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Xevo TQ MS with Atmospheric Pressure Photo Ionization (APPI) Source: The Ionization of Compounds with Diverse Structures Using Vitamins as a Model

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This is an Application Brief and does not contain a detailed

Experimental section.

Abstract

Atmospheric Pressure Photo Ionization (APPI) is an alternative mass spectrometry ionization technique, which has demonstrated complementary capabilities to the more widely used LC-MS ionization techniques of ElectroSpray Ionization (ESI) and Atmospheric Pressure Chemical Ionization (APCI). In this application brief we demonstrate that APPI can be used to analyze both compounds that would typically be ionized using ESI, and those that would typically be ionized using APCI, offering a streamlined approach to method development and routine analysis, as well as removing the possibility of probe misidentification.

Introduction

Atmospheric Pressure Photo Ionization (APPI) is an alternative mass spectrometry ionization technique,[1,2,3] which has demonstrated complementary capabilities to the more widely used LC-MS ionization techniques of ElectroSpray Ionization (ESI) and Atmospheric Pressure Chemical Ionization (APCI).

APPI offers the ability to ionize a broader range of compounds than those ionized by either ESI or APCI alone, particularly very non-polar compounds, and highly aromatic compounds or species that contain regions of electron delocalization. In addition, APPI can ionize the majority of compounds that would typically be analyzed using ESI or APCI. This provides analysts with the option of using only one ion source where they would typically switch between two different ion sources depending on the chemical features of the analytes under consideration.

The APPI ion source configuration is similar to that of an APCI source, except the corona discharge pin of the APCI source is replaced with a krypton vacuum ultraviolet (V-UV) discharge lamp in APPI, as shown in Figure 1.

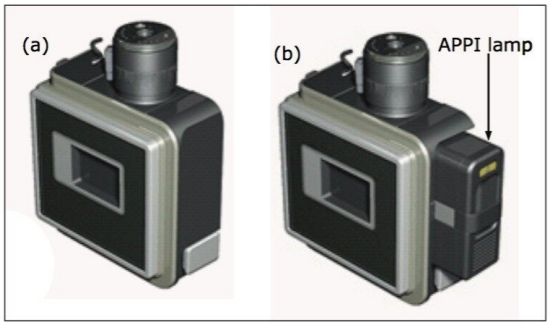

Figure 1. The APPI source has a krypton V-UV lamp in place of the APCI source corona discharge pin

The Waters Xevo family of MS systems and SYNAPT G2 share a common source platform, as shown in Figure 2. Waters' Engineered Simplicity means that the APPI source is easily fitted in place of the ESI source, utilizing the Xevo and SYNAPT G2 novel tool-free source exchange, as shown in Figure 3.

