SUPPORTING INFORMATION

On the Reactivity of 2-Methylene-3-quinuclidinone in Water

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1. General Information

Reagents and solvents were used as obtained by the chemical supplier. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Ascend 400 spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrometer. Chemical shifts are reported in ppm relative to the residual non-deuterated solvent peak for ^{1}H and the carbon peak of the deuterated solvent for ^{13}C. ^{1}H NMR data are reported as follows: chemical shift, integral, multiplicity (s = singlet, d = doublet, t = triplet, quint = quintet, m = multiplet, br = broad) and coupling constants (Hz). LCMS were performed on Agilent 1100 system equipped with an electrospray interface, a UV diode array detector and an XBridge C18 (3.0 × 50 mm) column with a gradient of acetonitrile (5-97 %) in 10 mM aqueous ammonium bicarbonate (pH 9), or heptafluorobutyric acid (pH 4) for ion paring. Elemental analyses were performed by DB Lab A/S in Odense, Denmark. Energy minimisation of compound **5** was done using the MM2 force field with the standard setup in Chem3D (ver. 15.1.0.144).

2. X-ray structure of Compound 6

SXRD data were collected at 200K on a Bruker Apex II diffractometer with graphite-monochromated MoK(α) radiation. The crystal structures were determined by direct methods and refined by full matrix least-squares analyses with anisotropic temperature factors for all atoms except protons. Proton positions were calculated using known molecular geometries.

Crystal data	
Chemical formula	C16H28N2O4·2(Cl)·2(O)
Mr	415.30
Crystal system, space group	Monoclinic, *P*21/*n*
Temperature (K)	200
a, b, c (Å)	6.8849 (2), 12.7925 (4), 10.5447 (3)
β (°)	92.633 (1)
V (Å^3)	927.74 (5)
Z	2
Radiation type	Mo *K*α
μ (mm^{-1})	0.37
Data collection	
Diffractometer	Kappa Apex II
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	75222, 1925, 1915
R_{int}	0.021
$(\sin \theta/\lambda)_{max}$ (Å^{-1})	0.628

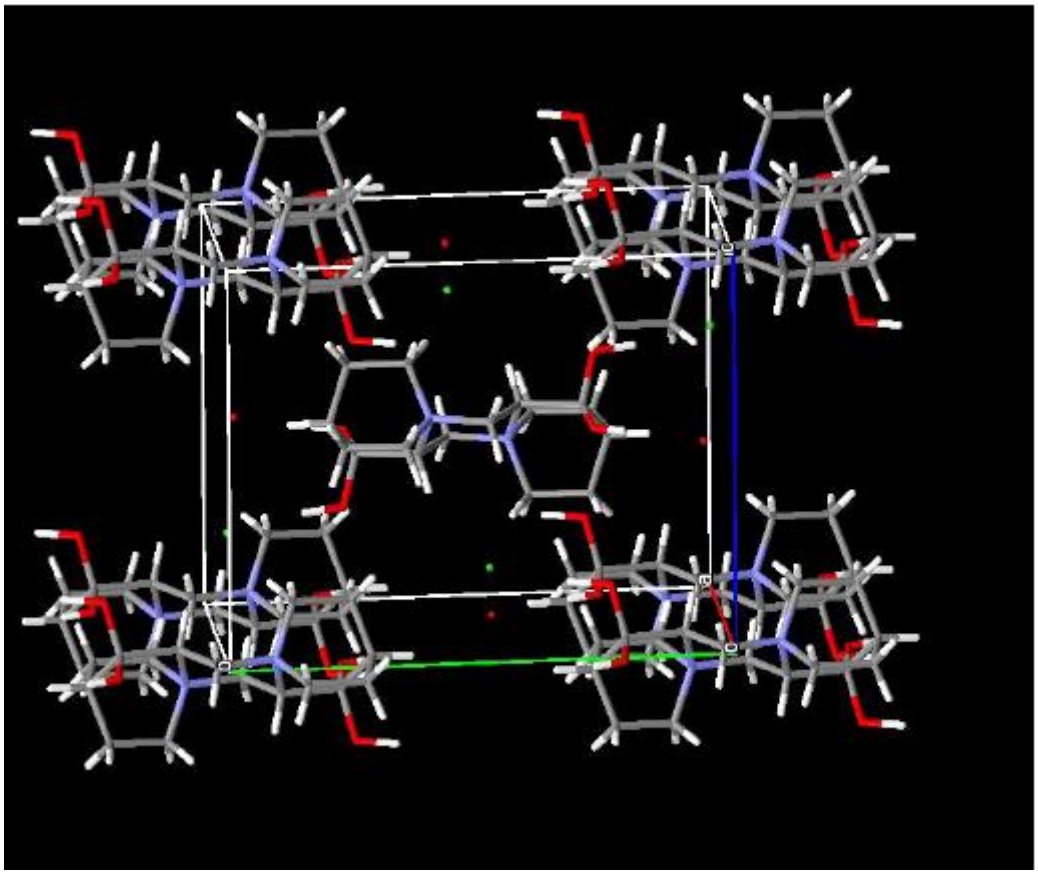
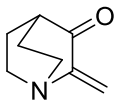


