Electrospray ionization and atmospheric pressure ionization mass spectrometry of stable organic radicals[a]

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The ionization behavior of stable radicals (1–8) was studied under electrospray ionization (ESI) and atmospheric pressure ionization (APCI) conditions. With APCI, using methanol + water as the solvent, most radicals were protonated to give the radical cation $[M + 1]^{+\bullet}$, whereas TEMPO (5) was detected as the M^+ ion and galvinoxyl (1) gave the $[M + 2]^+$ ion. Using benzene as the solvent, all radicals (1–8) were efficiently oxidized to give M^+ as the base peak. Under ESI conditions using methanol + water with 1% acetic acid, stable radicals (5–7) gave $[M + 1]^{+\bullet}$, (2–4) M^+, whereas (1) and (8) showed the $[M + 2]^+$ ion as the base peak. Using the ESI source as a controlled-current electrolytic cell, radicals (2–8) were efficiently oxidized to give M^+ ions. Radical (1) was detected as the $[M + 2]^+$ ion. Detection limits for the radicals (1–8) were between 3×10^{-9} mol L^{-1} and 5×10^{-7} mol L^{-1} using APCI or ESI in the full-scan mode. In most cases detection limits were lower using the tandem mass spectrometry (MS/MS) full-scan mode.
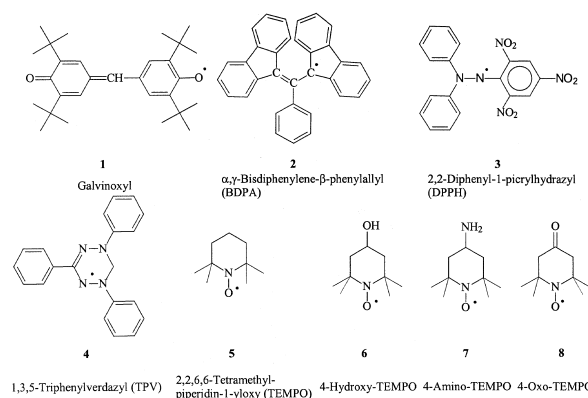
Introduction

With the introduction of electrospray ionization (ESI) by Fenn *et al.*[1] and Alexandrov *et al.*,[2] it became possible to analyze reaction solutions directly by mass spectrometry. There have been some examples in the literature indicating that this technique can be helpful in determining reaction mechanisms or characterizing intermediates in chemical reactions.[3–10]

Radicals are reactive intermediates in many industrially important processes and in synthetically useful reactions, as well as in biological systems. However, methods for the investigation of radicals are limited. In order to develop a new method to detect transient radicals in solutions, we began to study the ionization behavior of some stable radicals under ESI and atmospheric pressure chemical ionization (APCI) conditions using various solvents. Galvinoxyl (**1**), α,γ-bisdiphenylene-β-phenylallyl (**2**, BDPA), 2,2-diphenyl-1-picrylhydrazyl (**3**, DPPH), 1,3,5-triphenylverdazyl (**4**, TPV), 2,2,6,6-tetramethylpiperidine-1-yloxy (**5**, TEMPO), 4-hydroxy-TEMPO (**6**), 4-amino-TEMPO (**7**) and 4-oxo-

TEMPO (**8**) were selected since they contained different functional groups as well as a radical centered on either a C, N or O atom.

