SHIMADZU

LC-MS Application Data Sheet No. 003

LC-MS analysis for vitamin D_3 using atmospheric pressure chemical ionization

Vitamins are essential to maintaining health. Since the body is either completely incapable of producing certain vitamins or incapable of producing vitamins in sufficient quantity to meet its needs, problems occur when sufficient quantities of vitamins are not obtained through food.

Vitamins are broadly separated into two categories: water soluble and fat soluble. Compared to water soluble vitamins, extreme care is required in adjusting the intake of fat soluble vitamins (A, D, E, and K). The reason for this is simple. Although excess water soluble vitamins can be purged through the kidneys, fat soluble vitamins are stable, heat resistant, and do not dissolve in water. These vitamins therefore cannot be purged through the kidneys. As a result, fat soluble vitamins accumulate in the liver and excessive levels of these vitamines can cause problems. For example, vitamin D promotes absorption of calcium ions from the intestines and re-absorption of calcium ions from bones and kidneys. The action of vitamin D therefore increases the concentration of calcium ions in the blood. As a result, although soft bones and deformations (called osteoperosis) occur when vitamin D is lacking, excessive intake of the vitamin can cause side-affects such as kidney stones and calcification of the joints.

Although electrospray ionization (ESI), an atmospheric pressure ionization method, is often used as an LC-MS interface, atmospheric pressure chemical ionization (APCI), another type of atmospheric pressure ionization, is more applicable to low polarity compounds. An example of analysis of vitamin D3, a low polarity compound, is introduced here. Since the LCMS-QP8000 has both electrospray and atmospheric pressure chemical ionization probes as standard equipment, analysis over a wide range--from low polarity compounds to high polarity compounds and ionic compounds--is possible.

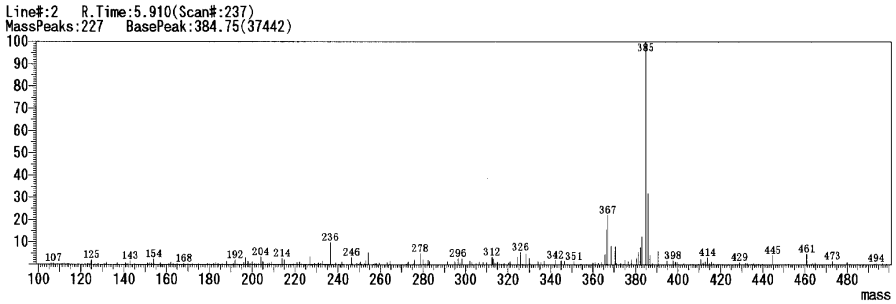

Fig. 1 Mass spectrum of Vitamin D_3

With atmospheric pressure chemical ionization, the mobile phase is first vaporized and reaction ions are generated by corona discharge. Next, ion-molecule reactions between the reaction ions and the target compound under atmospheric pressure cause ionization of the target compound. Polar solvents, such as methanol or water, included in the mobile phase will act as reaction ions.

Fig. 1 shows the mass spectrum obtained for vitamin D3 and Fig. 2 shows the results of selective ion detection (SIM) for the protonated molecule. Although other components appear on the actual chromatogram, analysis is possible without the influence of these components when selectivity is provided by SIM. In addition, the component with a retention time of 8 minutes on the mass chromatogram does not even appear on the UV chromatogram (Fig. 3). Finally, when a calibration curve is created (Fig. 4), good linearity can be obtained on the ppb level.

