

# **The PhoTorrent™ Atmospheric Pressure Photoionization (APPI) Source Utilized for High Efficiency Photoionization of Testosterone and 25-OH Vitamin D3**

Frenny Ruparelia, **Ellie Majdi**, Joshua Sha Ye

IONICS Mass Spectrometry, 32 Nixon Rd, Bolton, ON, Canada L7E 1W2

## **Novel Aspects**

Modular APPI Source assembly, compatible and interchangeable with existing high performance dual probe ion source hardware

## **Introduction**

Atmospheric Pressure Photoionization (APPI) is commonly used to ionize compounds of interest for environmental research and is becoming popular in clinical research. It is an effective ionization method for many compounds which are not conducive to electrospray ionization (ESI) or atmospheric chemical ionization (APCI), leading to enhanced sensitivity and lower background for a wide range of compounds. The IONICS PhoTorrent™ APPI source is a modular assembly, easily interchangeable with the ESI or APCI hardware on a high performance dual probe ion source. This study demonstrates the characteristics of the IONICS modular PhoTorrent APPI source for 2 clinical compounds (Testosterone and 25-OH Vitamin D3). Performance under a range of conditions, including various total flow rates and dopant levels, was determined.

## **Methods**

The IONICS PhoTorrent APPI source module achieves high efficiency, broad range photoionization with the aid of a 10.6eV lamp from Syagen Technologies. The PhoTorrent APPI module was mounted onto the dual probe source of an IONICS 3Q 320 LC-MS/MS, in place of the ESI/APCI probes. Standards for Testosterone and 25-Hydroxy Vitamin D3 were purchased from Sigma and prepared in concentrations ranging from 0.0001 -10 ng/mL (Testosterone) and 0.1-100 ng/mL (25-OH Vitamin D3). Standards were dissolved in 20/80 water/methanol with 0.1% formic acid. The injection volumes were 10 µL. Duplicate injections were made for each concentration. The samples were eluted using a Shimadzu UHPLC with a C18 column using a mobile phase of 20/80 water/methanol, 0.1% formic acid, 5mM ammonium acetate.

## Results

The IONICS PhoTorrent APPI source demonstrated sensitivity to as low as 100 ag/ $\mu$ L for Testosterone while maintaining excellent linearity ( $R^2 = 0.9997$ ) over the range of 0.0001-10 ng/mL. This was achieved using a total solvent flowrate for Testosterone of 300  $\mu$ L/min. A dopant of toluene to 10% of the total flow rate was added to enhance photoionization.

The 25-OH Vitamin D3 achieved a sensitivity as low as 100 fg/ $\mu$ L while maintaining linearity ( $R^2 = 0.9999$ ) over the range of 0.1-100 ng/mL. The 25-OH Vitamin D3 was run with a total flowrate of 500  $\mu$ L/min, again with a 10% dopant of Toluene.

In general, it was observed that increasing flow rate provided sharper LC peaks, resulting in the expected increase in signal to noise ratio. Minimal variation in peak area was observed across a total flow range of 100-500  $\mu$ L/min. The PhoTorrent APPI source therefore demonstrates high linearity and excellent LLOQ's, and is well suited to flow rates of 100-500  $\mu$ L/min.

Development of the modular PhoTorrent APPI Source assembly facilitates switching between modes, from APPI to ESI and APCI, increasing the number and range of compounds that can be analyzed effectively with minimal hardware changes.