Electronic Supplementary Material (ESI) for RSC Advances.

# High Throughput Mass Spectrometry-based Characterisation of *Arabidopsis thaliana* GROUP H Glycosyltransferases

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## Phylogenetic classification of Arabidopsis thaliana

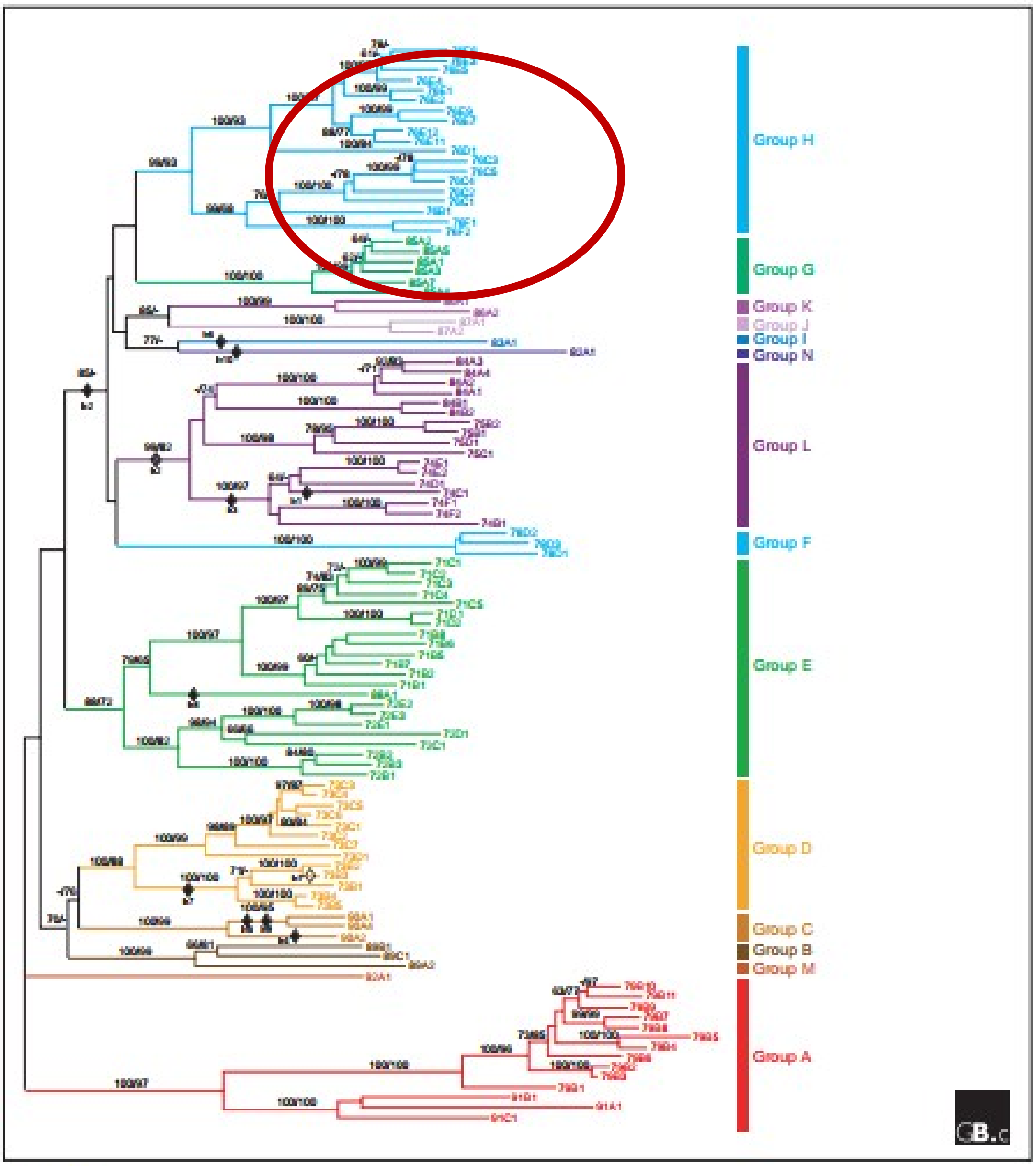


**Fig 1: Phylogenetic grouping of *Arabidopsis thaliana* UGTs (Ross et al., 2001)**

# RESULTS AND DISCUSSION

## Protein purification; SDS PAGE

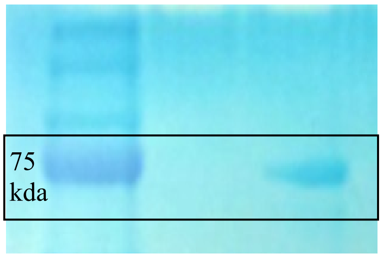


**Fig 2a**: **Lane 1: Protein ladder; Lane 3:Purified 76E5 MW ( 80kda)**

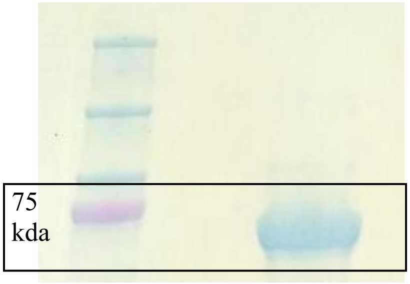


**Fig 2b: Lane 1: Protein ladder Lane 3:Purified 76E1 (MW 65kda)**

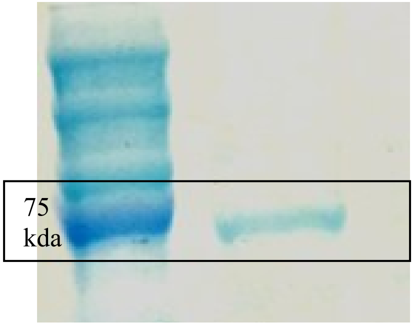


**Fig 2c: Lane 1: Protein ladder Lane 3:Purified 76D1 (MW 65kda)**

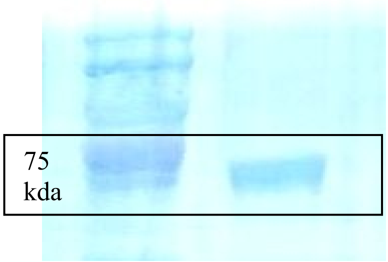
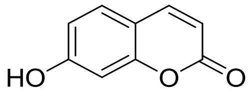
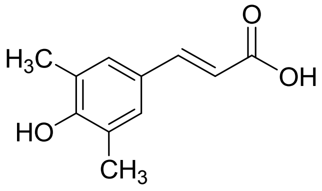
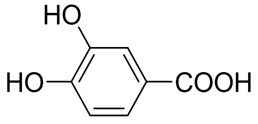
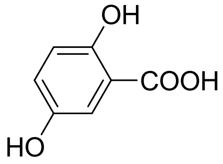
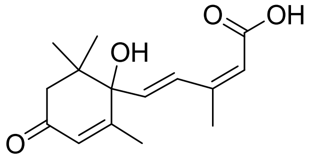
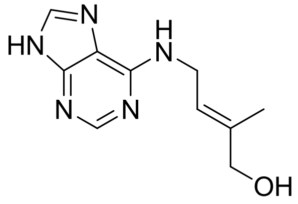
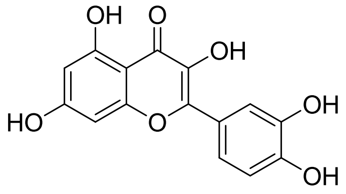
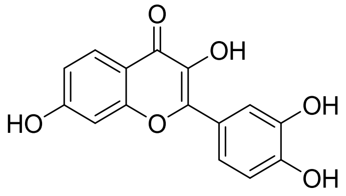
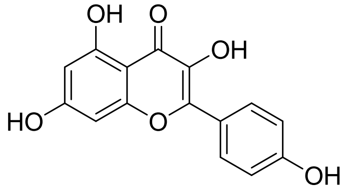


**Fig 2d: Lane 1: Protein ladder Lane 3:Purified 76E2 (MW 65kda)**

## ACCEPTOR LIBRARY USED

| | **Name** | **Structure** | **Molecular weight** |
|---|---|---|---|
| 1. | Umbelliferone |  | **162.0** |
| 2. | Sinapic acid |  | **224.0** |
| 3. | 3,4-dihydroxybenzoic acid |  | **154.0** |
| 4. | 2,5-dihydroxybenzoic acid |  | **154.0** |
| 5. | Abscisic acid |  | **264.0** |
| 6. | Zeatin |  | **219.0** |
| 7. | Quercetin |  | **302.0** |
| 8. | Fisetin |  | **286.0** |
| 9. | Kaempferol |  | **286.0** |

