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J. Am. Soc. Mass Spectrom. (2017) 28:724–728
DOI: 10.1007/s13361-016-1581-z

SHORT COMMUNICATION

Effects of Solvent and Ion Source Pressure on the Analysis of Anabolic Steroids by Low Pressure Photoionization Mass Spectrometry

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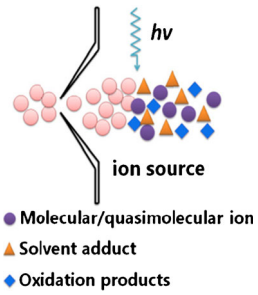

Abstract. Solvent and ion source pressure were two important factors relating to the photon induced ion-molecule reactions in low pressure photoionization (LPPI). In this work, four anabolic steroids were analyzed by LPPI mass spectrometry. Both the ion species present and their relative abundances could be controlled by switching the solvent and adjusting the ion source pressure. Whereas $M^{\bullet+}$, MH^+, $[M - H_2O]^+$, and solvent adducts were observed in positive LPPI, $[M - H]^-$ and various oxidation products were abundant in negative LPPI. Changing the solvent greatly affected formation of the ion species in both positive and negative ion modes. The ion intensities of the solvent adduct and oxygen adduct were selectively enhanced when the ion source pressure was elevated from 68 to 800 Pa. The limit of detection could be decreased by increasing the ion source pressure.

Keywords: Low pressure photoionization, Mass spectrometry, Anabolic steroid

Received: 18 July 2016/Revised: 28 November 2016/Accepted: 14 December 2016/Published Online: 24 January 2017

Introduction

Photoionization (PI) was first described approximately 60 years ago [1]. Owing to its favorable characteristics in contrast with other available ionization sources such as electrospray ionization (ESI) (i.e., softness, no polarity discrimination, and reduced ion suppression), PI has been widely adopted by scientists for many mass spectrometric applications [2–9]. Low pressure photoionization (LPPI) in a vacuum has attracted increasing attention for on-line and in situ analyses of gaseous samples in recent years [10–13]. In addition, LPPI has also been applied to liquid samples. Based on the direct EI interface, Zimmermann and coworkers developed a flow injection method for liquid samples with a dual electron and PI ion source that works under vacuum [14]. Syage et al. reported a heated injector combined with syringe injection and capillary infusion for the introduction of liquid into a vacuum and subsequent photoionization [15]. Recently, our group constructed an ultrasonic nebulizer system for a LPPI source, in which analytes in solvents and various matrices were extracted via ultrasonic nebulization [16]. The desolvated chemicals from the system can be ionized by direct ionization, and ion–molecule reactions such as charge exchange and proton transfer can be initiated by an ionized dopant.

In addition to molecular/quasi-molecular ions, solvent adducts and oxidation products are also formed by ion–molecule reactions in LPPI and the solvent used greatly affects the formation of these ions [16]. Moreover, the photon-induced ion–molecule reactions under low pressure are highly dependent on the ion source pressure [17, 18]. The importance of the ion source pressure in ion formation in LPPI has been studied by Li et al. under moderate pressure [17]. They found that the ions intensities increased rapidly as the ion source pressure was increased from 2 to 30 Pa because the number density of the analyte increased. When the ion source pressure was increased from 60 to 100 Pa, the ion intensities decreased as a result of excessive ion–molecule reactions at higher pressures. However, there have been few studies on ion formation and ion–molecule reactions at higher pressure [19].

In this paper, four anabolic steroids with low polarity were selected as target compounds to study solvent adduct formation and oxidation reactions with different solvents over an extended pressure range (68–800 Pa) in LPPI.

Chengyuan Liu and Yanan Zhu contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s13361-016-1581-z) contains supplementary material, which is available to authorized users.

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Experimental

Reagents and Sample Preparation

Estradiol (E2), dehydroepiandrosterone (DHEA), methyltestosterone (MT), and progesterone (P) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Their structures are shown in Sipplementary Figure S1. All these steroids were >99% pure and used without further purification. HPLC grade methanol and acetonitrile were obtained from Merck (Darmstadt, Germany). Water was purified in a Milli-Q system (Millipore, Billerica, MA, USA). Toluene (purity >99%) for use as a dopant was purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). Nitrogen (purity 99.9%) and compressed air was purchased from Nanjing Special Gas Factory Co., Ltd. (Nanjing, China).

