

# JMS Letters

Dear Sir,

## Occurrence of radical molecular ions in atmospheric pressure chemical ionization mass spectra of heterocyclic compounds

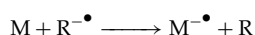
It is well-known that the use of atmospheric pressure ionization (API) techniques, such as electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI), is associated with the formation of even-electron ions,<sup>1</sup> for example, protonated molecules  $[M + H]^+$ , adducts with alkali metal ions  $[M + Na]^+$  and  $[M + K]^+$ , adducts with ammonium ions  $[M + NH_4]^+$  in the positive-ion mode and deprotonated molecules  $[M - H]^-$  and some adducts with small inorganic anions in the negative-ion mode. The relative abundances of fragment ions are generally low to negligible and mostly they have also even number of electrons, otherwise even-electron rule would be contradicted. Odd-electron ions do not typically occur in ESI/APCI mass spectra, but some exceptions are known,<sup>2</sup> for example, radical molecular ions  $M^{\bullet-}$  formed by an electron capture<sup>3</sup> or charge exchange mechanisms<sup>4,5</sup> in the negative-ion APCI mode, and radical molecular ions  $M^{\bullet+}$  formed by the charge exchange mechanism in case of nonpolar polyaromatic hydrocarbons.<sup>6,7</sup>

In the present communication, we would like to highlight few well-pronounced examples on the formation of radical molecular ions both in the positive-ion and negative-ion APCI modes using an ion trap Esquire 3000 (Bruker Daltonics, Bremen, Germany) or a single quadrupole Platform (Micromass, Manchester, UK) under the following experimental conditions: the mass range  $m/z$  50–1000, the target mass  $m/z$  250 and the compound stability 20% for alkyl series from C2 to C4, the target mass  $m/z$  350 and the compound stability 100% for alkyl series from C5 to C10, pressure of the nebulizing gas 15 psi, the drying gas flow rate 4 l/min, temperature of the ion source 350 °C and temperature of drying gas 300 °C. The samples were dissolved in acetonitrile and delivered into the system by an infusion pump at the flow rate of 5  $\mu$ l/min. In some experiments, different solvents (methanol, benzene, or water) were used to compare results with the measurements in acetonitrile.

Unlike the conventional electron ionization, the formation of odd-electron molecular ions is quite unusual in API mass spectra. The preference of radical ion formation depends on the structure, the ionization mode, and the experimental conditions, for example solvents used for the analysis. In ESI, the occurrence of molecular radical ions is very rare and it is usually attributed to the electrochemical processes on the electrospray needle or different oxidation states of metal ions. On the other hand, the presence of radical molecular ions in the latest API technique, atmospheric pressure photoionization (APPI), is relatively common due to the different ionization mechanism.<sup>5</sup> The presence of molecular radical ions is not typical for APCI, but some examples have been reported for polyaromatic hydrocarbons without other functionalities in the positive-ion mode<sup>6,7</sup> or for compounds with the high electron affinity and low gas-phase proton affinity in the negative-ion mode.<sup>8</sup> The formation of radical molecular ions  $M^{\bullet+}$  in the positive-ion API mode can be explained by a charge exchange mechanism<sup>2</sup>:



where M denotes a molecule of analyte, R denotes a reactant gas. The mechanism of radical molecular ion  $M^{\bullet-}$  in the negative-ion mode<sup>2,5</sup> can be attributed either to the charge exchange mechanism:



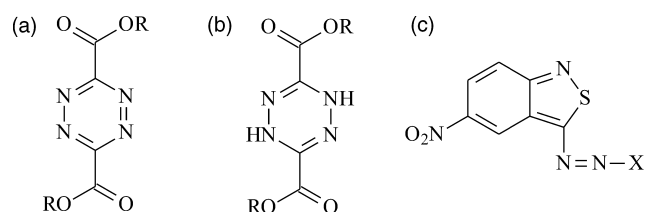
or preferably to the electron capture mechanism due to the presence of thermal electrons in the corona discharge:



