Article

Development of Validated Stability-Indicating Method for Determination of Vortioxetine in Bulk and Pharmaceutical Formulation by Hplc-Dad, Stress Degradation Kinetics Studies and Detection of Degradation Products by LC-ESI-QTOF-MS

Karol Wróblewski[1,2,3*], Małgorzata Szultka-Młyńska[4], Daria Janiszewska[4], Anna Petruczynik[5], Bogusław Buszewski[4,6]

[1]Department of Experimental and Clinical Pharmacology, University of Rzeszów, Kopisto 2a, 35-959 Rzeszow, Poland; karolw222@wp.pl

[2]Laboratory for Innovative Research in Pharmacology, University of Rzeszów, Kopisto 2a, 35-959 Rzeszow, Poland

[3]Interdisciplinary Center for Preclinical and Clinical Research, University of Rzeszów, Werynia 2A, 36-100 Kolbuszowa, Poland

[4]Department of Environmental Chemistry and Bioanalytics, Nicolaus Copernicus University, Faculty of Chemistry Gagarina 7, 87-100 Torun, Poland; szultka.malgorzata@wp.pl, janiszewska_daria@doktorant.umk.pl

[5]Department of Inorganic Chemistry, Medical University of Lublin, Chodźki 4a, 20-093 Lublin, Poland; annapetruczynik@poczta.onet.pl

[6]Centre for Modern Interdisciplinary Technologies, Nicolaus Copernicus University, Wileńska 4, 87-100 Torun, Poland; bbusz@chem.umk.pl

* Correspondence: karolw222@wp.pl

Supplementary Material

Table S1. Lazar toxicity predictions (https://lazar.in-silico.ch/predict).

Figure S1. Q-TOF MS/MS spectra of VOR and its degradation product DP1-DP7.

Figure S2. Fragmentation pattern of the [M+H]+ ions of VOR and its degradation product DP1-DP7.

Table S1. Lazar toxicity predictions (https://lazar.in-silico.ch/predict).

Compound		*Acute toxicity (Daphnia magna)*	*Carcinogenicity (Mouse)*	*Carcinogenicity (Rat)*	*Carcinogenicity (Rodents)*	*Mutagenicity (Salmonella typhimurium)*
DP1	*Type*	regression	classification	classification	classification	classification
	Prediction	14.8 mg/L	non-carcinogenic	non-carcinogenic	non-carcinogenic	non-mutagenic
	95% Prediction interval	1.56 -142 mg/L	NA	NA	NA	NA

Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

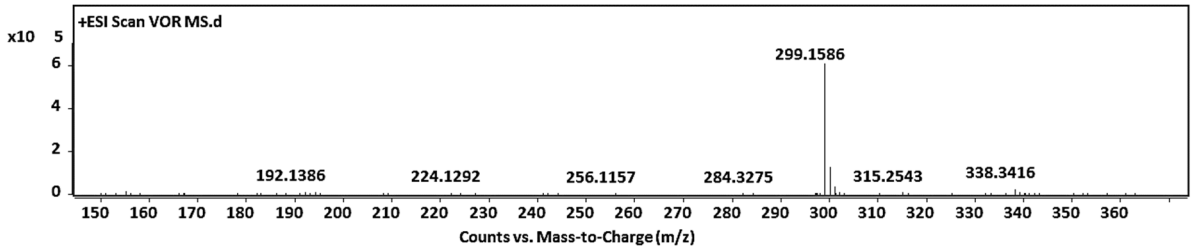
	Probability	NA	non-carcinogenic: 0.207 carcinogenic: 0.066	carcinogenic: 0.13 non-carcinogenic: 0.143	carcinogenic: 0.06 non-carcinogenic: 0.213	mutagenic: 0.255 non-mutagenic: 0.29
DP2	*Type*	regression	classification	classification	classification	classification
	Prediction	2.36 mg/L	non-carcinogenic	**carcinogenic**	non-carcinogenic	mutagenic; attention: measured activity: non-mutagenic
	95% Prediction interval	0.111 - 50.5 mg/L	NA	NA	NA	NA
	Probability	NA	non-carcinogenic: 0.244 carcinogenic: 0.0892	carcinogenic: 0.193 non-carcinogenic: 0.141	carcinogenic: 0.0989 non-carcinogenic: 0.136	mutagenic: 0.254 non-mutagenic: 0.191
DP3	*Type*	regression	classification	classification	classification	classification
	Prediction	17.5 mg/L	non-carcinogenic	**carcinogenic**	**carcinogenic**	**mutagenic**
	95% Prediction interval	0.172 – 1780.0 mg/L	NA	NA	NA	NA
	Probability	NA	non-carcinogenic: 0.153 carcinogenic: 0.141	carcinogenic: 0.191 non-carcinogenic: 0.103	carcinogenic: 0.246 non-carcinogenic: 0.0481	mutagenic: 0.184 non-mutagenic: 0.15
DP4	*Type*	regression	classification	classification	classification	classification
	Prediction	47.1 mg/L	non-carcinogenic	**carcinogenic**	**carcinogenic**	non-mutagenic