Stock solutions of each steroid (100 µg/mL) were prepared in methanol/water (1:1, v:v) and acetonitrile/water (1:1, v:v), respectively. The stock solution of four steroids (80 ng/mL for E2 and DHEA, 40 ng/mL for MT and P) was prepared in acetonitrile/water (1:1, v:v) for the simultaneous detection of multiple steroids at parts per billion (ng/mL) levels. In the control experiment, methanol/water (1:1, v:v) and acetonitrile/water (1:1, v:v) were used to acquire the background mass spectra.

Instrumentation

All experiments were carried out on a modified Platform LCZ quadrupole mass spectrometer (Micromass, Manchester, UK) with an ultrasonic nebulization system, which has been described in detail elsewhere [16]. The temperature and pressure of the ion source were held at 115 °C and 68 Pa, respectively, unless stated otherwise. The ultrasonic nebulization system included an ultrasonic nebulizer (model 402AI; Yuwell Medical Equipment and Supply Corp., Suzhou, China) with a frequency of 1.7 MHz, a nebulization cell, and a transfer tube. The transfer tube consisted of a heating tube and a water-cooled condenser tube. Aerosols of the sample solution were formed in the nebulization cell, and carried through the transfer tube by the carrier gas (390 mL/min). To improve the photoionization efficiency, the vaporized sample was mixed with gaseous toluene [20], which was transferred from a bubbler to the exit of a T-shaped tube by the auxiliary gas at a constant flow rate of 40 mL/min (Supplementary Figure S2). The angle and distance between the exit of T-shaped tube and cone of mass spectrometer were optimized to 45° and 3 mm, respectively. Next, the mixture was introduced into the ion source of the mass spectrometer, and ionized by the photons (10.6 eV) emitted from a krypton discharge lamp (PKS 106; Heraeus Ltd., Hanau, Germany). For signal stability, the mass spectra were generally recorded for 2 s with a delay of 5 s after the valve was turned on. Each experiment was repeated three times.

Results and Discussion

The positive mass spectra of the four steroids in methanol/water and acetonitrile/water were mainly characterized by molecular ions ($M^{+\bullet}$), protonated molecular ions (MH^+), water-loss fragments ($[M - H_2O]^+$), and solvent adducts ($[M + S]^{+\bullet}$ and $[M + S + H]^+$) (Figure 1). As a nonpolar compound, E2 in methanol/water only produced M^+ and $[M - H_2O]^+$. When methanol was replaced with acetonitrile, intense $[M + CH_3CN]^{+\bullet}$ and dehydrated acetonitrile adduct $[M + CH_3CN + H - H_2O]^+$ peaks were observed in addition to $M^{+\bullet}$ and $[MH - H_2O]^+$. For DHEA dissolved in methanol/water, the mass spectra showed abundant $[M - H_2O]^+$, MH^+, and $[MH\text{-}2H_2O]^+$ ions. In addition to these ions, $M^{+\bullet}$ and $[M + CH_3CN + H]^+$ were observed in acetonitrile/water. The mass spectra of MT and P were relatively simple. The most noticeable characteristic was the relative abundances of solvent adducts of MT and P in acetonitrile/water (i.e., $[M + CH_3CN + H]^+$) became dominant compared with that in the methanol/water spectra. The mass peaks and their relative abundances are given in Supplementary Table S1.