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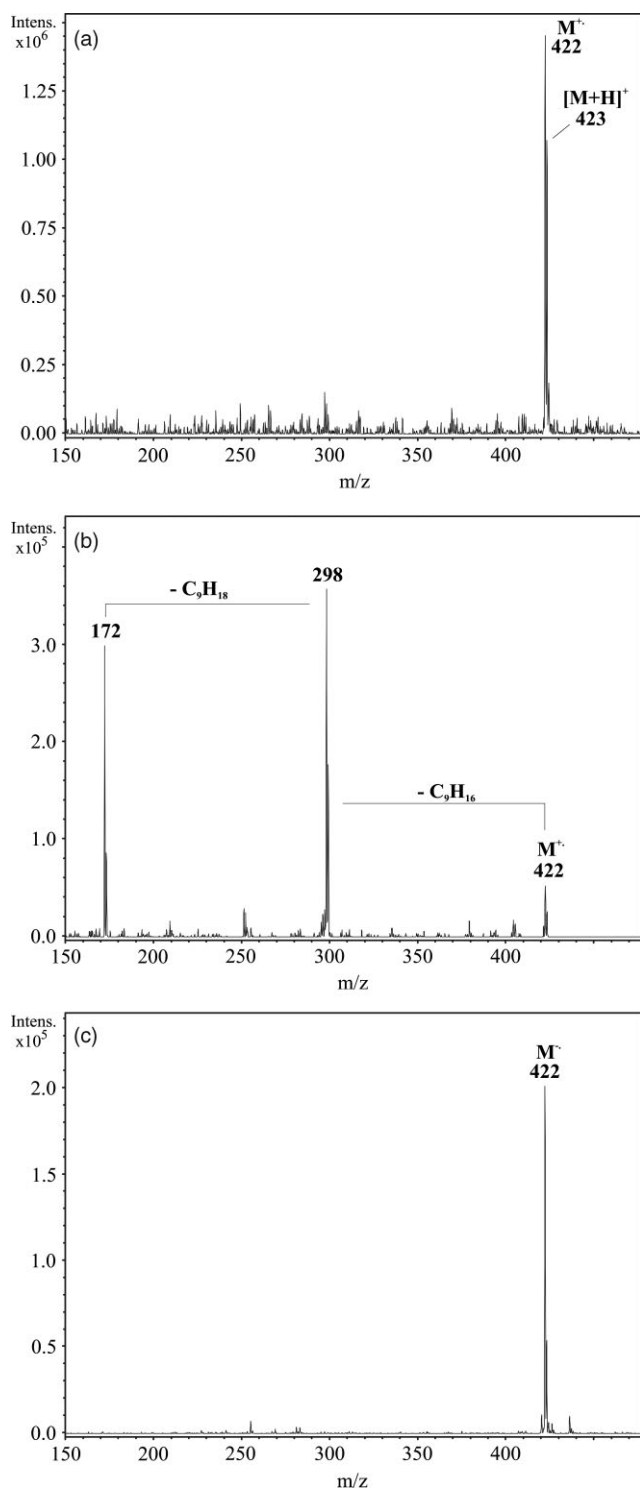
Figure 1 shows the structures of three series of compounds studied in our work, dialkyl 1,2,4,5-tetrazine-3,6-dicarboxylate derivatives (Fig. 1(a)) and dialkyl 1,4-dihydro-1,2,4,5-tetrazine-3,6-dicarboxylate derivatives (Fig. 1(b)) with different lengths of alkyl chains from C2 to C10 and eleven 3,3-disubstituted 1-(5-nitro-2,1-benzisothiazol-3-yl)triazenes (Fig. 1(c)). Typical APCI mass spectra of tetrazines (Fig. 2) and dihydrotetrazines (Fig. 3) illustrate an unusual presence of odd-electron molecular ions in both polarity modes. To the best of our knowledge, this is the first report, where radical molecular ions are the base peaks in both polarity modes for one class of nitrogen-containing heterocyclic compounds. Positive-ion APCI mass spectra of tetrazines exhibit both the protonated molecule  $[M + H]^+$  and radical molecular ions  $M^{\bullet+}$ , as illustrated in Fig. 2(a). The ratio of these ions depends on the alkyl chain length (Fig. 4(a)). The peak of the protonated molecule is prevailing for short alkyl chains (C2 and C3) and it also has an important contribution for long alkyl chains (e.g. 95% for C10), while radical molecular ions are dominating for medium alkyl chain length. The contribution of  $[M + H]^+$  ion is very low, for example it is below 2% in case of C5 and C6. Figure 4 represents the net relative ion abundances after subtracting <sup>13</sup>C contribution to the intensity of  $[M + H]^+$  ion. The situation is somewhat different for dihydrotetrazines (Figs 3(a) and 4(b)), because the  $[M + H]^+$  is always the base peak of spectra accompanied by  $M^{\bullet+}$  at relative abundances between 12 and 44%. The ratio of these ions again depends on the alkyl chain length, as shown in Fig. 4(b). The relative abundances of fragment ions in the full scan spectra of both tetrazine (Fig. 2(a)) and dihydrotetrazine (Fig. 3(a)) derivatives are very low, so tandem mass spectra (MS/MS) have to be used for the identification of alkyl chain length based on the characteristic neutral losses of one and consecutively two alkene moieties (Fig. 2(b)). This fragmentation behavior is identical for both compound classes, therefore only one example is shown for C9 tetrazines (Fig. 2(b)). Negative-ion APCI mass spectra of both tetrazine (Fig. 2(c)) and dihydrotetrazine (Fig. 3(b)) contain only radical ions. Meaningful MS/MS spectra could not be recorded regardless of the collision energy.

