Austin Chromatography

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Austin Publishing Group

Review Article

The Ionization Technology of LC-MS, Advantages of APPI on Detection of PPCPs and Hormones

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Received: April 01, 2015; **Accepted:** May 18, 2015; **Published:** May 25, 2015

Abstract

This short review introduces the basic components and mechanism of Atmospheric Pressure Photoionization (APPI) and its application to detect Pharmaceutical and Personal Care Products (PPCPs) and hormones in environmental samples.

Keywords: APPI; LC-MS; PPCPs and hormones

Introduction

Mass Spectrometry (MS) has been an ideal detector for Liquid Chromatography (LC) because of its sensitivity, selectivity and accuracy. MS was first couple with LC in 1970s, but until the invention of Atmospheric Pressure Ionization (API) in 1990s, MS becomes a widespread detector for LC. Nowadays, the two most widely used API for LC is Electrospray Ionization (ESI) and Atmospheric Pressure Chemical Ionization (APCI). ESI occurs in the liquid phase and its application is mainly on the polar compounds. While APCI occurs in the gas phase and can be employed to ionized less polar molecules. Atmospheric Pressure Photoionization (APPI) is a relatively less popular ionization technology for LC-MS. Compared to the ESI and APCI, APPI is the last soft ionization technique that cans ionize less polar and nonpolar molecules which are poorly amenable to ESI and APCI. On the basis of the polarity and molecular weight of target compounds, the application of different ionization technology is shown in Figure 1.

Besides ionization technique and mass spectrometer, LC technique also plays an important role on the selectivity and sensitivity of LC–MS analysis. Although reversed-phase LC is most commonly used, there are also many other LC techniques, including ion-pair, ion exchange, affinity and size exclusion chromatography. The separation efficiency of LC is dependent on the column diameter and solid-phase material. The organic mobile phase includes methanol, acetonitrile or a combination of these two solvents, while the aqueous phase is water with the addition of formic acid, acetic acid, ammonium hydroxide, and ammonium format and ammonium acetate to adjust pH.

Pharmaceuticals and Personal Care Products (PPCPs) and hormones are Endocrine Disrupting Compounds (EDCs), which exist in the ecosystem at very low concentration (ng/L) but have adverse effect on wild animals and humans. The detection of these challenge compounds requires proper sample preparation and sophisticated instrument. Combinations of the latest technologies in LC and MS offer powerful approaches to the analysis of these challenge compounds in minimal amounts in complex matrices. The successful operation of LC–MS requires experienced personnel and a clear understanding of the operational parameters. The instrumental parameters, analytes characteristics and eluent composition directly influence the signal response. This short review briefly introduces the technology of APPI and focus on its application on environmental samples, especially for detection of PPCPs and hormones.

APPI is based on the interaction of a photon beam created by a discharge lamp with the vapors of a nebulizer liquid solution [1]. The most widely used lamp for APPI is krypton lamp, which can produce photons of 10.03 and 10.64 eV in a 4:1 ratio [2]. The reason to choose krypton is mainly because the energy of photons is higher than most analytes and lowers than commonly used solvents. Besides krypton, xenon and argon were also used. Xenon was employed in the early years of APPI [3], but its low energy of produced photons (8.4 eV) prohibits its employment. Because argon produces more energetic photons (11.7 eV) than krypton, argon lamps give about 100 time's higher intensities in the solvent ions and produce more abundant molecular ions than krypton lamps [2]. Considering the ionization efficiency of the analytes as a function of the flow rate, krypton lamps produce a better S/N ratio at a low solvent flow rate, whereas argon lamps are better at higher solvent flow rate [1].

The mechanism of APPI

The Molecules (M) absorbs a photon *(E=hv)* and become an electronically excited molecule: $M+hv \rightarrow M^*$ (1). If the Ionization Energy (IE) of molecule is lower than the energy of photon, *IE<hv*, the molecule releases and energetic electron and becomes the corresponding odd-electron caution (2).