1
Galvinoxyl

2
α,γ-Bisdiphenylene-β-phenylallyl (BDPA)

3
2,2-Diphenyl-1-picrylhydrazyl (DPPH)

4
1,3,5-Triphenylverdazyl (TPV)

5
2,2,6,6-Tetramethyl-piperidin-1-yloxy (TEMPO)

6
4-Hydroxy-TEMPO

7
4-Amino-TEMPO

8
4-Oxo-TEMPO

Experimental

Mass spectrometry

All experiments were performed on a Finnigan LCQ (Thermoquest–Finnigan, San Jose, CA, USA) quadrupole ion trap mass spectrometer equipped with a standard ESI and APCI ion source. For some ESI experiments, the standard fused-silica spray capillary (100 μm ID, 200 μm OD, Polymicro, Phoenix, AZ, USA) was replaced by a stainless

steel capillary (110 µm ID, 240 µm OD, Hamilton, Bonaduz, Switzerland). Sample solutions (*c.* 1×10^{-5} mol L^{-1}) were infused using the syringe pump of the LCQ. ESI operation used a spray voltage of + 5 kV, a heated capillary temperature of 200°C and a liquid flow rate of 3 µL min^{-1}, whereas in APCI mode, the vaporizer temperature was set to 450°C, the capillary temperature was 150°C, the liquid flow rate was 50 µL min^{-1} and a corona discharge current of 5 µA was employed. In both ESI and APCI modes, positively charged ions were analyzed. The auxiliary gas flow was disabled and all other parameters (sheath gas flow, ion guide voltages) were optimized to yield a maximum analyte ion signal. Data analysis used the LCQ-Navigator (Version 1.1) software package.

Flow injection analysis

Determination of detection limits of the stable radicals **1–8** was performed using flow injection analysis (FIA). Samples were diluted in an appropriate solvent to concentrations of 5×10^{-5} mol L^{-1}, 1×10^{-5} mol L^{-1}, 5×10^{-6} mol L^{-1}, 1×10^{-6} mol L^{-1}, 5×10^{-7} mol L^{-1}, 1×10^{-7} mol L^{-1}, 5×10^{-8} mol L^{-1}, 1×10^{-8} mol L^{-1}, 5×10^{-9} mol L^{-1} and 1×10^{-9} mol L^{-1} just prior to analysis. A solvent flow was delivered by an HPLC pump (P4000, Thermoquest–Thermo Separation Products (TSP), San Jose, CA, USA) and directed through an autosampler (AS3000, Thermoquest–TSP) to the ion source of the mass spectrometer. To ensure proper operation of the pump, a 0.5 µm frit (Upchurch, Oak Harbour, WA, USA) was introduced into the flow path between pump and autosampler to yield a reasonable back pressure. For low flow rates (1–10 µL min^{-1}) the frit was replaced by an Acurate ICP-500 flow splitter (fixed split-ratio 1 : 500, LC-Packings, Amsterdam, Netherlands). To avoid dead volumes and to achieve a good peak shape, a 50 µm ID fused-silica capillary (365 µm OD, Polymicro) was used to connect the autosampler to the mass spectrometer. In the APCI mode, methanol + water (50 : 50, v/v) was used as the solvent, at a flow rate of 100 µL min^{-1}, whereas in the ESI mode 1% (by volume) acetic acid was added to the solvent and the flow rate set to 10 µL min^{-1}.

The mass spectrometer was scanned from *m/z* 50 to approximately 1.1 times the mass of the analyte. Volumes of 50 µL in the APCI mode and 2.5 µL in the ESI mode were injected for analysis. For each sample concentration, three injections were made and the ion chromatogram of a selected ion was analyzed to determine the signal-to-noise ratio (S/N). The detection limits of the method were then calculated considering the limit to be an S/N of 10. This experiment was done in normal mass spectrometer full-scan mode and also in tandem mass spectrometry (MS/MS) scan mode after collision-induced dissociation (CID) of a selected ion. For CID the excitation amplitude of the ion trap was adjusted to decrease the parent ion signal to approximately 10–20% of its initial value.

Chemicals

The stable radicals **1–3** and **5–8** were obtained from Aldrich (Steinheim, Germany). 1,3,5-Triphenylverdazyl (**4**) was synthesized according to a literature procedure[11] and characterized by IR to confirm its structure.

