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# Fragmentation Outcome Modelling: Prototype software for prediction of CID fragment ions for small molecule structures

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# Fragmentation Outcome Modelling: Prototype software for prediction of CID fragment ions for small molecule structures

## 1. Introduction

Fragmentation Outcome Modelling is a new approach to the prediction of CID fragment ions and has been successfully applied to a series of pharmaceutical structures generating complete fragmentation including rearrangement mechanisms. This expert system uses a series of prediction models that encapsulate the outcome of fragmentation mechanisms that could result in the breaking of one, two or three bonds. In this paper, midazolam and its 1-hydroxy and 4-hydroxy metabolites were used as test compounds to highlight the advantages of fragmentation outcome modelling compared to other commercially available fragmentation prediction software products.

## 2. Method and Materials

MS/MS spectra of Midazolam and its metabolites were generated on high mass accuracy systems including Shimadzu LCMS-IT-TOF, Waters Synapt G2 and Thermo LTQ-Orbitrap. Fragmentation Outcome Modelling software was developed by Shimadzu Corporation, Japan. The input file for each structure was supplied as an MDL V2000 MOL file. Comparative analyses were performed using three commercially available software products which are described as 'Product A', 'Product B' and 'Product C'. Each software application was optimised to give the greatest number of predicted fragment ions. Fragment Outcome Modelling software functions equally for positive and negative ion data and can also be applied to either accurate or nominal mass data.

## 3. Results

MS/MS of Midazolam, 1-OH Midazolam and 4-OH Midazolam gave 8, 11 and 10 fragment ions respectively. The fragmentation of each molecule was unique with each fragment ion being a unique mass. For comparative purposes the total number of fragment ions successfully predicted for each software package is expressed as a percentage of the total number of experimental fragment ions.

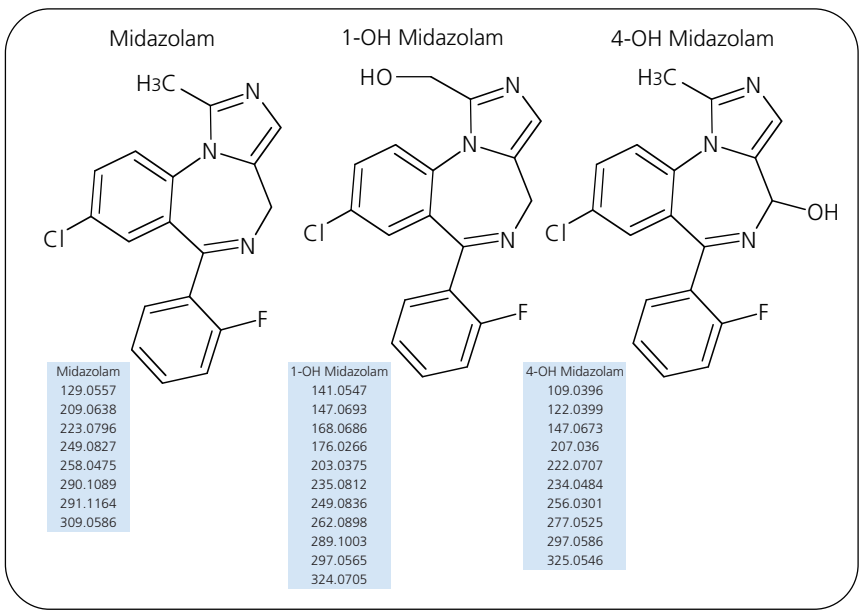


Fig. 1 Structures of Midazolam, 1-OH Midazolam and 4-OH Midazolam with corresponding experimental mass fragmentation data

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## 3-1. Midazolam

Fragmentation Outcome Modelling prototype software examples are shown as yellow shadowed representations of the parent molecule image. Atoms covered by the shadow image are present in the proposed fragment species. The left hand table gives data relating to the fragments with theoretical *m/z*, formula, electron type (odd or even), number of steps taken to generate the fragment and the number of unique images that can be created to represent the selected fragment species. The right hand table gives data on the precursor species with the total number of precursors and a line of example data for the current precursor with precursor formula, neutral loss formula and precursor theoretical *m/z*. Clicking on cells in the table allows the user to iterate through data for each precursor.