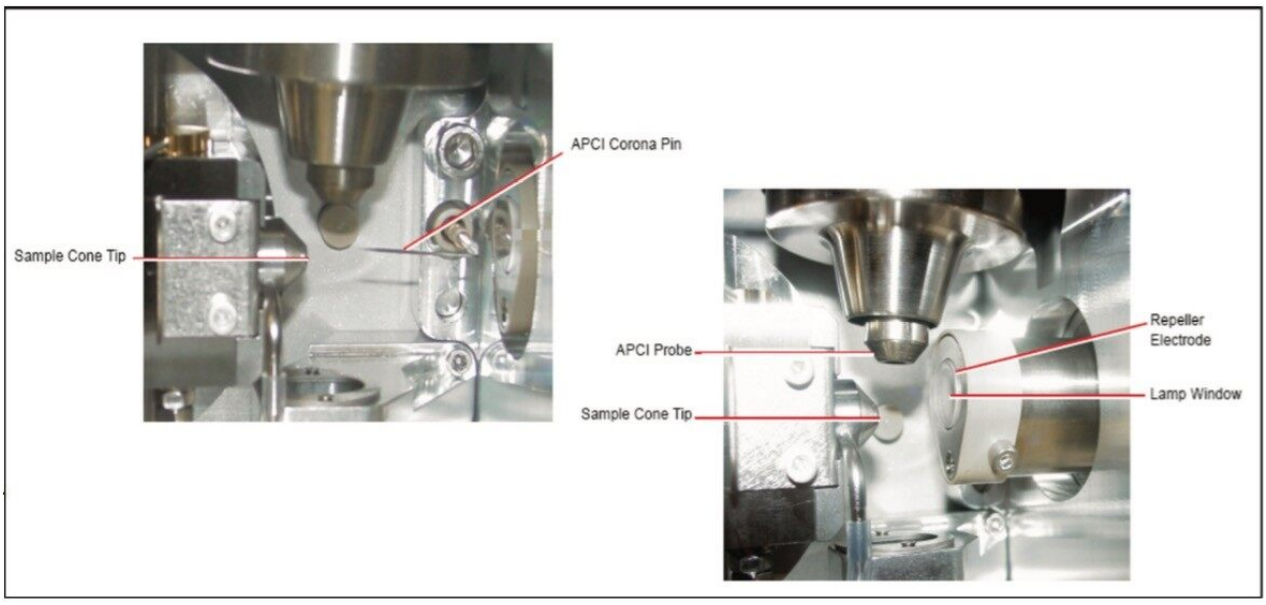

Figure 2. (a) The standard ESI ZSprayTM source; (b) The APPI ZSpray source.

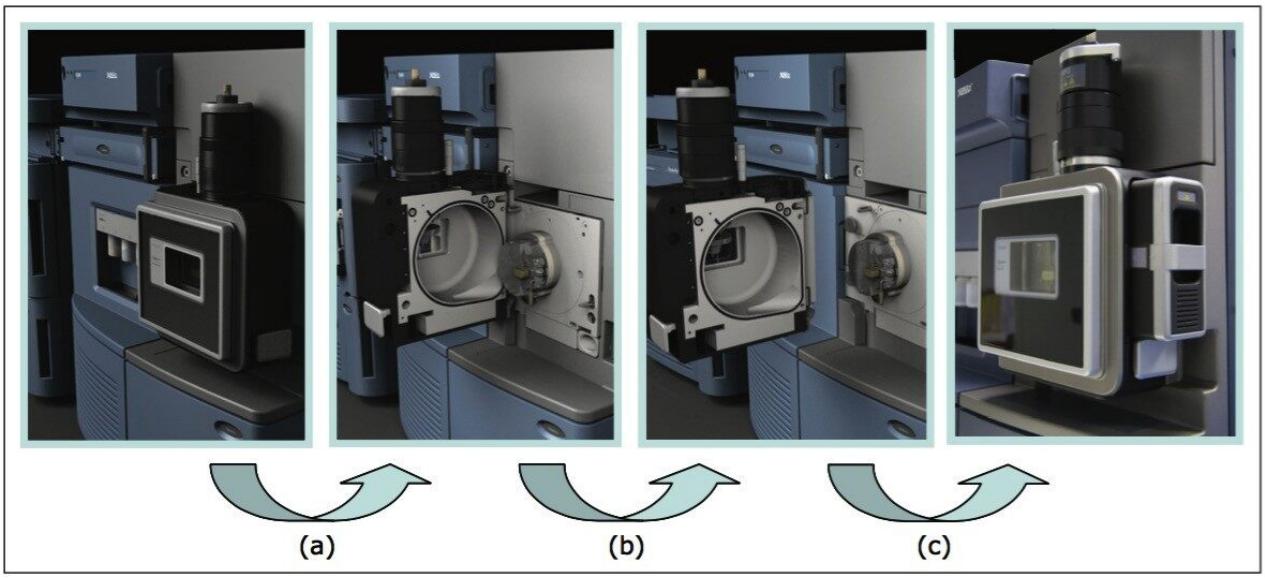

Figure 3. (a) The ESI source housing is easily opened; (b) The source housing is then removed; (c) The APPI source housing slips simply into place.

In this technical note we demonstrate that APPI can be used to analyze both compounds that would typically be ionized using ESI[4,5] and those that would typically be ionized using APCI,[6] thus offering the analyst a streamlined approach to method development and routine analysis, as well as removing the possibility of probe misidentification.

Experimental

Fat-soluble and water-soluble vitamin compounds were used to demonstrate the behavior and performance of the APPI ion source. Data for the fat-soluble vitamins were acquired in full scan MS mode using direct infusion from the on-board Xevo TQ MS fluidics system, and also in Multiple Reaction Monitoring (MRM) mode using the Waters ACQUITY UPLC System, coupled with Xevo TQ MS. Data for the water-soluble vitamins were acquired in MRM mode only.

