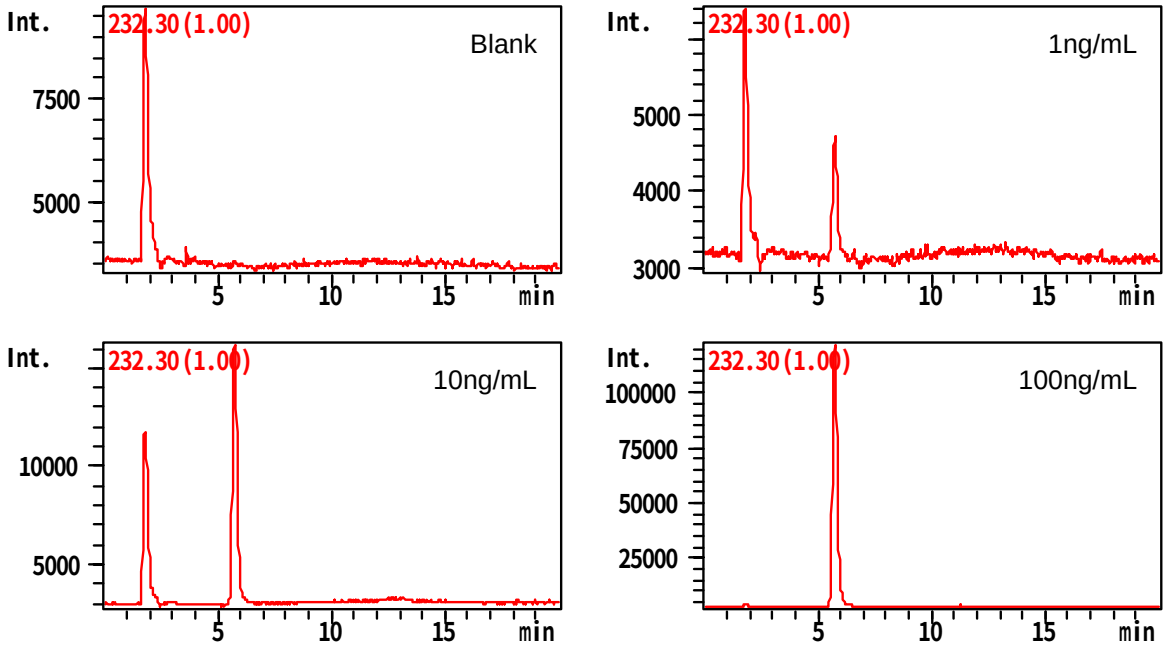

Fig.3 SIM chromatograms of fenfluramine

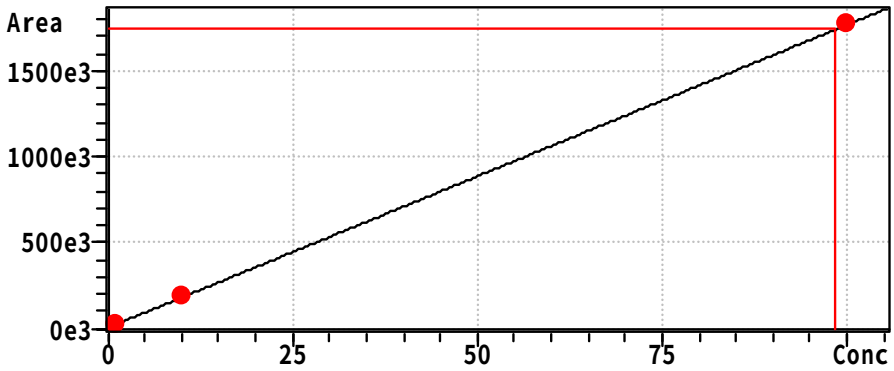

Fig.4 Calibration curve of fenfluramine

Table 1 Analytical conditions for LC-MS

Column	: Phenomenex LUNA 5u C18(2) (2.0 mmI.D. x 150 mmL)		
Mobile phase A	: 0.1% trifluoroacetic acid in water		
Mobile phase B	: 0.1% trifluoroacetic acid in acetonitrile		
Time program	: 30%B (0 min) - 80%B (15 min) - 80%B (20 min)		
Flow rate	: 0.2 mL/min		
Injection volume	: 2 µL	**Column temperature**	: 30 °C
Probe voltage	: +4.5 kV (ESI-Positive mode)		
CDL temperature	: 250 °C	**Block heater temperature**	: 200 °C
Nebulizing gas flow	: 1.5 L/min		
Drying gas flow	: 0.2 MPa		
CDL voltage	: Scan-mode		
Q-array DC voltage	: Scan-mode	**Q-array RF voltage**	: Scan-mode
Scan range	: m/z 100 - 400 (1.0 sec/scan)		
SIM	: 232.3 (1.0 sec/ch, fenfluramine)		

SHIMADZU CORPORATION **International Marketing Division**
3. Kanda-Nishikicho 1-chome, Chiyoda-ku, Tokyo 101-8448, Japan
Phone: 81 (3) 3219-5641 Fax. 81 (3) 3219-5710
Cable Add. SHIMADZU TOKYO