| | | | |
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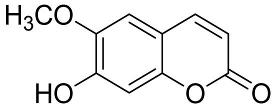
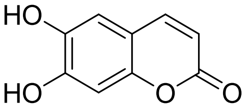
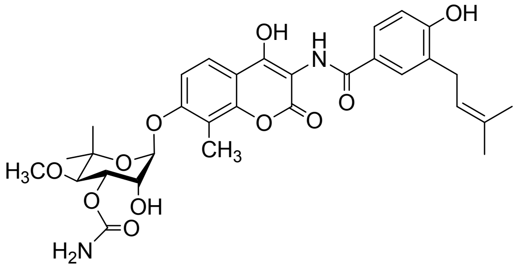
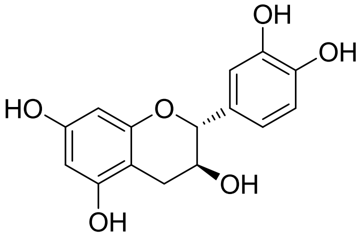
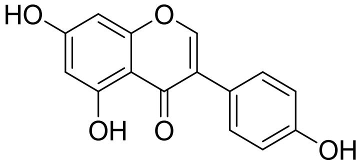
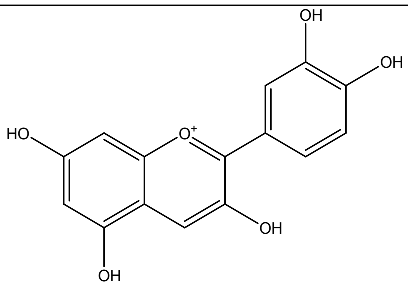
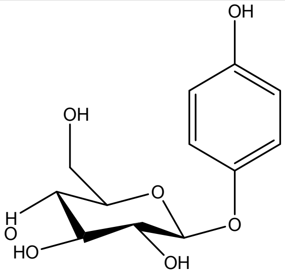
| 10. | Scopoletin |  | **192.0** |
| 11. | Esculetin |  | **178.0** |
| 12. | Novobiocin |  | **613.0** |
| 13 | Catechin |  | **290.0** |
| 14 | Genistein |  | **270.0** |
| 15 | Cyanidin |  | **286.0** |
| 16 | Arbutin |  | **272.0** |

| | | | |
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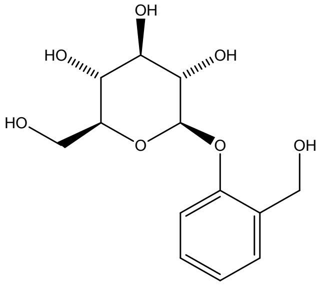
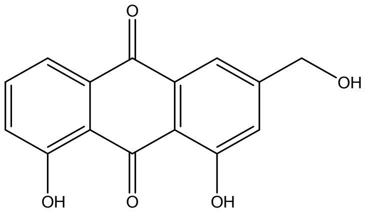
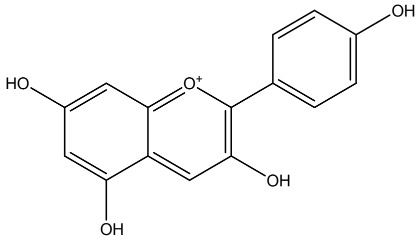
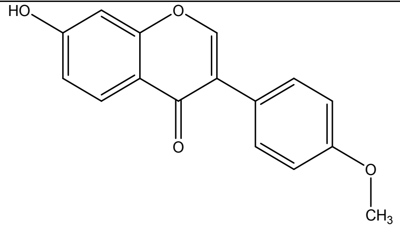
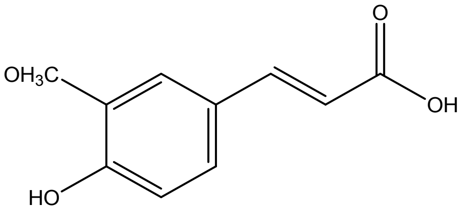
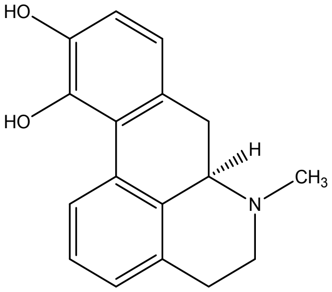
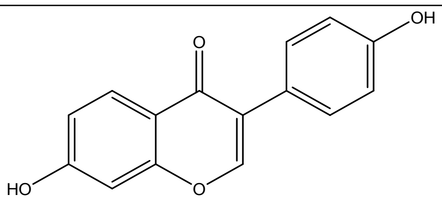
| 17 | Salicin |  | **286.0** |
| 18 | Aloe-emodin |  | **270.0** |
| 19 | Pelargonidin |  | **271.0** |
| 20 | Formononetin |  | **268.0** |
| 21 | Ferulic acid |  | **194.0** |
| 22 | Apomorphine |  | **267.0** |
| 23 | Daidzein |  | **254.0** |

| | | | |
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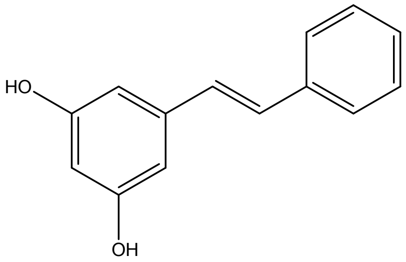
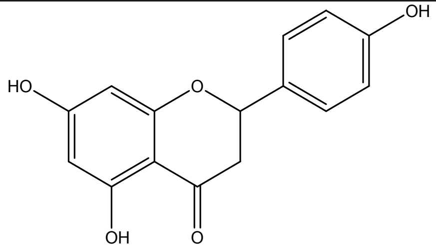
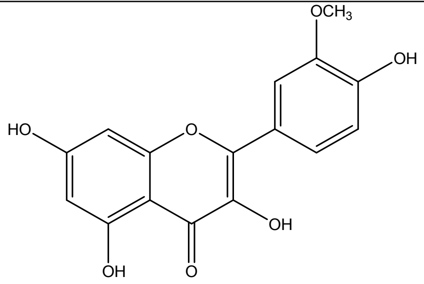
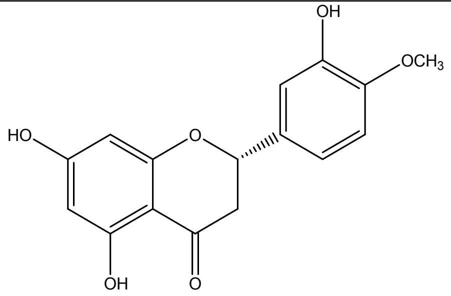
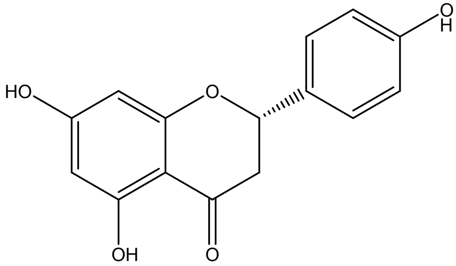
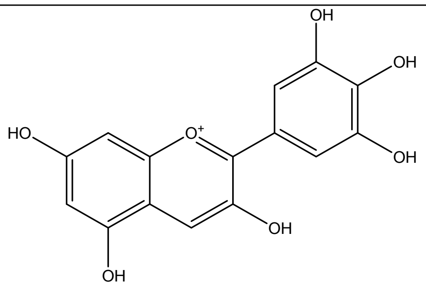
| 24 | Resveratrol |  | **228.0** |
| 25 | Apigenin |  | **270.0** |
| 26 | Isorhamnetin |  | **316.0** |
| 27 | Hesperetin |  | **302.0** |
| 28 | Naringenin |  | **272.0** |
| 29 | Delphinidin |  | **303.0** |

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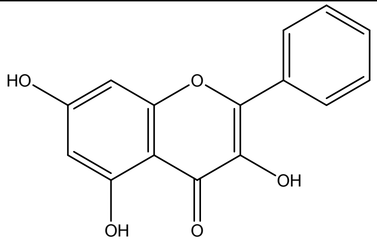
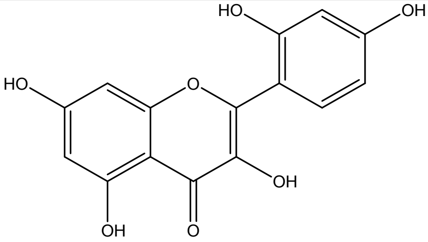
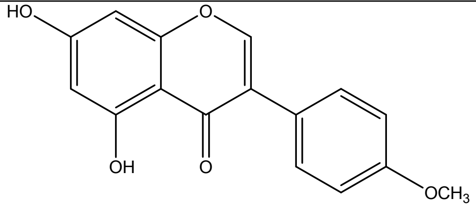
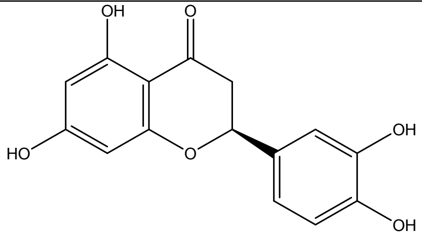
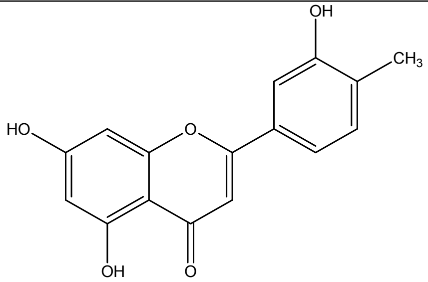
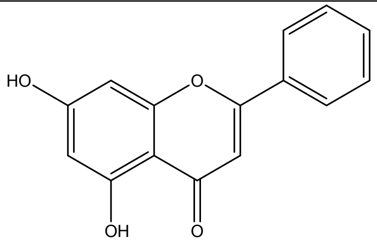
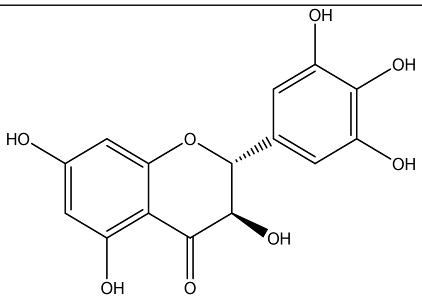
| 30 | Galangin |  | **270.0** |
| 31 | Morin |  | **302.0** |
| 32 | Biochanin A |  | **284.0** |
| 33 | Eriodictyol |  | **288.0** |
| 34 | Diosmetin |  | **300.0** |
| 35 | Chrysin |  | **254.0** |
| 36 | Dihydromyricetin |  | **320.0** |