	95% Prediction interval	3.12 – 713.0 mg/L	NA	NA	NA	NA
	Probability	NA	non-carcinogenic: 0.263 carcinogenic: 0.0	carcinogenic: 0.222 non-carcinogenic: 0.0415	carcinogenic: 0.263 non-carcinogenic: 0.0	mutagenic: 0.0709 non-mutagenic: 0.215
DP5	*Type*	regression	classification	classification	classification	classification
	Prediction	17.3 mg/L	non-carcinogenic	**carcinogenic**	**carcinogenic**	**mutagenic**
	95% Prediction interval	0.24 -1240.0 mg/L	NA	NA	NA	NA
	Probability	NA	non-carcinogenic: 0.17 carcinogenic: 0.124	carcinogenic: 0.21 non-carcinogenic: 0.084	carcinogenic: 0.253 non-carcinogenic: 0.0413	mutagenic: 0.171 non-mutagenic: 0.162
DP6	*Type*	regression	Warnings: cannot create prediction: only one similar compound for threshold 0.2 in the training set (Threshold: 0.2).	classification	Warnings: cannot create prediction: only one similar compound for threshold 0.2 in the training set (Threshold: 0.2).	classification
	Prediction	34.6 mg/L		non-carcinogenic		non-mutagenic
	95% Prediction interval	3.26 -367.0 mg/L		NA		NA
	Probability	NA		carcinogenic: 0.0829 non-carcinogenic: 0.167		mutagenic: 0.132 non-mutagenic: 0.168

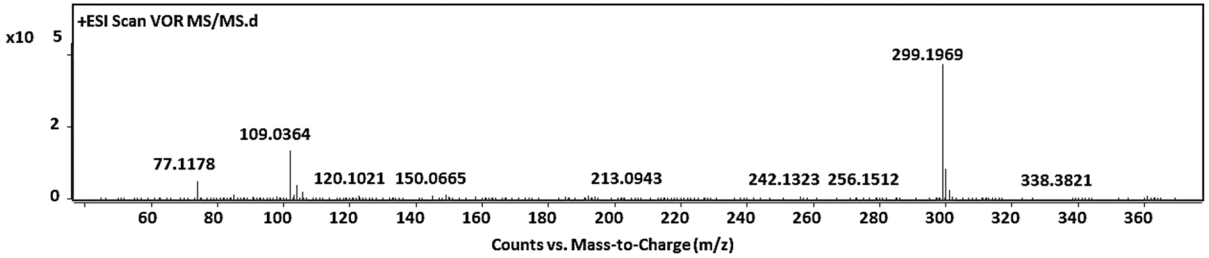
DP7	*Type*	regression	classification	classification	classification	classification
	Prediction	45.4 mg/L	non-carcinogenic	**carcinogenic**	**carcinogenic**	non-mutagenic
	95% Prediction interval	Warnings: Insufficient number of neighbors (2) for regression model, using weighted average of similar substances (no prediction interval available).	NA	NA	NA	NA
	Probability	NA	non-carcinogenic: 0.217 carcinogenic: 0.0611	carcinogenic: 0.167 non-carcinogenic: 0.11	carcinogenic: 0.151 non-carcinogenic: 0.127	mutagenic: 0.0628 non-mutagenic: 0.253

NA-not applicable

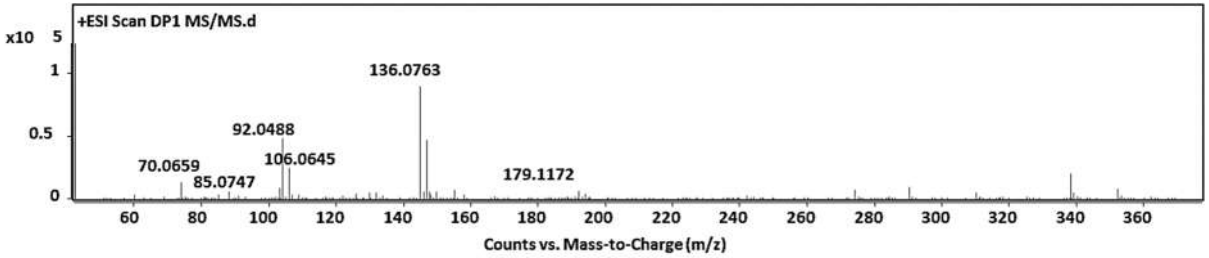
(a)



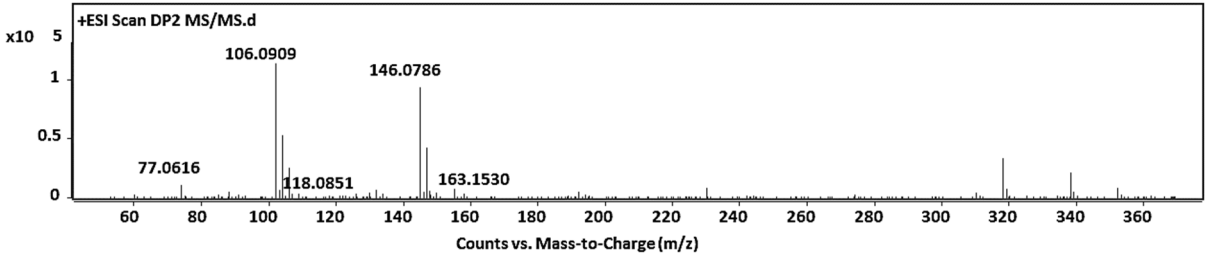
(b)


+ESI Scan VOR MS/MS.d
x10 5
2
0
299.1969
109.0364
77.1178
120.1021
150.0665
213.0943
242.1323
256.1512
338.3821
60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360
Counts vs. Mass-to-Charge (m/z)

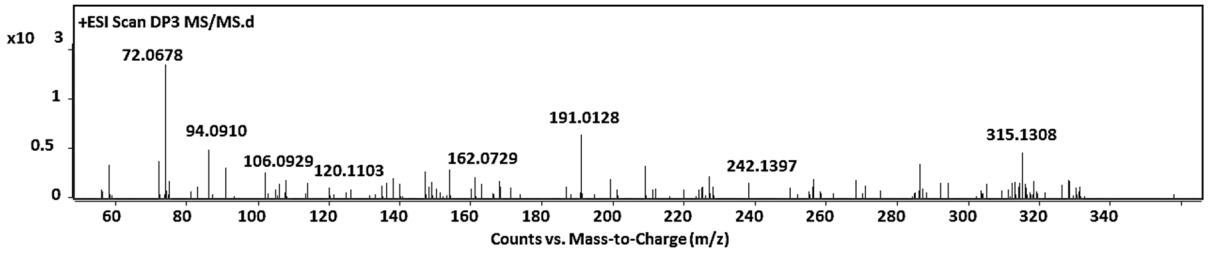
(c)


