Identification of novel pyrimidine ring cleavage metabolites of Buspirone via Spectral Similarity correlation score

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1:Introduction

Buspirone is an anxiolytic psychoactive drug of the azapirone chemical class, and is primarily used to treat generalized anxiety disorder (GAD). Buspirone is a common model compound for xenobiotic metabolism. Here we describe the application of a Spectral Similarity Correlation methodology (‘S Score’) to the analysis of data generated from a human microsomal incubation of Buspirone. Non-targeted data-dependent MSMS data was collected from a human microsomal incubate using a Shimadzu LCMS IT-TOF system. The ‘S scores’ were derived from the correlation of fragment ion and neutral loss species of unknown chromatographic peaks to those of Buspirone. This approach was unbiased and success was not influenced any user interaction.

2:Method and Materials

Buspirone (30µM) was incubated with human liver microsomes and NADPH at 37ºC for 45 minutes. 5µL of incubate was injected using a LCMS IT-TOF system (Shimadzu, Kyoto, Japan) onto a 2.1 x 50 mm C18 BEH 1.7um; A - water + 0.1 % formic acid; B - acetonitrile + 0.1 % formic acid. Flow 0.6ml/min @ 65ºC. Gradient: 2%B 0min, 40%B 8min, 90%B 9-10min, 2%B 10.5min. The Spectral Similarity scoring was performed using MetID Solutions software (v1.2, Shimadzu, Kyoto, Japan). Two data files were used in the analysis. The t0 sample was used to supply reference fragment ion and neutral loss data for Buspirone, and a t45 sample was treated as the unknown.

3:Results

The Spectral Simplicity scores generated by Met ID Solutions software were derived from a comparison of unknown MS^2 spectra against an experimental MS^2 spectrum of Buspirone. Figure 1 is an example MS^2 spectrum of Buspirone and figure 2 shows the common bond cleavages and products.

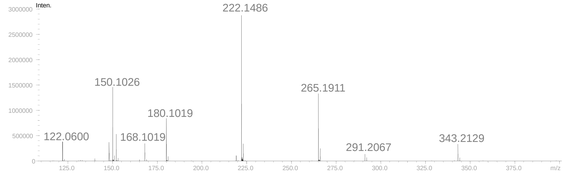
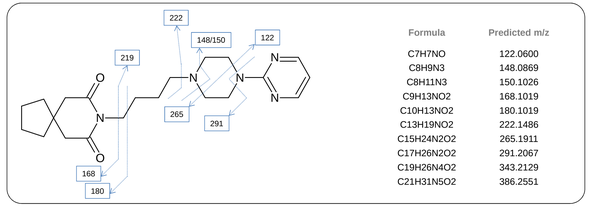


Figure 1. Buspirone MS^2 Data (low energy fragmentation; theoretical mass values shown).



Formula	Predicted m/z
C7H7NO	122.0600
C8H9N3	148.0869
C8H11N3	150.1026
C9H13NO2	168.1019
C10H13NO2	180.1019
C13H19NO2	222.1486
C15H24N2O2	265.1911
C17H26N2O2	291.2067
C19H26N4O2	343.2129
C21H31N5O2	386.2551

Figure 2. Buspirone fragmentation.

3-1: Identification of novel metabolites by the software

Spectral similarity is a process that uses MS^n data to identify expected and unexpected metabolites of a pharmaceutical drug in an non-targeted analysis. The process inspects the MS^n fragments and neutral loses produced by the parent drug and then looks for molecular ions in the data that demonstrate a statistical similarity with the parent. This similarity suggests common neutral loss or fragment ions, which in turn suggests at least partial structural similarity.

The benefit of this process is that it requires no prior knowledge of the cleavages that may occur in the parent drug and therefore no explicit fragment or neutral loses need to be specified for the analysis. This means that even unlikely cleavages will be used when identifying drug metabolites. In addition, when the structure of the parent drug is known, identifying common fragments or neutral loses will help identify the modified area in an expected metabolite, therefore assisting in the process of structural elucidation.

For unexpected metabolites the common fragments and neutral loses will identify part of the metabolites empirical formula and therefore since the unknown is only a sub-section of the metabolite’s complete empirical formula, the mass being investigated is reduced resulting in considerably fewer possible empirical formulae generated using mass alone.

Initial data analysis by MetID Solutions software was performed without a search for ‘expected’ metabolites and 47 peaks were returned with positive S Score’s indicating likely metabolites. Re-analysis by the software with a list of expected metabolites containing all previously described Phase 1 metabolites reduced the number of candidate ‘unknown’ peaks to 12.

Manual scrutiny of the data indicated that some of the unknown peaks could be attributed to combinations of dealkylation and oxidative metabolic reactions but two peaks (m/z 438.2707 & 350.2531 m/z, 2.31 & 2.45 min) could not be assigned to previously described metabolites of Buspirone.