Methanol, acetonitrile, dichloromethane, benzene, acetic acid and lithium trifluoromethanesulfonate were purchased from Fluka (Deisenhofen, Germany). All solvents were of HPLC-grade quality. Dichloromethane and acetonitrile were dried over molecular sieves (3 Å) by use of the dynamic method. All chemicals were used without further purification. Deuterated methanol (CD_3OD) was obtained from Deutero (Kastellaun, Germany) and deuterated water from Merck (Darmstadt, Germany).

Results and discussion

Atmospheric pressure chemical ionization mass spectrometry

With methanol + water (50 : 50, v/v) as a reagent gas, most stable radicals were detected as the $[M + H]^{+\bullet}$ radical cation. Only radical **5** gave the molecular ion M^+, whereas radical **1** was found to give the $[M + 2]^+$ ion. Figure 1 shows the mass spectrum of 1,3,5-triphenylverdazyl (**4**) as an example. The radical cation $[M + H]^{+\bullet}$ is the base peak. Table 1 lists the relative intensities of the M^+, $[M + 1]^{+\bullet}$ and the $[M + 2]^+$ ions, corrected for the calculated isotope contributions of the M^+ and the $[M + H]^{+\bullet}$ peaks. Different aqueous solvent mixtures were tested but none were found that changed the ionization behavior of the radicals.

Fenselau *et al.*[12] proposed benzene as a reagent gas for chemical ionization mass spectrometry to ionize analytes by charge exchange. Thus, benzene was used for APCI-MS of

Table 1. Relative intensities of the M^+, $[M + 1]^{+\bullet}$ and $[M + 2]^+$ signals (corrected for calculated isotope contributions) of the radicals 1–8. APCI conditions: continuous infusion via syringe pump, 50 µL min^{-1}, c. 1×10^{-5} mol L^{-1}, solvent methanol + water (50 : 50, v/v), vaporizer temperature 450°C.

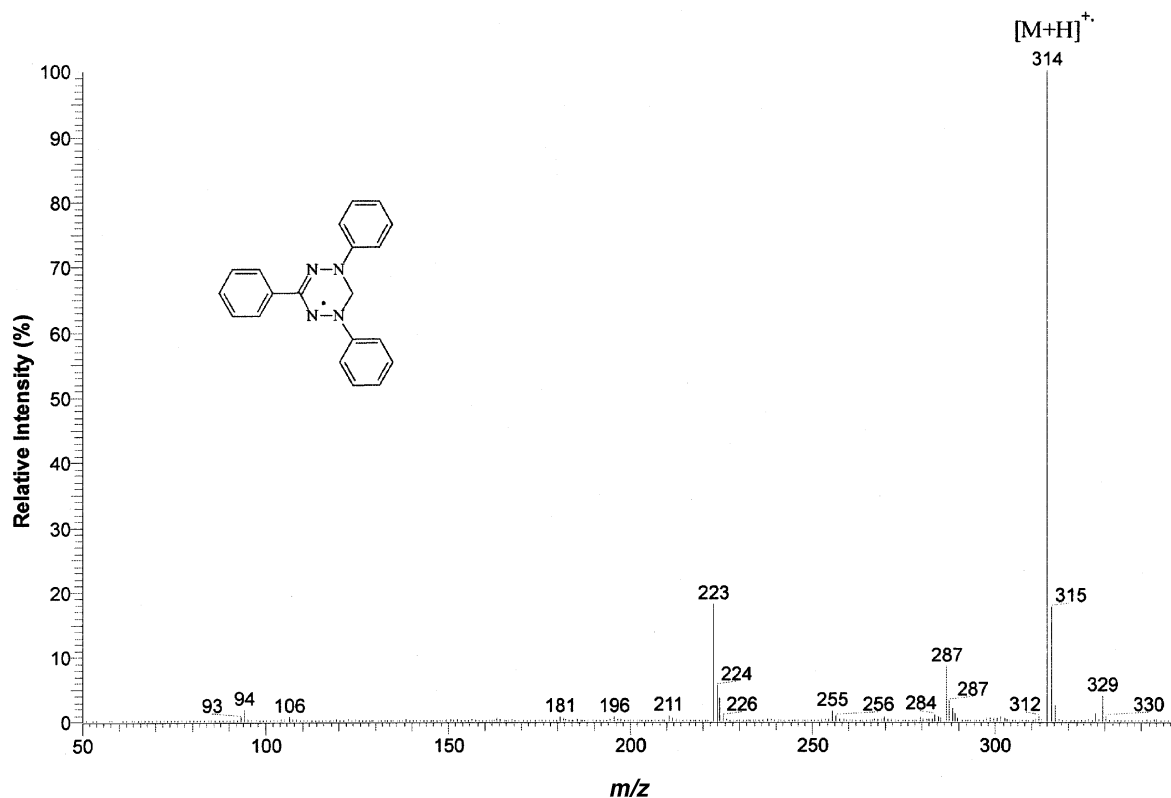| Radical	Rel. intensity (corrected for isotope contributions)		
	M^+	$[M + 1]^{+\bullet}$	$[M + 2]^+$
1	0%	1%	100%
2	49%	100%	0%
3	0%	100%	9%
4	0%	100%	0%
5	100%	1%	12%
6	74%	100%	4%
7	9%	100%	1%
8	2%	100%	0%