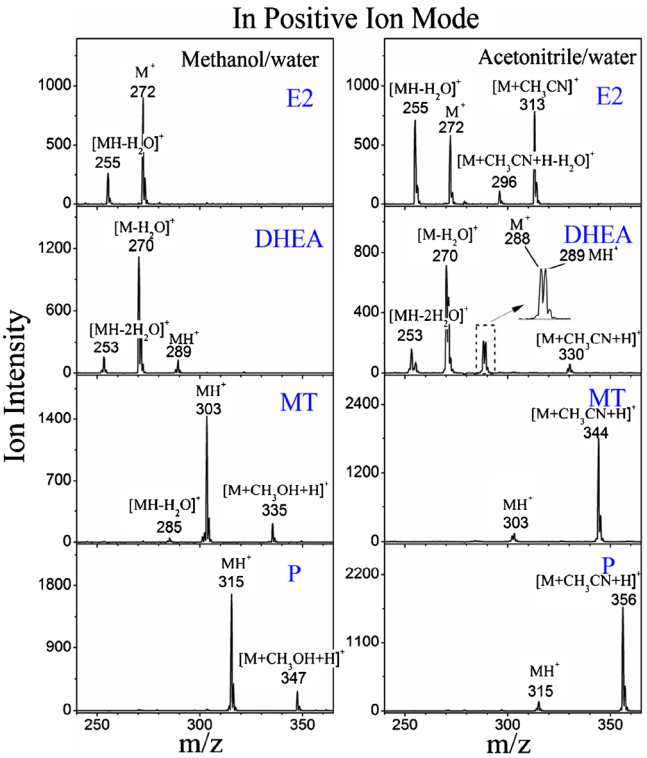

Figure 1. Mass spectra of the four anabolic steroids obtained by LPPI using acetonitrile/water or methanol/water as the solvent in positive ion mode

In LPPI, ions form via different ionization mechanisms to those in atmospheric pressure photoionization (APPI) [21]. For example, the M^+ ions in LPPI are mainly formed by direct photoionization and charge exchange between the analyte and toluene molecular ions (Eqs. 1–3), whereas MH^+ ions are produced by proton transfer from toluene molecular ions to the analyte (Eq. 4) [14]. Note that Eqs. 1–4 have been discussed previously [16]. Two forms of solvent adducts were observed, with $[M + S]^{+\bullet}$ for E2 and $[MH + S]^+$ for DHEA, MT, and P. The $[M + S]^{+\bullet}$ ion, such as the $[M + CH_3CN]^{+\bullet}$ of E2, could be formed through combination of the E2 molecular ion with neutral CH_3CN (Eq. 5) because no acetonitrile molecular ion $CH_3CN^{+\bullet}$ was found in the background spectrum of acetonitrile/water (Supplementary Figure S3). By comparison, both protonated solvent molecules, SH^+ and MH^+, were obtained and contributed to the production of

$[M + S + H]^+$ (Eq. 6 and 7). In addition to Eq. 7, the binding reaction of protonated solvent molecules (SH^+) and neutral solvent molecules (S) was facilitated by high binding energy. For example, the $[CH_3OH+H]^+$-CH_3OH binding energy is –33 kcal/mol [22]. Because the number density of neutral S is much higher than that of the analyte molecule M, formation of $SH^+ – S$ was more prominent than that of $SH^+ – M$. Thus Eq. 7 may not be the dominant pathway for $[M + S + H]^+$ formation. Compared with the spectra of E2 and DHEA, the solvent adduct was more abundant in the mass spectra of MT and P, which could be related to the presence of a conjugated keto group in MT and P. Moreover, acetonitrile facilitated formation of the solvent adduct to a larger extent than methanol and water, with no water adduct observed at all. This could be attributed to the higher proton affinity (PA) of acetonitrile (779.2 kJ/mol) compared with the PAs of methanol (754.3 kJ/mol) and water (691 kJ/mol). The importance of Eq. 6 for the formation of $[M + S + H]^+$ was validated by the positive relationship between the solvent–steroid adduct generation efficiency and PA of the solvent.

$$M(\text{analyte}) + h\nu \rightarrow M^{\bullet+} + e^- \quad (1)$$

$$C_7H_8 + h\nu \rightarrow C_7H_8^{\bullet+} + e^- \quad (2)$$

$$C_7H_8^{\bullet+} + M \rightarrow M^{\bullet+} + C_7H_8 \quad (3)$$

$$C_7H_8^{\bullet+} + M \rightarrow MH^+ + C_7H_7^{\bullet} \quad (4)$$

$$M^{\bullet+} + S(\text{solvent}) \rightarrow [M + S]^{\bullet+} \quad (5)$$

$$MH^+ + S \rightarrow [M + S + H]^+ \quad (6)$$

$$M + SH^+ \rightarrow [M + S + H]^+ \quad (7)$$

Figure 2 shows the negative LPPI mass spectra of the four steroids in methanol/water and acetonitrile/water. Oxidation products ($[M + 2O]^{\bullet-}$) and quasi-molecular ions ($[M – H]^-$) were observed in all spectra, as also reported for negative-ion APPI [21] and desorption APPI [23]. In addition, $[M – H + O]^-$ for MT and $[M – H + O]^-$/$[M – H + 3O]^-$ for P were observed in methanol/water. When acetonitrile/water was used as the solvent, $[M + 43]^-$ ions at *m/z* 331 and 345 were observed for DHEA and MT, respectively, and may be produced from the $[M – H + CO_2]^-$ ions. For P, a relatively weak $[M – H – H_2O]^-$ peak was observed. The dominant mass peaks and their relative abundances are shown in Supplementary Table S1.