For tetrazines,  $m/z$  values of ions observed in both polarity modes are identical, for example  $M^{\bullet+}$  at  $m/z$  422 (Fig. 2(a)) and  $M^{\bullet-}$  at  $m/z$  422 (Fig. 2(c)). Surprisingly, the difference between radical ions observed in both polarity modes is  $\Delta m/z$  2 for dihydrotetrazines (compare Fig. 3(a) and (b)), which is explained by the presence of  $M^{\bullet+}$  ion in the positive-ion mode and the formation of more stable oxidation product  $[M - 2]^{\bullet-}$  in the negative-ion mode formed by the loss of H<sub>2</sub>. It has been confirmed that the reduction process takes place in the APCI ion source by a simple experiment. After recording the negative-ion APCI spectrum, the polarity is switched to the positive-ion mode still using the identical sample solution from the infusion pump. The polarity is switched between positive and negative modes for several times and the spectra were identical without any change, which confirms that the sample is reduced in the ion source. Moreover, the sample



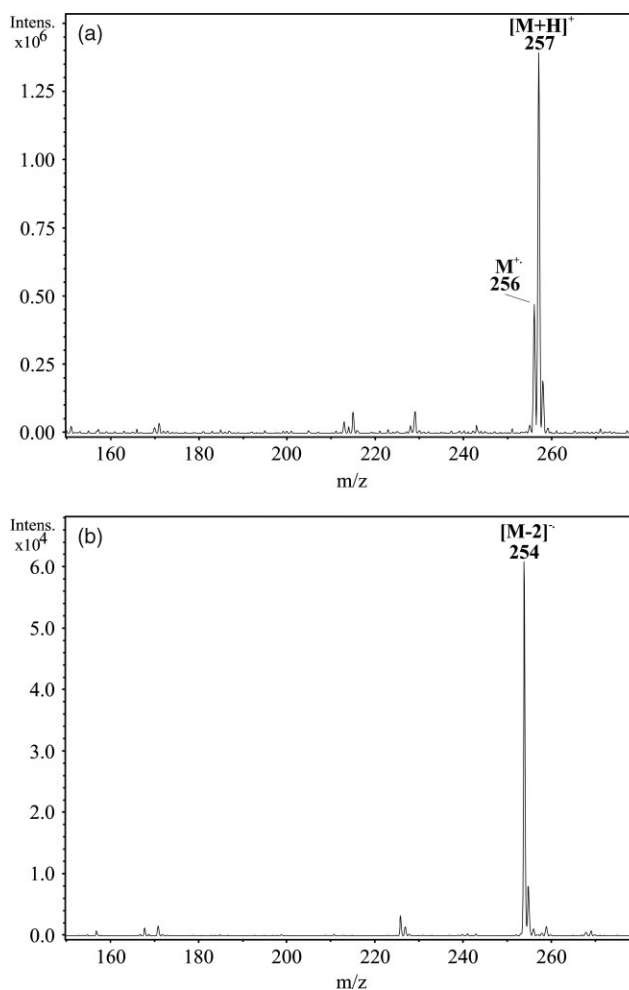
**Figure 1.** Structures of compounds studied: (a) tetrazine derivatives, (b) dihydrotetrazine derivatives, (c) 3,3-disubstituted 1-(5-nitro-2,1-benzisothiazol-3-yl) triazenes. R = (CH<sub>2</sub>)<sub>n</sub>CH<sub>3</sub>, where n = 1–9;