Figure 1. APCI mass spectrum of radical 4. **For conditions see Table 1.**

the radicals **1–8**. All analytes were ionized to give the molecular ion M^+ as the base peak. Results are summarized in Table 2.

Table 2. Relative intensities of the M⁺, [M + 1]⁺• and [M + 2]⁺ signals (corrected for calculated isotope contributions) of the radicals 1–8, **APCI conditions: continuous infusion via syringe pump, 50 μL min⁻¹,** *c.* **1 × 10⁻⁵ mol L⁻¹, solvent benzene, vaporizer temperature 450°C.**

Radical	Rel. intensity (corrected for isotope contributions)		
	M^+	$[M + 1]^{+•}$	$[M + 2]^+$
1	100%	59%	40%
2	100%	3%	1%
3	100%	2%	0%
4	100%	1%	0%
5	100%	13%	0%
6	100%	35%	0%
7	100%	8%	0%
8	100%	59%	1%

Electrospray ionization mass spectrometry

Using an acidified mixture of methanol + water (50 : 50, v/v containing 1% acetic acid), the stable radicals showed rather different ionization behavior. Radicals **2–4** were detected as the M^+ ion and **5–7** as the $[M + H]^{+•}$ radical cation, whereas **1** and **8** gave the $[M + 2]^+$ ion as the base peak. The signals from TEMPO (**5**) and 4-hydroxy-TEMPO (**6**) were strongly influenced by the source parameters, i.e. sample flow rate, sample concentration and spray voltage. Lower analyte solution flow rates (concentrations) resulted in a higher abundance of the M^+ ion, whereas higher flow rates (concentrations) favored the $[M + H]^{+•}$ signal. High ESI voltages gave intense M^+ ions, whereas low ESI voltages gave intense $[M + H]^{+•}$ signals. The ion distribution was also influenced by source gas flows and source geometry, but this aspect