In contrast to negative APPI mass spectra [24], solvent adducts such as $[M – H + CH_3OH]^-$ and $[M – H + CH_3CN]^-$ were not observed in the negative LPPI mass spectra, but the oxygen adduct $[M + 2O]^{\bullet-}$ was abundant (Figure 2). It is accepted that O_2 is readily ionized by thermal electrons to produce the superoxide ion $O_2^{\bullet-}$ (Eq. 8), which is a very important ion in negative APPI [21]. The superoxide ion was observed in the background mass spectra (Supplementary Figure S4). The $[M–H]^-$, $[M – H + O]^-$, and $[M – H + 3O]^-$ ions were speculated to form through Eqs. 9–11 between the analyte molecule M and superoxide ion $O_2^{\bullet-}$ based on the ionization mechanism in APPI [21, 24]. Note that $[M – H]^-$ could also be formed through dissociative electron capture (Eq. 13) [25] in the case of absence of oxygen (Supplementary Figure S5). Because the $M^{\bullet-}$ ion was not detected, the oxygen adduct $[M + 2O]^{\bullet-}$ was presumed to be generated through

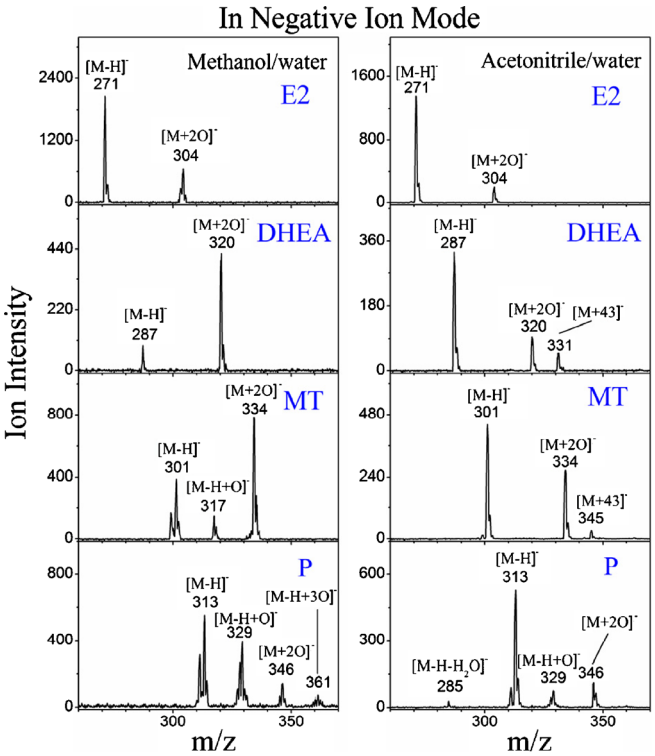

Figure 2. Mass spectra of E2, DHEA, MT, and P obtained by LPPI mass spectrometry in negative ion mode using acetonitrile/water or methanol/water as the solvent

combination of the analyte molecule M and superoxide ion $O_2^{\bullet-}$ (Eq. 12). Moreover, the steroids dissolved in methanol/water were more susceptible to oxidation than those in acetonitrile/water, which agrees with an earlier study using APPI [21]. This is likely attributable to the fact that light from a krypton discharge lamp corresponds to a much lower absorption cross-section for methanol than for acetonitrile [22]. This means fewer photons are adsorbed by the solvent, and more thermal electrons form (Eq. 2). These thermal electrons can combine with O_2 (Eq. 8) for subsequent oxidation reactions when using methanol/water as the solvent. This was validated by the background mass spectrum of methanol/water, which showed more abundant superoxide ion $O_2^{\bullet-}$ at *m/z* 32 than the background spectrum of acetonitrile/water (Supplementary Figure S4).