Figure 1. Unit cell view of the crystal structure.

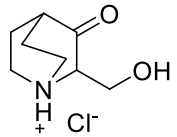
3. Synthetic Procedures



MQ

Methylene-2-quinuclidinone is commercially available in technical quality but can be synthesised in high purity from compound **6**.

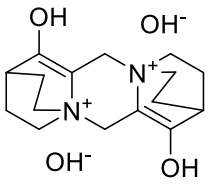
Compound **6** (5.0 g, 23.8 mmol) was added to a stirred mixture of dichloromethane (50 mL) and water (40 mL). K_2CO_3 (13.4 g, 96.4 mmol) was added in portions resulting in a clear solution within a few minutes. The mixture was allowed to stir at room temperature over night resulting in a pale yellow organic phase and a clear aqueous layer. Water was added (30 mL) and the phases were separated. The aqueous phase was further extracted with dichloromethane (3 x 30 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated to dryness to furnish 2.50 g (77%) of a yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 5.83 (s, 1 H) 5.25 (s, 1 H) 3.05 - 3.29 (m, 2 H) 2.81 - 3.05 (m, 2 H) 2.59 (quint, 1 H) 1.90 - 2.06 (m, 4 H). ^{13}C NMR (150 MHz, $CDCl_3$) δ ppm 204.0, 152.2, 113.2, 48.2, 40.1, 24.9. LCMS ESI+ (m/z): 138 $[M+H]^+$



Compound 1

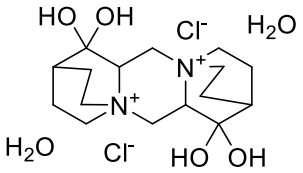
2-Methylenequinuclidin-3-one (511 mg, 3.73 mmol) was dissolved in water (18 mL) and 1.8 mL glacial acetic acid was added. LCMS after 3 hours showed small amounts of MQ left. The mixture stirred for an additional hour at room temperature and solid K_2CO_3 (2.72 g, 19.7 mmol) was added to basify the solution. The reaction mixture was concentrated on a rotavapor and dried over the weekend under high vacuum. The off-white solid was crushed to a fine powder with a spatula and pentane (50 mL) was added and the suspension was stirred for 20 minutes and decanted. The process was repeated (2x50 mL). The organic phase was concentrated to a colourless oil and redissolved in warm pentane (30 mL) and stored in the freezer. After 1 hour white crystals had been formed. The pentane was decanted off and the residual crystals were dried to give the product as a white powder (111 mg, 19%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.99-3.91 (1H, m), 3.76-3.67 (1H, m), 3.40-3.32 (1H, m), 3.18-2.94 (3H, m), 2.92-2.80 (1H, m), 2.47-2.42 (1H, m), 2.34-2.19 (1H, br s), 2.08-1.88 (4H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 219.3, 70.4, 58.8, 48.5, 41.6, 39.9, 26.9, 24.7. LCMS ESI+ m/z 156 $[M+H]^+$.

