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Comparison of ESI and APCI Sources in Q-TOF Mass Spectrometer in Photodegradation Study of Selected Psychotropic Drugs

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Summary. Ultra high-performance liquid chromatography (UHPLC) coupled with high-resolution quadrupole time-of-flight (Q-TOF) mass spectrometry was used for the preliminary photodegradation study of nine new generation psychotropic drugs. Based on the above method, two ionization sample modes — electrospray and atmospheric pressure chemical ionization were used for the registration of photodegradation profiles of the selected drugs. Multivariate chemometric analysis showed that electrospray ionization (ESI) method is more specific than atmospheric pressure chemical ionization (APCI) in high-resolution mass spectrometry (HR-MS) analysis of the analyzed psychotropic drugs. It was noticed that, with the use of ESI method, more potential photodegradation products can be identified and lower limits of its detection can be obtained.

Key Words: PCA, ESI, APCI, Q-TOF, mass spectrometry

Introduction

Mass spectrometry is one of the most frequently applied analytical methods these days, and even listing of disciplines using this technique may be difficult: toxicology, pharmacy, pharmacology, proteomics, chemistry, and many others. The major principle of mass spectrometry is measurement of mass to charge ratio (m/z) of a molecule in an ionized form; therefore, not surprising is the fact that choice of a proper ionization technique is substantial. Unlike several dozen years ago, nowadays, plenty of various ion sources offering different parameters (such as sensitivity and selectivity) are available, but still, ionization is often the most challenging stage of a whole analytical procedure. Nowadays, the two most frequently applied "soft" ion sources are electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI). The second one, despite numerous advantages, cannot be coupled with liquid chromatography. Among ion sources suitable for liquid chromatography–mass spectrometry (LC–MS) connections, the

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most popularly mentioned are ESI and atmospheric pressure chemical ionization (APCI). In ESI, ions are formed by injecting sample containing solution through high-potential gradient at atmospheric pressure. Coaxially flowing hot nitrogen causes evaporation of solvents followed by concentration of charged species on the surface of droplets and in consequence the so-called Coulomb explosion, leading to formation of desolvated ions. ESI is mostly applied to liquid samples and is a method of choice in the case of small polar compounds; however, it is less useful for analysis of weakly polar analytes. Worth noticing is that ESI is the "softest" ion source, causing very little fragmentation. It also enables analysis of very large molecules because of possibility of creation of multiply charged ions. In APCI, analytes are ionized indirectly in a corona discharge and, of course, also at atmospheric pressure but usually at higher temperature, which may be problematic in the case of thermally labile molecules. Another disadvantage is much narrower molecular weight range than in the case of ESI (<1000 Da). APCI offers better ionization efficiency of low polar compounds and lower ion suppression or matrix effects [1–8].

There are many available papers comparing APCI and ESI on various fields. Conclusions of study on pesticides are not surprising — APCI was more sensitive in the case of neutral and basic compounds, while ESI was more suitable for analysis of anionic and cationic molecules [9]. Although matrix influence is more significant than in APCI, ESI is better source for diuron, irgarol, and their degradation products analysis [10]. Wick et al. in their study on biocides, ultraviolet (UV) filters, and benzothiazoles in wastewater and sludge found APCI more efficient and less susceptible to ion suppression [11]. This source gives better effects in the case of analysis of polyaromatic amines and nitroamines with the use of triple quadrupole. Dinitropyrene was weakly ionized by ESI, probably as a consequence of lack of proton donating/accepting functional groups, and APCI was better source also for diaminopyrene [12]. Similarly, ESI turned out to be useless for analysis of polycyclic aromatic hydrocarbons (PAHs) by quadrupole time-of-flight (Q-TOF) [13]. In the study by Garcia-Ac et al. on pharmaceuticals in wastewater, best selectivity and signal-to-noise ratio were achieved with the use of ESI. In the case of methotrexate, APCI was extremely inefficient (ion intensity not sufficient for identification), probably because of low volatility and possible thermal degradation [14]. ESI outperforms APCI also in the case of analysis of cyclophospamide and ifosfamide in effluents [15], estrogens and progestogens in environmental samples [16], and determination of fluoroquinolones in chicken tissues — three to four times better sensitivity [17].