+ESI Scan DP1 MS/MS.d
x10 5
1
0.5
0
136.0763
92.0488
106.0645
70.0659
85.0747
179.1172
60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360
Counts vs. Mass-to-Charge (m/z)

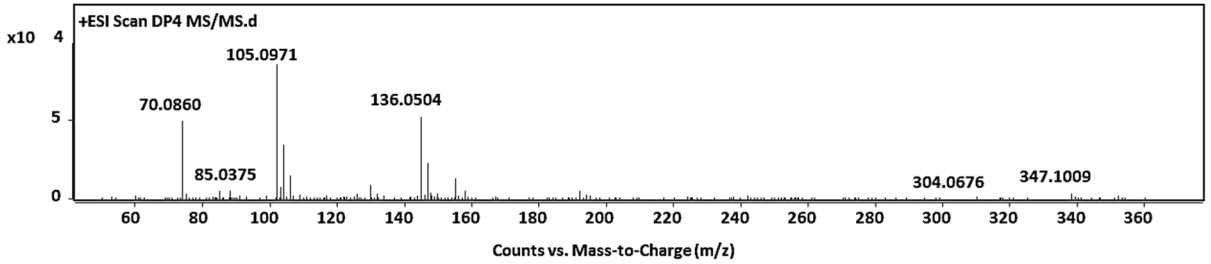
(d)


+ESI Scan DP2 MS/MS.d
x10 5
1
0.5
0
106.0909
146.0786
77.0616
118.0851
163.1530
60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360
Counts vs. Mass-to-Charge (m/z)

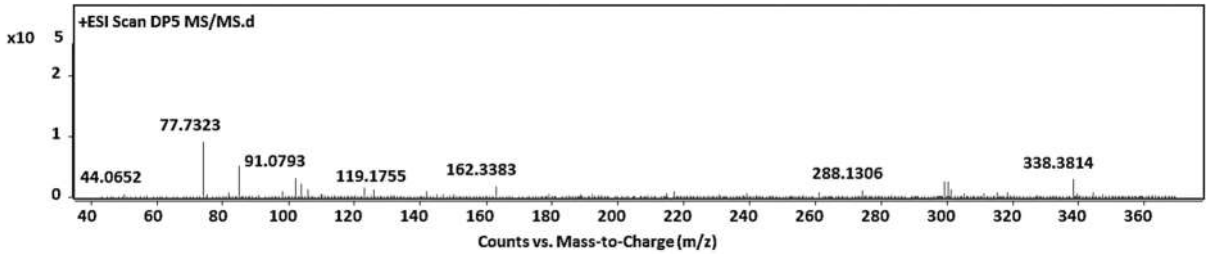
(e)


+ESI Scan DP3 MS/MS.d
x10 3
1
0.5
0
72.0678
94.0910
106.0929
120.1103
162.0729
191.0128
242.1397
315.1308
60 80 100 120 140 160 180 200 220 240 260 280 300 320 340
Counts vs. Mass-to-Charge (m/z)

(f)


+ESI Scan DP4 MS/MS.d
x10 4
5
0
105.0971
70.0860
136.0504
85.0375
304.0676
347.1009
60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360
Counts vs. Mass-to-Charge (m/z)

(g)


+ESI Scan DP5 MS/MS.d
x10 5
2
1
0
77.7323
44.0652
91.0793
119.1755
162.3383
288.1306
338.3814
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360
Counts vs. Mass-to-Charge (m/z)

(h)