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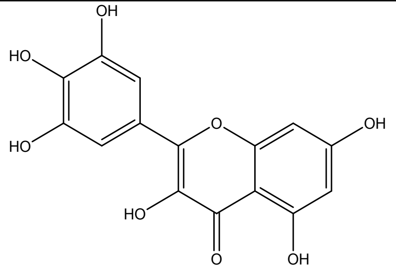
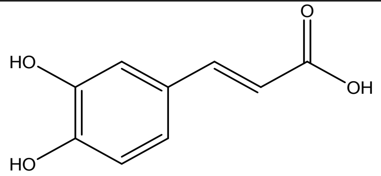
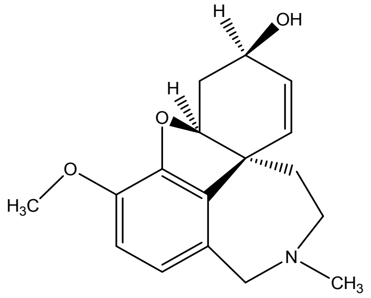
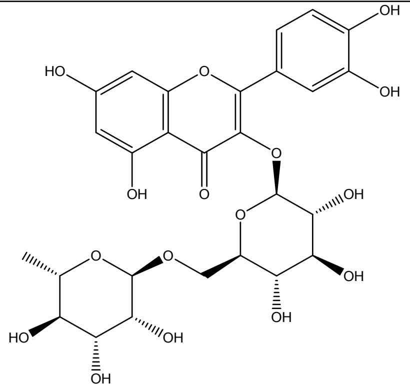
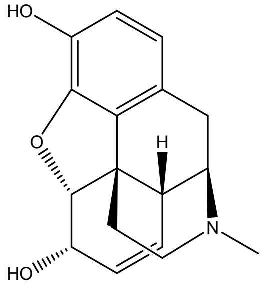
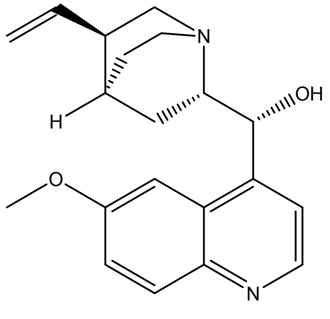
| 37 | Myricetin |  | **318.0** |
| 38 | Caffeic acid |  | **180.0** |
| 39 | Galantamine |  | **287.0** |
| 40 | Rutin |  | **611.0** |
| 41 | Morphine |  | **285.0** |
| 42 | Quinine |  | **324.0** |

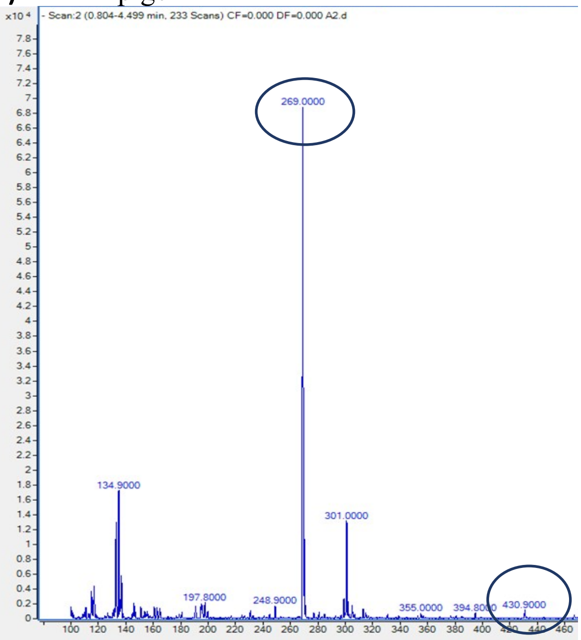
**Table 1: Library of acceptor compounds screened**

## MASS SPECTRA SUMMARISED IN GAR SCREEN

### A) 76E1 Acceptor screening result

MS spectra; UDP Glucose (MW 162) was added to the test aglycones. m/z of target peaks (glycosylated products) are shown in bold in the red boxes below.

**i)** Apigenin



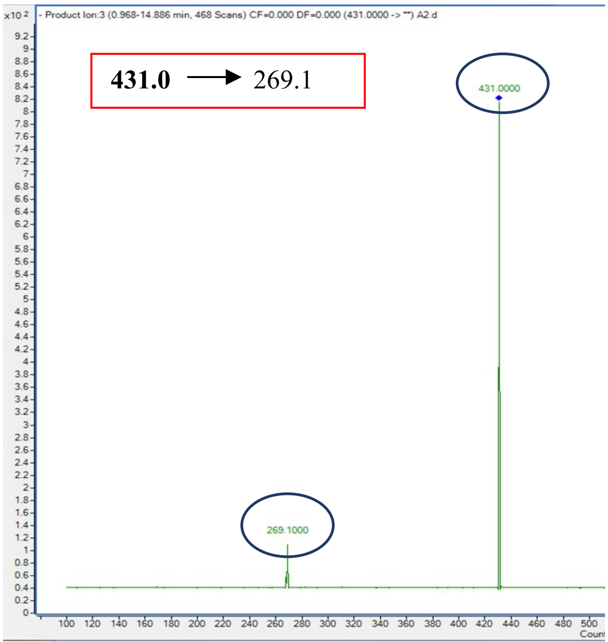
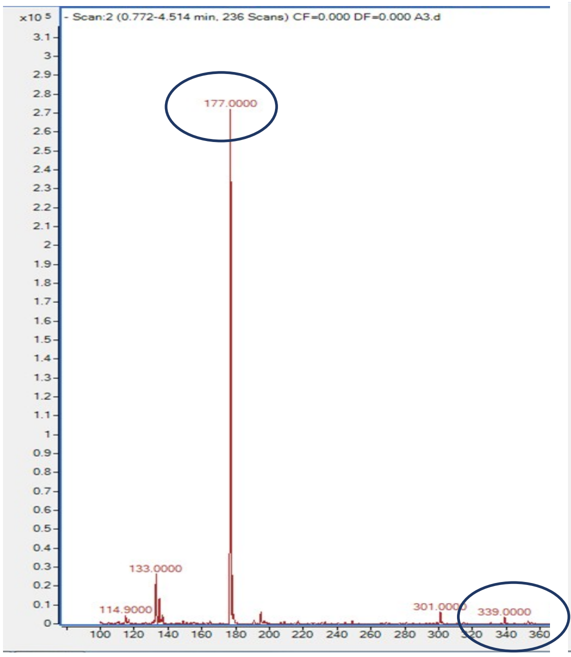


**Fig 3a: MS spectrum of 76E1 glycosylation of apigenin (Full scan) Fig 3b: MS spectrum showing fragmentation of apigenin glucoside (Product ion scan)**

MS/MS was used to fragment apigenin glucoside (MW 431). The observation of apigenin (MW 269) confirms the glucoside formation, and hence the glycosylation process.

**ii) Esculetin**



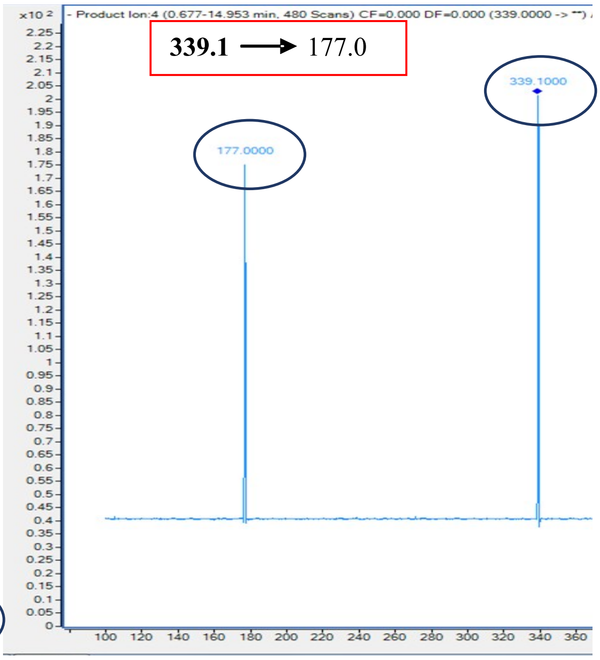
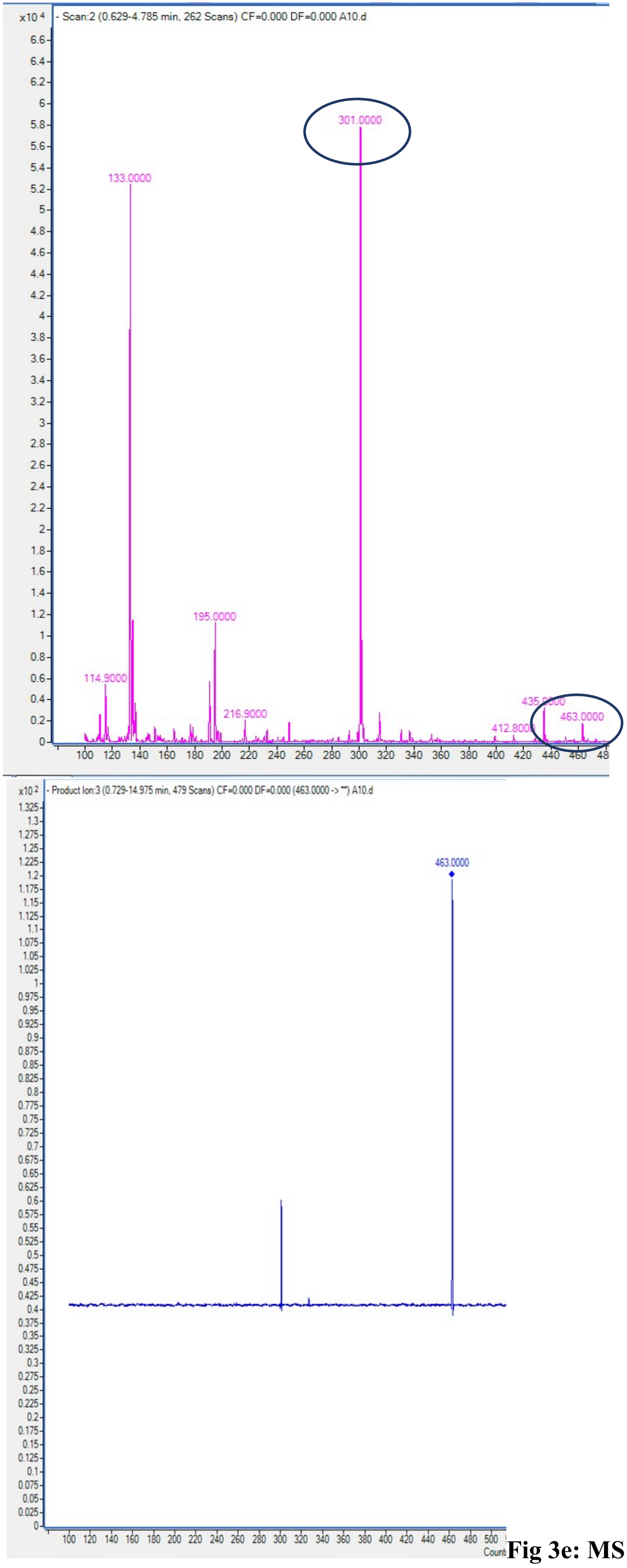