$M^* \rightarrow M^{\bullet+} + e^-$ (2)

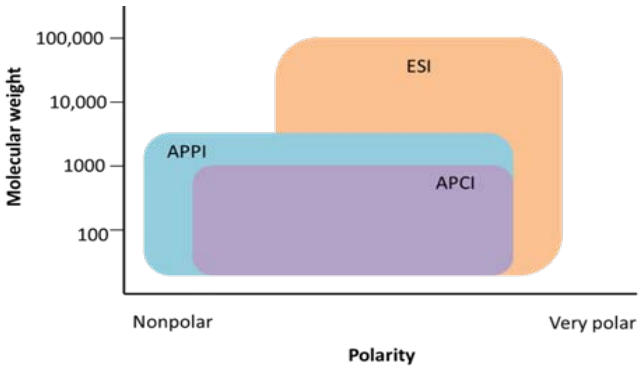

Figure 1: Application of API techniques for compounds on the basis of polarity and molecular weight.

Citation: Wang C. The Ionization Technology of LC-MS, Advantages of APPI on Detection of PPCPs and Hormones. Austin Chromatogr. 2015;2(2): 1032.

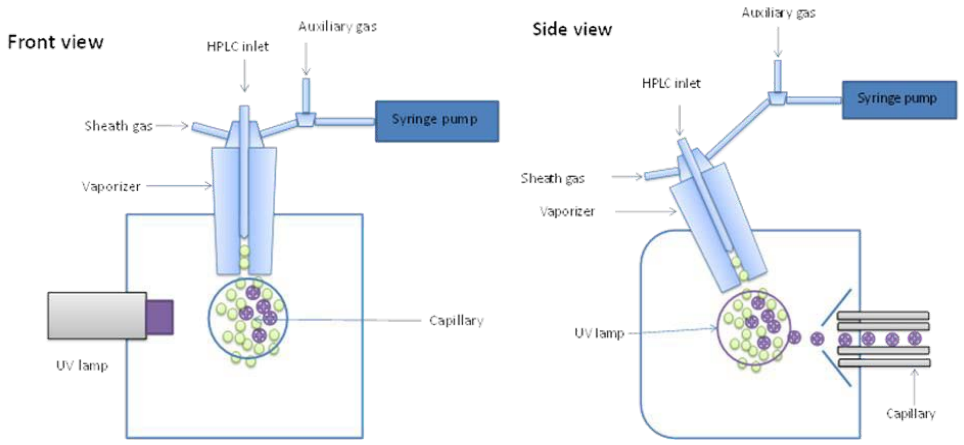

Figure 2: Dopant gas delivery system. A Thermo Ion MAX source housing equipped with Syagen technology APPI source.

If the *IE>hv*, M* may undergo a de-excitation process, such as photodissociation (3), photon emission (4) or collision quenching (5).

$M^* \rightarrow A + B$ (3)

$M^* \rightarrow M + hv$ (4)

$M^* + C \rightarrow M + C^*$ (5)

In such situation, the use of a preferentially ionized substance, dopant (D), has been used to promote the ionization of M. Compared to analytes, the dopant is added in large quantities and it acts as an intermediate between the photons and the analytes. The dopant is photo ionized first (6) and charge exchange with analytes subsequently if the Electron Affinity (EA) of the analyte is higher than the EA of dopant (7). If the Proton Affinity (PA) of analytes is higher than the PA of the deprotonated dopant ion, solvent molecules can act as intermediate between the dopant ion and the analytes (8,9).

$D + hv \rightarrow D^{\bullet+}$ (6)

$D^{\bullet+} + M \rightarrow D + M^{\bullet+}$ if $EA_M > EA_D$ (7)

$D^{\bullet+} + nS \rightarrow [D\text{-}H]^{\bullet} + [Sn + H]^+$ if $PA_S > PA_{[D\text{-}H]^{\bullet}}$ (8)

$[Sn + H]^+ + M \rightarrow nS + [M+H]^+$ if $PA_M > PA_S$ (9)

The most frequently used dopants are toluene, actone, anisole, and chlorobenzene, etc. Dopant can be introducing to system through gas phase delivery (Figure 2). Syringe Pump is used to deliver dopant into system.