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In pharmaceutical and pharmacological sciences, ESI was found more suitable than APCI for analysis of metabolites of antimalarial artemisin derivatives [18] with the use of QTRAP, fentanyl, and norfentanyl in biological samples (also QTRAP; Verplaetse and Tytgat [19]) and identification of phase II metabolites of apomorphine, dobutamine, and entacapone (triple quadrupole). In this last case, ESI was the only ionization source able to identify sulfate conjugates and the most efficient in general [5]. Wang et al. recommend using ESI for determination of illicit drugs in the oral fluid [20], and Rybak et al., for analysis of the urinary estrogens [21]. On the other hand, APCI was more efficient in ionization of sulfonamides [22] and sulfonate esters genotoxic impurities in drug substances [4] and less susceptible to matrix effect during analysis of estrogens in biological samples [23]. Although studies on matrix effects during analysis of plasma samples confirmed that the use of APCI gives better sensitivity, authors suggest the use of ESI because of potentially present thermally labile metabolites [24].

As it can be seen, generally accepted differences in applicability of ESI and APCI do not always correspond with the facts. In the case of analysis of samples containing numerous unknown compounds, comparison of utility of each ion source would be desirable, which of course may be problematic because of large amounts of data that have to be analyzed. In such case, application of chemometric methods may be helpful. In literature data, only one paper was found concerning the comparison of ESI and APCI with the use of principal component analysis (PCA), perfect prognosis (PP), classification and regression trees (CART), partial least squares (PLS), and multiple linear regression (MLR) method. Caetano et al. compared the two ion sources on over 400 molecules, but the obtained results did not allow to conclude which method could be preferred [25].

The aim of this study was a multivariate chemometric comparison of ionization source efficiency in Q-TOF high-resolution mass spectrometry during analysis of nine psychotropic drugs. The experiment is intended as a preliminary study before further research of their unknown photodegradation mechanism. For this purpose, a new analytical method with the use of ultrahigh-performance liquid chromatography (UHPLC) gradient chromatography combined with APCI/ESI–Q-TOF mass spectrometry was developed. The obtained results were inspected by PCA, and optimal ionization source for further photodegradation studies of these psychotropic drugs was selected.

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Experimental

Materials

Agomelatine (*N*-[2-(7-methoxynaphthalen-1-yl)ethyl]acetamide), sertindole (1-[2-[4-[5-chloro-1-(4-fluorophenyl)indol-3-yl]piperidin-1-yl]ethyl]imidazolidin-2-one), tiapride hydrochloride (*N*-[2-(diethylamino) ethyl]-2-methoxy-5-methylsulfonylbenzamide hydrochloride), asenapine maleate (5-chloro-2,3,3a,12b-tetrahydro-2-methyl-1*H*-dibenz(2,3-6,7)oxepino(4,5-c)pyrrole maleate), toloxatone (5-(hydroxymethyl)-3-(3-methylphenyl)-1,3-oxazolidin-2-one), loxapine succinate (8-chloro-6-(4-methylpiperazin-1-yl)benzo[b] [1,4] benzoxazepine succinate), tandospirone (3a,4,7,7a-hexahydro-2-(4-(4-(2-pyrimidinyl)-1-piperazinyl)butyl)-4,7-methano-1*H*-isoindole-1,3(2*H*)-dione), formic acid for LC–MS, and water for LC–MS were purchased from from Sigma-Aldrich (St. Louis, USA). Hypergrade acetonitrile for LC–MS was purchased from Merck (Darmstadt, Germany). Brintellix 10 mg tablets (H. Lundbeck A/S, Denmark) — vortioxetine hydrobromide (1-[2-(2,4-dimethylphenyl)sulfanylphenyl]piperazine hydrobromide) and Edronax 4 mg tablets (Pfizer Europe, Great Britain) — reboxetine methanosulfonate ((2*R*)-2-[(*R*)-(2-ethoxyphenoxy)-phenylmethyl]morpholine methanosulfonate) were obtained in the local pharmacy.

