Rapid Determination of Vitamin D_3 in Poultry Feed Supplements

Application Note

Food and Flavors

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Introduction

Vitamin D is an essential constituent in both human and animal nutrition. Vitamin D_3, one of the biologically active forms of vitamin D, plays a central role in calcium and phosphate homeostasis and is essential for the proper development and maintenance of bone. Livestock diets deficient in vitamin D have been implicated in a number of growth abnormalities including seizures, bone fracturing, thin or soft egg shells, decreased egg production and hatching, and diseases of the liver and kidneys.[1, 2] As a result, proper regulation of the supplemental vitamin D_3 enriched in animal feeds is critically important.

Traditional methods for analyzing vitamin D in feed extracts rely on gas chromatographic/mass spectrometric (GC/MS) techniques because of the high sensitivity and specificity of these techniques in complex matrices. However, feed analysis utilizing GC/MS assays often require extensive and time-consuming sample preparation and derivatization prior to analysis.[3]

Atmospheric pressure ionization (API) techniques coupled with ion trap mass spectrometric detection are particularly well suited for the analysis of liposoluble vitamins, such as vitamins A, D, and E. These API/ion trap techniques provide a sensitive analytical method that eliminates the sample preparation/derivatization that is typically required for many GC/MS assays. Furthermore, flow injection analysis combined with the multiple MS stage capability of the ion trap mass spectrometer permits the development of a rapid, sensitive, and specific analytical method for detecting vitamin D_3 in animal feed.

This note demonstrates the use of flow injection analysis using the power of MS^n to rapidly screen for the presence of vitamin D_3 in a poultry feed extract without the need for chromatographic separation.

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Experimental

All experiments were done using an Agilent 1100 Series LC/MSD Trap system composed of a binary pump, a vacuum degasser, and an autosampler. The system was operated with the atmospheric pressure chemical ionization (APCI) source in the positive ion mode.

Reagent grade chemicals and HPLC grade solvents were used in preparing mobile phases and standards.

Results and Discussion

Figure 1 shows the full scan mass spectrum obtained from flow injection analysis of a poultry feed extract enriched with vitamin D_3. The mass spectrum shows a peak at *m/z* 385 that suggests the presence of vitamin D_3 in the feed extract. However, because the spectrum is very complex and shows a peak at almost every mass, the data cannot be used to conclusively establish the presence of vitamin D_3 in the sample.

By using the ion trap mass spectrometer, one can quickly and easily design an experiment to specifically select the desired precursor ion at *m/z* 385 in a complex matrix such as that of animal feed, and then isolate and fragment it to generate the full scan product ion spectrum corresponding to vitamin D_3. Figure 2 shows the full scan MS/MS spectrum obtained from a second flow injection analysis of the enriched feed extract. MS/MS of *m/z* 385 produces predominantly a product ion at *m/z* 367, which corresponds to the elimination of a single water molecule. Water-loss peaks are considered structurally nonspecific because they are a commonly observed neutral loss and occur in many different types of compounds. In order to confirm the presence of vitamin D_3 in the feed extract, more specific information related to the structure of vitamin D_3 is necessary. To accomplish this, a simple MS^3 experiment can be designed to automatically perform an additional MS stage on the *m/z* 367 water-loss product ion.

Figure 3 shows a comparison of the product ion spectra obtained from MS^3 flow injection analysis following the transition *m/z* 385 → 376 → (Figure 3b) done on the feed extract sample, as well as on a pure standard of vitamin D_3 (Figure 3a). The MS^3 spectrum of pure vitamin D_3 (Figure 3a) shows a unique "fingerprint-type" pattern that is rich in structurally specific product ions.