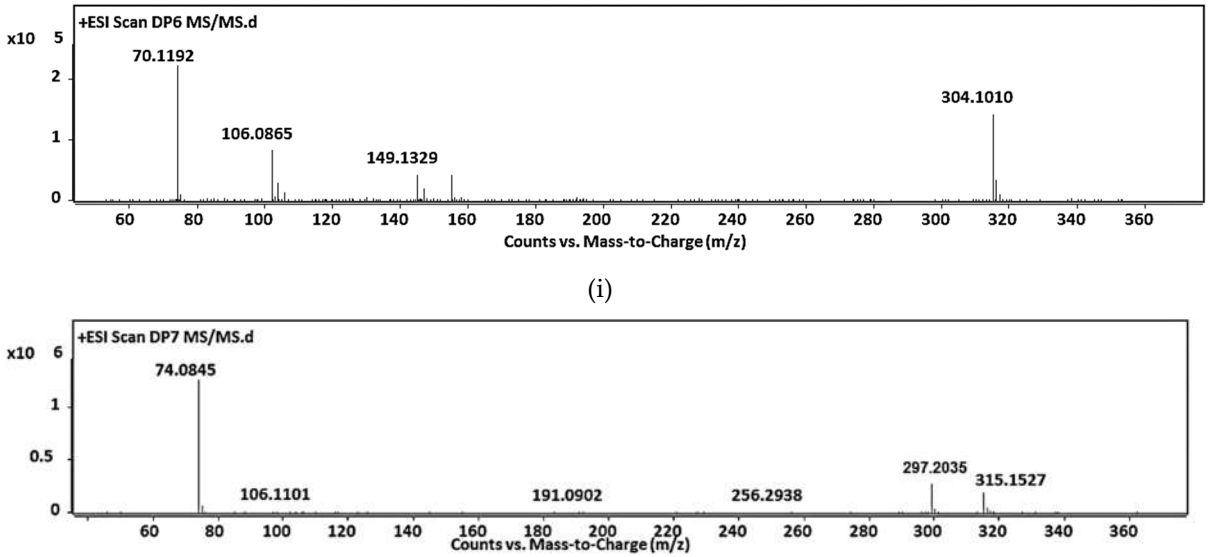
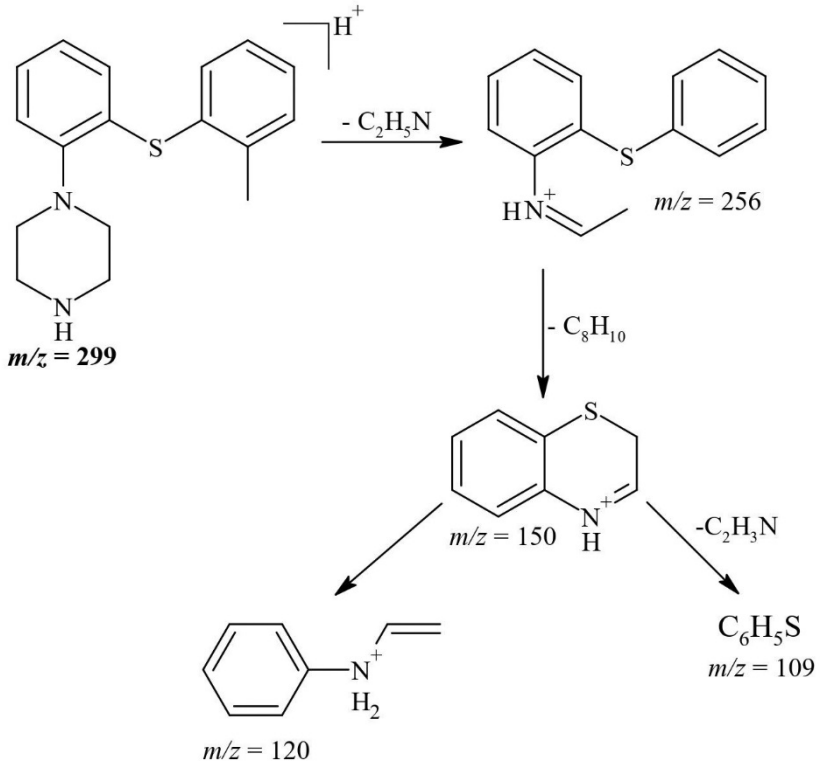
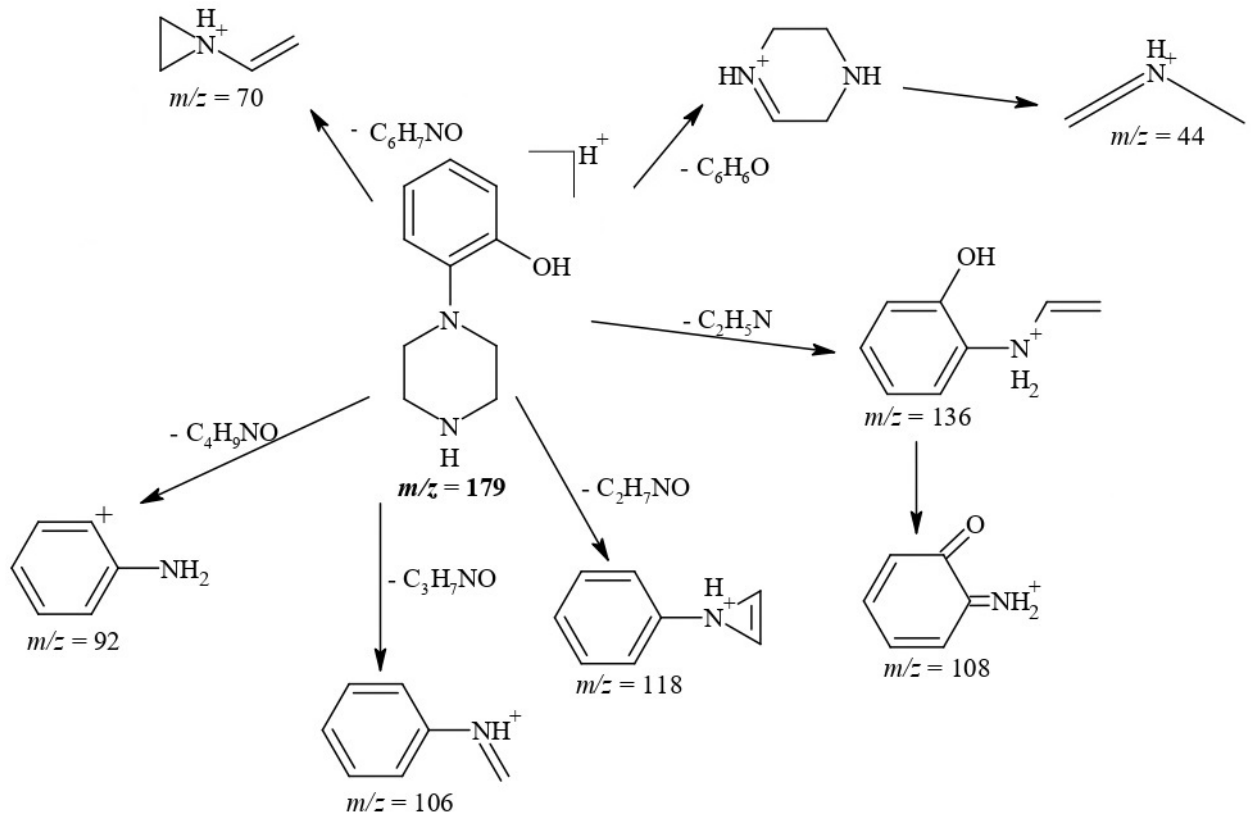


Figure S1. Q-TOF MS/MS spectra of VOR and its degradation product DP1-DP7.