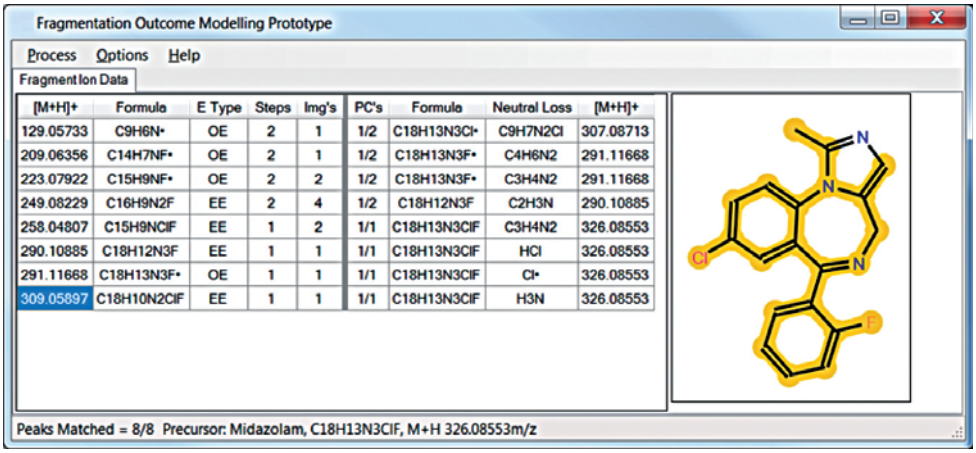



Fig. 2 Protonated fragment formed by loss of NH3 from the imidazole ring

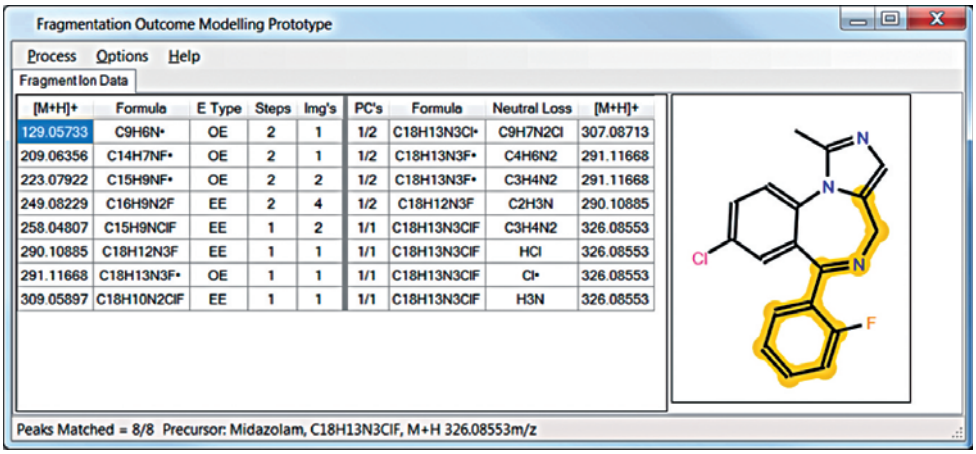
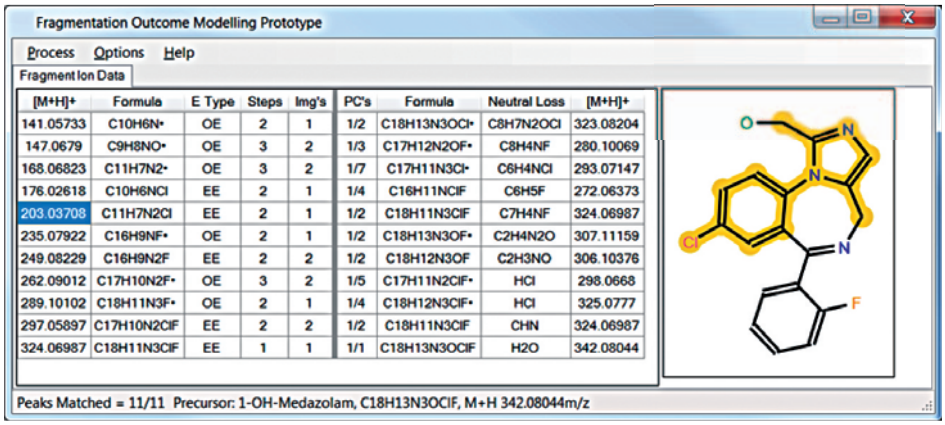