X = N(CH<sub>2</sub>CH<sub>2</sub>OH)C<sub>6</sub>H<sub>5</sub>, N(CH<sub>2</sub>CH<sub>2</sub>CN)C<sub>6</sub>H<sub>5</sub>, N(CH<sub>2</sub>CH<sub>2</sub>OH)C<sub>6</sub>H<sub>4</sub>-*m*-Cl, N(CH<sub>2</sub>CH<sub>2</sub>CN)C<sub>6</sub>H<sub>4</sub>-*m*-CH<sub>3</sub>, N(CH<sub>3</sub>)C<sub>6</sub>H<sub>5</sub>, N(CH<sub>2</sub>CH<sub>3</sub>)C<sub>6</sub>H<sub>5</sub>, N(C<sub>6</sub>H<sub>5</sub>)<sub>2</sub>, N(CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>)C<sub>6</sub>H<sub>5</sub>, N(CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>)C<sub>6</sub>H<sub>5</sub>, N(CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>)C<sub>6</sub>H<sub>4</sub>-*m*-CH<sub>3</sub>, N(CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>)C<sub>6</sub>H<sub>4</sub>-*m*-Cl.



**Figure 2.** APCI mass spectra of C9 tetrazine derivative: (a) positive-ion spectrum, (b) positive-ion MS/MS spectrum of  $[M+H]^+$  ion at  $m/z = 422$ , (c) negative-ion spectrum.

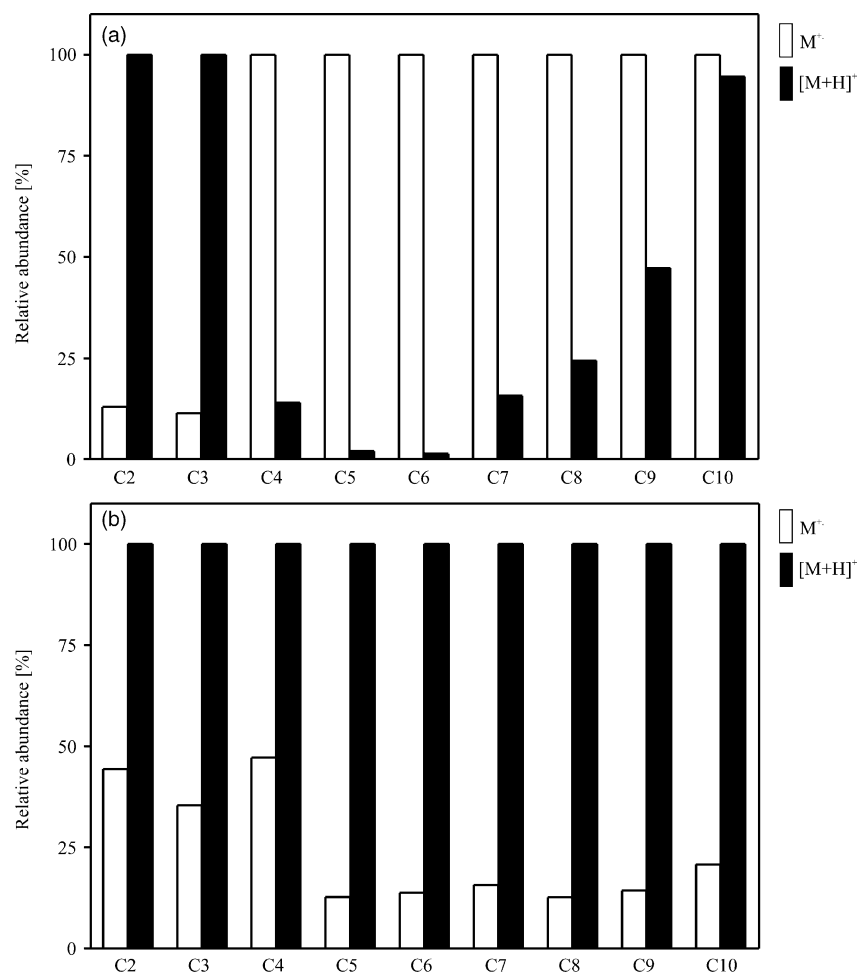
structures have been previously confirmed by nuclear magnetic resonance spectroscopy. Such behavior is rarely observed in APCI mass spectra,<sup>9</sup> but the electrochemical oxidation/reduction is known for ESI.<sup>10–12</sup> The similar behavior was previously observed in case of on-line electropolymerization of aniline,<sup>9</sup> but they could eliminate the reduction by using acetonitrile as an aprotic solvent. In our case, no notable effect of the solvent type has been found and  $[M-2]^-$  ions are the base peaks in all studied solvents (acetonitrile, methanol, or benzene). This phenomenon can be probably explained by the fact that the structures of corresponding tetrazines are better



**Figure 3.** APCI mass spectra of C3 dihydrotetrazine derivative: (a) positive-ion spectrum, (b) negative-ion spectrum.

stabilized by an extensive conjugation system in comparison to their dihydrotetrazine analogs.