APPI is a mass-flow sensitive technique, which means that the ionization efficiency is dependent on the total flow (LC eluent plus dopant) in front of the lamp. A greater response from compounds is seen when lower flow rates are used. For this reason, a total flow rate of around 0.3 mL/min was used in each case, and longer columns (150 mm long) were employed to best utilize the resolution of the ACQUITY UPLC System.

For the MRM acquisitions a mix of six water-soluble vitamins was prepared in water, and a mix of fat-soluble vitamins was prepared in 50:50 methanol:water. All compounds were at a concentration of 20 pg/µL, except vitamin B12, which was present at 2 pg/µL. In addition, for the MRM acquisitions a dopant solution, comprising 50:50 toluene:methanol, was infused into the LC eluent flow using the fluidics system. The dopant infusion was triggered simultaneously with the chromatographic acquisition by enabling Method Events in the MS Method File, and using the Method Events schedule shown in Figure 4. This provides a stable and easily controlled dopant delivery system that avoids the need to use additional pumps with the system. The dopant flow rate was maintained at 20 µL/min.

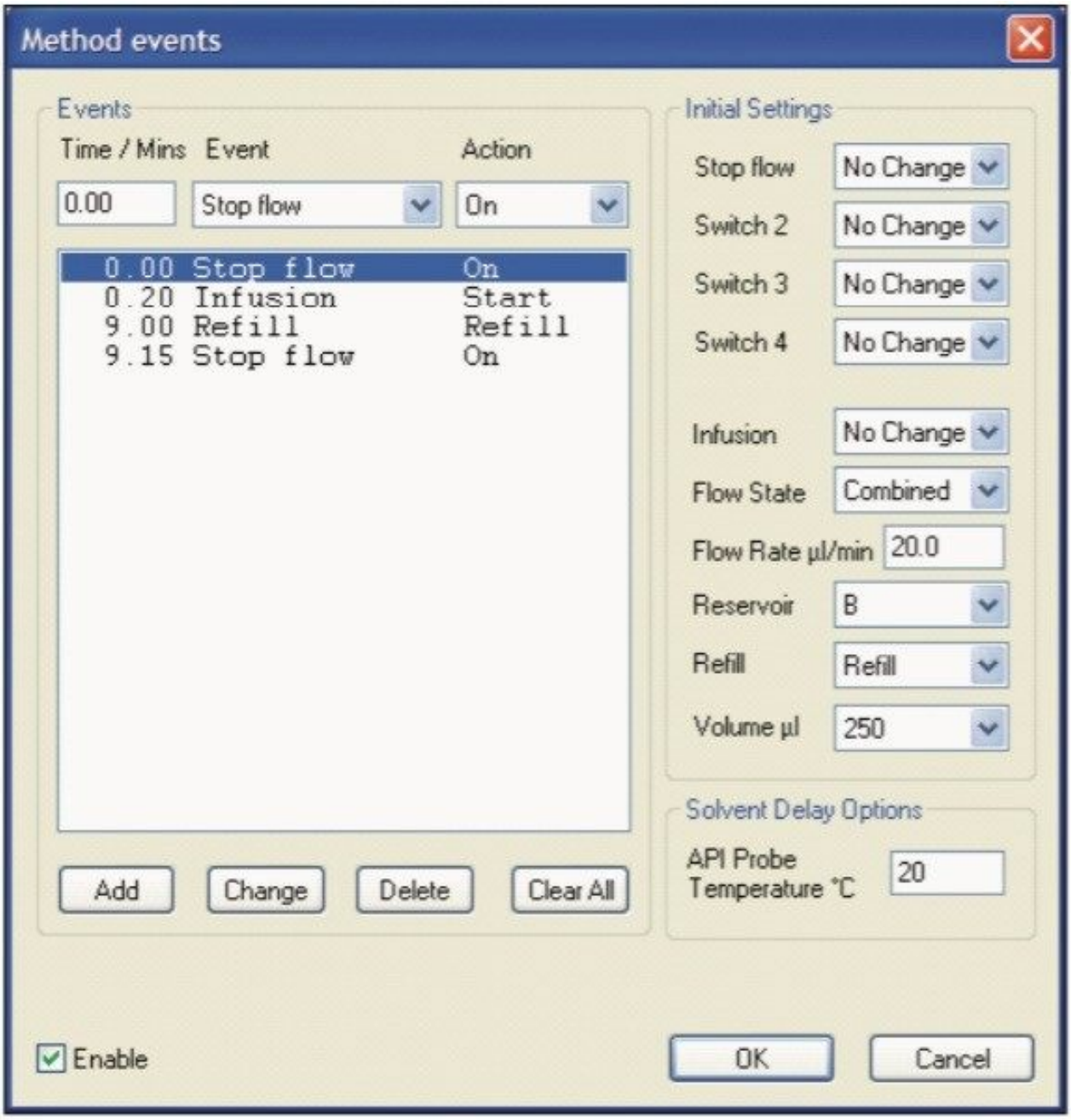

Figure 4. The Method events schedule was used to trigger the infusion of dopant during APPI MRM data acquisition.

For the acquisition of full scan MS data, fat-soluble vitamin standards were initially prepared to a concentration of 500 pg/µL in 50:50 methanol:toluene. These individual standard solutions were then infused directly into the mass spectrometer, using the fluidics system, at a flow rate of 200 µL/min. The standard solutions were prepared in a mixture of methanol and toluene so that the dopant was already

present in the solution to aid ionization.

MS conditions

MS System:	Waters Xevo TQ MS
Ionization mode:	APPI positive polarity
Repeller voltage:	0.9 kV
Desolvation gas:	Nitrogen, 1000 L/Hr,
Cone gas:	Nitrogen, 10 L/Hr
APCI Probe temp:	550 ˚C
Source temp:	150 ˚C