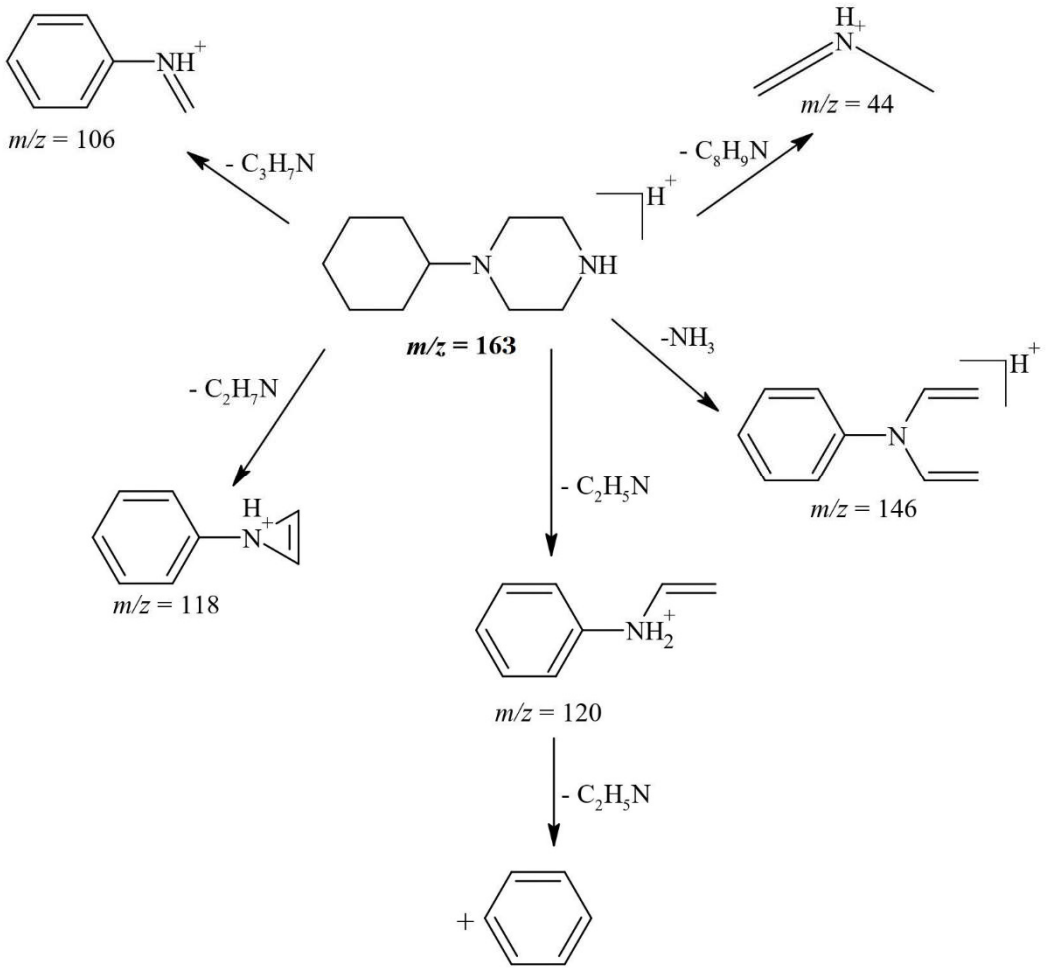
(a)



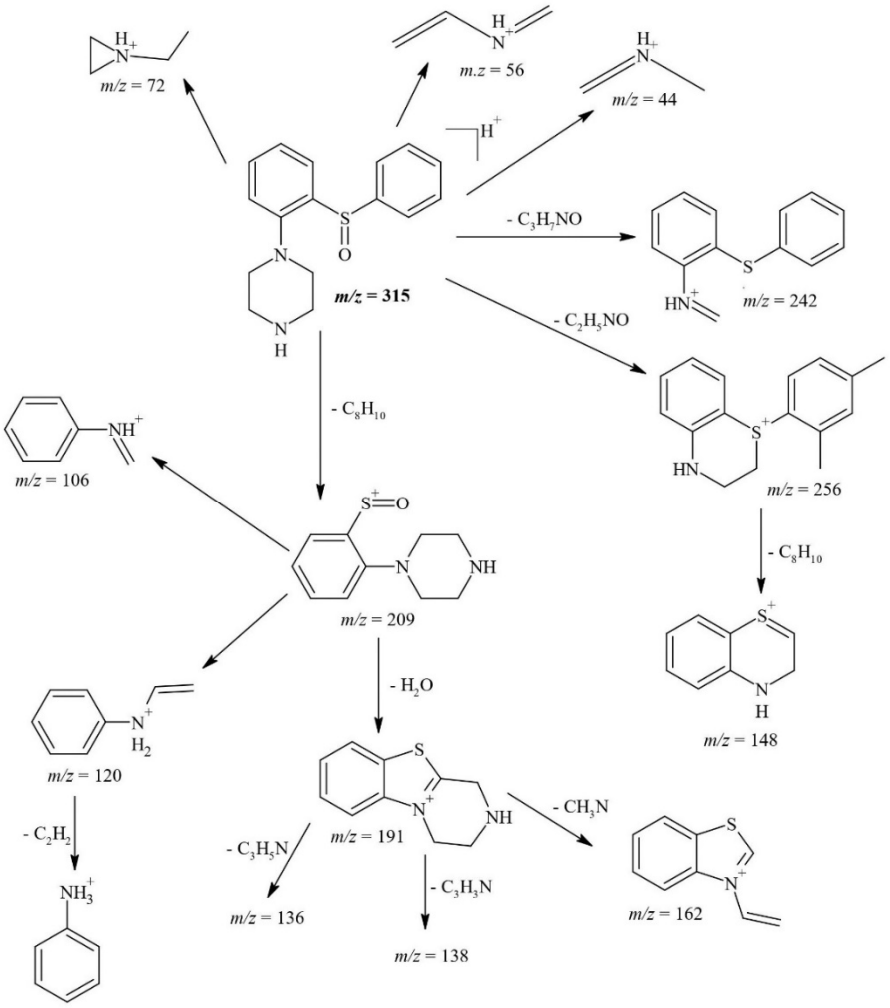
(b)