Application of APPI

High Performance Liquid Chromatography Mass Spectrometry (HPLC-MS) has been shown as a valuable alternative for detection of PPCPs and EDCs to overcome the drawbacks of GC-MS [4-14]. ESI, APCI and APPI are the three most common ionization techniques coupled with liquid chromatography [1]. ESI and APCI have both been widely used for analysis of polar molecules in the aqueous environmental samples in many studies [7,8,11]. Several studies that described multi-target detection of up to 74 compounds by ESI have been recently published in the literature [11,15]. However, ESI and APCI also have many critical limitations. For example, some steroids, and generally nonpolar compounds, such as Polycyclic Aromatic Hydrocarbons (PAHs), are poorly ionized or cannot be ionized by ESI or APCI [16]. Therefore, it is not surprising that most of the studies using ESI are focused on the most polar, easily ionizable pharmaceuticals. Only a handful of studies have tried to detect steroid hormones that are difficult to ionize by ESI or APCI with marginal results [17,6]. Not surprisingly, there is abundant literature for compounds amenable to ESI but reports are scarce for those that present an ionization challenge. The critical issue is that the most EDC active compounds are not well ionized by ESI.

APPI is a technique that has the capability to ionize compounds with a wide range of polarities while being remarkably tolerant of matrix components of HPLC additives. The rapidly growing number of publications in this area clearly demonstrates the advantages of atmospheric pressure photoionization [18,19,1]. At the beginning, APPI was introduced as a complement of ESI and APCI. So far, APPI has been proved to be a valuable tool for analytes which are poorly ionized or not ionized by ESI and APCI. In particular APPI was shown to be able to detect steroid hormones down to several ng/L and had been proven to have much higher sensitivity than ESI. For example, Viglino and coworkers developed a fully automated online method using LC-APPI-MS/MS to simultaneously detect selected natural and synthetic hormones at concentrations as low as 5 ng/L [20]. Yamanoto et al. compared detection of steroidal hormones using ESI and APPI and they found that APPI displayed higher sensitivity than ESI for most of the unconjugated steroids examined, with much greater sensitivity for testosterone and 4-androstene-3, 17-dione [21]. Wang compared ionization efficiency of HESI, APCI and APPI on 23 PPCPs and hormones. Results indicate that APPI using toluene as dopant provides exceptional ionization capabilities for a broad range of compounds, in particular for hormones and sterols compared to APCI and HESI [22].

Conclusion

Although APPI is a relatively less popular atmospheric pressure ionization technology for LC-MS, its ability of ionization a broad range of compounds, especially high ionization efficiency for less polar and nonpolar compounds, extended the LCMS's application. APPI makes LCMS become a powerful approach to analysis of EDCs. Also, its remarkable tolerance to complex matrix makes it more

applied in environmental research and many other research areas.