**Fig 3c: MS spectrum of 76E1 glycosylation of esculetin (Full scan) 3d: MS spectrum showing fragmentation of esculetin glucoside (Product ion scan)**

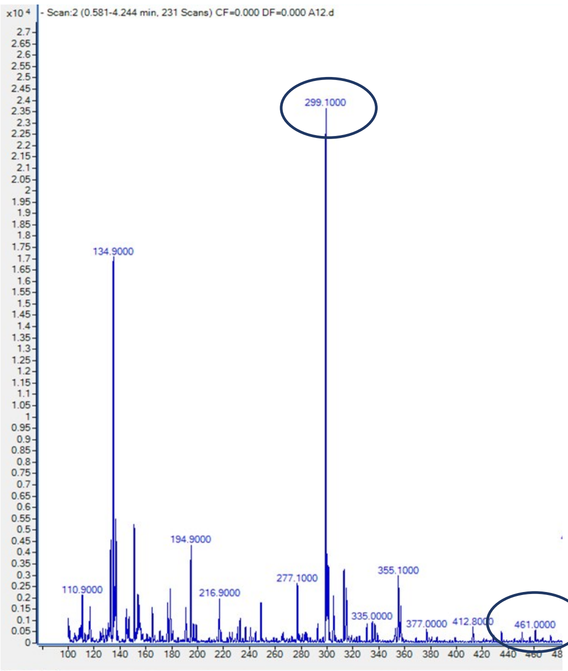
### iii) Quercetin



**463.0** → 301.0

Fig 3e: MS spectrum of 76E1 glycosylation of quercetin (Full scan) 3f: MS spectrum showing fragmentation of quercetin glucoside (Product ion scan)

### iv) Diosmetin



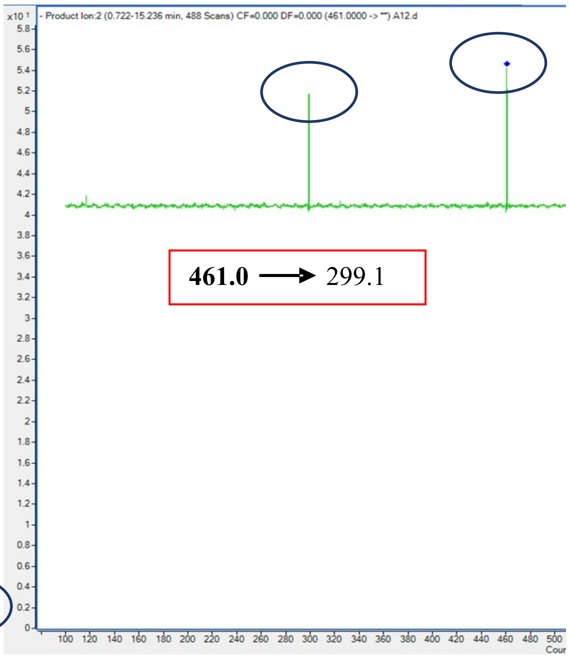
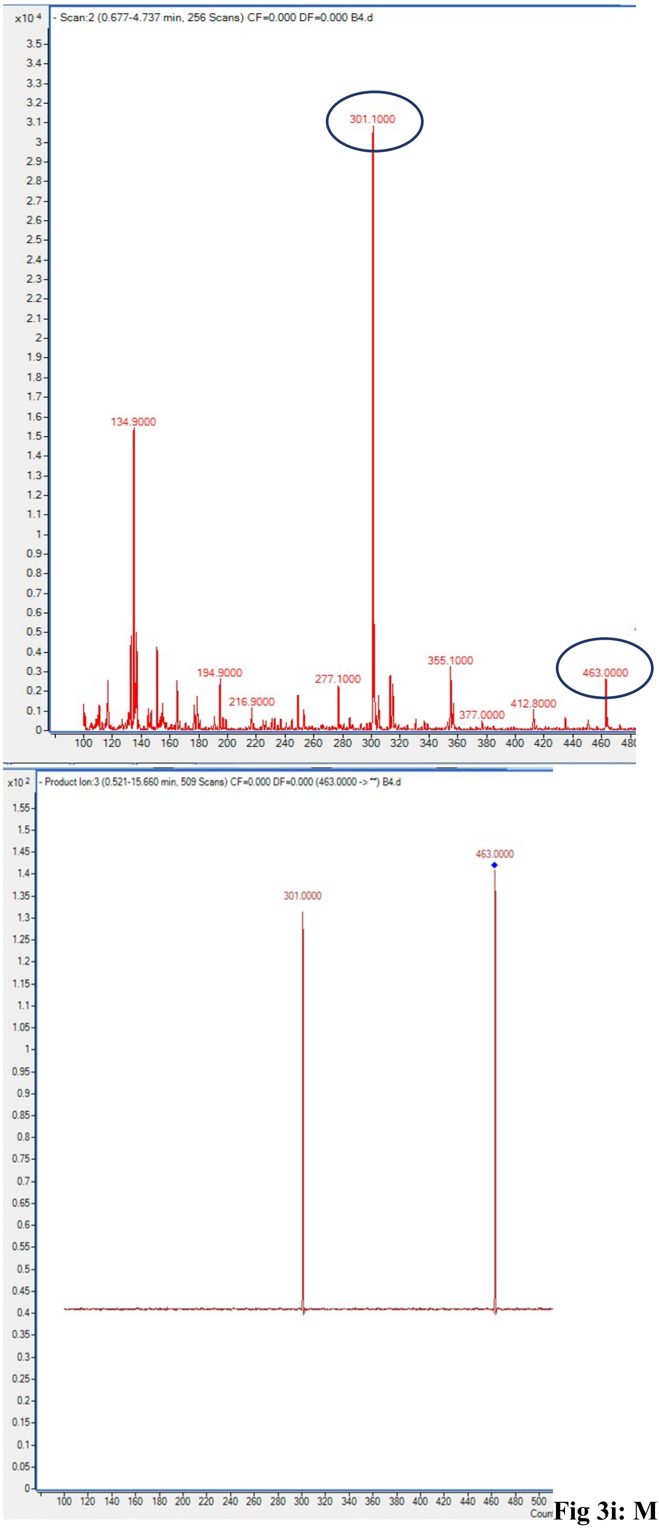


**Fig 3g: MS spectrum of 76E1 glycosylation of diosmetin (Full scan)     3h: MS spectrum showing fragmentation of diosmetin glucoside (Product ion scan)**

## v) Hesperetin



**463.0** → 301.0

**Fig 3i: MS spectrum of 76E1 glycosylation of hesperetin (Full scan) 3j: MS spectrum showing fragmentation of hesperetin glucoside (Product ion scan)**

