Supporting Information

An Algorithm for Thorough Background Subtraction of High Resolution LC/MS Data: Application to Obtain Clean Product Ion Spectra from Non-selective Collision-induced Dissociation Experiments

Haiying Zhang1, Mary Grubb1, Wei Wu2, Jonathan Josephs1, William G. Humphreys1

1Biotransformation, 2Protein Therapeutics Development,
Bristol-Myers Squibb Research and Development, Pennington, NJ 08534

Address correspondence to:
Haiying Zhang, Ph.D.
Bristol-Myers Squibb Research and Development
311 Pennington-Rocky Hill Road, Pennington, NJ 08534
E-mail: haiying.zhang@bms.com. Tel: (609) 818-3537.

Contents:

Page S2. **Table S1.** Summary of ions detected in background-subtracted spectra for buspirone metabolites in human plasma obtained with QToF in MS^E experiments.

Page S3. **Table S2.** Summary of ions detected in background-subtracted spectra for clozapine metabolites in human plasma obtained with LTQ Orbitrap in source CID experiments.

Table S1. Summary of ions detected in background-subtracted spectra for buspirone metabolites in human plasma obtained with QToF in MS^E experiments. The structures proposed are based on knowledge of published literature.[29-32] Bold ions indicate some of the characteristic fragments of the mono-oxygenated metabolites.

Metabolite ID	RT	MH+ Ions	Ions in background-subtracted CID spectrum	Proposed structure
M1	16.4	418.2344	418.2360, 281.1917, 238.1534, 180.1119, 166.1184, 138.0943	dihydroxy buspirone
M2	17.1	402.2404	402.2402, 359.2066, 307.2019, 281.1888, 238.1529, 220.1508, 219.1722, 196.1103, **184.1191, 178.1428**, 166.1086, 165.1359, 150.1278, 148.1127, 122.1028	hydroxy buspirone
M3	17.9	402.2413	402.2413, 307.2004, 281.1888, 238.1533, 220.1462, **219.1726**, 196.1148, 192.1546, **178.1424**, 150.1279, 148.1122, 122.1030, 98.1345, 98.0972	hydroxy buspirone
M4	18.5	418.2348	418.2321, 400.2252, 293.1873, 281.1925, 267.1743, 238.1529, 148.1122, 122.1044	dihydroxy buspirone
M5	18.9	418.2341	418.2333, 308.2318, 281.1829, 238.1503, 166.1201, 138.0941	dihydroxy buspirone
M6	19.2	418.2340	418.2343, 293.1866, 281.1874, 265.1967, 238.1531, 222.1584, 196.1107, 154.0850, 152.1310, 122.1013, 98.1020	dihydroxy buspirone
M7	19.5	418.2350	356.2412, 338.2316, 287.1887, 217.1582, 194.1715, 192.1599, 164.1658, 162.1120, 152.1317, 148.1131, 124.1439. 122.1031, 95.1234	dihydroxyl buspirone
M8	19.9	402.2416	402.2404, 359.1988, 281.1891, 246.1789, 238.1537, 220.1454, **219.1688**, 192.1541, 168.1248, 165.1320, 150.1280, 148.1123, **139.1052**, 122.1031, 98.1344	6'-oxa-buspirone
M9	20.5	418.2349	418.2336, 281.1901, 238.1528, 210.1628, 196.1067, 184.1164, 168.1209, 166.1192, 164.1031, 139.1067, 138.0940, 121.0988, 98.1342, 95.1231	5,6'-dihydroxy buspirone
M10	21.4	402.2391	402.2424, 359.2019, 307.2018, 281.1885, 238.1522, 220.1479, 219.1711, **210.1612**, 196.1124, 192.1531, 184.1148, 168.1229, 166.1079, 150.1281, 148.1129, **139.1034**, 122.1026, 98.1352, 95.0990	6'-hydroxy buspirone
M11	22.4	360.2328	360.2308, 265.1964, 222.1593, 180.1216, 168.1233, 152.1327, 122.1032	N,N-desethyl buspirone
M12	22.7	418.2338	418.2338, 400.2231, 374.2086, 306.1949, 293.1871, 281.1873, 267.1751, 238.1529, 210.1629, 196.1140, 177.1317, 175.1276, 168.1244, 162.1126, 148.1120, 139.1023, 122.1039, 120.0873, 108.0912, 95.1252	6',N-dihydroxy buspirone
M13	23.2	384.2318	384.2315, 222.1596, 217.1581, 180.1229, 162.1118, 152.1308, 148.1097, 147.0955, 146.0989, 134.1025, 122.1038, 120.0892	dehydrogenated buspirone
M14	23.5	402.2396	402.2400, 265.1960, 235.1595, 222.1604, 180.1212, 168.1236, **166.1197**, 164.1054, 152.1325, **138.0937**, 109.1359, 98.1336, 95.1235	5-hydroxy buspirone
buspirone	24.4	386.2479	386.2465, 343.2076, 291.2071, 265.1959, 222.1601, 219.1681, 180.1200, 168.1228, 152.1323, 150.1283, 148.1123, 140.1335, 122.1028, 109.1362, 98.1347, 95.1241, 95.0994	parent
M15	25.5	402.2418	402.2417, 384.2320, 358.2201, 290.2027, 277.1954, 265.1960, **251.1825**, 222.1605, 180.1215, **177.1325**, 168.1230, 162.1123, 152.1323, 148.1128, 140.1345, 122.1031, 120.0880, 108.0916, 98.1350, 95.1244	buspirone N-oxide

Table S2. Summary of ions detected in background-subtracted spectra for clozapine metabolites in human plasma obtained with LTQ Orbitrap in source CID experiments. The structures proposed are based on knowledge of published literature.[33,34]

Metabolite ID	RT	MH+ Ions	Ions in background-subtracted CID spectrum	Proposed structure
M1	14.0	343.1320, 172.0686*	312.0892, 286.0736, 243.0315, 208.0629	hydroxy clozapine
M2	17.5	632.2056, 316.6059*	632.2057, 503.1590, 359.1095, 302.0523, 259.0092, 224.0386	clozapine + GSH - 2H
M3	18.6	632.2040, 316.6058*	686.1249#, 632.2051, 615.1799, 575.1451, 503.1629, 446.1038, 359.1097, 328.0671, 302.0508, 282.0794, 259.0082, 223.0324	clozapine + GSH - 2H
M4	19.6	313.1213, 157.0640*	313.1202, 296.0936, 270.0779, 253.0516, 244.0622, 227.0359, 209.0699, 192.0673, 164.0486	norclozapine
clozapine	21.2	327.1376, 164.0726*	327.1370, 296.0950, 270.0791, 253.0533, 227.0371, 209.0710, 192.0682, 164.0495	parent
M5	23.1	685.2588**, 343.1323, 172.0697*	343.1318, 325.1216, 296.0946, 282.0785, 270.0782, 268.0629, 256.0631, 243.0555, 227.0367, 215.0374, 205.0757, 192.0679, 181.0757, 164.0491	clozapine N-oxide

* doubly charged species
** dimer species
# $[M-H+Fe]^+$ ion