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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.

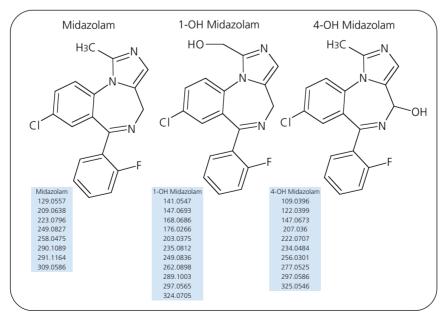


Fig. 1 Structures of Midazolam, 1-OH Midazolam and 4-OH Midazolam with corresponding experimental mass fragmentation data



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.

Fragmention	n Data								
[M+H]+	Formula	E Type	Steps	Img's	PC's	Formula	Neutral Loss	(M+H)+	
129.05733	C9H6N•	OE	2	1	1/2	C18H13N3CI+	C9H7N2CI	307.08713	N
209.06356	C14H7NF•	OE	2	1	1/2	C18H13N3F•	C4H6N2	291.11668	
223.07922	C15H9NF•	OE	2	2	1/2	C18H13N3F•	C3H4N2	291.11668	
249.08229	C16H9N2F	EE	2	4	1/2	C18H12N3F	C2H3N	290.10885	
258.04807	C15H9NCIF	EE	1	2	1/1	C18H13N3CIF	C3H4N2	326.08553	
290.10885	C18H12N3F	EE	1	1	1/1	C18H13N3CIF	HCI	326.08553	
291.11668	C18H13N3F•	OE	1	1	1/1	C18H13N3CIF	CI•	326.08553	
309.05897	C18H10N2CIF	EE	1	1	1/1	C18H13N3CIF	H3N	326.08553	

Fig. 2	Protonated fra	agment formed	by loss	of NH3 fron	n the imidazole ring
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Fragmention	Options Hel								
[M+H]+	Formula	E Type	Steps	Img's	PC's	Formula	Neutral Loss	[M+H]+	
129.05733	C9H6N+	OE	2	1	1/2	C18H13N3CI+	C9H7N2CI	307.08713	
209.06356	C14H7NF•	OE	2	1	1/2	C18H13N3F•	C4H6N2	291.11668	
223.07922	C15H9NF•	OE	2	2	1/2	C18H13N3F•	C3H4N2	291.11668	
249.08229	C16H9N2F	EE	2	4	1/2	C18H12N3F	C2H3N	290.10885	
258.04807	C15H9NCIF	EE	1	2	1/1	C18H13N3CIF	C3H4N2	326.08553	
290.10885	C18H12N3F	EE	1	1	1/1	C18H13N3CIF	HCI	326.08553	
291.11668	C18H13N3F•	OE	1	1	1/1	C18H13N3CIF	CI-	326.08553	
309.05897	C18H10N2CIF	EE	1	1	1/1	C18H13N3CIF	H3N	326.08553	
									↓

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



3-2. 1-OH Midazolam

rocess	Options Hel	lp							
agment lo	n Data								
(M+H]+	Formula	E Type	Steps	Img's	PC's	Formula	Neutral Loss	[M+H]+	
1.05733	C10H6N•	OE	2	1	1/2	C18H13N3OCI+	C8H7N2OCI	323.08204	0-
47.0679	C9H8NO-	OE	3	2	1/3	C17H12N2OF•	C8H4NF	280.10069	
8.06823	C11H7N2-	OE	3	2	1/7	C17H11N3CI-	C6H4NCI	293.07147	
6.02618	C10H6NCI	EE	2	1	1/4	C16H11NCIF	C6H5F	272.06373	
3.03708	C11H7N2CI	EE	2	1	1/2	C18H11N3CIF	C7H4NF	324.06987	
5.07922	C16H9NF•	OE	2	1	1/2	C18H13N3OF+	C2H4N2O	307.11159	
9.08229	C16H9N2F	EE	2	2	1/2	C18H12N3OF	C2H3NO	306.10376	
2.09012	C17H10N2F•	OE	3	2	1/5	C17H11N2CIF•	HCI	298.0668	
9.10102	C18H11N3F•	OE	2	1	1/4	C18H12N3CIF•	HCI	325.0777	F
7.05897	C17H10N2CIF	EE	2	2	1/2	C18H11N3CIF	CHN	324.06987	
4.06987	C18H11N3CIF	EE	1	1	1/1	C18H13N3OCIF	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