Dopant:	50:50 toluene:methanol
*Collision gas pressure:	Argon at 2.6 x 10^{-3} mBar
*Collision gas flow:	0.15 mL/min
*Collision energy:	3 eV (full scan acquisitions)

* When running the Xevo TQ MS, the collision gas remains on at all times, irrespective of the acquisition mode in use, to aid in the collimation and transmission of ions through the instrument.

The MRM transitions, along with cone voltage and collision energy values used, are shown in Table 1 for water-soluble vitamin compounds and in Table 2 for fat-soluble vitamin compounds.

Water-soluble vitamin compound	MRM transition	Cone voltage (V)	Collision energy (eV)
Thiamine (B1)	265.2 > 122.1	19	15
Pyridoxine (B6)	170.1 > 152.1	20	13
Nicotinamide (B3)	123.1 > 80.0	33	18
Cyanocobalamin (B12)	989.6 > 916.5	100	65
Biotin (B7)	245.1 > 227.1	22	15
Riboflavin (B2)	377.2 > 243.1	38	22

Table 1. MRM, cone voltage, and collision energy information for water-soluble vitamin compounds.

Fat-soluble vitamin compound	MRM transition	Cone voltage (V)	Collision energy (eV)
Retinol (A)	269.3 > 93.0	30	21
Menaquinone (K2)	445.4 > 187.1	25	27
δ-Tocopherol (E)	402.4 > 177.1	42	26
Ergocalciferol (D2)	397.4 > 379.4	22	12
Cholecalciferol (D3)	385.4 > 367.4	27	14
Phylloquinone (K1)	451.4 > 187.1	35	27

Table 2. MRM, cone voltage, and collision energy information for fat-soluble vitamin compounds.

Results and Discussion

Toluene was used as a dopant, to promote ionization of the analyte molecules. The dopant molecule has an ionization energy lower than that of the photons emitted by the krypton discharge lamp, i.e. lower than 10 eV. Toluene molecules absorb the photons, which causes them to ionize forming a toluene radical cation and an electron, as shown in Figure 5. In positive ion mode the radical cations subsequently react with solvent molecules, analyte molecules, or other species within the ion source, thus forming positive ions for the

analytes of interest. In negative ion mode, the electrons interact similarly, ultimately forming negatively charged analyte ions.