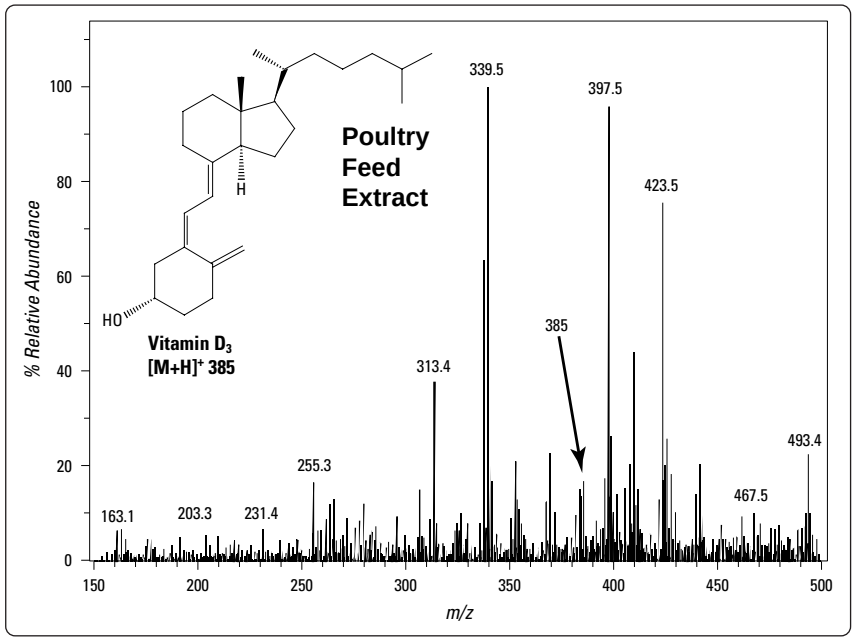

Figure 1. Full scan mass spectrum of a vitamin D_3 enriched poultry feed extract from a 10 µL loop injection in the positive APCI mode.

ANALYSIS METHOD

Flow injection

Flow rate:	0.6 ml/min
Injection volume:	10 µl
Mobile phase:	A = 0.05% Acetic Acid in Water B = Methanol

50/50% MeOH/H_2O

MS Conditions

Source:	APCI
Drying gas flow:	10 l/min
Nebulizer:	40 psig
Drying gas temperature:	350°C
Skim 1:	15.0 V
Cap Exit Offset:	60.0 V
Averages:	2
ICC:	On
Max Accu Time:	200 ms
Target:	50000
Ion mode:	Positive

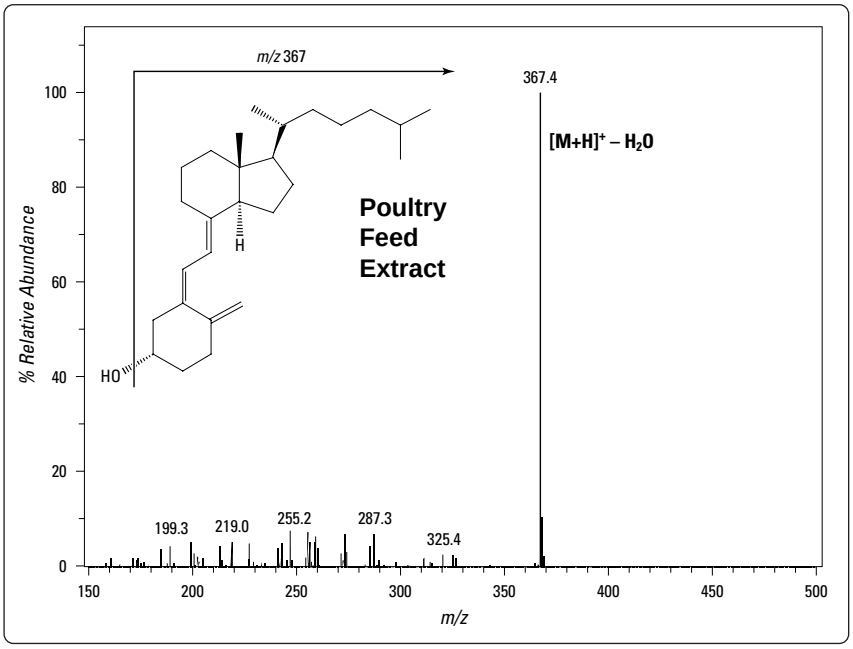

Figure 2. Full scan MS/MS spectrum (MS/MS of *m/z* 385) of a vitamin D_3 enriched poultry feed extract showing a predominance of the non-specific water-loss peak at *m/z* 367 from a 10 µL loop injection in the positive APCI mode.