will not be discussed here. These results clearly indicate that protonation and oxidation processes are competitive reactions in this case. With radical **7**, the protonation process dominates due to the high basicity of the amino group. As the radicals **2–4** were readily oxidized to the M^+ ion, even in acidified solutions, they are likely to have very low oxidation potentials, so that the oxidation process dominates compared with the protonation process. It was, however, still unclear why some radicals (**1** and **8**) gave an $[M + 2]^+$ ion. Using CD_3OD and D_2O as the solvent, these radicals gave an $[M + 4]^+$, i.e. $[MD + D]^+$ ion. From this it
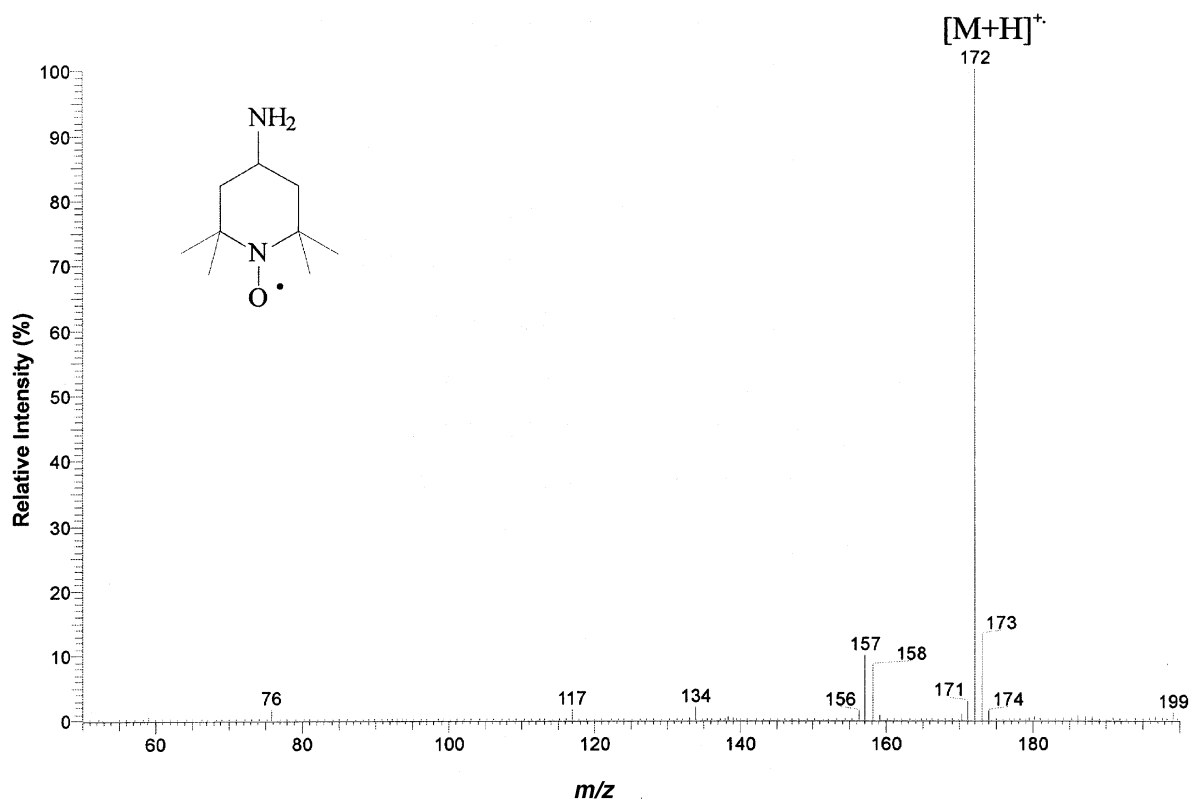
Figure 2. ESI mass spectrum of radical 7. For conditions see Table 3.

was concluded that radicals **1** and **8** must be reduced by hydrogen transfer in solution and/or under the source conditions used. In the case of galvinoxyl (**1**) there is experimental evidence that the radical is reduced in solution.

The ESI source can be used as a controlled-current electrolytic cell as shown by van Berkel *et al.*[13] This is done by using a stainless steel spray capillary and a dichloromethane + acetonitrile mixture (50 : 50, v/v) as the solvent

Table 3. Relative intensities of the M$^+$, [M + 1]$^{•+}$ and [M + 2]$^+$ signals (corrected for calculated isotope contributions) of the radicals 1–8. ESI conditions: continuous infusion via syringe pump, 3 μL min^{-1}, *c.* 1 × 10^{-5} mol L^{-1}, solvent methanol + water (50 : 50, v/v) with 1% (by volume) acetic acid added, spray voltage + 5 kV.