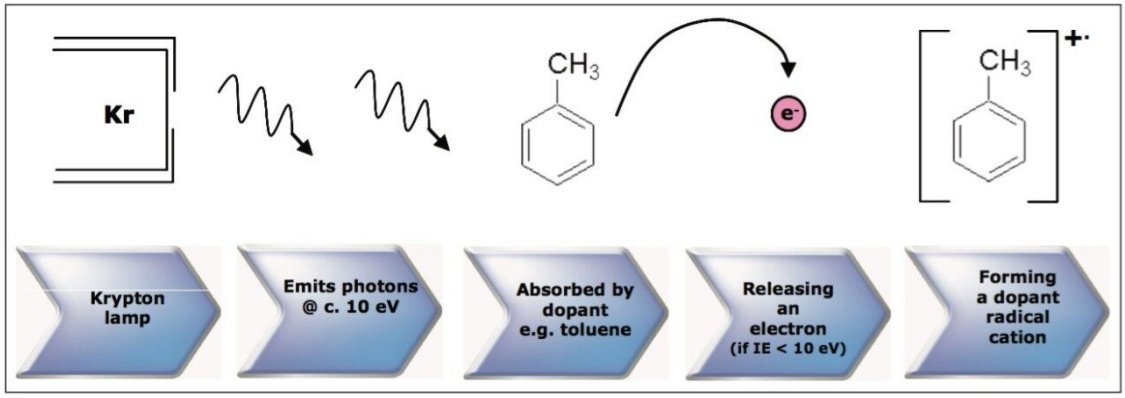

Figure 5. Schematic illustrating the action of a dopant in APPI.

Both water-soluble and fat-soluble vitamin compounds were successfully analyzed using an ACQUITY UPLC System coupled to a Xevo TQ MS with dopant-assisted APPI. Figure 6 shows the chromatograms acquired for a mixed solution of water-soluble vitamin compounds, with vitamins B1, B2, B3, B6, and B7 at 20 pg/µL and vitamin B12 at 2 pg/µL; alongside chromatograms acquired for a mixed solution of fat-soluble vitamins with all compounds at 20 pg/µL.