To prepare the HCl salt, 350 µL (1.4 mmol) of 4M HCl in dioxane was added to a solution of the product (108 mg, 0.7 mmol) in DCM (10 mL). The mixture was immediately concentrated and dried *in vacuo* to give the product as a white solid (110 mg, 83%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.92 (1H, br s), 5.72 (1H, br s), 4.30 (1H, t J=5.6 Hz), 3.93 (2H, d J=5.6 Hz), 3.68-3.56 (1H, m), 3.51-3.28 (3H, m), 2.64-2.59 (1H, m), 2.21-2.11 (2H, m), 2.09-1.97 (2H, m). LCMS ESI+ (m/z): 156 $[M+H]^+$.



Compound 5

2-Methylenequinuclidin-3-one (500 mg) was dissolved in water (1 mL) and the reaction mixture was stirred at room temperature for 24 hours. Addition of acetone (6 mL) precipitated the product. The solid material was filtered off and washed with acetone (2 mL) and dried *in vacuo* to give a white powder. Yield 273 mg (48%). ^{1}H NMR (400 MHz D_2O) δ ppm 4.29 (2H, m), 3.63 (1H, m), 3.40-3.25 (3H, m), 2.69 (1H, m), 2.15-2.05 (2H, m), 2.00-1.90 (2H, m). ^{13}C NMR (100 MHz D_2O) δ ppm: 170.2, 98.9, 59.3, 57.6, 50.6, 34.5, 22.0, 21.8. Calculated: C 61.9; H 8.44; N 9.03; O 20.6. Measured: C 62.8; (±0.5); H 8.22 (±1.0); N 9.12 (±1.0). LCMS ESI+ (m/z): 275, 138.



Compound 6

2-Methylenequinuclidin-3-one (100 mg, 0.72 mmol) was dissolved in water (2 mL) and stirred at room temperature for 18 hours before 100 µL of a saturated NaCl solution was added. The mixture was stirred for 5 minutes followed by addition of 1 mL of a HCl (1 M). The mixture was stirred for 10 minutes, and the precipitate was collected by suction filtration. The solid was washed with acetonitrile (2 x 1 mL) and dried *in vacuo* to yield the product as a white solid (130 mg, 85%). ^{1}H NMR (400 MHz D_2O) δ ppm: 4.25-3.40 (m, 12 H),

2.45-2.05 (m, 10H). ^{13}C NMR (100 MHz D_2O) δ ppm: 92.3, 64.9, 59.8, 54.3, 48.6, 32.1, 19.2, 19.1. Calculated: C 45.8; H 7.69; N 6.68; Cl 16.9; O 22.9. Measured: C 46.1; (±0.5); H 7.75 (±1.0); N 6.71 (±1.0); Cl 16.5.

4. NMR studies

The NMR studies were recorded on a Bruker 600 MHz spectrometer using a 5 mm ^{1}H/^{13}C/^{15}N/^{31}P inverse detection CryoProbe, equipped with z-gradient. MQ was dissolved in 600 µL borate buffer (pH 9), phosphate buffer (pH 7.5) or phthalate buffer (pH 4) and transferred to 5 mm NMR tubes. The assignment of ^{1}H and ^{13}C resonances were obtained from 1D proton, carbon and self-diffusion experiments as well as from 2D TOCSY, HSQC and HMBC experiments from the Bruker pulse sequence library. Mixing time of 120 ms were used for TOCSY experiments. The shifts of the atoms of the second moiety are identical for the dimers due to rotational symmetry.

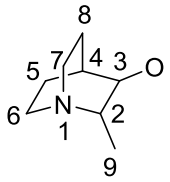


Table 1. ^{1}H and ^{13}C NMR chemical shifts of MQ and derivatives at pH 9 (25°) at a concentration of 42 mg/mL MQ.