m/z = 70
- C6H7NO
H+
HN
NH
- C6H6O
m/z = 44
OH
N
N
H
m/z = 179
- C2H5N
OH
N+
H2
m/z = 136
- C4H9NO
NH2
m/z = 92
- C3H7NO
- C2H7NO
H
N+
m/z = 118
O
NH2+
m/z = 108
NH+
m/z = 106

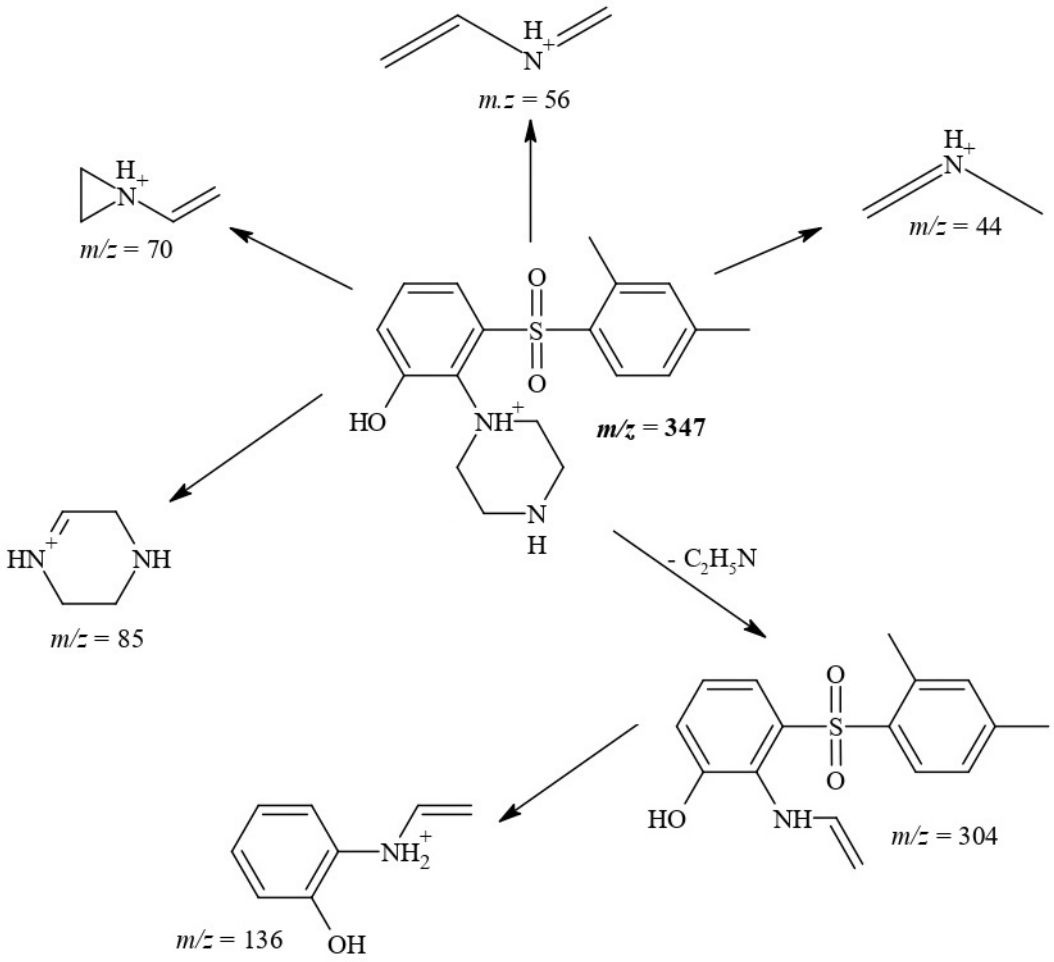
(c)


m/z = 106
- C3H7N
m/z = 44
- C8H9N
m/z = 163
-NH3
m/z = 146
- C2H7N
m/z = 118
- C2H5N
m/z = 120
- C2H5N