## vi) Naringenin

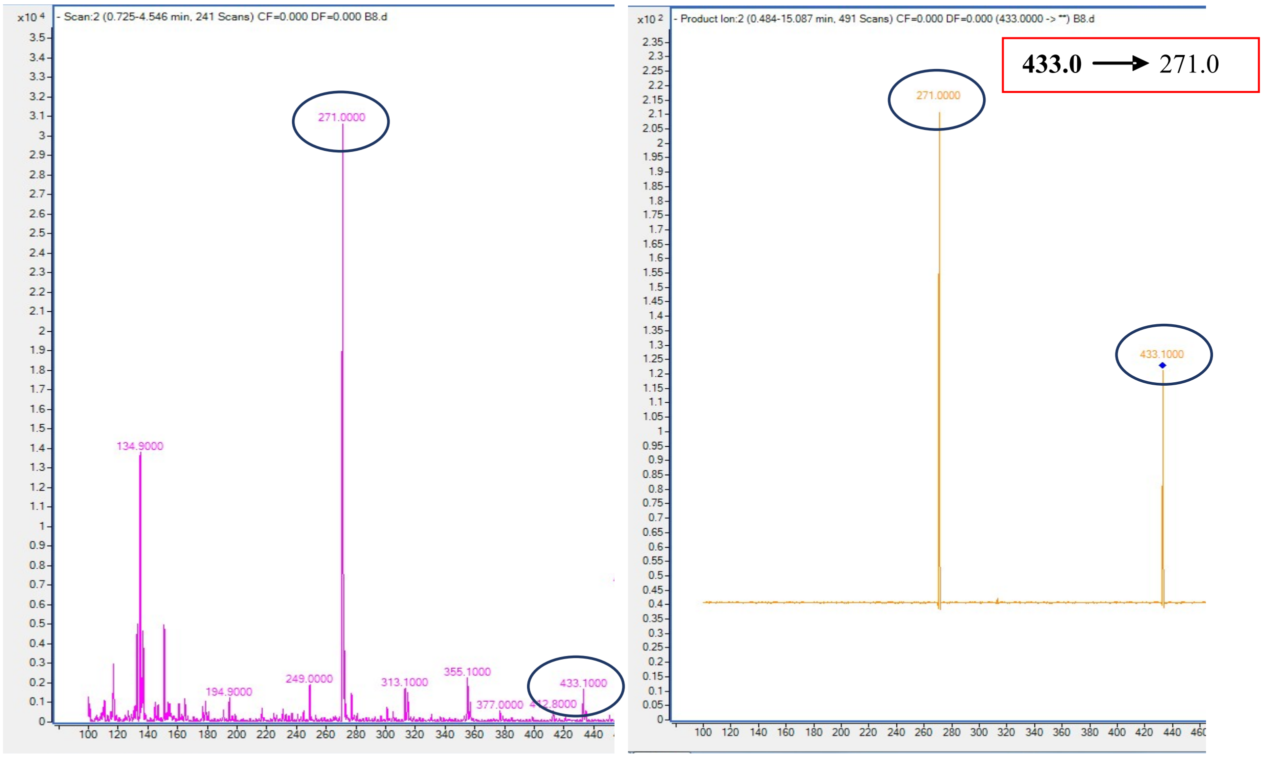


**Fig 3k: MS spectrum of 76E1 glycosylation of naringenin (Full scan) 3l: MS spectrum showing fragmentation of naringenin glucoside (Product ion scan)**

### vii) Eriodictyol

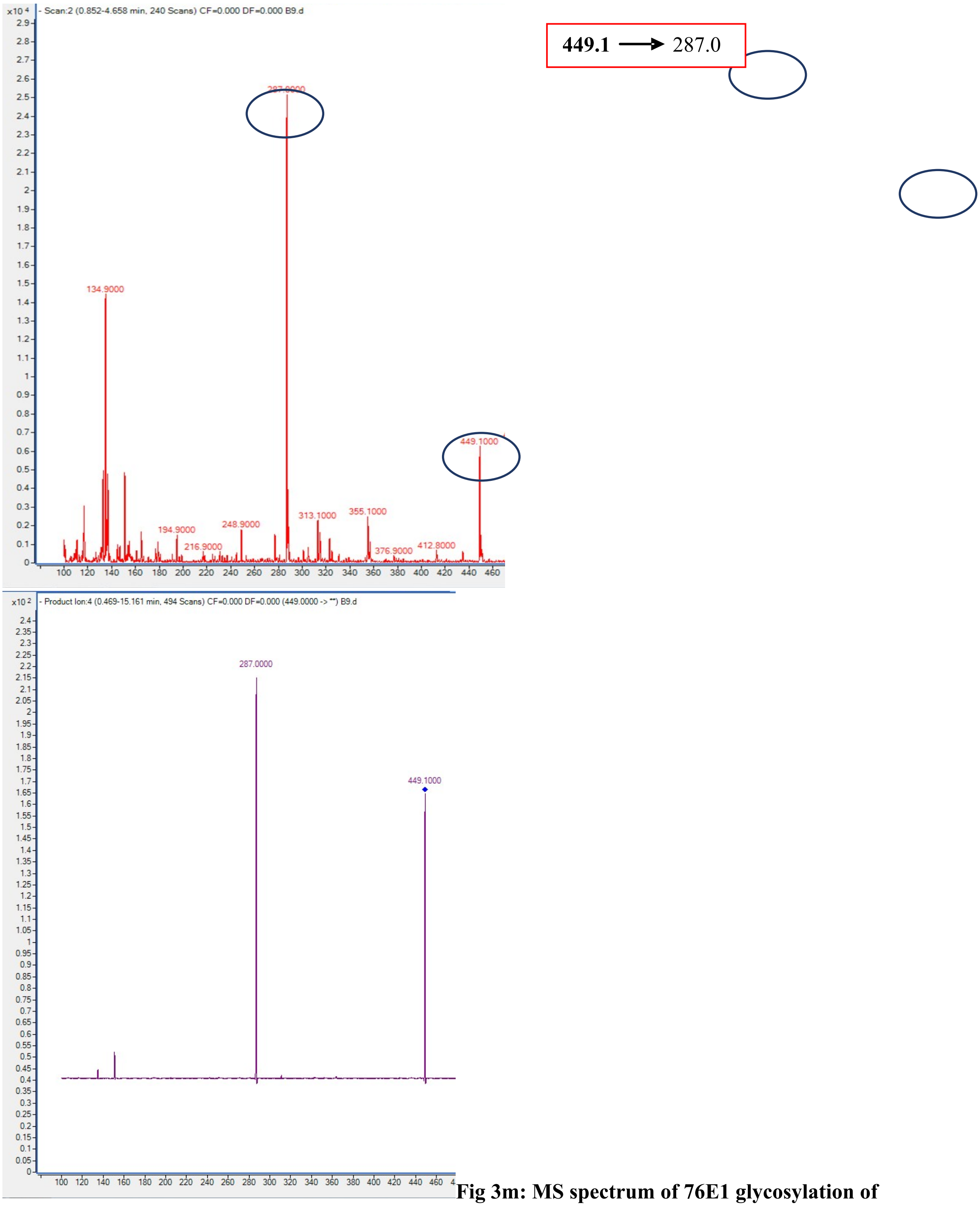


**Fig 3m: MS spectrum of 76E1 glycosylation of eriodictyol (Full scan) 3n: MS spectrum showing fragmentation of eriodictyol glucoside (Product ion scan)**

### viii) Kaempferol

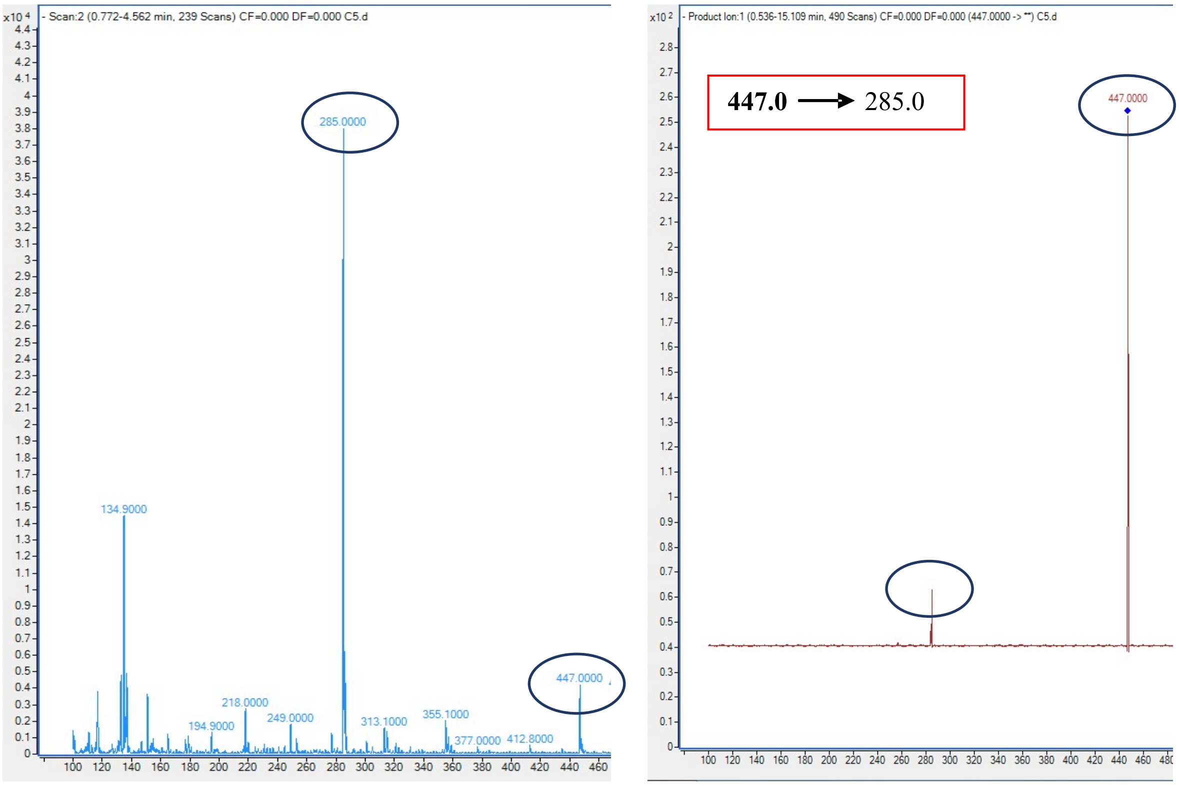


Fig 3o: MS spectrum of 76E1 glycosylation of kaempferol (Full scan) 3p: MS spectrum showing fragmentation of kaempferol glucoside (Product ion scan)