Options He	lp							
n Data								
Formula	E Type	Steps	Img's	PC's	Formula	Neutral Loss	[M+H]+	
C10H6N+	OE	2	1	1/2	C18H13N3OCI+	C8H7N2OCI	323.08204	0N
C9H8NO+	OE	3	2	1/3	C17H12N2OF+	C8H4NF	280.10069	
C11H7N2+	OE	3	2	1/7	C17H11N3CI+	C6H4NCI	293.07147	
C10H6NCI	EE	2	1	1/4	C16H11NCIF	C6H5F	272.06373	
C11H7N2CI	EE	2	1	1/2	C18H11N3CIF	C7H4NF	324.06987	
C16H9NF•	OE	2	1	1/2	C18H13N3OF+	C2H4N2O	307.11159	
C16H9N2F	EE	2	2	1/2	C18H12N3OF	C2H3NO	306.10376	
C17H10N2F•	OE	3	2	1/5	C17H11N2CIF•	HCI	298.0668	
C18H11N3F•	OE	2	1	1/4	C18H12N3CIF•	HCI	325.0777	
C17H10N2CIF	EE	2	2	1/2	C18H11N3CIF	CHN	324.06987	
C18H11N3CIF	EE	1	1	1/1	C18H13N3OCIF	H2O	342.08044	
	Data Formula C10H6N• C9H8NO• C11H7N2• C10H6NCI C11H7N2CI C16H9NF• C16H9N2F C17H10N2CIF C18H11N3F• C17H10N2CIF	Data E Type Formula E Type C10H6N* OE C9H8NO* OE C11H7N2* OE C10H6NCI EE C10H6NF OE C10H9NF* OE C16H9NF* OE C16H9N2F EE C17H10N2F* OE C18H11N3F* OE	Data E Type Steps Formula E Type Steps C10H6N+ OE 2 C9H8NO+ OE 3 C11H7N2+ OE 3 C10H6NCI EE 2 C11H7N2CI EE 2 C16H9NF+ OE 2 C16H9NF+ OE 2 C16H9NZF EE 2 C17H1002F+ OE 3 C18H11N3F+ OE 2 C17H10N2CIF EE 2	Formula E Type Steps Img's C10H6N+ OE 2 1 C9H8NO- OE 3 2 C11H7N2- OE 3 2 C11H7N2- OE 3 2 C10H6NCI EE 2 1 C1H7N2CI EE 2 1 C16H9NF- OE 2 1 C16H9N2F EE 2 2 C17H10N2F OE 3 2 C18H11N3F- OE 2 1 C17H10N2CIF EE 2 1	Data E Type Steps Img's PC's C10H6N• OE 2 1 1/2 C9H8NO• OE 3 2 1/3 C11H7N2• OE 3 2 1/7 C10H6NC1 EE 2 1 1/2 C11H7N2C1 EE 2 1 1/2 C16H9NF• OE 2 1 1/2 C16H9N2F EE 2 2 1/2 C17H10N2F• OE 3 2 1/5 C18H11N3F• OE 2 1 1/4 C17H10N2CF EE 2 1 1/4	Data E Type Steps Img's PC's Formula C10H6N+ OE 2 1 1/2 C18H13N3OCI- C9H8NO+ OE 3 2 1/3 C17H12N2OF- C11H7N2+ OE 3 2 1/7 C17H11N3CI- C10H6NCI EE 2 1 1/4 C16H11NCIF C10H6NCI EE 2 1 1/2 C18H11N3CIF C16H9NF+ OE 2 1 1/2 C18H13N3OF- C16H9NFF EE 2 1 1/2 C18H13N3OF- C16H9NFF OE 2 1 1/2 C18H13N3OF- C16H9NFF OE 2 1 1/2 C18H12N3OF C17H10N2CF OE 3 2 1/5 C17H11N2CIF- C18H11N3F+ OE 2 1 1/4 C18H11N3CIF- C17H10N2CIF EE 2 2 1/2 C18H11N3CIF-	Data E Type Steps Img's PC's Formula Neutral Loss C10H6N+ OE 2 1 1/2 C18H13N3OC+ C8H7N2OCI C9H8NO+ OE 3 2 1/3 C17H12N2OF+ C8H4NF C11H7N2+ OE 3 2 1/7 C17H11N3CI+ C6H4NCI C10H6NCI EE 2 1 1/4 C16H11NCIF C6H5F C11H7N2CI EE 2 1 1/2 C18H11N3CIF C6H5F C16H9NF+ OE 2 1 1/2 C18H13N3OF+ C2H4N2O C16H9NF+ OE 2 1 1/2 C18H13N3OF+ C2H4N2O C16H9NF+ OE 2 1 1/2 C18H13N3OF+ C2H4N2O C16H9NF+ OE 2 1/2 C18H12N3OF+ C2H3NO C17H10N2CF+ OE 3 2 1/5 C17H11N2CIF+ HCI C18H11N3F+ OE 2 1	Data E Type Steps Img's PC's Formula Neutral Loss [M+H]+ C10H6N+ OE 2 1 1/2 C18H13N3OCH C8H7N2OCI 323.08204 C9H8NO+ OE 3 2 1/3 C17H12N2OF+ C8H7N2OCI 323.08204 C9H8NO+ OE 3 2 1/3 C17H12N2OF+ C8H4NF 280.10069 C11H7N2- OE 3 2 1/7 C17H11N3CH C6H4NCI 293.07147 C10H6NCI EE 2 1 1/4 C18H11N3CF C6H4NCI 293.07147 C10H6NCI EE 2 1 1/2 C18H11N3CF C7H4NF 324.06987 C16H9NF- OE 2 1 1/2 C18H11N3OF+ C2H3NO 306.10376 C16H9NF- OE 2 1 1/2 C18H12N3OF C2H3NO 306.10376 C16H9NF- OE 3 2 1/5 C17H11N2CIF+ HCI 298.0668