$$O_2 + e^- \rightarrow O_2^{\bullet-} \quad (8)$$

$$M + O_2^{\bullet-} \rightarrow [M–H]^- + HO_2^{\bullet} \quad (9)$$

$$M + O_2^{\bullet-} \rightarrow [M–H + O]^- + HO^{\bullet} \quad (10)$$

$$[M–H + O]^- + O_2 \rightarrow [M–H + 3O]^- \quad (11)$$

$$M + O_2^{\bullet-} \rightarrow [M + 2O]^{\bullet-} \quad (12)$$

$$M + e^- \rightarrow [M–H]^- + H \quad (13)$$

The ionization pressure in LPPI is important in ion formation because of the varied number densities of the analytes and corresponding ion–molecule reactions. Here, the pressure effect was studied from 68 to 800 Pa. LPPI mass spectra of E2 dissolved in acetonitrile/water were obtained at various ion source pressures (68, 200, 400, 600, and 800 Pa) in both positive and negative ion

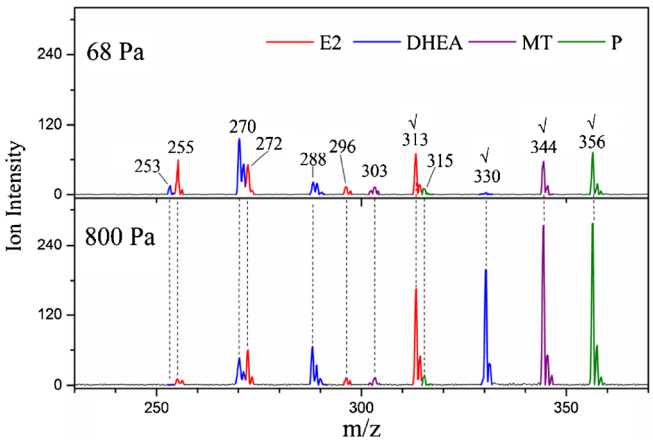

Figure 3. LPPI mass spectra of a steroid mixture (E2, DHEA, MT and P) in acetonitrile/water at parts per billion (ng/ml) levels with the ion source pressure set at 68 Pa or 800 Pa. Solvent–steroid adducts are indicated by check marks

modes (Supplementary Figure S6a). The total ion intensities of all mass signals (i.e., all detected ions of E2) and those of the adduct ions in positive (i.e., $[M + CH_3CN]^{\bullet+}$) and negative (i.e., $[M + 2O]^{\bullet-}$) ion modes were plotted against the ion source pressure (Supplementary Figure S6b and c). The total ion intensities in both positive and negative ion modes initially decreased and then increased as the ion source pressure was increased from 68 to 800 Pa. Previous experiments have demonstrated that excess solvent will reduce the sensitivity of analyte detection [16]. Therefore, the initial decrease may be attributed to the negative effect of the increased solvent volume. Further pressure elevation will increase the number densities of the analyte, dopant, and oxygen, which will enhance the signal. Although the total signal intensity did not increase, the intensities of the solvent adduct $[M + CH_3CN]^{\bullet+}$ and oxygen adduct $[M + 2O]^{\bullet-}$ were enhanced, especially as the ion source pressure was increased from 400 to 800 Pa. These enhancements were attributed to increased ion–molecule collisions. At low pressure, the ion–molecule collision rate was directly proportional to the ion source pressure. Thus, more binding reactions of $M^{\bullet+} - CH_3CN$ and $M - O_2^{\bullet-}$ would occur as the ion source pressure increases. In addition, previous studies have demonstrated that collisions at higher pressure allow for release of excess internal energy of molecular ions leading to less fragmentation in He^M-Penning ionization [26]. This is why the yield of $[M - H_2O]^+$, a typical fragment of M^+ for E2, greatly decreased as the ion source pressure was increased from 78 to 800 Pa (Supplementary Figure S7a). At the same time, there was more M^+ left for subsequent adduct $[M + CH_3CN]^+$ formation than at lower pressure. The yield of the fragment $[M - H_2O]^+$ for DHEA showed the same trends (Supplementry Figure S7b). Supplementary Figure S8 shows the relationship between the ion source pressure and adduct yield (solvent adduct and oxygen adduct), which is defined as the ratio of the adduct ion intensity over that of the total signal. Similar results were obtained for all four steroids and adduct yield clearly increased with increases of the ion source pressure, especially from 400 to 800 Pa.