Radical	Rel. intensity (corrected for isotope contributions)		
	M$^+$	[M + 1]$^{•+}$	[M + 2]$^+$
1	0%	1%	100%
2	100%	0%	0%
3	100%	8%	0%
4	100%	0%	0%
5	44%	100%	72%
6	74%	100%	42%
7	3%	100%	2%
8	3%	2%	100%

Table 4. Relative intensities of the M$^+$, [M + 1]$^{+•}$ and [M + 2]$^+$ signals (corrected for calculated isotope contributions) of the radicals 1–8. ESI conditions: continuous infusion via syringe pump, 3 μL min^{-1}, *c.* 1 × 10^{-5} mol L^{-1}, solvent dichloromethane + acetonitrile (50 : 50, v/v) with 1 mmol L^{-1} lithium trifluoromethanesulfonate, spray voltage + 5 kV.

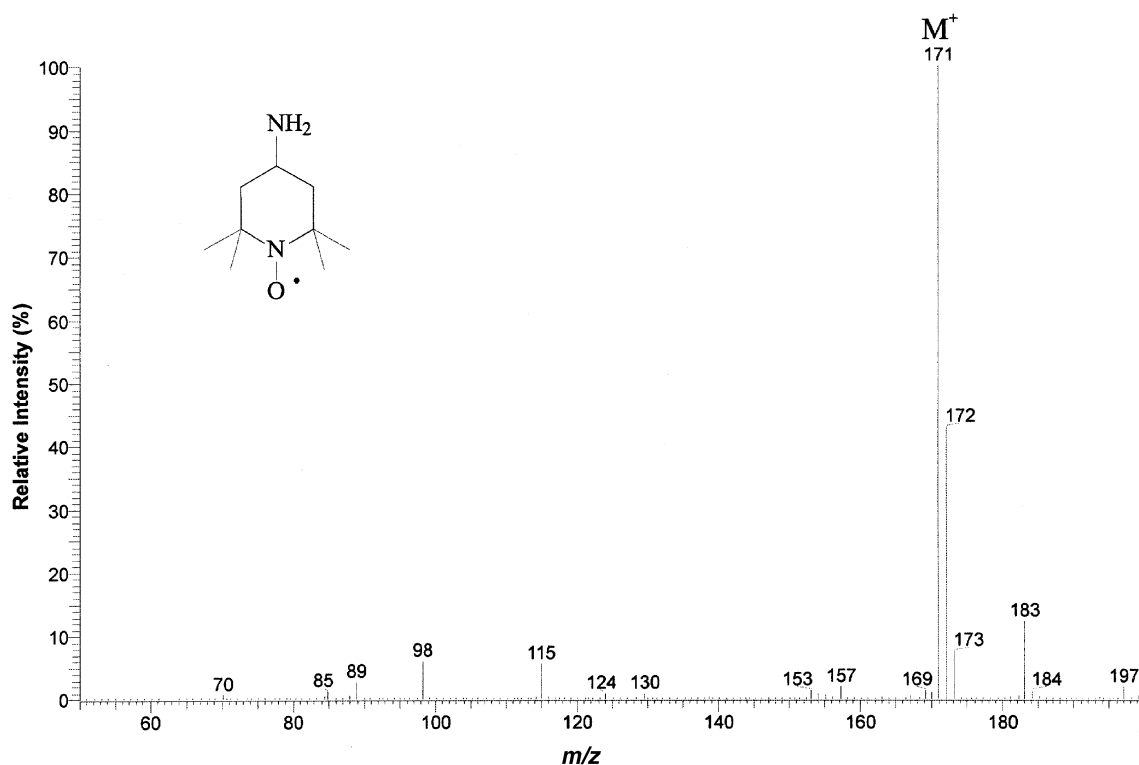| Radical	Rel. intensity (corrected for isotope contributions)		
	M$^+$	[M + 1]$^+$	[M + 2]$^+$
1	0%	1%	100%
2	100%	0%	0%
3	100%	0%	0%
4	100%	0%	9%
5	100%	0%	1%
6	100%	5%	1%
7	100%	32%	7%
8	100%	1%	4%