MQ	**2**	**3**	**4**	**5, 8**	**6, 7**	**9**
^{13}C ^{1}H	148.4	207.0	38.5 2.63	21.8 2.10; 1.95	45.9 3.11; 2.91	113.5 5.89; 5.37
Cmp 1	**2**	**3**	**4**	**5, 8**	**6, 7**	**9**
^{13}C ^{1}H	68.5 Appears as a triplet in ^{13}C due to exchange of H with D	222.0	38.2 2.47	21.8 and 22.7 2.09; 1.95	46.1 and 39.5 3.06; 2.87 and 3.13; 2.85	57.3 3.88
Cmp 5	**2**	**3**	**4**	**5, 8**	**6, 7**	**9**
^{13}C ^{1}H	98.9	170.2	34.5 2.69	21.8 and 22.0 2.09; 1.94	57.6 3.35; 3.37 59.3 3.28; 3.63	50.6 4.29

Table 2. ^{1}H and ^{13}C NMR chemical shifts of MQ and derivatives at pH 7.5 (25°C) at a concentration of 42 mg/mL MQ. Two isomers are seen for 4 but only one is reported in the table.

MQ	**2**	**3**	**4**	**5, 8**	**6, 7**	**9**
^{13}C ^{1}H	148.4	207.0	38.5 2,63	22.31 2.10; 1.95	45.9 3.11; 2.91	113.5 5,89; 5.37
Cmp 1	**2**	**3**	**4**	**5, 8**	**6, 7**	**9**
^{13}C ^{1}H	68.5 Appears as a triplet in ^{13}C due to exchange of H with D	222.0	38.2 2.47	22.3 2.09; 1.95	46.1 3.06; 2.87	57.3 3.88
Cmp 5	**2**	**3**	**4**	**5, 8**	**6, 7**	**9**
^{13}C ^{1}H	98.9	170.2	34.5 2.69	22.3 2.09; 1.94	57.6 3.35 59.3 3.28; 3.63	50.6 4.29
Cmp 4	**2**	**3**	**4**	**5**	**6**	**9**
	64.5 Not seen in 1D carbon but observed in HMBC	225.0	40.42 2.56	26.4 2.14; 1.99		63.0 4.195; 3.93

Table 3. ^{1}H and ^{13}C NMR chemical shifts of MQ and derivatives at pH 4 and (25°C) at a concentration of 42 mg/mL MQ. Two isomers are seen for 4 and 6 but only one is reported in the table.

MQ	**2**	**3**	**4**	**5, 8**	**6, 7**	**9**
^{13}C ^{1}H	150.2	208.8	40.3 2,62	24.1 2.08; 1.93	47.7 3.10; 2.92	115.3 5,89; 5.37
Cmp 1	**2**	**3**	**4**	**5, 8**	**6, 7**	**9**
^{13}C ^{1}H	70.3 Appears as a triplet in ^{13}C due to exchange of H with D	223.7	40.0 2.47	24.1 2.08; 1.95	47.9 3.06; 2.87	59.2 3.88
Cmp 5	**2**	**3**	**4**	**5, 8**	**6, 7**	**9**
^{13}C ^{1}H	101.0	171.8	36.3 2.68	23.9 2.07; 1.94	59.3 3.34 61.0 3.25; 3.61	52.1 4.20
Cmp 6	**2**	**3**	**4**	**5, 8**	**6, 7**	**9**
	65.0 Appears as a triplet in ^{13}C due to exchange of H with D	94.9	32.7 2.3	19.5 2.04; 2.17	49.1 3.36; 4.01	60.6 3.75; 3.67
Cmp 4	**2**	**3**	**4**	**5, 8**	**6, 7**	**9**
	64.3 Appears as a triplet in ^{13}C due to exchange of H with D	225.2	40.7 2.55	26.8 2.14; 1.99		63.3 4.18; 3.90

Table 4. ^{1}H and ^{13}C NMR chemical shifts of MQ and derivatives at pH 4 (5°C) at a concentration of 1.5 mg/mL MQ. Two isomers are seen for 6 but only one is reported in the table.

