FORMIC ACID–AMMONIUM FORMATE BUFFER SYSTEM FOR LC/MS ANALYSIS OF VITAMIN D

Stephen C. Roemer, Subhra Bhattacharya, and Deva Puranam Fisher Chemical – Thermo Fisher Scientific

Abstract

Acidic mobile phase solutions consisting of 0.05% formic acid and 10 mM ammonium formate in methanol and water were developed for analysis of vitamin D using LC/MS. Proper retention/elution of 25-OH vitamin D_2 through reverse phase columns and trace level MS detection was achieved with these solutions due to their optimal ionic strength, low pH, and interference-free baseline.

Introduction

A challenge for clinical research laboratories has been the variability within different methodologies for accurate measurement of vitamin D in biological fluids, although LC/MS is positioned best to standardize the process by providing high specificity and sensitivity via suitable chromatography and MS detection (1). In the present work, an acidic buffer system for mobile phase solutions was evaluated for trace level analysis of 25-OH vitamin D_2 using ESI-LC/MS.

Methods

Stock solutions of 25-OH vitamin D_2 (NIST, Cat. # SRM 2972) were obtained in ethanol and stored at -20°C. Fisher Chemical mobile phases had the following formulations: 10 mM ammonium formate and 0.05% formic acid in water (MB123) and in methanol (MB122).

An Agilent 1100 series LC equipped with the Model SL single quadrupole mass spectrometry detector (MSD) was used for the vitamin D assay. Zorbax SB-C8 column (2.1 mm x 150 mm, 3.5 micron pore size) was selected. The sample elution was performed with column temperature of 50°C at a flow rate of 0.25 ml/min for 30 min using the gradient profile in Table 1. ESI-MS data was generated in positive mode with capillary voltage 3500V and fragmentation voltage 70V. Approximately two nanogram of 25-OH D_2 standard was loaded on column per injection.

Time (min)	% MB123	% MB122
2	60	40
10	15	85
12	15	85
20	0	100
30	0	100

Table 1
LC gradient for vitamin D_2 assay.

Results and Discussion

The selected ion monitoring (SIM) chromatogram of 25-OH vitamin D_2 and the corresponding mass spectra (Figs. 1, 2) show that the mass baseline (noise level) contributed by the acidic (pH 3.5) mobile phases is very low in positive mode of MSD. Also, no tailing of m/z 395 peak was observed using this method. An earlier LC/MS study indicated that a formic acid–ammonium formate buffer system contributed to repression of peak tailing of amines resulting in improved signal intensity (2). Ongoing research shows this buffer system to be suitable for other compounds such as various hormones and immunosuppressive drugs which require acidic conditions for enhancing LC/MS detection.