The effects of three parameters on the  $M^{+\bullet}/[M+H]^+$  ratio of C10 tetrazine and dihydrotetrazine have been studied: (1) corona voltage in the range from 1.5 to 10 kV with a step of 0.5 kV, (2) ion source temperature from 350 to 500 °C with the step of 25 °C (lower temperature cannot be used at this flow rate), and (3) different sample solvents – acetonitrile, methanol, benzene, water mixtures of water with acetonitrile (1:1, v/v) or methanol (1:1, v/v). No clear effect can be recognized for altered corona voltage values except for significantly decreased absolute ion intensities for values far from the optimal value for this parameter (about 3–4 kV). Ion source temperature can cause the decomposition of the sample, especially for tetrazine derivatives. Therefore, only the lowest values of temperature (i.e. 350 °C) can be used. As expected, the strongest effect on the competition between  $M^{+\bullet}$  and  $[M+H]^+$  ion formation is observed for different solvents. Experiments with water and aqueous acetonitrile or methanol have failed completely because of extremely fast hydrolysis within few seconds, so only data for measurements in acetonitrile, methanol, or benzene are presented in Table 1 for C10 derivatives of tetrazine and dihydrotetrazine together with thermochemical data for these solvents. Thermochemical data of our analytes cannot be found in any database, but they can be estimated based on comparison with similar compounds,<sup>13</sup> ionization energy can be approximately 9 eV and proton affinity should be higher than 900 kJ/mol. In both cases, the highest preference for the radical molecular ion formation is observed for benzene as the solvent with the lowest ionization energy and proton affinity. Benzene  $C_6H_6^{+\bullet}$  reactant ions is known to be capable of both proton transfer and charge transfer depending on the gas-phase basicity of the analyte.<sup>2</sup> The highest preference of



**Figure 4.** Comparison of relative abundances of  $M^{+\bullet}$  and  $[M+H]^+$  ions in positive-ion APCI mass spectra of: (a) tetrazine derivatives, (b) dihydrotetrazine derivatives. Contributions of  $^{13}\text{C}$  isotopic peaks to the intensity of  $[M+H]^+$  are subtracted.

**Table 1.** Relative abundances of  $M^{+\bullet}$  and  $[M+H]^+$  ions measured in different organic solvents for C10 derivatives of tetrazine and dihydrotetrazine and some thermochemical data for these solvents<sup>13</sup>

Solvent	Ionization energy (eV)	Proton affinity (kJ/mol)	Gas-phase acidity (kJ/mol)	Gas-phase basicity (kJ/mol)	Relative abundance (%)			
					Tetrazine		Dihydrotetrazine	
					$M^{+\bullet}$	$[M+H]^+$	$M^{+\bullet}$	$[M+H]^+$
Acetonitrile	12.20	779	1524	748	53	100	13	100
Methanol	10.84	754	1593	724	15	100	4	100
Benzene	9.24	750	1672	725	100	20	100	85

$[M+H]^+$  ion formation is found in methanol. Identical trends are observed for both tetrazines and dihydrotetrazines.

The last example of compounds providing radical ions in APCI is a group of eleven 1-(5-nitro-2,1-benzisothiazol-3-yl) triazine derivatives with different alkyl substituents (Fig. 1(c)). Positive-ion APCI spectra of these derivatives follow the established rules with the presence of  $[M+H]^+$  ions together with several abundant fragment ions useful for the identification of the substituent type and the common base structure of these compounds.<sup>14</sup> No radical ions are observed in the positive-ion mode, but the radical molecular ions  $M^{+\bullet}$  are formed in negative-ion APCI spectra without the presence of  $[M-H]^-$  ions. On the basis of our previous knowledge that the nitro group may be responsible for the electron capture mechanism

in the negative-ion mass spectrometric measurements similarly to conventional chemical ionization, a similar role of the nitro group has been assumed in this case. An analog molecule to Fig. 1(c) has been synthesized but without the nitro group in the position 5. Surprisingly, the radical molecular anion does not disappear, but the relative ratio of  $M^{+\bullet}$  and  $[M-H]^-$  ions is approximately 1:1 suggesting that the nitro group is not the only structural subunit responsible for the electron capture mechanism, but the heterocyclic ring structure also contributes to the radical ion formation. Finally, the nitro group is replaced by a sulfonic acid group that is known to dissociate easily both in aqueous solutions and gas phase as well to  $\text{RSO}_3^-$  and  $\text{H}^+$  (or  $\text{Na}^+$  for sodium sulfonates).<sup>15–17</sup> In this case, the radical ions completely disappear and  $[M-H]^-$  ion is the base peak of the spectrum with significantly improved sensitivity.