## ix) Fisetin

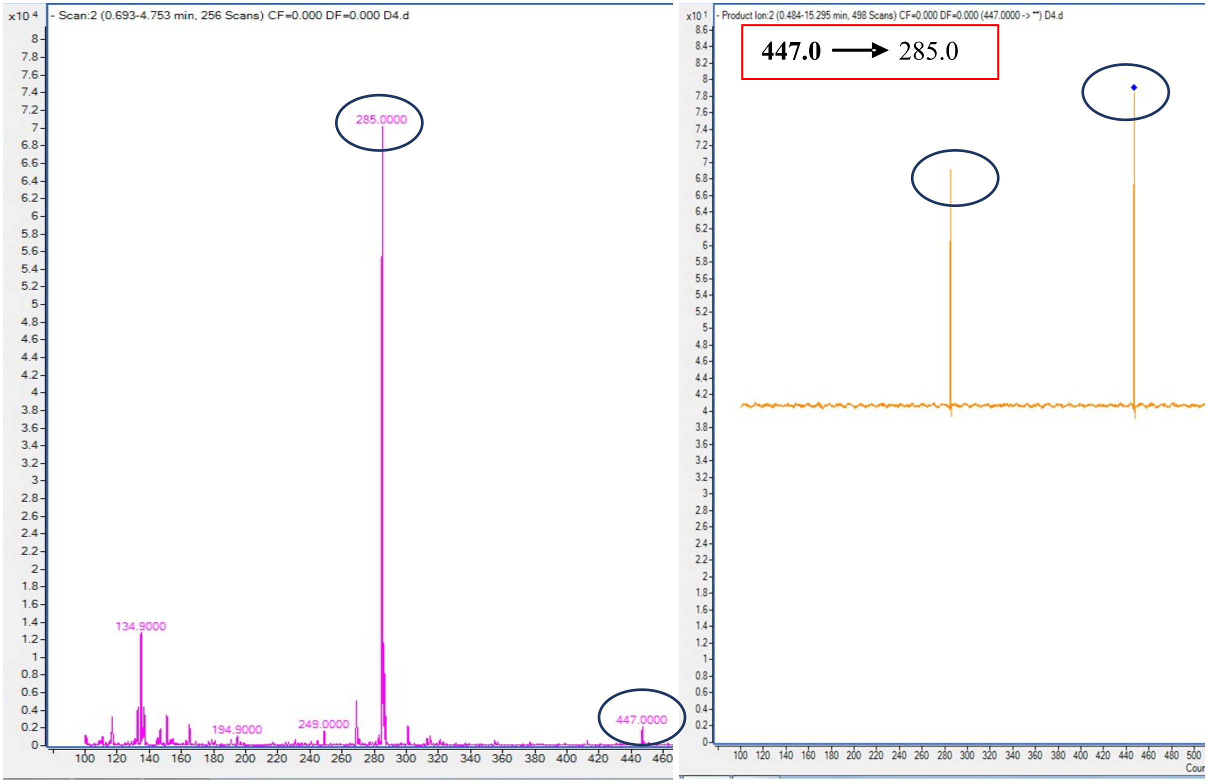


Fig 3q: MS spectrum of 76E1 glycosylation of fisetin (Full scan) 3r: MS spectrum showing fragmentation of fisetin glucoside (Product ion scan)

## B) 76E2 Acceptor screening

## i) Quercetin

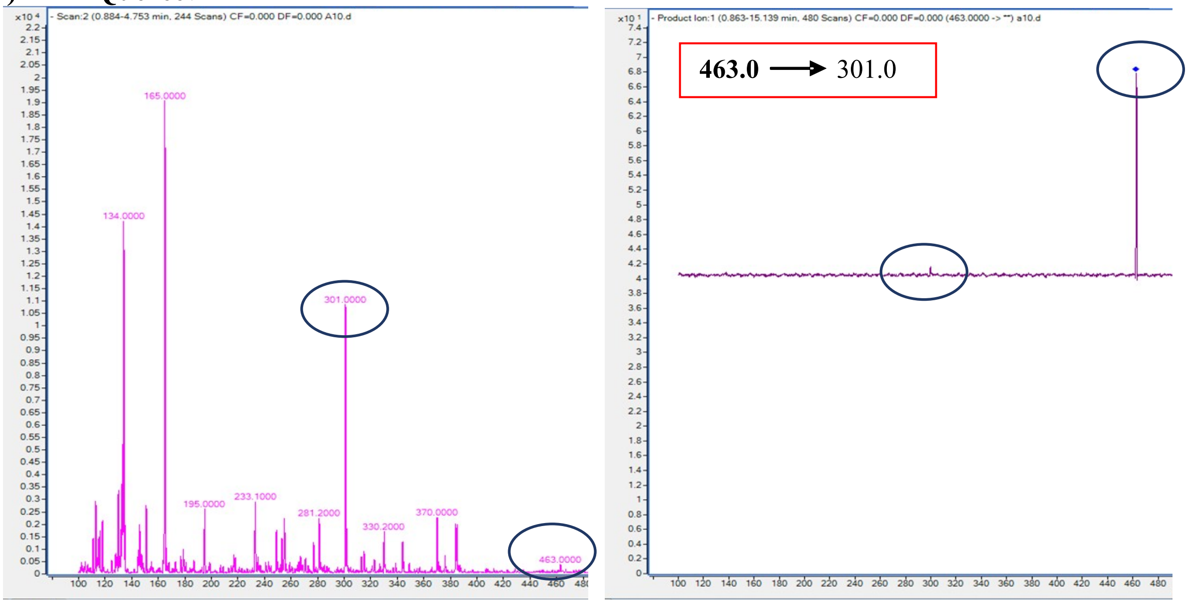


**Fig 4a: MS spectrum of 76E2 glycosylation of quercetin (Full scan) 4b: MS spectrum showing fragmentation of quercetin glucoside (Product ion scan)**

## ii) Myricetin

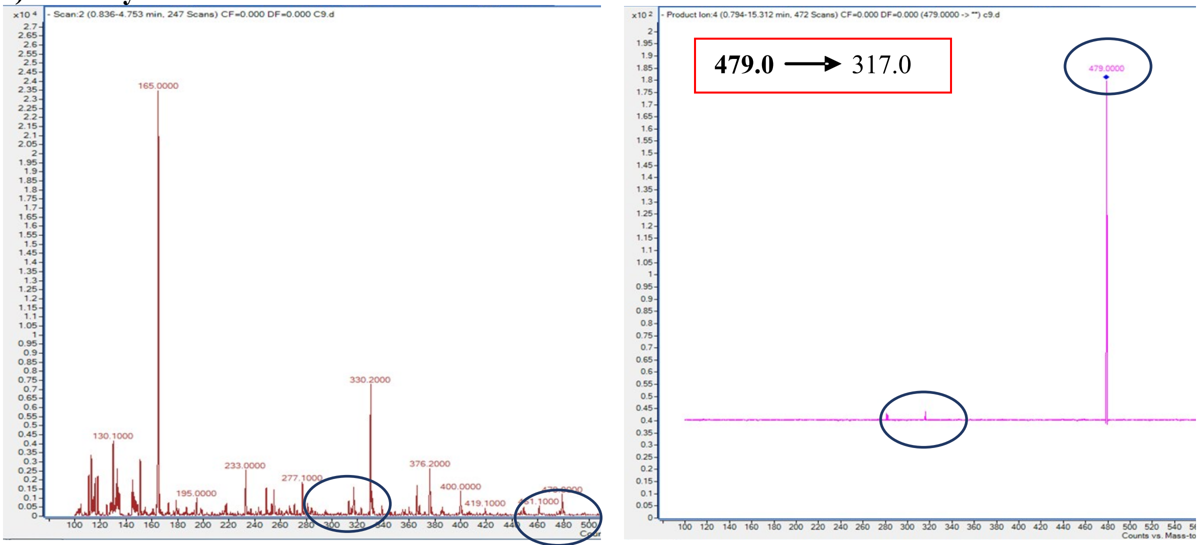


**Fig 4c: MS spectrum of 76E2 glycosylation of myricetin (Full scan) 4d: MS spectrum showing fragmentation of myricetin glucoside (Product ion scan)**

## iii) Fisetin

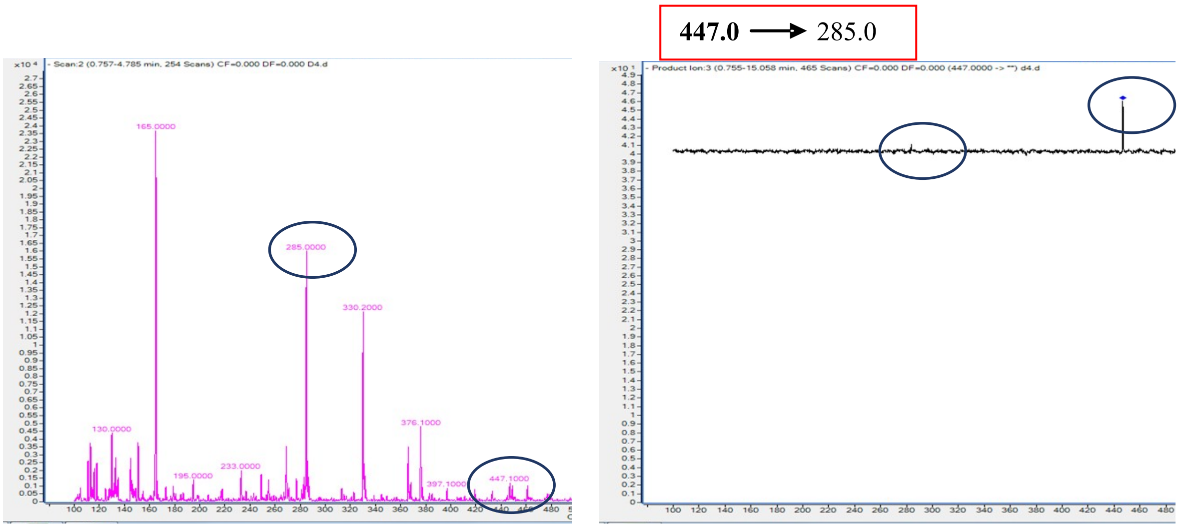


**Fig 4e: MS spectrum of 76E2 glycosylation of fisetin (Full scan) 4f: MS spectrum showing fragmentation of fisetin glucoside (Product ion scan)**