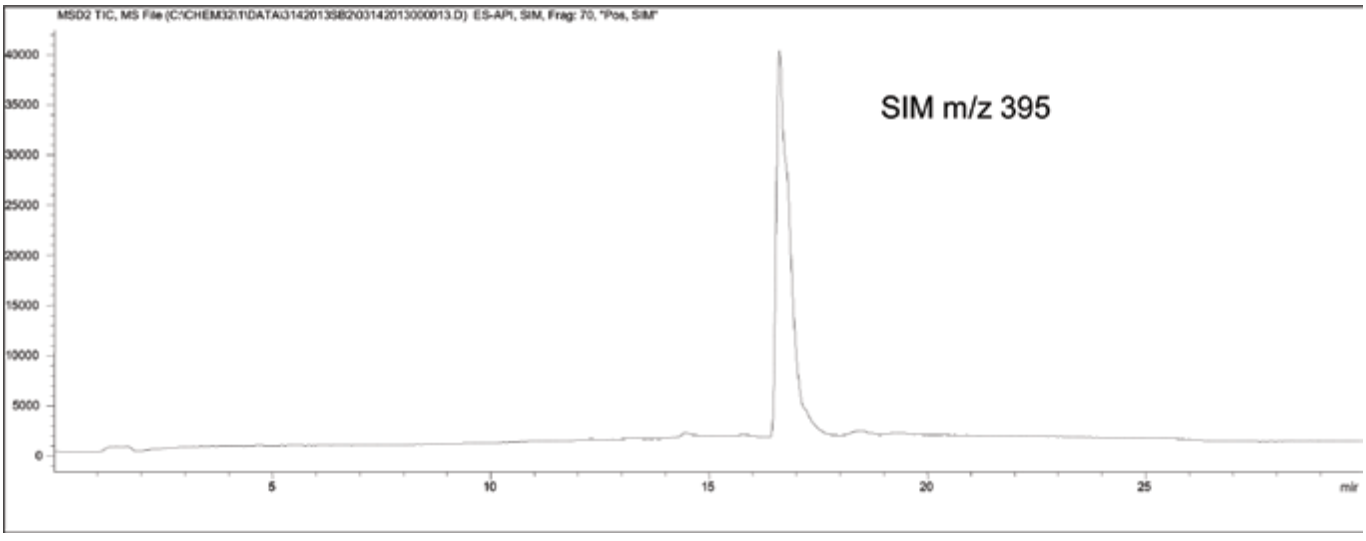

Figure 1
SIM of m/z 395 (25-OH vitamin D_2, 2.29 ng on column) using acidic mobile phases.

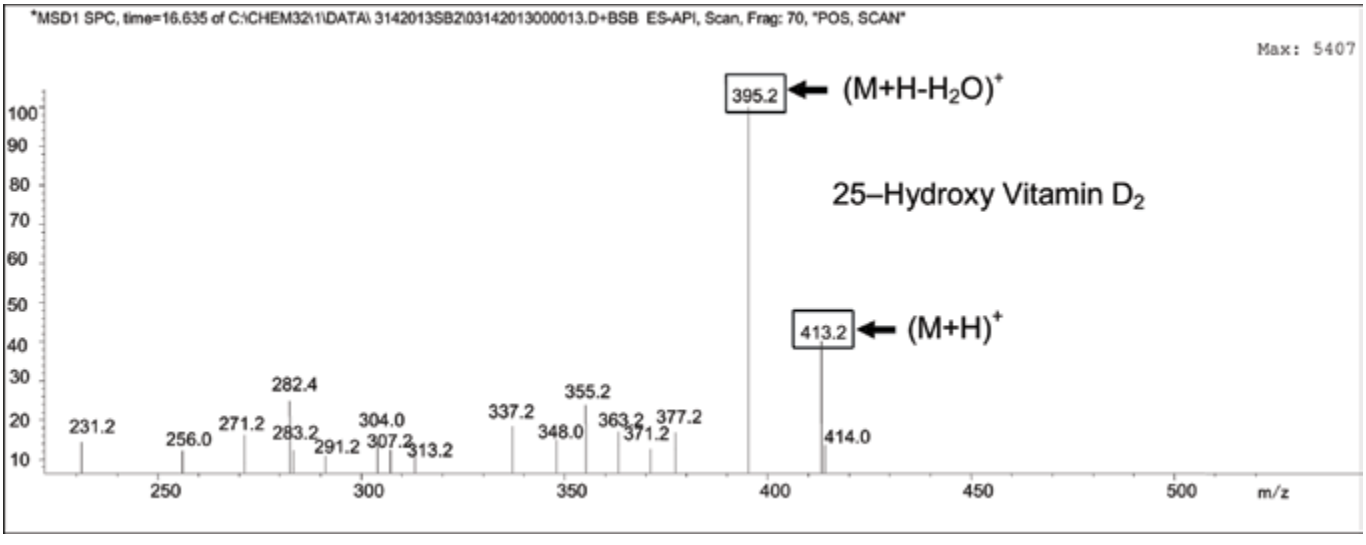

Figure 2
Corresponding mass spectra of the characteristic ions m/z 395 and m/z 413.

References

1. Grebe, S. and Singh, R.J. *Clin. Biochem.* Rev. 32, 5-31 (2011).
2. Iida, J. and Murata, T. *Analytical Sci.* 6, 269-272 (1990). ■