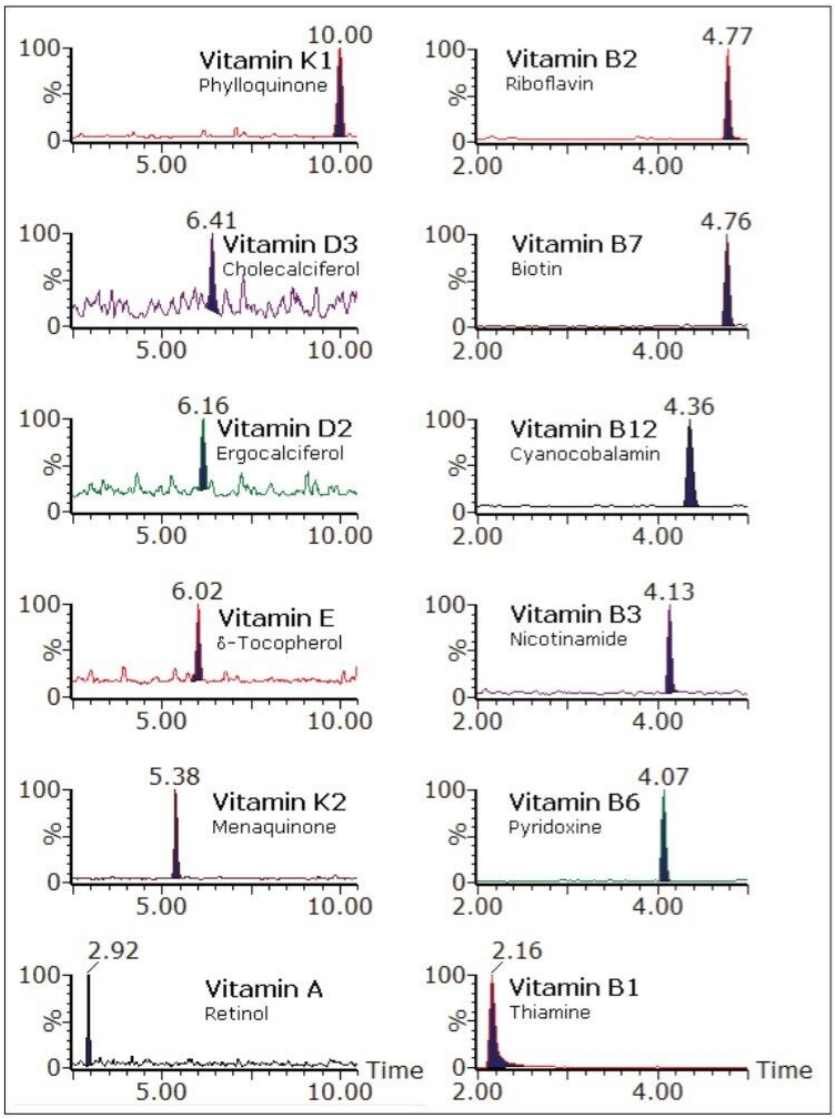

Figure 6. Chromatograms for a solution of six water-soluble vitamin compounds, and a solution of six fat-soluble vitamin compounds, all acquired using the ACQUITY UPLC System coupled to Xevo TQ MS with APPI.

Figure 7 shows the structures and monoisotopic masses of the vitamin compounds used in this technical note, illustrating that they have wide-ranging masses and diverse structures. The mass spectra acquired from infusion of four of the fat-soluble vitamin compounds provide information about the ionization processes taking place within the APPI source. Figure 8 shows the mass spectra acquired for [II] cholecalciferol (vitamin D3), [III] ergocalciferol (vitamin D2), [IV] δ-tocopherol (vitamin E), and [V] menaquinone (vitamin K2).

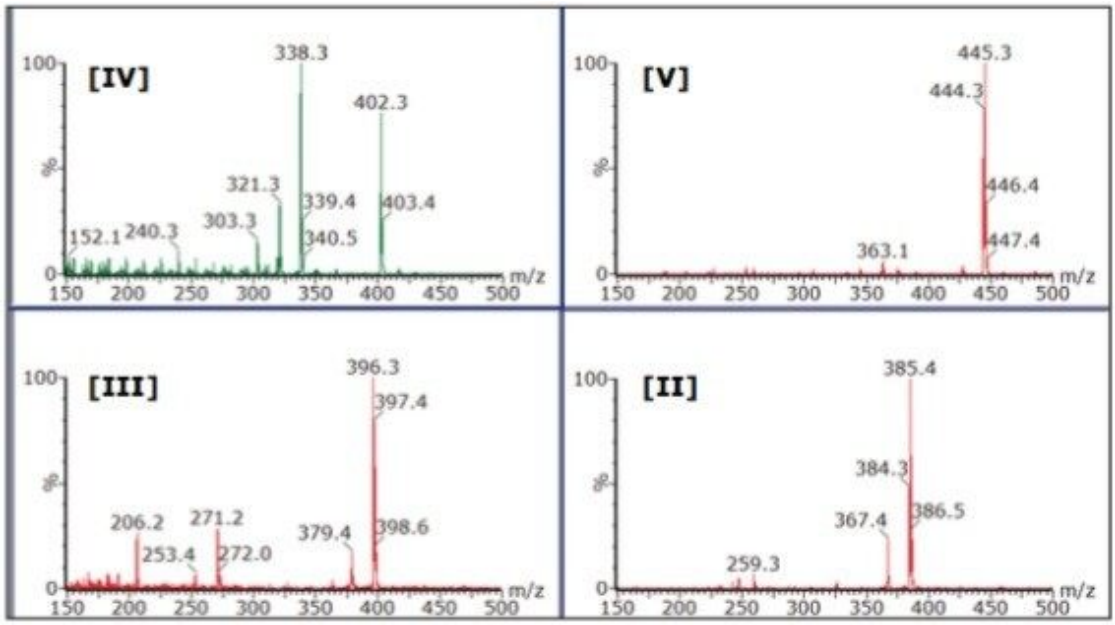

Figure 7. Structures and monoisotopic masses for the vitamin compounds used in this technical note.