Sample Preparation

Stock solutions of all the analyzed compounds were prepared in acetonitrile at concentration of 0.5 mg mL^{-1}. In the case of drugs obtained from pharmaceutical formulations (vortioxetine and reboxetine), the equivalents of active compounds were weighed and, after 5 min of ultrasonic sweeping with acetonitrile, centrifuged at 10,000 RPM for 5 min. The working solution of the analyzed drugs at concentration 10 μg mL^{-1} was prepared by diluting all stock solutions in water in one sample.

For the photodegradation test, the working solution was placed in quartz-capped cells (l = 1 cm) mounted horizontally in photostability chamber Atlas Suntest CPS+ (Linsengericht, Germany) and irradiated for 4 h with full UV–Vis spectrum (D65). The irradiance was set to 750 W m^{-2} which corresponds to dose of 2700 kJ m^{-2} h^{-1}. The temperature in the chamber was controlled and kept below 35 °C. The dark control sample was also performed by exposing the analyzed drugs in quartz cell wrapped in aluminum foil for the same period of time.

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All the samples including the working solution before irradiation (Std), dark control sample (Cont), and working solution after irradiation (Deg) were analyzed in seven replications in ESI as well as in APCI mode.

UHPLC–Q-TOF Analysis

UHPLC–MS analysis was performed with the use of Agilent Accurate-Mass Q-TOF LC/MS G6520B system with dual electrospray (DESI) and atmospheric pressure chemical ionization (APCI) source and Infinity 1290 ultra-high-pressure liquid chromatography system consisting of binary pomp G4220A, FC/ALS thermostat G1330B, autosampler G4226A, DAD detector G4212A, TCC G1316C module (Agilent Technologies, Santa Clara, USA), and Hibar RP-18e (2.1 × 50 mm, dp = 2 μm) HR column (Merck, Darmstadt, Germany). A mixture of acetonitrile (A) and water which contained 5% of acetonitrile (B) with addition of 0.1% solution of formic acid in both media was used as a mobile phase. The gradient elution was carried out at constant flow 0.3 mL min^{-1} from 0%A (100%B) 0–0.5 min and then 0%A to 70%A 0.5–9.5 with 2.5 min isocratic post time (0%A). The injection volume was 2 μL, and the column temperature was maintained at 35 °C. MassHunter workstation software in version B.04.00 was used for the control of the system and data acquisition.

The MS detector was tuned in a positive mode with the use of Agilent APCI-L tuning mix in extended dynamic range (2 GHz). To ensure accuracy in masses measurements, reference mass correction was also used and mass 121.0508 and 922.0097 (Agilent ES TOF reference mix solution) were used as lock masses. The main parameters were optimized, and the following settings were applied for ESI and APCI experiments: gas temp., 300 °C; drying gas, 9 L min^{-1}; nebulizer pressure, 35 psig; capillary voltage, 3000 V; fragmentor voltage, 200 V; skimmer voltage, 65 V; octopole 1; and radio frequency (RF) voltage, 750 V. For APCI, additionally, vaporizer temperature (325 °C) and corona voltage (6 V) were optimized. Data acquisition was performed in TOF (MS) mode with spectral parameters: mass range of 60–950 m/z and acquisition rate of 2 spectra per second.

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Data Analysis

Mass Hunter Qualitative Analysis software version B.06.00 (Agilent) with built-in molecular feature extraction (MFE) algorithm was used for data background ion noise cleaning and to extract the list of the ions. The MFE parameters were optimized, and the following settings were applied: maximum 1 charge state of the analyzed ions, more than 5000 counts for compound filter, and isotope model: common organic molecules with peak spacing tolerance 0.0025 m/z.

In order to execute a multivariate chemometric analysis, the obtained results after MFE extraction were next exported to the Mass Profiler Professional software (MPP v.12.6, Agilent and Strand Life Sciences Pvt. Ltd.). Using MPP software, the data was normalized, aligned, and filtered with the use of built-in MPP filtration including sample frequency, abundance, and moderated *t*-test. The obtained data was finally used to perform the principal component analysis (PCA).