References

1. Marchi I, Rudaz S, Veuthey JL. Atmospheric pressure photoionization for coupling liquid-chromatography to mass spectrometry: a review. Talanta. 2009; 78: 1-18.
2. Short LC, Cai SS, Syage JA. APPI-MS: effects of mobile phases and VUV lamps on the detection of PAH compounds. J Am Soc Mass Spectrom. 2007; 18: 589-599.
3. Locke DC, Dhingra BS, Baker AD. Liquid-phase photoionization detector for liquid chromatography. Analytical Chemistry. 1982; 54: 447-450.
4. Gardinali PR, Zhao X. Trace determination of caffeine in surface water samples by liquid chromatography--atmospheric pressure chemical ionization--mass spectrometry (LC-APCI-MS). Environ Int. 2002; 28: 521-528.
5. Gentili A, Perret D, Marchese S, Mastropasqua R, Curini R, Di Corcia A. Analysis of free estrogens and their conjugates in sewage and river waters by solid-phase extraction then liquid chromatography-electrospray-tandem mass spectrometry. Chromatographia. 2002; 56: 25-32.
6. Ingrand V, Herry G, Beausse J, de Roubin MR. Analysis of steroid hormones in effluents of wastewater treatment plants by liquid chromatography-tandem mass spectrometry. J Chromatogr A. 2003; 1020: 99-104.
7. Cahill JD, Furlong ET, Burkhardt MR, Kolpin D, Anderson LG. Determination of pharmaceutical compounds in surface- and ground-water samples by solid-phase extraction and high-performance liquid chromatography-electrospray ionization mass spectrometry. J Chromatogr A. 2004; 1041: 171-180.
8. Castiglioni S, Bagnati R, Calamari D, Fanelli R, Zuccato E. A multiresidue analytical method using solid-phase extraction and high-pressure liquid chromatography tandem mass spectrometry to measure pharmaceuticals of different therapeutic classes in urban wastewaters. J Chromatogr A. 2005; 1092: 206-215.
9. Schlüsener MP, Bester K. Determination of steroid hormones, hormone conjugates and macrolide antibiotics in influents and effluents of sewage treatment plants utilising high-performance liquid chromatography/tandem mass spectrometry with electrospray and atmospheric pressure chemical ionisation. Rapid Communications in Mass Spectrometry. 2005; 19: 3269-3278.
10. Martnez Bueno MJ, Agera A, Gomez MJ, Hernando MD, Garca-Reyes JF, Fernndez-Alba AR. Application of Liquid Chromatography/Quadrupole-Linear Ion Trap Mass Spectrometry and Time-of-Flight Mass Spectrometry to the Determination of Pharmaceuticals and Related Contaminants in Wastewater. Analytical Chemistry. 2007; 79: 9372-9384.
11. Gros M, Petrovic M, Barcelo D. Tracing pharmaceutical residues of different therapeutic classes in environmental waters by using liquid chromatography/quadrupole-linear ion trap mass spectrometry and automated library searching. Anal Chem. 2009; 81: 898-912.
12. Laven M, Alsberg T, Yu Y, Adolfsson-Erici M, Sun H. Serial mixed-mode cation- and anion-exchange solid-phase extraction for separation of basic, neutral and acidic pharmaceuticals in wastewater and analysis by high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry. J Chromatogr A. 2009; 1216: 49-62.
13. Huerta-Fontela M, Galceran MT, Ventura F. Fast liquid chromatography-quadrupole-linear ion trap mass spectrometry for the analysis of pharmaceuticals and hormones in water resources. J Chromatogr A. 2010; 1217: 4212-4222.
14. Jian-lin W, Jie L, Zongwei C. Determination of triclosan metabolites by using in-source fragmentation from high-performance liquid chromatography/negative atmospheric pressure chemical ionization ion trap mass spectrometry. Rapid Communications in Mass Spectrometry. 2010; 24: 1828-1834.
15. Lopez-Serna R, Perez S, Ginebreda A, Petrovic M, Barcelo D. Fully automated determination of 74 pharmaceuticals in environmental and waste waters by online solid phase extraction-liquid chromatography-electrospray-tandem mass spectrometry. Talanta. 2010; 83: 410-424.
16. Hanold KA, Fischer SM, Cormia PH, Miller CE, Syage JA. Atmospheric pressure photoionization. 1. General properties for LC/MS. Anal Chem. 2004; 76: 2842-2851.
17. Jeannot R, Sabik H, Sauvard E, Dagnac T, Dohrendorf K. Determination of endocrine-disrupting compounds in environmental samples using gas and liquid chromatography with mass spectrometry. J Chromatogr A. 2002; 974: 143-159.
18. Raffaelli A, Saba A. Atmospheric pressure photoionization mass spectrometry. Mass Spectrom Rev. 2003; 22: 318-331.
19. Bos SJ, van Leeuwen SM, Karst U. From fundamentals to applications: recent developments in atmospheric pressure photoionization mass spectrometry. Anal Bioanal Chem. 2006; 384: 85-99.
20. Viglino L, Aboulfadl K, Prevost M, Sauve S. Analysis of natural and synthetic estrogenic endocrine disruptors in environmental waters using online preconcentration coupled with LC-APPI-MS/MS. Talanta. 2008; 76: 1088-1096.
21. Yamamoto A, Kakutani N, Yamamoto K, Kamiura T, Miyakoda H. Steroid Hormone Profiles of Urban and Tidal Rivers Using LC/MS/MS Equipped with Electrospray Ionization and Atmospheric Pressure Photoionization Sources. Environmental Science & Technology. 2006; 40: 4132-4137.
22. Wang C, Gardinali PR. Comparison of multiple API techniques for the simultaneous detection of microconstituents in water by on-line SPE-LC-MS/MS. J Mass Spectrom. 2012; 47: 1255-1268.

Austin Chromatogr - Volume 2 Issue 2 - 2015
ISSN 2379-7975 | www.austinpublishinggroup.com

Citation: Wang C. The Ionization Technology of LC-MS, Advantages of APPI on Detection of PPCPs and Hormones. Austin Chromatogr. 2015;2(2): 1032.