Fig. 3 Protonated radical fragment formed by ring opening and cleavage across imidazole and diazepine rings combined with the loss of a fluorine radical

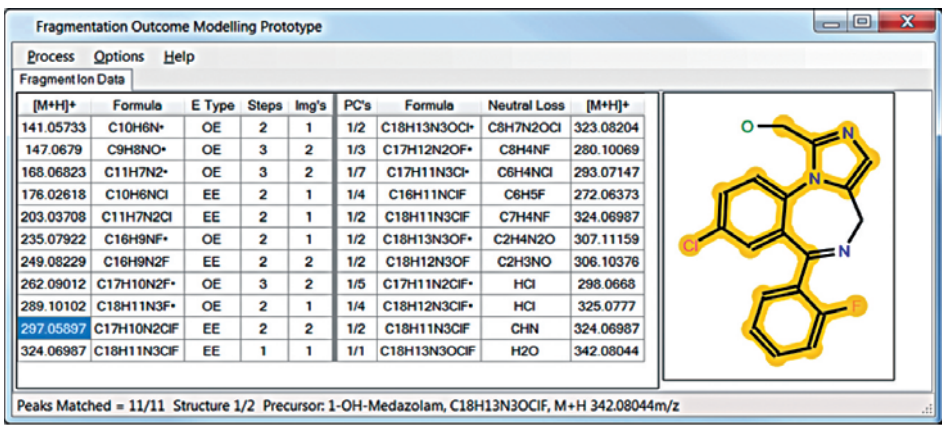
# Fragmentation Outcome Modelling: Prototype software for prediction of CID fragment ions for small molecule structures

## 3-2. 1-OH Midazolam




| [M+H]+ | Formula | E Type | Steps | Img's | PC's | Formula | Neutral Loss | [M+H]+ |
|---|---|---|---|---|---|---|---|---|
| 141.05733 | C10H6N• | OE | 2 | 1 | 1/2 | C18H13N3OCl• | C8H7N2OCl | 323.08204 |
| 147.0679 | C9H8NO• | OE | 3 | 2 | 1/3 | C17H12N2OF• | C8H4NF | 280.10069 |
| 168.06823 | C11H7N2• | OE | 3 | 2 | 1/7 | C17H11N3Cl• | C6H4NCl | 293.07147 |
| 176.02618 | C10H6NCl | EE | 2 | 1 | 1/4 | C16H11NClF | C6H5F | 272.06373 |
| 203.03708 | C11H7N2Cl | EE | 2 | 1 | 1/2 | C18H11N3ClF | C7H4NF | 324.06987 |
| 235.07922 | C16H9NF• | OE | 2 | 1 | 1/2 | C18H13N3OF• | C2H4N2O | 307.11159 |
| 249.08229 | C16H9N2F | EE | 2 | 2 | 1/2 | C18H12N3OF | C2H3NO | 306.10376 |
| 262.09012 | C17H10N2F• | OE | 3 | 2 | 1/5 | C17H11N2ClF• | HCl | 298.0668 |
| 289.10102 | C18H11N3F• | OE | 2 | 1 | 1/4 | C18H12N3ClF• | HCl | 325.0777 |
| 297.05897 | C17H10N2ClF | EE | 2 | 2 | 1/2 | C18H11N3ClF | ClHN | 324.06987 |
| 324.06987 | C18H11N3ClF | EE | 1 | 1 | 1/1 | C18H13N3OClF | H2O | 342.08044 |