Results and Discussion

The photodegradation of drugs is the up-to-date problem in pharmaceutical science as it is strictly related to the concept of drug quality (which cannot be evaluated by the patient himself and strict regulations must be applied). Moreover, this phenomenon is really complex, and it is well-known that it is a resultant mainly of photooxidation and photolysis. As the reaction mechanism cannot be easily predicted, the investigation must be done very carefully and strict optimization of data collection must be applied.

Reversed-phase UHPLC chromatographic system coupled with Q-TOF high-resolution mass spectrometer (HR-MS) was employed for the qualitative analysis of nine psychotropic drugs (agomelatine, sertindole, tiapride, asenapine, toloxatone, loxapine, tandospirone, vortioxetine, and reboxetine — *Fig. 1*) and their photodegradation products. Broad range gradient elution with acetonitrile as modifier was used to achieve a wild range of retention and short time of the analysis *(Fig. 2)*. In order to achieve maximum selectivity of the spectrometric method, HR-MS analyzer was independently optimized for the two examined ion sources — APCI and ESI; however, in both cases, the same most effective spectra data acquisition method — TOF (MS) mode was used [26].

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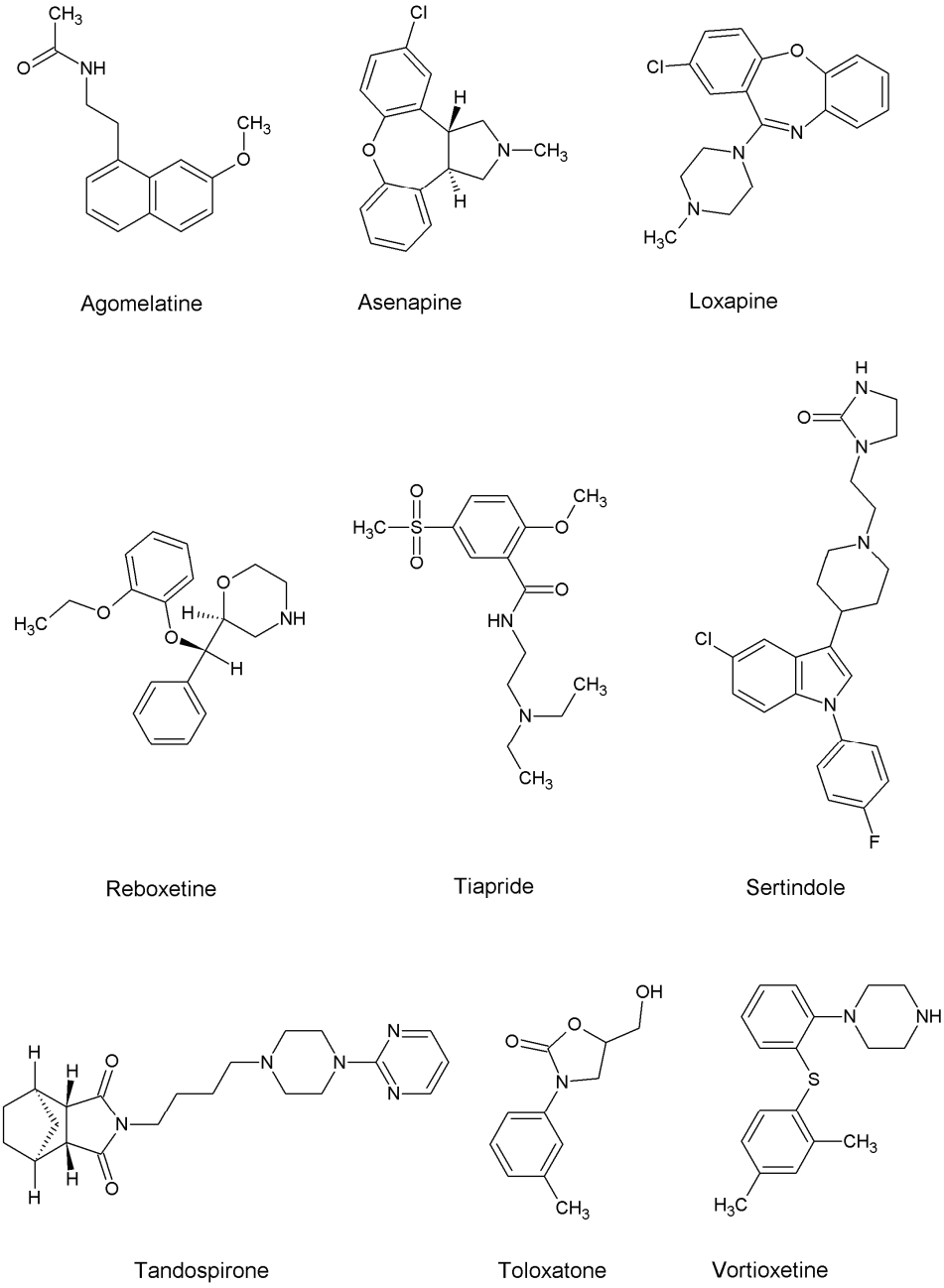