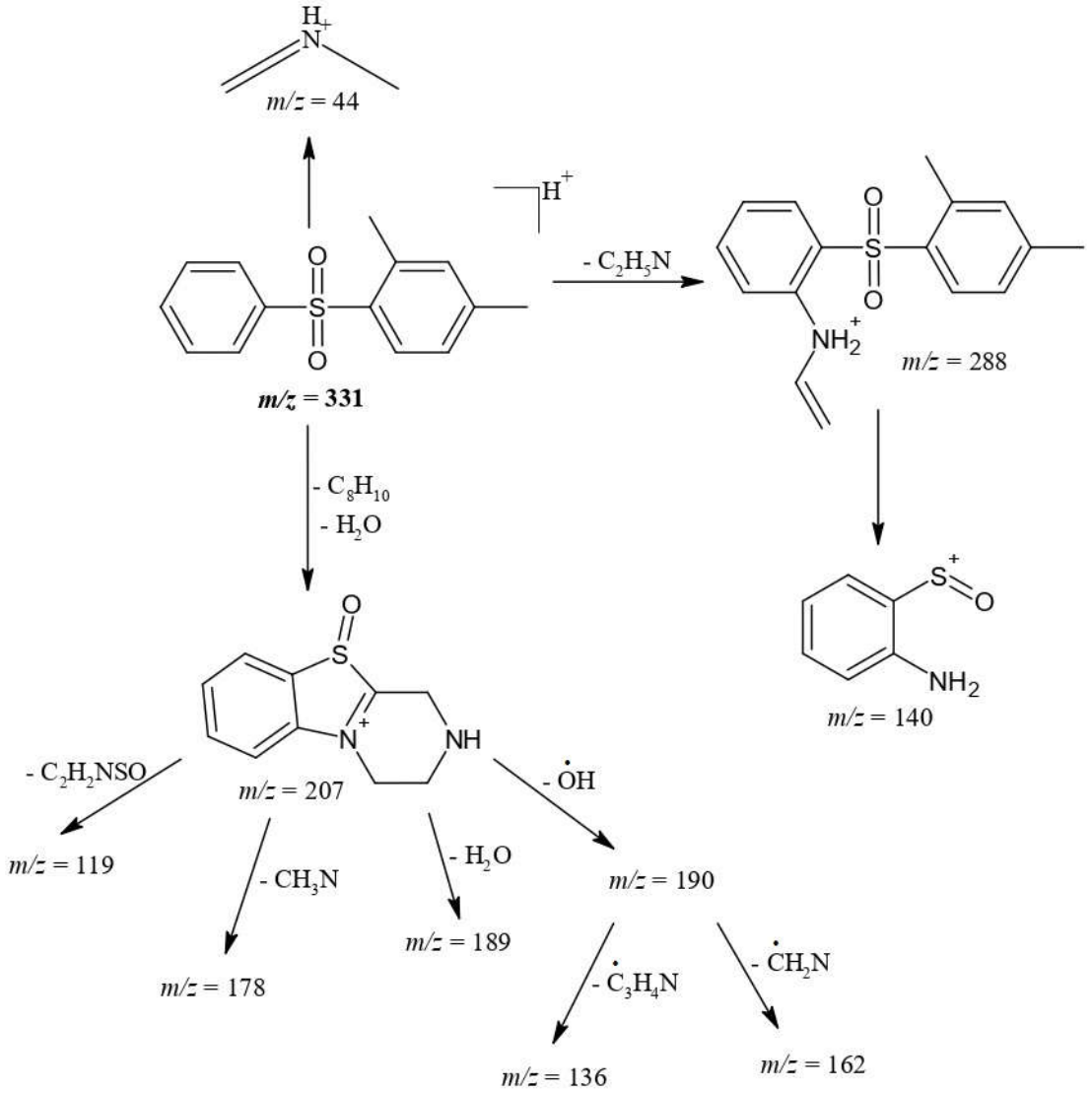
(d)


m/z = 72
m.z = 56
m/z = 44
- C3H7NO
m/z = 315
m/z = 242
- C2H5NO
- C8H10
m/z = 106
m/z = 256
- C8H10
m/z = 209
m/z = 148
- H2O
m/z = 120
m/z = 191
- CH3N
- C2H2
- C3H5N
- C3H3N
m/z = 136
m/z = 138
m/z = 162

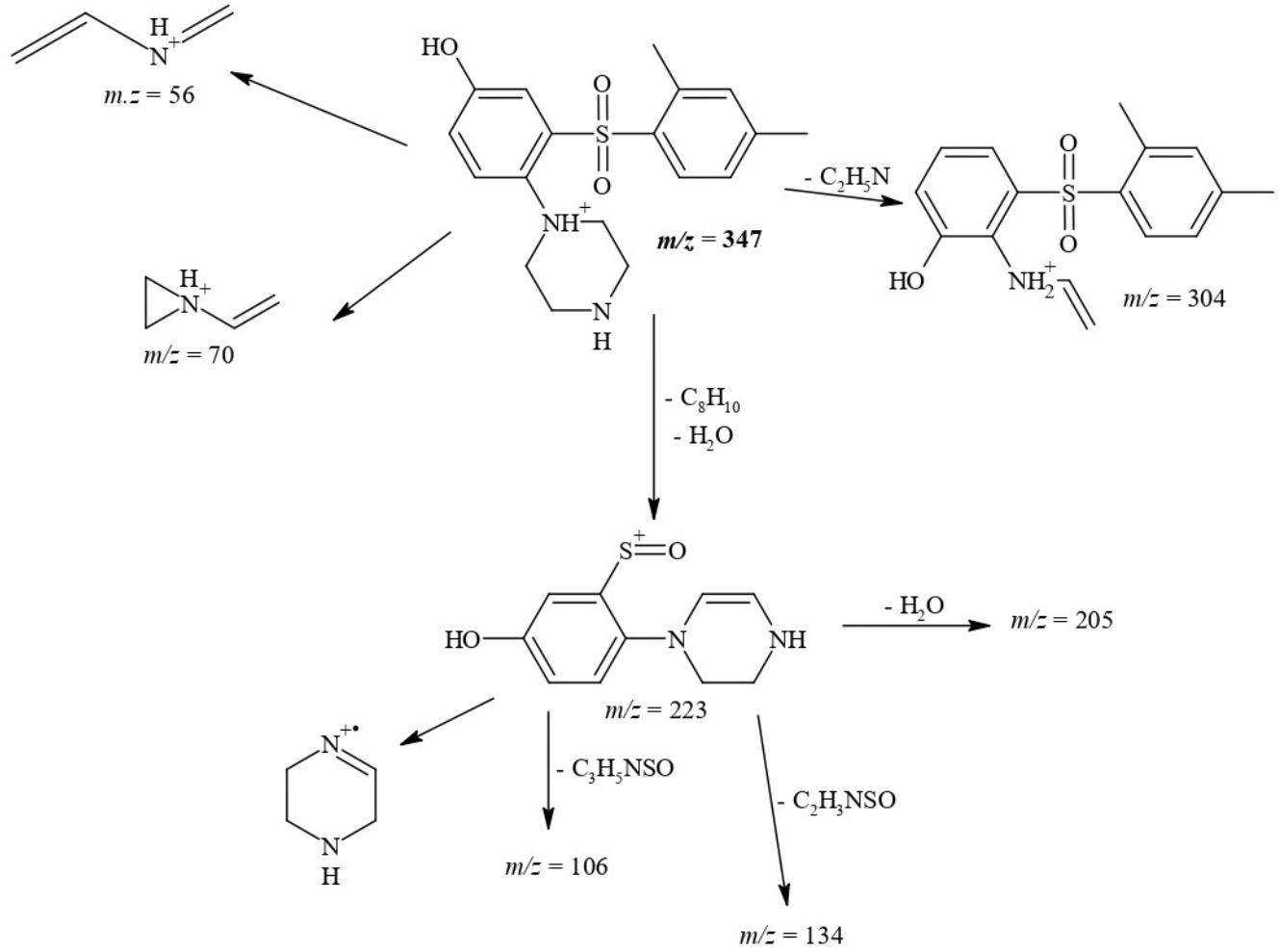
(e)