Lastly, to eveluate the real applicability of LPPI, the steroid mixture at parts per billion (ng/mL) levels (80 ng/mL E2 and DHEA, 40 ng/mL MT and P) was characterized in the positive LPPI mode at ion source pressures of 68 and 800 Pa (Figure 3). As the ion source pressure increased, the intensities of all acetonitrile adducts (labeled by check marks) greatly increased and the adduct ions became the dominant peaks at 800 Pa. This allowed for detection of anabolic steroids at low concentrations. The limit of detections (LODs) of the four studied steroids obtained by LPPI at 68 and 800 Pa are listed in Supplementary Table S2. The LODs in this study were determined using a LPPI source coupled with a single quadrupole mass spectrometer over 16 years, and the results for E2 were comparable to the detection limit (10 ng/mL) achieved by a LC/MS instrument equipped with an ESI source [27].

Conclusions

In this study, LPPI behaviors of four steroids were studied in two different solvents and ionization pressures ranging from 68 to 800 Pa. This study broadened the understanding of the ion–molecule reactions in LPPI and was useful for the control of ion distribution of target compound. In addition, the detection limit can be improved for selected ions by increasing the ion source pressure. The above results indicate that LPPI (68–800 Pa) has the potential for high-throughput analysis of steroids.

Acknowledgements

This work was supported by grants from the Natural Science Foundation of China (grant nos. U1432128 and 91545120) and Chinese Universities Scientific Fund.

References

1. Lossing, F., Tanaka, I.: Photoionization as a source of ions for mass spectrometry. J. Chem. Phys. **25**, 1031–1034 (1956)
2. Brutschy, B.: Reactions in molecular clusters following photoionization. J. Phys. Chem. **94**, 8637–8647 (1990)
3. Wilkerson Jr., C.W., Colby, S.M., Reilly, J.P.: Determination of polycyclic aromatic hydrocarbons using gas chromatography/laser ionization mass spectrometry with picosecond and nanosecond light pulses. Anal. Chem. **61**, 2669–2673 (1989)
4. Lubman, D.M.: Optically selective molecular mass spectrometry. Anal. Chem. **59**, 31A–40A (1987)
5. Köster, C., Grotemeyer, J.: Single-photon and multi-photon ionization of infrared laser-desorbed biomolecules. Org. Mass Spectrom. **27**, 463–471 (1992)
6. Schlag, E.W., Neusser, H.J.: Multiphoton mass spectrometry. Acc. Chem. Res. **16**, 355–360 (1983)
7. Hanold, K.A., Fischer, S.M., Cormia, P.H., Miller, C.E., Syage, J.A.: Atmospheric pressure photoionization. 1. General properties for LC/MS. Anal. Chem. **76**, 2842–2851 (2004)
8. Hanley, L., Zimmermann, R.: Light and molecular ions: the emergence of vacuum UV single-photon ionization in MS. Anal. Chem. **81**, 4174–4182 (2009)
9. Zimmermann, R.: Photo ionisation in mass spectrometry: light, selectivity, and molecular ions. Anal. Bioanal. Chem. **405**, 6901–6905 (2013)
10. Pan, Y., Hu, Y.H., Wang, J., Ye, L.L., Liu, C.Y., Zhu, Z.X.: Online characterization of isomeric/isobaric components in the gas phase of mainstream cigarette smoke by tunable synchrotron radiation vacuum ultraviolet photoionization time-of-flight mass spectrometry and photoionization efficiency curve simulation. Anal. Chem. **85**, 11993–12001 (2013)