Choices regarding MRM transitions should be made carefully, after considering the ionization processes taking place. If the parent ion regions of the spectra shown in Figure 8 are expanded, then the exact ratios of ions formed can be seen in more detail, as shown in Figure 9. This illustrates that some compounds form primarily radical cation parent ions (M^{+}.), such as d-tocopherol and ergocalciferol; whereas other compounds form primarily a protonated molecule ($[M+H]^{+}$) as the parent ion, for example menaquinone and cholecalciferol. However, in each case there is also a significant amount of the other type of ion formed as well, neither the radical cation nor the protonated molecule are formed in isolation. However, the relative distribution of parent ions remains constant and therefore quantitation is unaffected by this phenomenon.

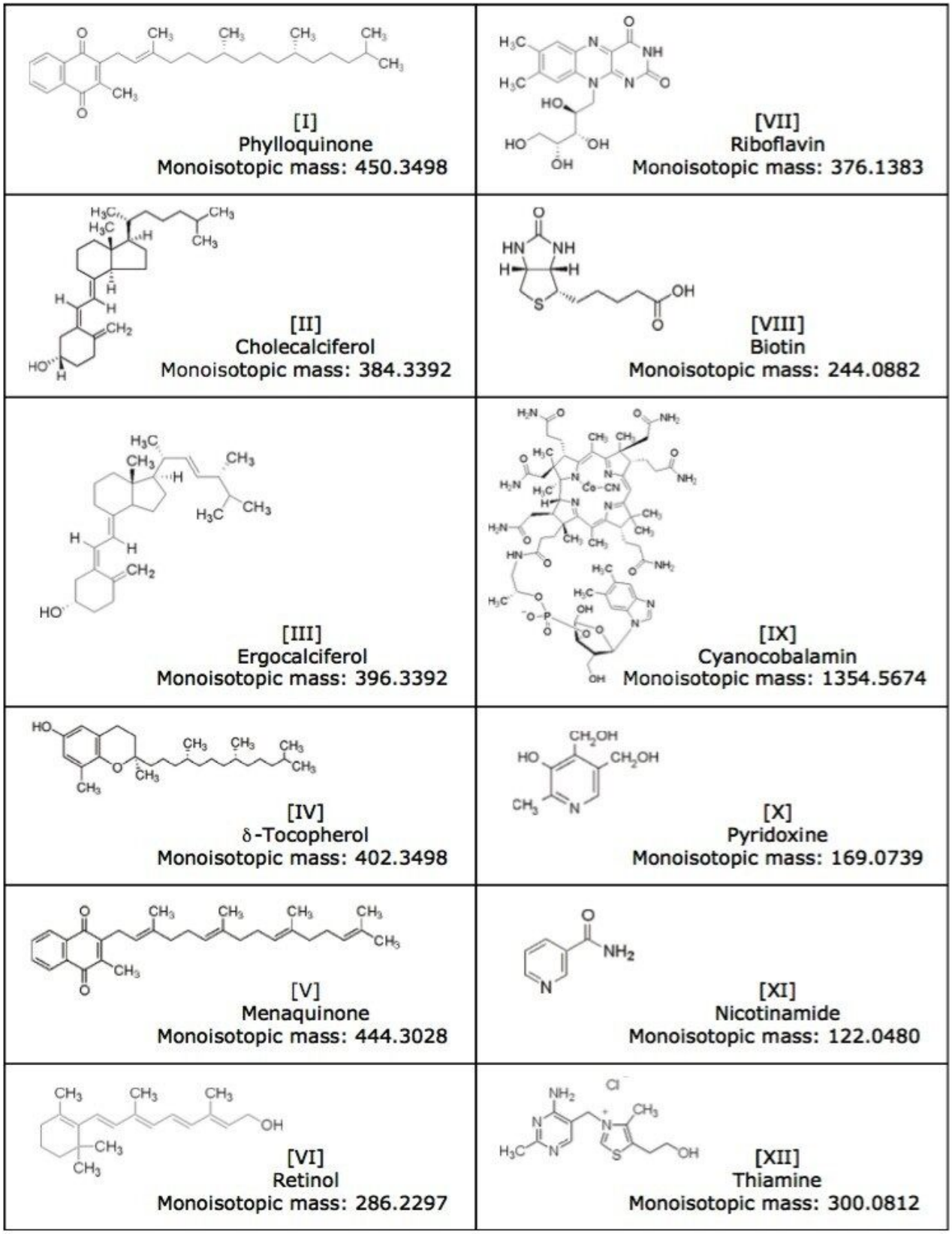

Figure 8. Mass spectra for four representative fat-soluble vitamin compounds. [II] cholecalciferol, [III]

ergocalciferol, [IV] δ-tocopherol, and [V] menaquinone.