Figure 3. ESI mass spectrum of radical 7. For conditions see Table 4.

with the addition of lithium trifluoromethanesulfonate (1 mmol L^{-1}) to enhance the solution conductivity and thus increase electrolysis efficiency. In this way the stable radicals **2–8** could be detected as the molecular ion M^+. Only galvinoxyl **1** was again detected as the $[M + 2]^+$ ion.

Figure 2 shows the ESI mass spectrum of 4-amino-TEMPO (**7**) in acidified methanol + water giving the radical cation $[M + H]^{+\bullet}$ as base peak. Under controlled current electrolysis conditions, however, the molecular ion M^+ was base peak (Figure 3). Tables 3 and 4 list the relative peak intensities in the molecular ion region of the spectra obtained under these conditions.

Determination of detection limits

Detection limits for the radicals **1–8** were determined by FIA. In APCI-MS full-scan mode, detection limits were between 3×10^{-9} mol L^{-1} and 5×10^{-7} mol L^{-1}, corresponding to a range of $6 \times 10^{-11} - 1 \times 10^{-8}$ g for an injection volume of 50 μL. In APCI-MS/MS full-scan mode, detection limits were between 2×10^{-9} mol L^{-1} and 4×10^{-7} mol L^{-1} ($4 \times 10^{-11} - 8 \times 10^{-9}$ g). Table 5 shows the results for the normal full-scan mode and Table 6 shows results for the MS/MS full-scan mode.

For ESI-MS full-scan mode the limits were determined to be between 1×10^{-8} mol L^{-1} and 6×10^{-7} mol L^{-1} ($4 \times 10^{-11} - 4 \times 10^{-10}$ g for a 2.5 μL injection). ESI-MS/MS gave limits ranging from 1×10^{-8} mol L^{-1} to 3×10^{-7} mol L^{-1} ($1 \times 10^{-11} - 2 \times 10^{-10}$ g). The ESI results are summarized in Tables 7 and 8.

These results show that the detection limits for APCI-MS, as well as those for ESI-MS, are good enough to detect transient radicals in reaction solutions, which occur in concentrations of approximately 10^{-6} mol L^{-1} to 10^{-8} mol L^{-1}. It should be noted that it appears to be possible to decrease the absolute detection limits in ESI mode by using lower flow rates.

Table 5. Detection limits for radicals 1–8 **in APCI-MS full-scan mode. Solvent methanol + water (50 : 50, v/v), vaporizer temperature 450°C, 100 μL min^{-1}.**

Radical	Observed ion	Observed mass	Detection limits	
		(m/z)	$(mol\ L^{-1})$	(g)
1	$[M + 2]^+$	423	3×10^{-9}	6×10^{-11}
2	$[M + H]^{+\bullet}$	418	5×10^{-7}	1×10^{-8}
3	$[M + H]^{+\bullet}$	395	5×10^{-7}	1×10^{-8}
4	$[M + H]^{+\bullet}$	314	4×10^{-7}	1×10^{-9}
5	M^+	156	2×10^{-7}	2×10^{-9}
6	$[M + H]^{+\bullet}$	173	3×10^{-7}	3×10^{-9}
7	$[M + H]^{+\bullet}$	172	1×10^{-7}	9×10^{-10}
8	$[M + H]^{+\bullet}$	171	3×10^{-7}	3×10^{-9}

Table 6. Detection limits for radicals 1–8 **in APCI-MS full-scan mode. Solvent methanol + water (50 : 50, v/v), vaporizer temperature 450°C, 100 μL min⁻¹.**