## C) 76E5 Acceptor screening

### i) Kaempferol

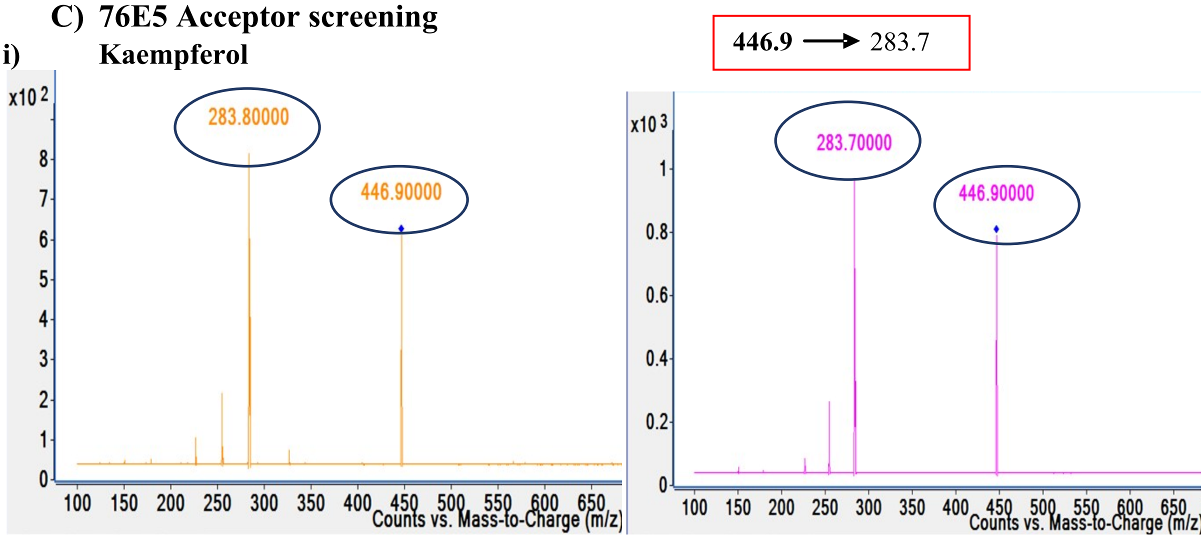


**Fig 5a: MS spectrum of 76E5 glycosylation of kaempferol (Full scan) 5b: MS spectrum showing fragmentation of kaempferol glucoside (Product ion scan)**

### ii) Quercetin

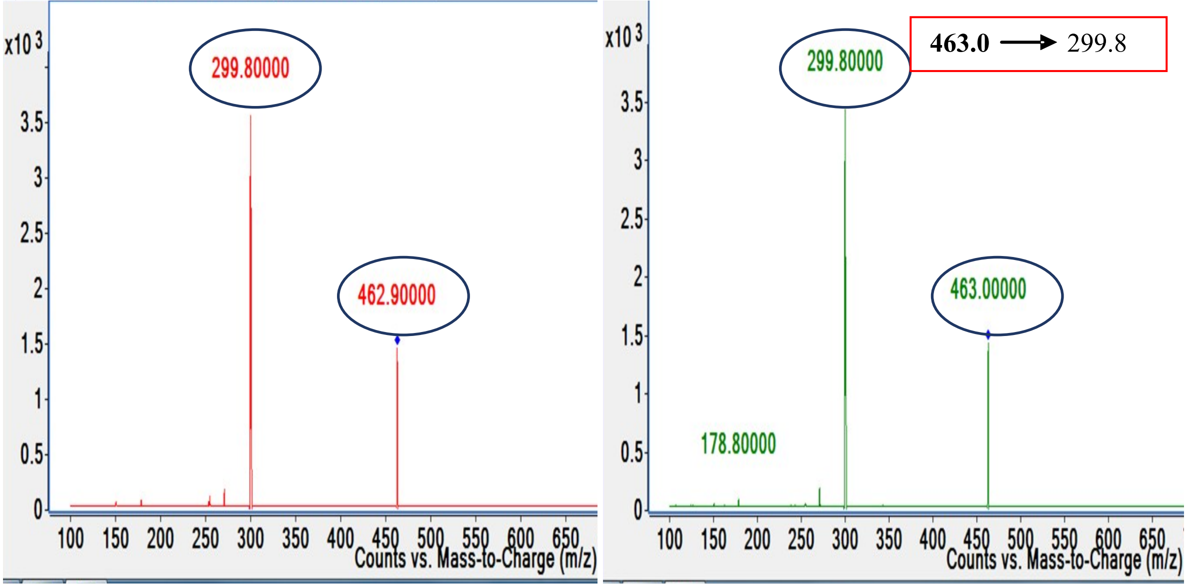


**Fig 5c: MS spectrum of 76E5 glycosylation of quercetin (Full scan) 5d: MS spectrum showing fragmentation of quercetin glucoside (Product ion scan)**

**iii) 2,5DHBA**

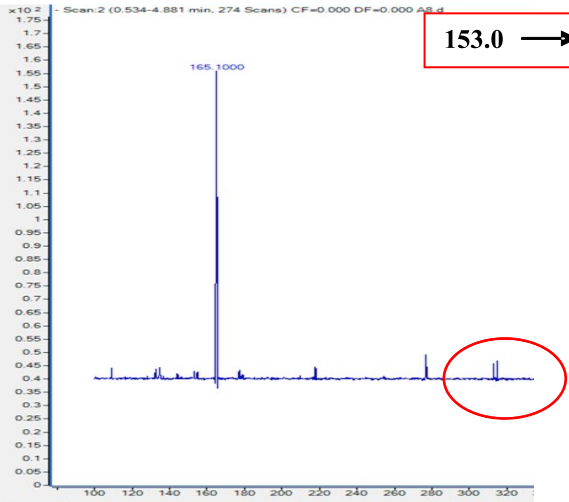


**Fig 5e: MS spectrum of 76E5 glycosylation of 2,5-dihydroxybenzoic acid (Full scan)**

**iv) Scopoletin**

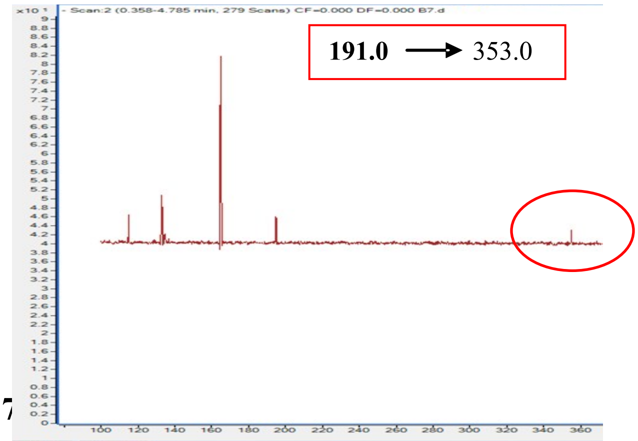


**Fig 5f: MS spectrum of 76E5 glycosylation of scopoletin (Full scan)**

**D) 7**

**i) 2,5 dihydrobenzoic acid**

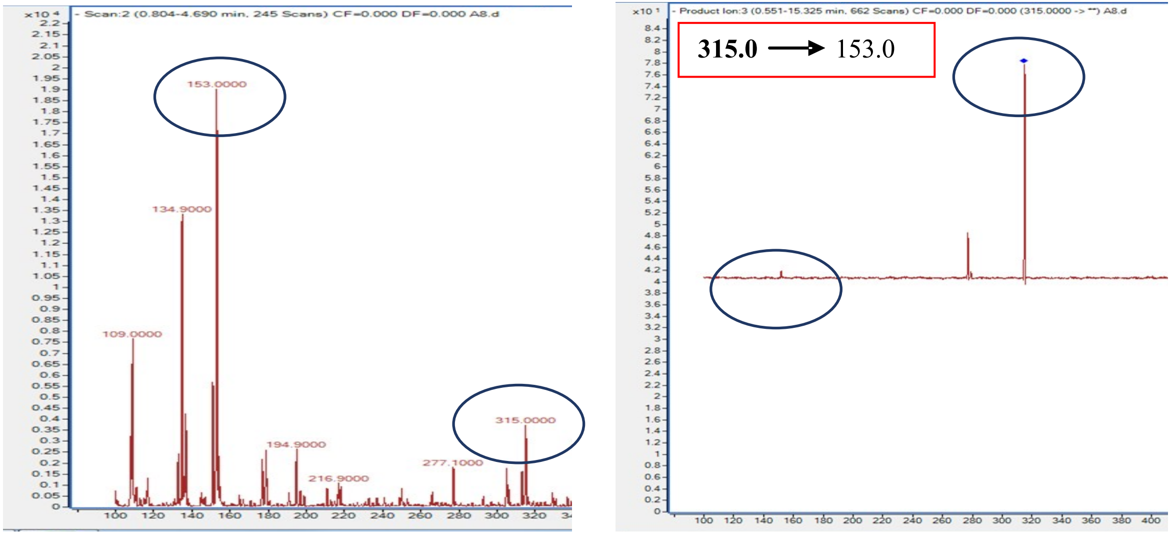


**Fig 6a: MS spectrum of 76E5 glycosylation of 2,5 dihydrobenzoic acid (Full scan) 6b: MS spectrum showing fragmentation of 2,5 dihydrobenzoic acid glucoside (Product ion scan)**