Cmp 1	**2**	**3**	**4**	**5, 8**	**6, 7**	**9**
^{13}C ^{1}H	69.7 Appears as a triplet in ^{13}C due to exchange of H with D	206.21	37.2 2.67	19.31 and 20.16 2.19; 2.09 and 2.21; 2.03	48.0 3.50; 3.35 42.0 3.56; 3.34	56.34 3.97; 3.90
Cmp 3	**2**	**3**	**4**	**5, 8**	**6, 7**	**9**
^{13}C ^{1}H	68.57 Appears as a triplet in ^{13}C due to exchange of H with D	92.61	32.8 2.07	18.6 1.82; 2.02 18.5 1.94; 1.77	47.1 3.29; 3.19 39.6 3.04; 3.23	54.68 3.87; 3.71
Cmp 6	**2**	**3**	**4**	**5, 8**	**6, 7**	**9**
^{13}C ^{1}H	64.9 Not seen in 1D carbon but observed in HMBC	92.3	32.07 2.17	19.05 19.15 2.07 2.04 1.89	59.8 3.63; 3.54 48.6 3.90; 3.26	54.3 3.80; 3.74

5. LCMS results

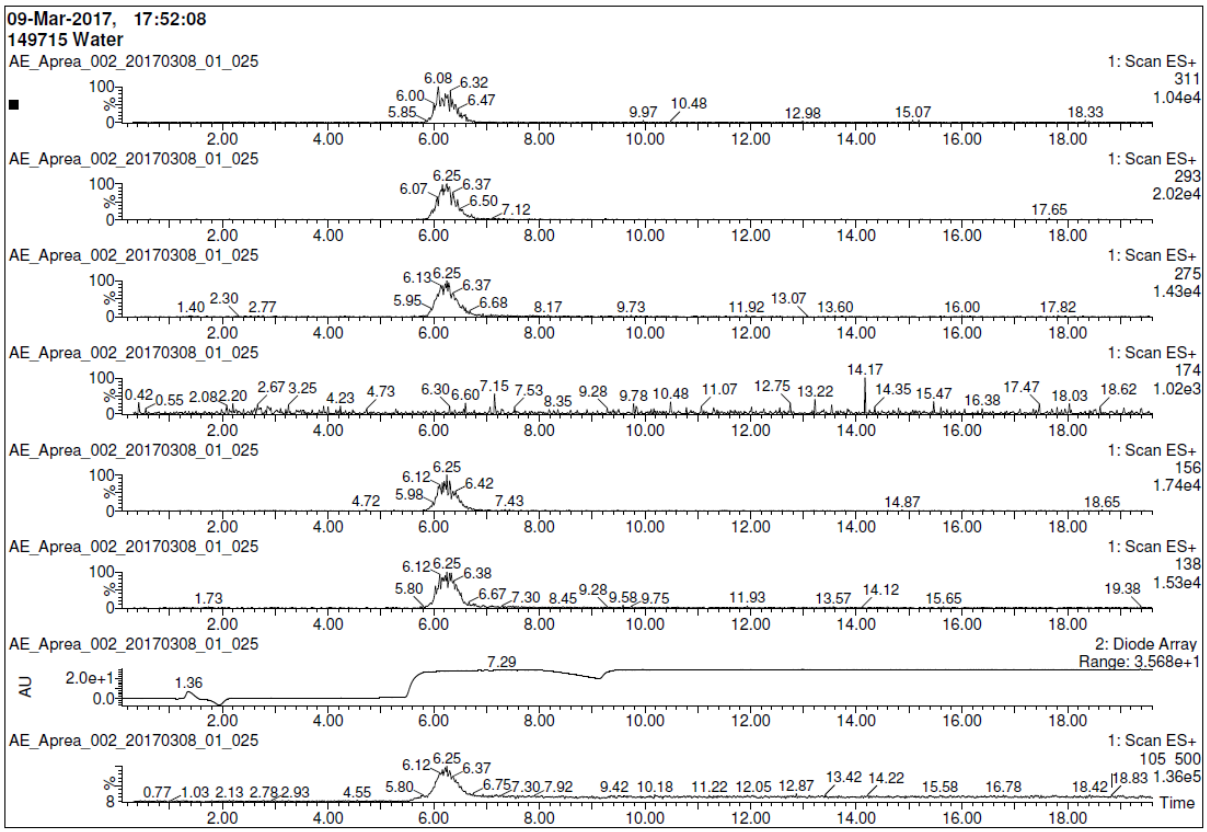


Figure 2. LCMS using pH 4 buffer recorded directly after dissolution of compound 6 in water.