Further analysis of the MS^n data supported the hypothesis that m/z 350.2531 at 2.45 min was a product of cleavage of the pyrimidine ring in Buspirone. The m/z 438.2707 ion at 2.31 min was tentatively identified as an intermediate in the metabolic cleavage of the pyrimidine ring as MS^2 data showed the m/z 350 ion as a fragment and MS^3 data of the m/z 350 ion at 2.31 min was identical to the m/z 350 ion at 2.45 min.

3-2: Proposed metabolite structure's

Figure 4 shows the MS^2 spectrum for the 350m/z metabolite (2.45 min). Assignment of the pyrimidine ring cleavage was based on the presence of the 308 and 333 fragment ions and the fact that the 222, 265 and 291 ions show parity with the fragmentation of Buspirone.

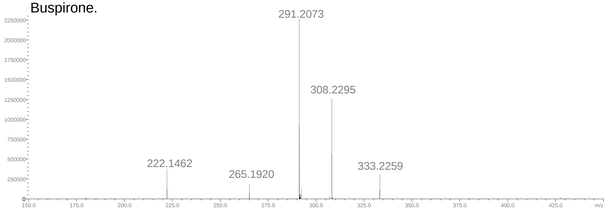
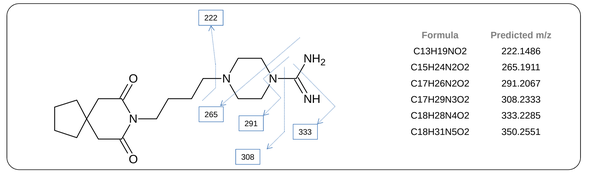


Figure 3. MS^2 of 350m/z metabolite (2.45 min).



Formula	Predicted m/z
C13H19NO2	222.1486
C15H24N2O2	265.1911
C17H26N2O2	291.2067
C17H29N3O2	308.2333
C18H28N4O2	333.2285
C18H31N5O2	350.2551

Figure 4. Proposed fragmentation of 350m/z metabolite (2.45 min).

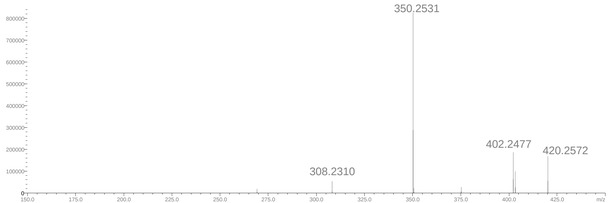


Figure 5. MS^2 of 438m/z metabolite (2.31 min).

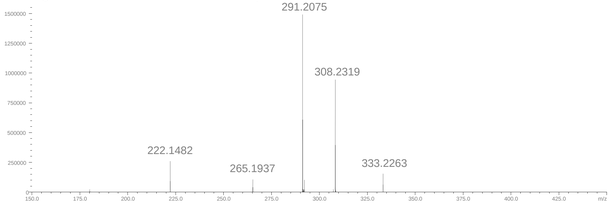
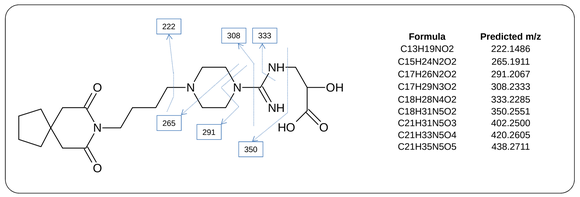


Figure 6. MS^3 of the 350m/z fragment ion from the 438m/z metabolite (2.31 min). Note the similarity with figure 3.



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C13H19NO2	222.1486
C15H24N2O2	265.1911
C17H26N2O2	291.2067
C17H29N3O2	308.2333
C18H28N4O2	333.2285
C18H31N5O2	350.2551
C21H31N5O3	402.2500
C21H33N5O4	420.2605
C21H35N5O5	438.2711

Figure 7. Proposed structure and fragmentation for 438m/z metabolite (2.31 min).

3-3: Supporting literature evidence for pyrimidine ring cleavage

Analysis of the literature showed that pyrimidine ring cleavage has been previously described for a structurally similar drug[1]. The metabolic cleavage of the pyrimidine ring results in a loss of C_3 relative to Buspirone. In the absence of prior knowledge of this metabolic step it is unlikely that a metabolism scientist would search for metabolites containing the C_3 mass difference.

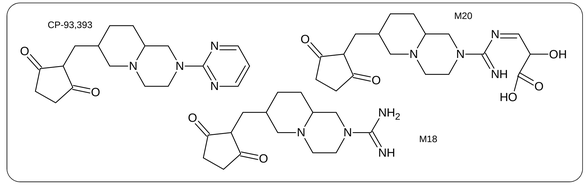

Figure 8. Pfizer drug CP-93,393 and pyrimidine ring metabolites reported by Prakesh[1]

4:Conclusions

An automated non-targeted approach using a scoring system based on the similarities between the fragment ion and neutral loss masses of parent drug and unknown metabolites was successful in identifying novel pyrimidine ring cleavage metabolites of Buspirone.

5:References

1) Prakash & Cui. Drug Metabolism & Disposition 1997 v25 (12) p1395-1406
2) Zhu *et al*. Drug Metabolism & Disposition 2005 v33 (4) p500-507