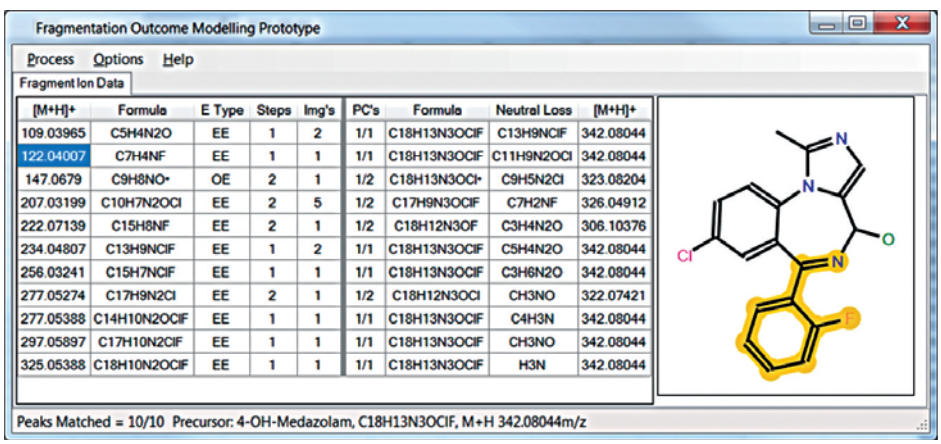
Fig. 4 Protonated fragment formed by ring opening and cleavage across the diazepine ring combined with the loss of water from the hydroxyl group




| [M+H]+ | Formula | E Type | Steps | Img's | PC's | Formula | Neutral Loss | [M+H]+ |
|---|---|---|---|---|---|---|---|---|
| 141.05733 | C10H6N• | OE | 2 | 1 | 1/2 | C18H13N3OCl• | C8H7N2OCl | 323.08204 |
| 147.0679 | C9H8NO• | OE | 3 | 2 | 1/3 | C17H12N2OF• | C8H4NF | 280.10069 |
| 168.06823 | C11H7N2• | OE | 3 | 2 | 1/7 | C17H11N3Cl• | C6H4NCl | 293.07147 |
| 176.02618 | C10H6NCl | EE | 2 | 1 | 1/4 | C16H11NClF | C6H5F | 272.06373 |
| 203.03708 | C11H7N2Cl | EE | 2 | 1 | 1/2 | C18H11N3ClF | C7H4NF | 324.06987 |
| 235.07922 | C16H9NF• | OE | 2 | 1 | 1/2 | C18H13N3OF• | C2H4N2O | 307.11159 |
| 249.08229 | C16H9N2F | EE | 2 | 2 | 1/2 | C18H12N3OF | C2H3NO | 306.10376 |
| 262.09012 | C17H10N2F• | OE | 3 | 2 | 1/5 | C17H11N2ClF• | HCl | 298.0668 |
| 289.10102 | C18H11N3F• | OE | 2 | 1 | 1/4 | C18H12N3ClF• | HCl | 325.0777 |
| 297.05897 | C17H10N2ClF | EE | 2 | 2 | 1/2 | C18H11N3ClF | ClHN | 324.06987 |
| 324.06987 | C18H11N3ClF | EE | 1 | 1 | 1/1 | C18H13N3OClF | H2O | 342.08044 |

Fig. 5 Protonated fragment formed by ring opening and loss of cyanide from the diazepine ring combined with the loss of water from the hydroxyl group