Fig. 1. Chemical structure of investigated psychotropic drugs

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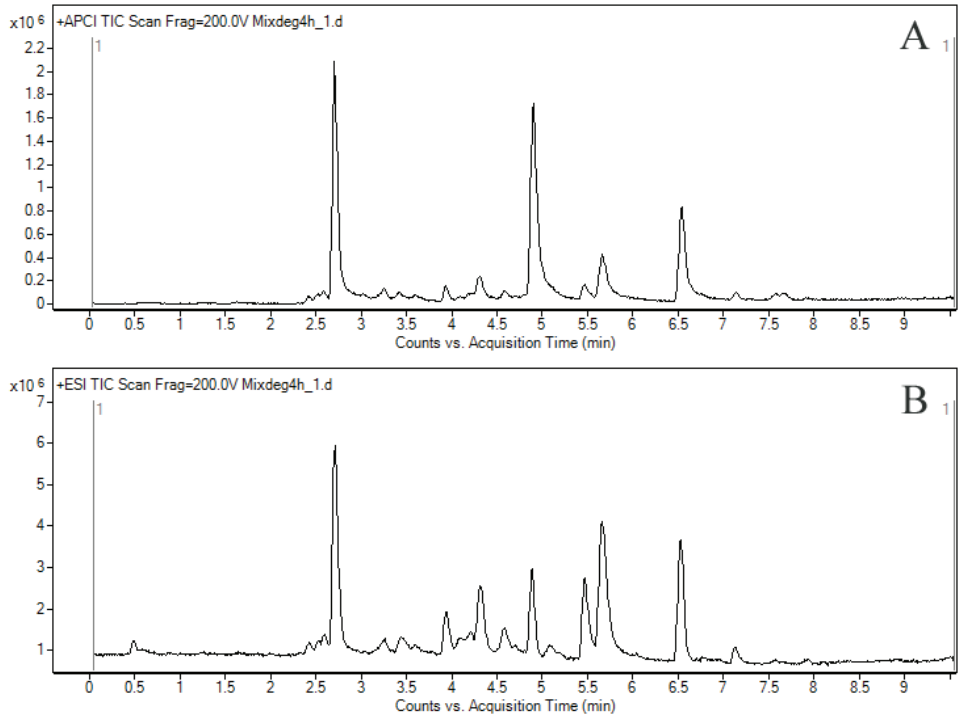

Fig. 2. UHPLC–Q-TOF total ion chromatograms obtained for the irradiated (4 h) sample of nine psychotropic drugs for APCI (A) and ESI (B) ionization