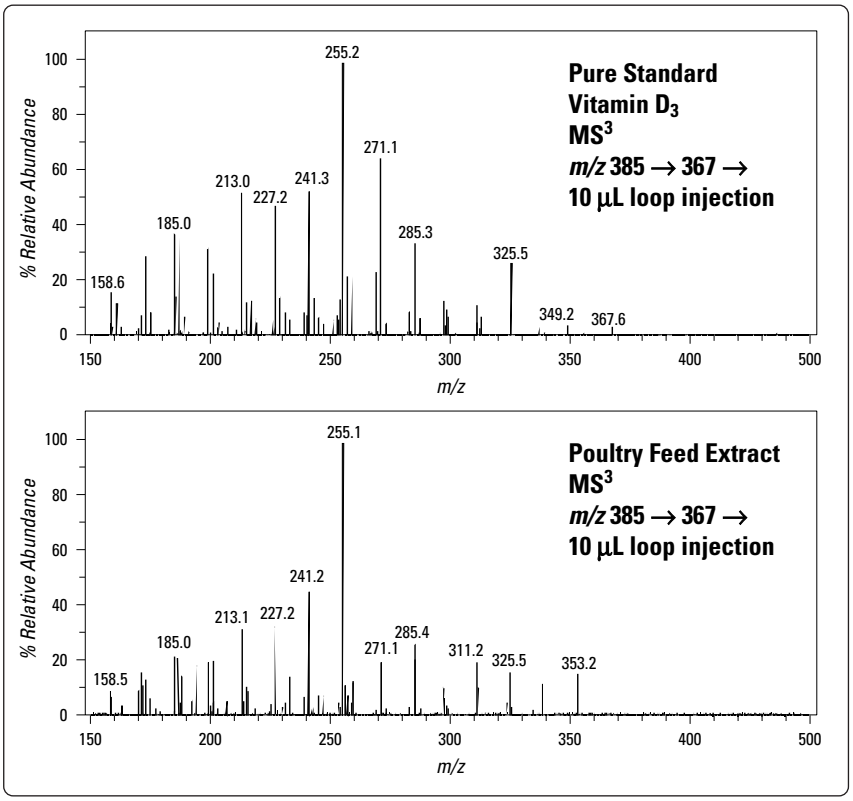

Figure 3. Comparison of product ion spectra from a pure standard of vitamin D_3 and an enriched poultry feed extract.

Following the loss of water in the first MS/MS process, the MS^3 spectrum of a pure standard of vitamin D_3 shows a successive hydrocarbon loss of 14 amu. Comparison with the MS^3 spectrum of the enriched feed extract (Figure 3b) shows a similar pattern of hydrocarbon losses, and thus good agreement between the two spectra. Such specific product ion information can be used to confirm the presence of vitamin D_3 in the complex feed matrix.

Conclusions

MS^n analysis using an ion trap mass spectrometer permits significantly more rapid method development, especially for determining target analytes in complex matrices. Full scan MS^3 experiments can be quickly and easily designed to automatically perform multiple stages of mass selection and fragmentation in order to produce a unique "fingerprint-type" fragmentation pattern that is specific to the molecular structure of the target analyte. In this manner, the unique capability of ion traps to perform multiple stage MS^n ensures unambiguous compound identification in complex matrices using simple flow injection analysis without chromatographic separation.

References

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