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Comparison of Atmospheric Pressure Photoionization and Atmospheric Pressure Chemical Ionization for the Analysis of Ubiquinones and Menaquinones

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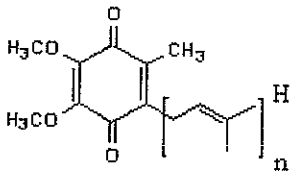
Atmospheric Pressure Photoionization (APPI) is a new gas phase ionization technique for LC/MS[1]. APPI offers a means to ionize non-polar samples that are not readily ionized by proton transfer or ion attachment in Atmospheric Pressure Chemical Ionization (APCI) or Electrospray (ES). There are three main means of producing an ion by APPI: (1) direct photoionization, (2) electron-transfer with a dopant ion, or (3) proton transfer with a dopant or other protic gas phase species. APPI makes use of an photoionizable dopant added to the vapor generated from the eluant to increase the ionization efficiency for the molecules in the vaporized LC eluant and also to influence the ionic species formed. This dopant is selected such that its ionic form will react by electron transfer or proton transfer with analyte species present in the ionization region. The high ion/molecule collision rate in the atmospheric pressure ionization region allows for highly efficient analyte ionization. We compare here the use of APPI and APCI for the analysis of ubiquinones and menaquinones (Figure 1).

All data were obtained on an API 365 triple quadrupole mass spectrometer outfitted with either an APCI or prototype APPI source[1] (SCIEX, Concord, Ontario). Heated nebulizer probe temperature was maintained at 400 °C. All solvents were HPLC grade. Sample standards were obtained commercially and used as received. Samples were analyzed by flow injection at 200 L/min with selected reaction monitoring (UQ_6: *m/z* 591 *m/z* 197; MK4: *m/z* 445 *m/z* 187). Toluene was used as the APPI dopant unless otherwise noted.

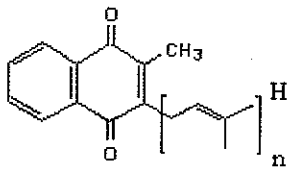
We found that APPI and APCI were equally sensitive for the analysis of ubiquinones as illustrated by the data in Figure 2. However, APPI was three times more sensitive than APCI when analyzing menaquinones (Figure 3). These latter compounds were not detected by us using ES-MS. We also noted that the APPI response was effected greatly by the source block offset potential. A potential between 2.5 and 3.0 kV appeared to be optimum for detection of both types of quinones (Figure 4).

The ionization process in APPI can be altered by using different dopants. In this case changing the dopant composition from toluene to acetone had no effect on sensitivity for either class of compounds (Figure 5). However, we did note that freshly distilled acetone provided less background noise than did anhydrous toluene used as received.

In general, APPI provided better sensitivity for the detection of menaquinones than did APPI. The technqiues were equally sensitive for the detection ubiquinones.



ubiquinone (UQ_6)



menaquinone (MK4)

Figure 1

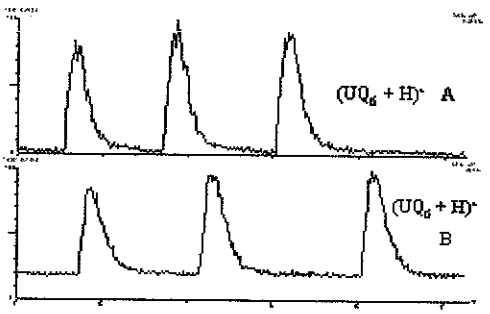


Figure 2. (A) APCI of UQ_6. (B) APPI of UQ_6. For both analysis 68 fmol/ L UQ_6, 200 L/min 50/50 ACN/H_2O, 20 L injected.

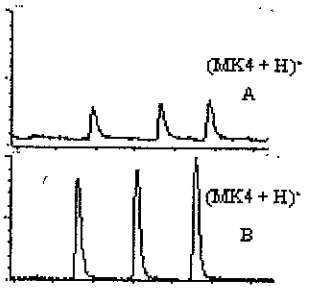


Figure 3. (A) APCI of MK4. (B) APPI of MK4. For both analysis 90 fmol/ L MK4, 200 uL/min 50/50 ACN/H_2O, 20 L injected.

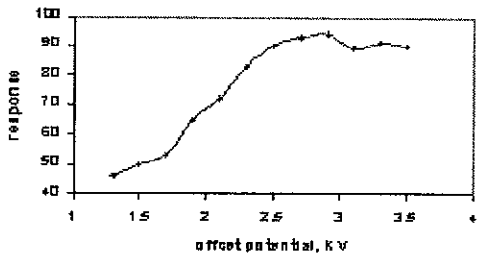


Figure 4 APPI response for UQ_6 as a function of source block offset potential.

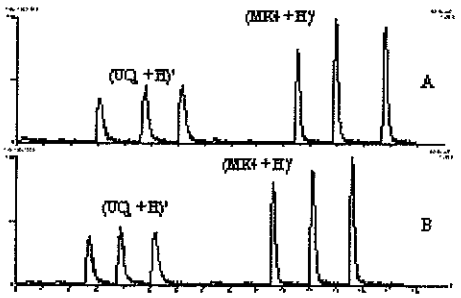


Figure 5. APPI of UQ_6, 68 fmol/ L and MK4, 90 fmol/ L in triplicate using (A) acetone and (B) toluene as the dopant. Both dopants were added at 20 L/min each.

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(1) Robb, D.B., Covey, R.T., Bruins, A.P. *Anal. Chem.* **2000**, 72, 3653-3659.