Photodegradation study was performed on the mixture solution of nine new generation psychotropic drugs in order to achieve maximum photoproducts in one sample. This strategy enabled optimization of HR-MS method ionization in one run without repetitions of the optimization procedure for each drug. However, due to a large number of the spectral data, it was necessary to use chemometric methods. For this purpose, all the chromatographic profiles (n = 42) obtained by the use of ESI and APCI were aligned with MPP software, and finally, 156 entities (specific ions) were received. After build-in MPP filtration including sample frequency, abundance, and moderated t-test (p = 0.05, FC = 5), 50 entities were finally selected for the chemometric study. These entities represent ions which belong to the potential photoproducts of the analyzed drugs, and on this set of data, the principal component analysis was performed *(Fig. 3)*. The PCA showed a visible categorization of the photodegraded samples (Deg) from the samples before degradation (Std) and control samples (Cont) for both

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ionization methods. However, for ESI method, this trend is much more visible. For APCI, samples after irradiation are located relatively close to control and standard samples, and at the same time, these two groups (Cont and Std) are significantly less correlated than the same groups of samples in ESI. Based on these results, it can be stated that ESI is a more specific method for HR-MS analysis of the photodegradation samples of the selected drugs. It should be also noticed that, in the PCA, the first two components explained up to 82.2% of the total variance.

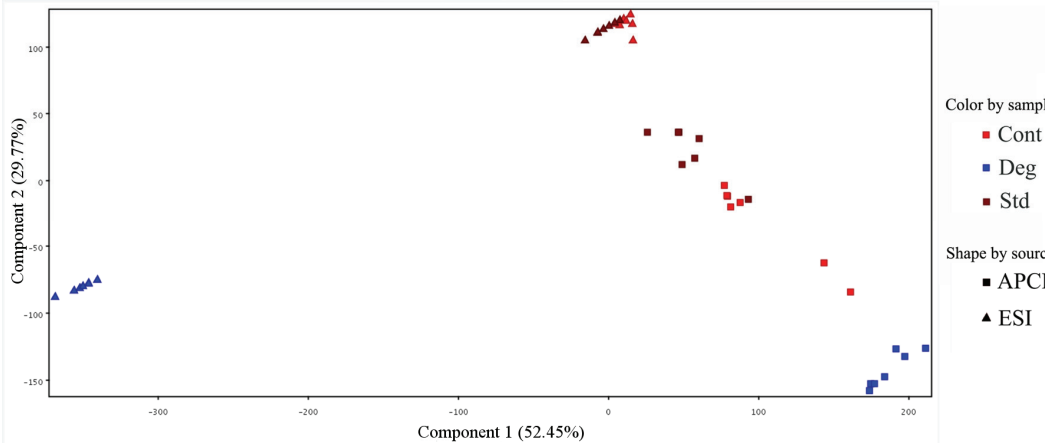

Fig. 3. Comparison of the sample ionization methods by PCA

Table I. Selected ions as potential photodegradation products of analyzed psychotropic drugs

Ions label	RT (min)	Measured mass (m/z)	ESI	APCI
D-1	2.59	376.23230	+	+
D-2	3.24	404.22617	+	+
D-3	3.52	284.12787	+	−
D-4	4.11	386.21796	+	−
D-5	4.16	455.20602	+	+
D-6	4.54	302.10513	+	−
D-7	5.55	314.10547	+	−
D-8	7.18	298.10422	+	−

+ observed, − not observed.