## 3-3. 4-OH Midazolam




| [M+H]+ | Formula | E Type | Steps | Img's | PC's | Formula | Neutral Loss | [M+H]+ |
|---|---|---|---|---|---|---|---|---|
| 109.03965 | C5H4N2O | EE | 1 | 2 | 1/1 | C18H13N3OClF | C13H9NClF | 342.08044 |
| 122.04007 | C7H4NF | EE | 1 | 1 | 1/1 | C18H13N3OClF | C11H9N2OCl | 342.08044 |
| 147.0679 | C9H8NO• | OE | 2 | 1 | 1/2 | C18H13N3OCl• | C9H5N2Cl | 323.08204 |
| 207.03199 | C10H7N2OCl | EE | 2 | 5 | 1/2 | C17H9N3OClF | C7H2NF | 326.04912 |
| 222.07139 | C15H8NF | EE | 2 | 1 | 1/2 | C18H12N3OF | C3H4N2O | 306.10376 |
| 234.04807 | C13H9NClF | EE | 1 | 2 | 1/1 | C18H13N3OClF | C5H4N2O | 342.08044 |
| 256.03241 | C15H7NClF | EE | 1 | 1 | 1/1 | C18H13N3OClF | C3H6N2O | 342.08044 |
| 277.05274 | C17H9N2Cl | EE | 2 | 1 | 1/2 | C18H12N3OCl | CH3NO | 322.07421 |
| 277.05388 | C14H10N2OClF | EE | 1 | 1 | 1/1 | C18H13N3OClF | C4H3N | 342.08044 |
| 297.05897 | C17H10N2ClF | EE | 1 | 1 | 1/1 | C18H13N3OClF | CH3NO | 342.08044 |
| 325.05388 | C18H10N2OClF | EE | 1 | 1 | 1/1 | C18H13N3OClF | H3N | 342.08044 |

Fig. 6 Protonated fragment formed by ring opening and cleavage across the diazepine ring

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## 3-4. Summary Table

Results are presented in the order of Midazolam, 1-OH Midazolam & 4-OH Midazolam: Product A 75%, 46%, 70%; Product B 25%, 9%, 50%; Product C 63%, 55%, 80%; Fragmentation Outcome Modelling 100%, 100%, 100%.

| | | Fragmentation Outcome Modelling | Product A | Product B | Product C |
|---|---|---|---|---|---|
| Midazolam | | | | | |
| 129.0557 | | √ | | | √ |
| 209.0638 | | √ | √ | | |
| 223.0796 | | √ | | | |
| 249.0827 | | √ | √ | | √ |
| 258.0475 | | √ | √ | √ | √ |
| 290.1089 | | √ | √ | √ | √ |
| 291.1164 | | √ | √ | | √ |
| 309.0586 | | √ | √ | | √ |
| | n | 8 | 6 | 2 | 5 |
| | % | 100 | 75 | 25 | 63 |
| 1-OH Midazolam | | | | | |
| 141.0547 | | √ | | | |
| 147.0693 | | √ | | | |
| 168.0686 | | √ | | | √ |
| 176.0266 | | √ | | | |
| 203.0375 | | √ | √ | | √ |
| 235.0812 | | √ | | | |
| 249.0836 | | √ | √ | | |
| 262.0898 | | √ | | | √ |
| 289.1003 | | √ | √ | | √ |
| 297.0565 | | √ | √ | | √ |
| 324.0705 | | √ | √ | √ | √ |
| | n | 11 | 5 | 1 | 6 |
| | % | 100 | 46 | 9 | 55 |
| 4-OH Midazolam | | | | | |
| 109.0396 | | √ | √ | √ | √ |
| 122.0399 | | √ | √ | √ | √ |
| 147.0673 | | √ | | | |
| 207.036 | | √ | √ | √ | √ |
| 222.0707 | | √ | | | |
| 234.0484 | | √ | √ | √ | √ |
| 256.0301 | | √ | | | √ |
| 277.0525 | | √ | √ | √ | √ |
| 297.0586 | | √ | √ | | √ |
| 325.0546 | | √ | √ | | √ |
| | n | 10 | 7 | 5 | 8 |
| | % | 100 | 70 | 50 | 80 |

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# 4. Conclusions

Fragmentation Outcome Modelling was the only application to successfully predict every fragment ion for each of the three examples given here. Typical processing time for each structure was approximately ten seconds.

There were marked differences between the capabilities of each respective software product tested and in general most packages were less successful with fragment ion predictions for the 1-OH metabolite.

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