(f)


m/z = 44
m/z = 331
- C2H5N
m/z = 288
- C8H10
- H2O
m/z = 140
m/z = 207
- C2H2NSO
m/z = 119
- CH3N
m/z = 178
- H2O
m/z = 189
- OH
m/z = 190
- C3H4N
m/z = 136
- CH2N
m/z = 162

(g)


m.z = 56
m/z = 70
m/z = 347
- C2H5N
m/z = 304
- C8H10
- H2O
m/z = 223
- H2O
m/z = 205
- C3H5NSO
m/z = 106
- C2H3NSO
m/z = 134

(h)

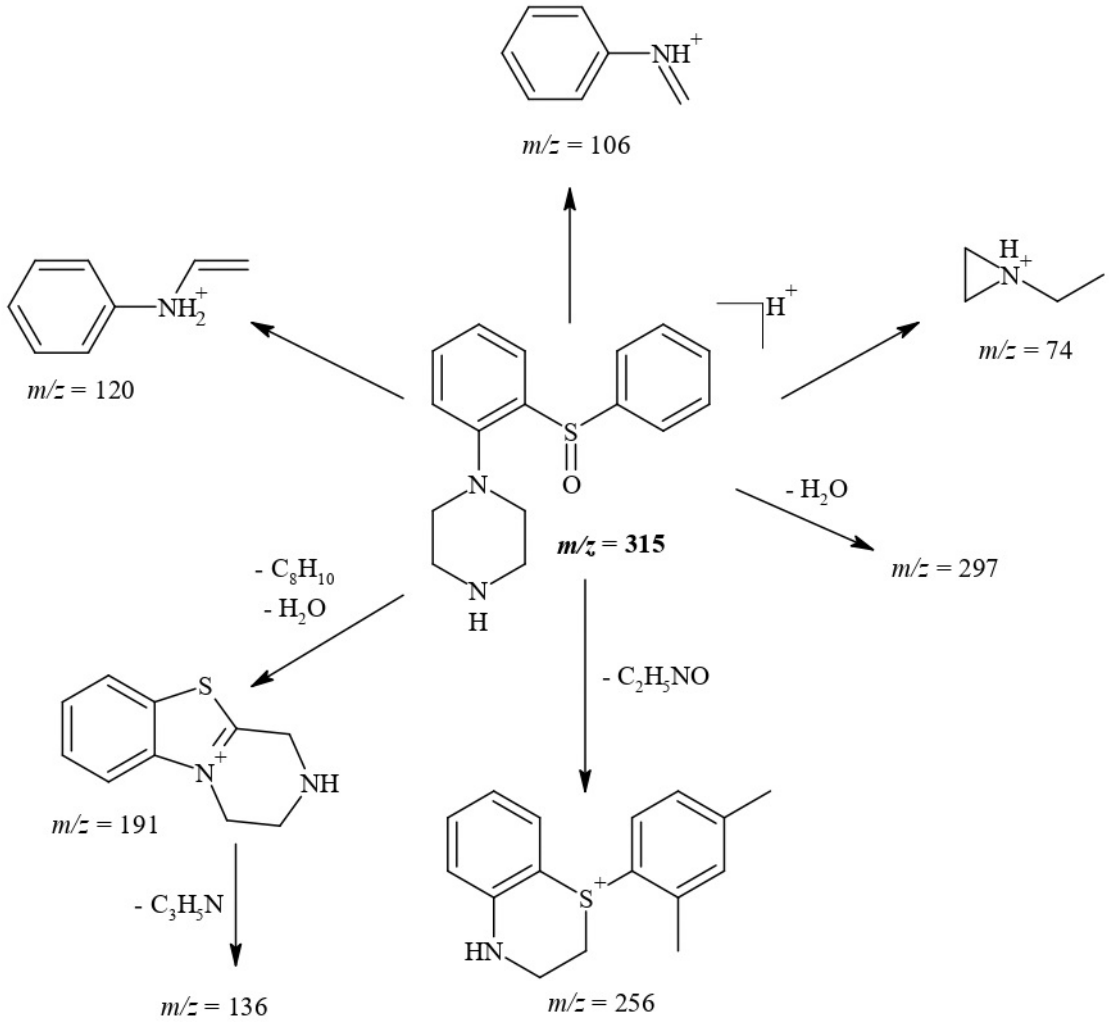


Figure S2. Fragmentation pattern of the [M+H]+ ions of VOR and its degradation product DP1-DP7.