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

Process	Options Help									
ragment lor	Data									
[M+H]+	Formula	E Type	Steps	Img's	PC's	Formula	Neutral Loss	[M+H]+		
09.03965	C5H4N2O	EE	1	2	1/1	C18H13N3OCIF	C13H9NCIF	342.08044		-
22.04007	C7H4NF	EE	1	1	1/1	C18H13N3OCIF	C11H9N2OCI	342.08044		Y
147.0679	C9H8NO-	OE	2	1	1/2	C18H13N3OCI+	C9H5N2CI	323.08204	~	N
07.03199	C10H7N2OCI	EE	2	5	1/2	C17H9N3OCIF	C7H2NF	326.04912		/"
22.07139	C15H8NF	EE	2	1	1/2	C18H12N3OF	C3H4N2O	306.10376		
34.04807	C13H9NCIF	EE	1	2	1/1	C18H13N3OCIF	C5H4N2O	342.08044		
56.03241	C15H7NCIF	EE	1	1	1/1	C18H13N3OCIF	C3H6N2O	342.08044	.	F
77.05274	C17H9N2CI	EE	2	1	1/2	C18H12N3OCI	CH3NO	322.07421	-	4
77.05388	C14H10N2OCIF	EE	1	1	1/1	C18H13N3OCIF	C4H3N	342.08044	F	
97.05897	C17H10N2CIF	EE	1	1	1/1	C18H13N3OCIF	CH3NO	342.08044		
25.05388	C18H10N2OCIF	EE	1	1	1/1	C18H13N3OCIF	H3N	342.08044		_"

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

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		V			1
209.0638		V	\checkmark		
223.0796		V			
249.0827		V	V		1
258.0475		V	V	V	1
290.1089		V	V	V	1
291.1164		V	\checkmark		Y
309.0586		V	V		1
	n	8	6	2	5
	%	100	75	25	63
1-OH Midazolam					
141.0547		V			
147.0693		V			
168.0686		V			×
176.0266		V			
203.0375		V	V		N
235.0812		V			
249.0836		V	V		
262.0898		V			N
289.1003		V	\checkmark		×
297.0565		V	V		N
324.0705		V	V	V	N
	n	11	5	1	6
	%	100	46	9	55
4-OH Midazolam					
109.0396		V	V	\checkmark	N
122.0399		V	\checkmark	V	N
147.0673		V			
207.036		V	V	\checkmark	N
222.0707		V			
234.0484		V	V	V	×
256.0301		V			N
277.0525		V	V	\checkmark	V
297.0586		V	V		N
325.0546		V	V		1
	n	10	7	5	8
	%	100	70	50	80



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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