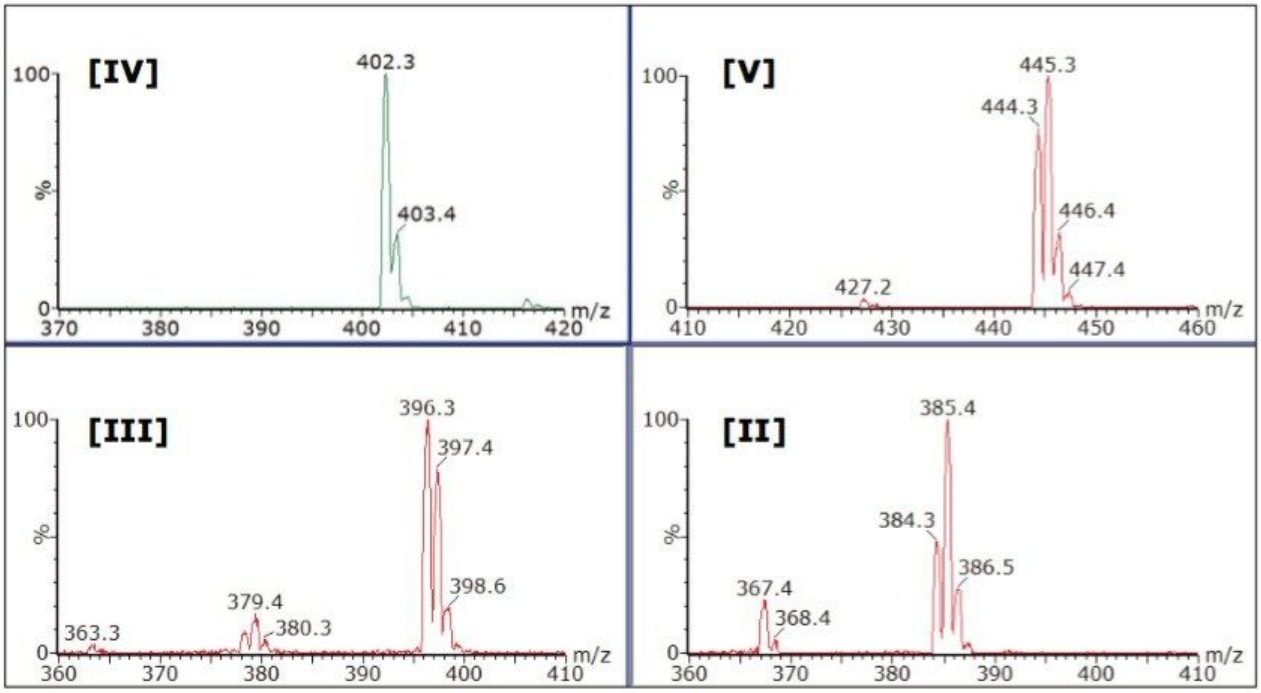

Figure 9. Expanded regions of the mass spectra for four representative fat-soluble vitamin compounds. [II] cholecalciferol; [III] ergocalciferol; [IV] d-tocopherol; [V] menaquinone.

Conclusion

This technical note demonstrates the suitability of APPI for ionizing a range of molecules with both polar and non-polar structures and a wide range of molecular masses. The analytical chemist is offered the possibility of ionizing a broader portfolio of molecules with dopant-assisted APPI than by ESI or APCI alone. This removes the requirement to switch between different ion sources when analyzing a selection of compounds with differing physicochemical properties, as illustrated with fat-soluble and water-soluble vitamin compounds.

The latest generation of Xevo MS and SYNAPT MS systems incorporate a common source housing engineered for all members of these classes of instrument, which provides quick and simple tool-free

exchange between different sources. In addition, the on-board fluidics system provides simple and reliable dopant delivery, avoiding the need for a separate syringe pump alongside the LC and mass spectrometer.

The APPI source complements the portfolio of ion sources for the already well-established atmospheric pressure ionization techniques provided by Waters, offering flexibility and greater choice in a challenging analytical arena.

References

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