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Print of window 80: MS Spectrum
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Sample Name : mq_precip
=====================================================================
Acq. Operator   : ATE                          Seq. Line :   1
Acq. Instrument : Instrument 1                  Location : Vial 92
Injection Date  : 2018-05-02 03:41:46               Inj :   1
                                               Inj Volume : Inj prog
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Last changed    : 2018-05-02 03:38:36  by ATE
                  (modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\STANDARD\ST1097A3.M
Last changed    : 2019-04-10 10:45:46  by ATE
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                  nm A: 0.1% TFA (pH 2), B: MeCN
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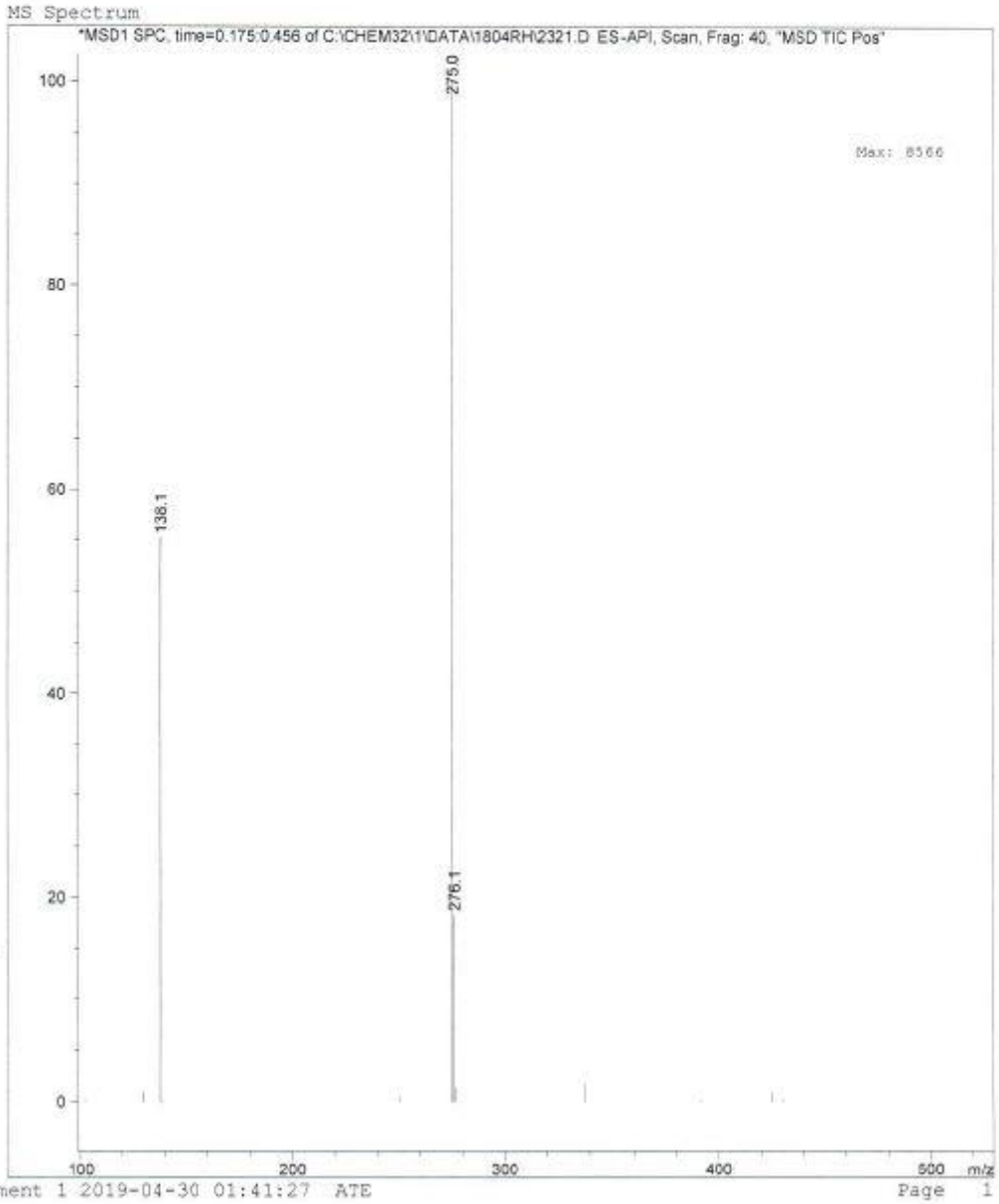