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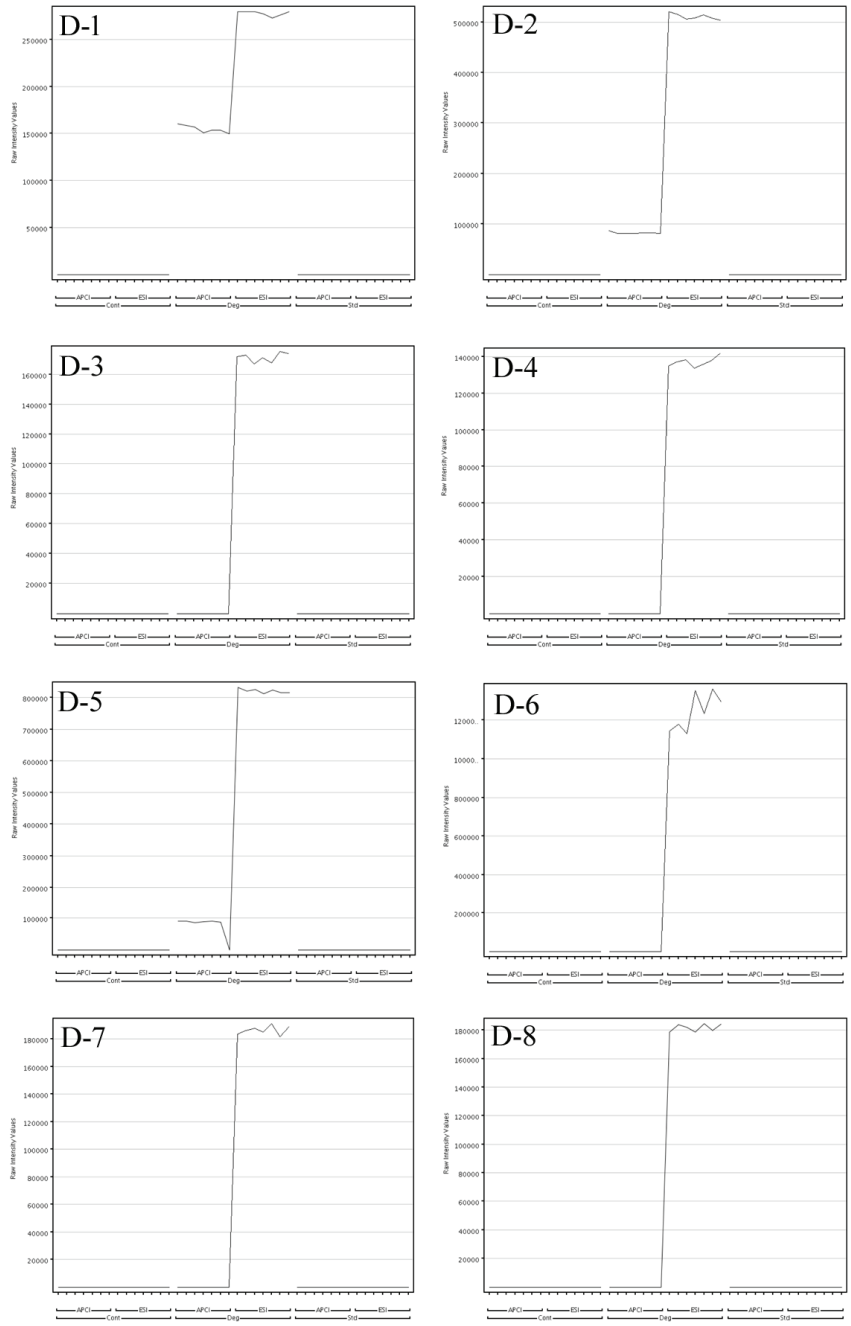

Fig. 4. Comparison of raw intensity profiles for the selected ions (subfigures labeled as in *Table I*)

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In order to make an additional comparison of the selectivity as well as sensitivity of ESI and APCI, eight ions ($p < 0.01$) from fifty entities obtained after statistical filtration were selected *(Table I)* and their raw intensity value profiles were compared *(Fig. 4)*. It can be clearly observed that many of the selected ions are not detected in APCI mode (D3, D4, and D6–8), or if they are detected (D-1, D-2, D-5), the sensitivity of the detection is much lower than in ESI mode.

Taking this into account, electrospray ionization can be recommended as a first choice method of ionization for HR-MS analysis for further research on photodegradation of the selected psychotropic drugs.

Conclusion

UHPLC–APCI/ESI-Q-TOF mass spectrometry method enabling the registration of specific photodegradation profiles of psychotropic drugs has been developed. Based on the above method, multivariate chemometric comparison of MS ionization source efficiency was performed.

PCA showed that an electrospray ionization method is more specific than APCI in HR-MS analysis of the photodegradated samples of the selected psychotropic drugs. In ESI mode, more potential photodegradation products can be identified and lower limits of their detection can be obtained.

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