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Yours,

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## References

- Levsen K, Schiebel HM, Terlouw JK, Jobst KJ, Elend M, Preiss A, Thiele H, Ingendoh A. Even-electron ions: a systematic study of the neutral species lost in the dissociation of quasi-molecular ions. *Journal of Mass Spectrometry* 2007; **42**: 1024.
- Carroll DI, Dzidic I, Horning EC, Stillwell RN. Atmospheric-pressure ionization mass spectrometry. *Applied Spectroscopy Reviews* 1981; **17**: 337.
- Hayen H, Jachmann N, Vogel M, Karst U. LC-electron capture-APCI(-)MS determination of nitrobenzoxadiazole derivatives. *Analyst* 2003; **128**: 1365.
- Bruins AP. Mass spectrometry with ion sources operating at atmospheric pressure. *Mass Spectrometry Reviews* 1991; **10**: 53.
- Bruins AP. Atmospheric-pressure-ionization mass spectrometry. I. Instrumentation and ionization techniques. *Trends in Analytical Chemistry* 1994; **13**: 37.
- Anacleto JF, Ramaley L, Boyd RK, Pleasance S, Quilliam MA, Sim PG, Benoit FM. Analysis of polycyclic aromatic compounds by supercritical fluid chromatography/mass spectrometry using atmospheric-pressure chemical ionization. *Rapid Communications in Mass Spectrometry* 1991; **5**: 149.
- Anacleto JF, Ramaley L, Benoit FM, Boyd RK, Quilliam MA. Comparison of liquid chromatography/mass spectrometry interfaces for the analysis of polycyclic aromatic compounds. *Analytical Chemistry* 1995; **67**: 4145.
- Kolakowski BM, Grossert JS, Ramaley L. The importance of both charge exchange and proton transfer in the analysis of polycyclic aromatic compounds using atmospheric pressure chemical ionization mass spectrometry. *Journal of the American Society for Mass Spectrometry* 2004; **15**: 301.
- Kertesz V, Van Berkel GJ. Surface-assisted reduction of aniline oligomers, N-phenyl-1,4-phenylenediimine and thionin in atmospheric pressure chemical ionization and atmospheric pressure photoionization. *Journal of the American Society for Mass Spectrometry* 2002; **13**: 109.
- Kertesz V, Van Berkel GJ, Granger MC. Study and application of a control led-potential electrochemistry – electrospray emitter for electrospray mass spectrometry. *Analytical Chemistry* 2005; **77**: 4366.
- Van Berkel GJ, Asano KG, Granger MC. Controlling analyte electrochemistry in an electrospray ion source with a three-electrode emitter cell. *Analytical Chemistry* 2004; **76**: 1493.
- Diehl G, Karst U. On-line electrochemistry – MS and related techniques. *Analytical and Bioanalytical Chemistry* 2002; **373**: 390.
- Linstrom PJ, Mallard WG (eds). NIST Standard Reference Database Number 69, June 2005 Release, <http://webbook.nist.gov/chemistry/>, downloaded on 21 August 2007.
- Přikryl J, Lyčka A, Bertolasi V, Holčápek M, Macháček V. Structure and reactivity of 1-(5-Nitrobenzo[c]-1,2-thiazol-3-yl)-3,3-disubstituted triazenes. *European Journal of Organic Chemistry* 2003; **22**: 4413.
- Holčápek M, Jandera P, Přikryl J. Analysis of sulphonated dyes and intermediates by electrospray mass spectrometry. *Dyes and Pigments* 1999; **43**: 127.
- Holčápek M, Jandera P, Zderadička P. High performance liquid chromatography – mass spectrometric analysis of sulphonated dyes and intermediates. *Journal of Chromatography A* 2001; **926**: 175.
- Holčápek M, Volná K, Ansorgová D. Effects of functional groups on the fragmentation of dyes in electrospray and atmospheric pressure chemical ionization mass spectra. *Dyes and Pigments* 2007; **75**: 156.