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Figure 3. LCMS of compound 5 using pH 9 buffer.

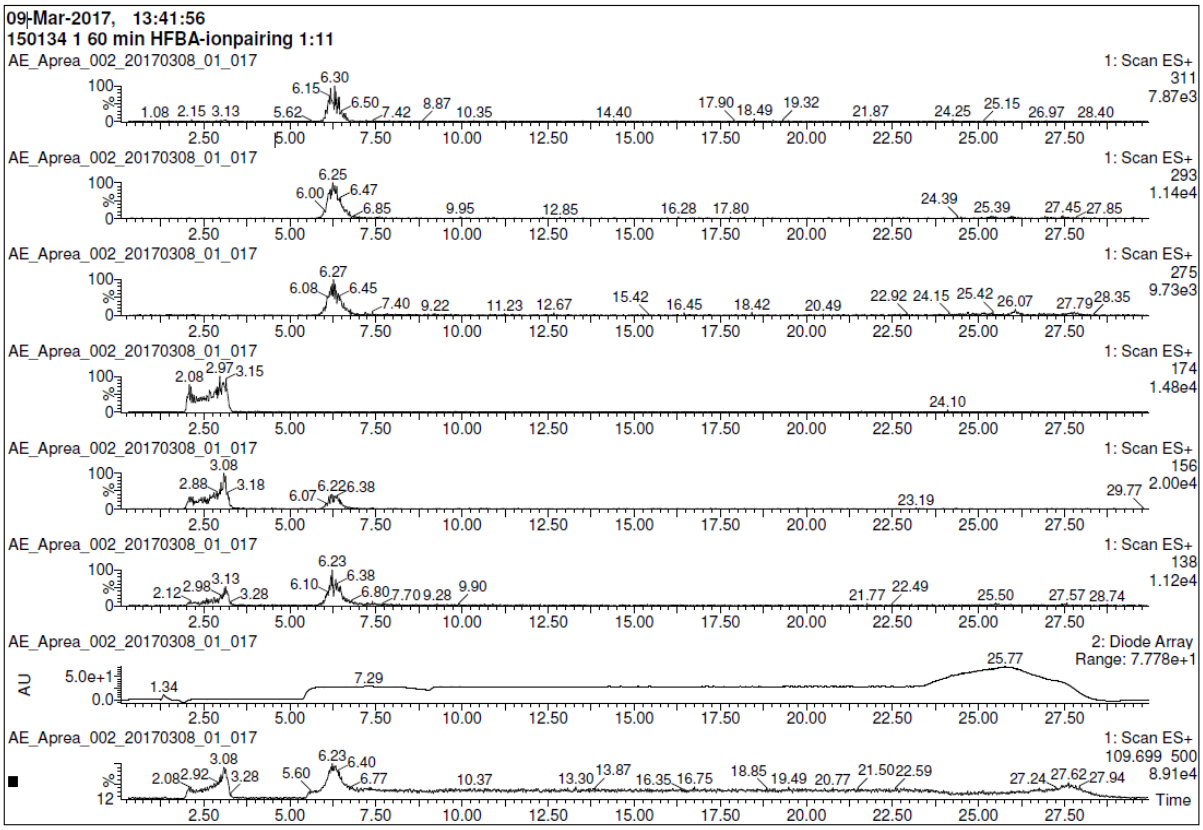

Figure 4. LCMS using pH 4 buffer recorded 60 min. after dissolution of MQ dissolved in pH 4 buffer.