Radical	Parent ion	Observed transition	Detection limits	
		(m/z)	$(mol\ L^{-1})$	(g)
1	$[M + 2]^+$	$423 \rightarrow 407$	2×10^{-9}	4×10^{-11}
2	$[M + H]^{+\bullet}$	$418 \rightarrow 339$	3×10^{-7}	6×10^{-9}
3	$[M + H]^{+\bullet}$	$395 \rightarrow 168$	4×10^{-7}	8×10^{-9}
4	$[M + H]^{+\bullet}$	$314 \rightarrow 222$	1×10^{-7}	2×10^{-9}
5	M^+	$156 \rightarrow 142$	3×10^{-8}	2×10^{-10}
6	$[M + H]^{+\bullet}$	$173 \rightarrow 158$	2×10^{-8}	3×10^{-10}
7	$[M + H]^{+\bullet}$	$172 \rightarrow 155$	1×10^{-8}	9×10^{-11}
8	$[M + H]^{+\bullet}$	$171 \rightarrow 156$	1×10^{-8}	9×10^{-11}

Table 7. Detection limits for radicals 1–8 **in ESI-MS full scan mode. Solvent methanol + water with 1% acetic acid (50 : 50, v/v), spray-voltage + 5 kV, 10 μL min⁻¹.**

Radical	Observed ion	Observed transition	Detection limits	
		(m/z)	$(mol\ L^{-1})$	(g)
1	$[M + 2]^+$	423	4×10^{-8}	4×10^{-11}
2	M^+	417	2×10^{-7}	2×10^{-10}
3	M^+	394	4×10^{-7}	4×10^{-10}
4	M^+	313	4×10^{-7}	3×10^{-10}
5	$[M + H]^{+\bullet}$	157	6×10^{-7}	2×10^{-10}
6	$[M + H]^{+\bullet}$	173	4×10^{-7}	2×10^{-10}
7	$[M + H]^{+\bullet}$	172	1×10^{-7}	4×10^{-11}
8	$[M + 2]^+$	172	5×10^{-7}	3×10^{-10}

Table 8. Detection limits for radicals 1–8 **in ESI-MS/MS full-scan mode. Solvent methanol + water with 1% acetic acid (50 : 50, v/v), spray-voltage + 5 kV, 10 μL min⁻¹.**

Radical	Parent ion	Observed transition	Detection limits	
		(m/z)	$(mol\ L^{-1})$	(g)
1	$[M + 2]^+$	$423 \rightarrow 407$	1×10^{-8}	1×10^{-11}
2	M^+	$417 \rightarrow 339$	2×10^{-7}	2×10^{-10}
3	M^+	$394 \rightarrow 168$	1×10^{-7}	1×10^{-10}
4	M^+	$313 \rightarrow 210$	3×10^{-7}	2×10^{-10}
5	$[M + H]^{+\bullet}$	$157 \rightarrow 142$	4×10^{-8}	2×10^{-11}
6	$[M + H]^{+\bullet}$	$173 \rightarrow 158$	5×10^{-8}	2×10^{-11}
7	$[M + H]^{+\bullet}$	$172 \rightarrow 155$	7×10^{-8}	3×10^{-11}
8	$[M + 2]^+$	$172 \rightarrow 154$	1×10^{-7}	4×10^{-11}

Conclusion

It was shown that stable radicals can be ionized by APCI and ESI mass spectrometry. The best method to detect radicals unambiguously seems to be oxidation to even-electron cations. This can best be done by the use of benzene as the reagent gas in APCI or by using the ESI source as a controlled-current electrolytic cell. In the latter case, all radicals, with the exeption of galvinoxyl (**1**), could be oxidized to give an even-electron ion as the base peak. The detection limits of the method were sufficiently good to allow the observation of transient radicals in reaction solutions.

Acknowledgment

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