11. Wang, Y., Huang, Q., Zhou, Z., Yang, J., Qi, F., Pan, Y.: Online study on the pyrolysis of polypropylene over the HZSM-5 zeolite with photoionization time-of-flight mass spectrometry. Energ. Fuel. **29**, 1090–1098 (2015)
12. Xie, Y., Hua, L., Hou, K., Chen, P., Zhao, W., Chen, W., Ju, B., Li, H.: Long-term real-time monitoring catalytic synthesis of ammonia in a microreactor by VUV-lamp-based charge-transfer ionization time-of-flight mass spectrometry. Anal. Chem. **86**, 7681–7687 (2014)
13. Kleeblatt, J., Schubert, J.K., Zimmermann, R.: Detection of gaseous compounds by needle trap sampling and direct thermal-desorption photoionization mass spectrometry: concept and demonstrative application to breath gas analysis. Anal. Chem. **87**, 1773–1781 (2015)
14. Schepler, C., Sklorz, M., Passig, J., Famiglini, G., Cappiello, A., Zimmermann, R.: Flow injection of liquid samples to a mass spectrometer with ionization under vacuum conditions: a combined ion source for single-photon and electron impact ionization. Anal. Bioanal. Chem. **405**, 6953–6957 (2013)
15. Syage, J.A., Cai, S.-S., Li, J., Evans, M.D.: Direct sampling of chemical weapons in water by photoionization mass spectrometry. Anal. Chem. **78**, 2967–2976 (2006)
16. Liu, C., Zhu, Y., Zhou, Z., Yang, J., Qi, F., Pan, Y.: Ultrasonic nebulization extraction/low pressure photoionization mass spectrometry for direct analysis of chemicals in matrices. Anal. Chim. Acta **891**, 203–210 (2015)
17. Hua, L., Wu, Q., Hou, K., Cui, H., Chen, P., Wang, W., Li, J., Li, H.: Single photon ionization and chemical ionization combined ion source based on a vacuum ultraviolet lamp for orthogonal acceleration time-of-flight mass spectrometry. Anal. Chem. **83**, 5309–5316 (2011)
18. Chen, P., Hou, K., Hua, L., Xie, Y., Zhao, W., Chen, W., Chen, C., Li, H.: Quasi-trapping chemical ionization source based on a commercial VUV lamp for time-of-flight mass spectrometry. Anal. Chem. **86**, 1332–1336 (2014)
19. Syage, J.A.: Mechanism of [M + H]+ formation in photoionization mass spectrometry. J. Am. Soc. Mass Spectrom. **15**, 1521–1533 (2004)
20. Robb, D.B., Covey, T.R., Bruins, A.P.: Atmospheric pressure photoionization: an ionization method for liquid chromatography-mass spectrometry. Anal. Chem. **72**, 3653–3659 (2000)
21. Kauppila, T.J., Kuuranne, T., Meurer, E.C., Eberlin, M.N., Kotiaho, T., Kostiainen, R.: Atmospheric pressure photoionization mass spectrometry. Ionization mechanism and the effect of solvent on the ionization of naphthalenes. Anal. Chem. **74**, 5470–5479 (2002)
22. Short, L.C., Cai, S.-S., Syage, J.A.: APPI-MS: effects of mobile phases and VUV lamps on the detection of PAH compounds. J. Am. Soc. Mass Spectrom. **18**, 589–599 (2007)
23. Luosujärvi, L., Arvola, V., Haapala, M., Saarela, V., Franssila, S., Kotiaho, T., Kostiainen, R., Kauppila, T.J.: Desorption and ionization mechanisms in desorption atmospheric pressure photoionization. Anal. Chem. **80**, 7460–7466 (2008)
24. Kauppila, T.J., Kotiaho, T., Kostiainen, R., Bruins, A.P.: Negative ion-atmospheric pressure photoionization-mass spectrometry. J. Am. Soc. Mass Spectrom. **15**, 203–211 (2004)
25. Song, L., Wellman, A.D., Yao, H., Bartmess, J.E.: Negative ion-atmospheric pressure photoionization: electron capture, dissociative electron capture, proton transfer, and anion attachment. J. Am. Soc. Mass Spectrom. **18**, 1789–1798 (2007)
26. Klee, S., Derpmann, V., Wißdorf, W., Klopotowski, S., Kersten, H., Brockmann, K.J., Benter, T., Albrecht, S., Bruins, A.P., Dousty, F.: Are clusters important in understanding the mechanisms in atmospheric pressure ionization? Part 1: Reagent ion generation and chemical control of ion populations. J. Am. Soc. Mass Spectrom. **25**, 1310–1321 (2014)
27. Díaz-Cruz, M.S., LópezdeAlda, M.J., López, R., Barceló, D.: Determination of estrogens and progestogens by mass spectrometric techniques (GC/MS, LC/MS and LC/MS/MS). J. Mass Spectrom. **38**, 917C923 (2003)