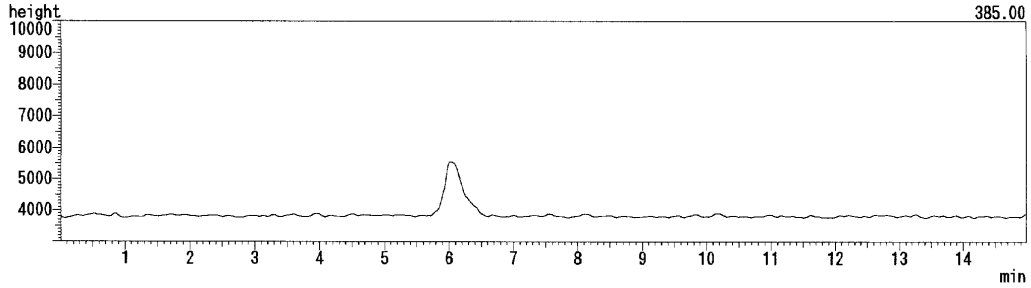

Fig. 2 SIM chromatogram of vitamin D_3 at m/z 385

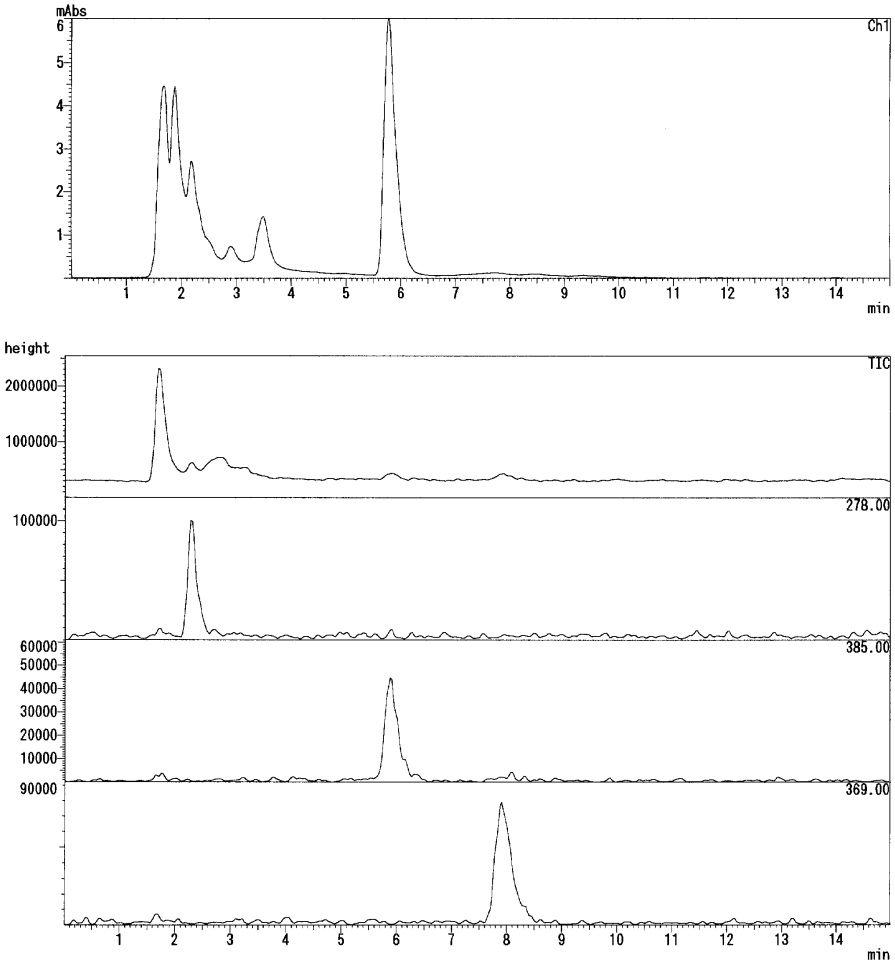

Fig. 3 UV and mass chromatograms of vitamin D_3

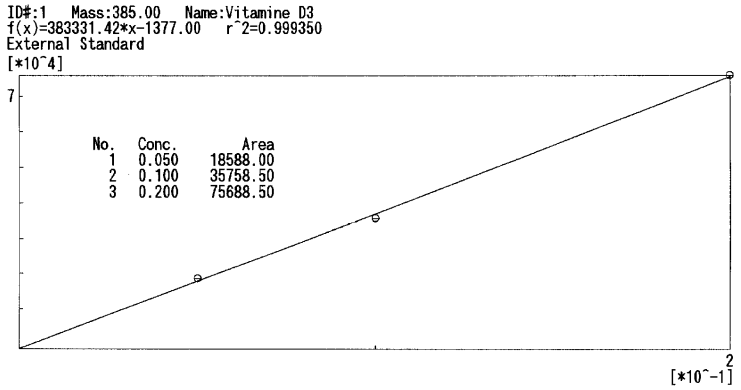

Fig. 4 Calibration curves for vitamin D_3

Table 1 Analytical conditions for LC-MS

Column	: STR ODS-II (2.0 mmI.D. x 150 mm)		
Mobile phase	: methanol	**Flow rate**	: 0.2 ml/min
Injection volume	: 20 □l	**Column oven temp.**	: 40 °C
APCI probe voltage	: +3.5 kV (APCI-Positive mode)	**APCI probe temp.**	: 400 °C
Nebulizing gas flow	: 2.5 L/min		
CDL voltage	: -60 V	**DEFs voltage**	: +30 V

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