**ii) Quercetin**

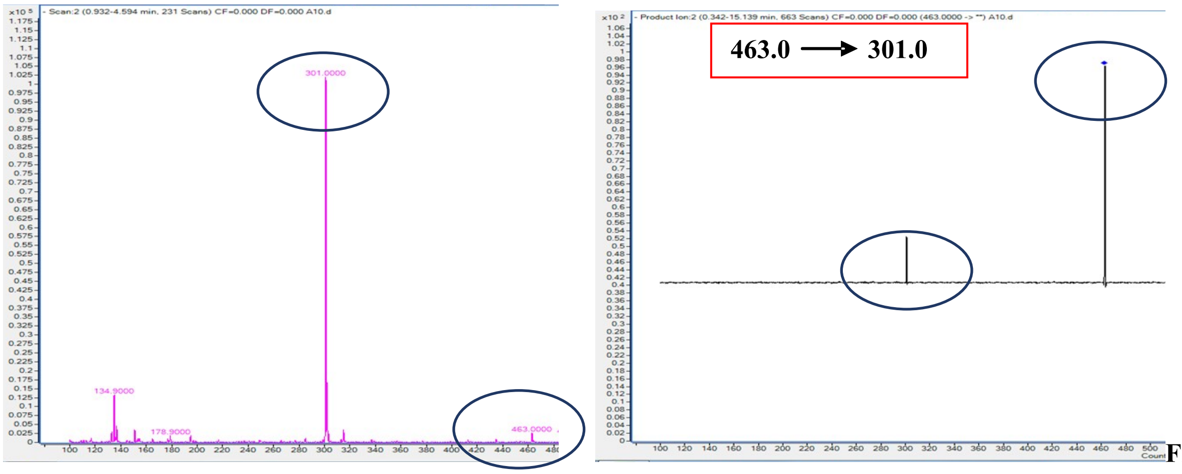
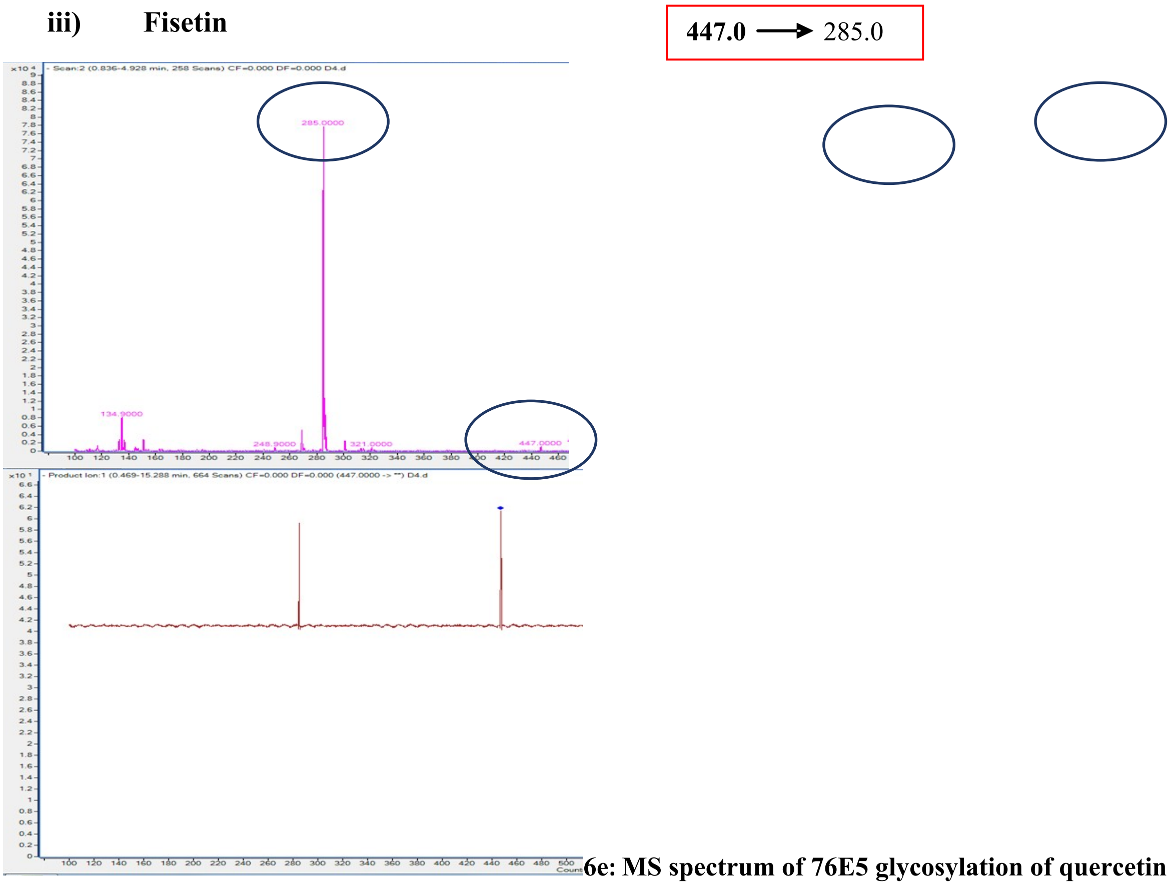


**Fig 6c: MS spectrum of 76E5 glycosylation of quercetin (Full scan) 6d: MS spectrum showing fragmentation of quercetin glucoside (Product ion scan)**

### iii) Fisetin

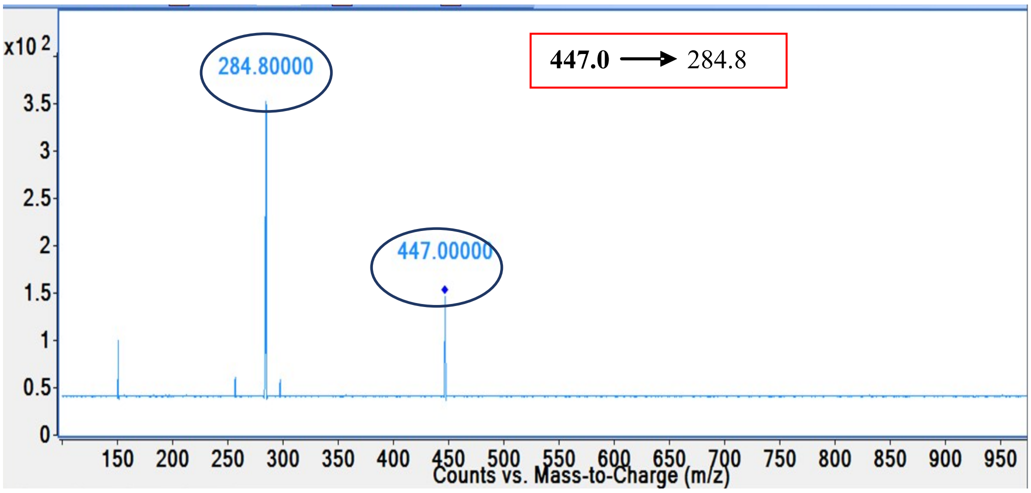


**6e: MS spectrum of 76E5 glycosylation of quercetin (Full scan) 6f: MS spectrum showing fragmentation of quercetin glucoside (Product ion scan)**

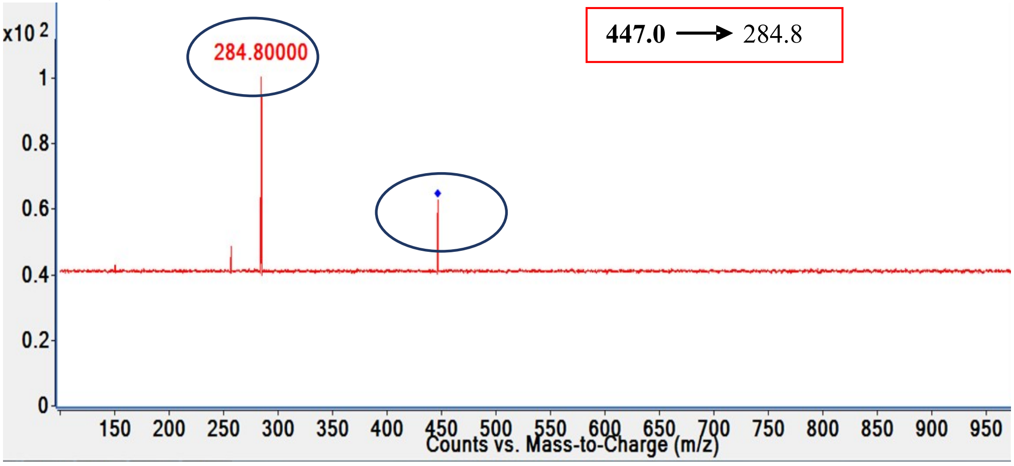
## DONOR SCREENING RESULT AND GLYCOSYLATION POSITION

### A) 76E1 Donor screening results

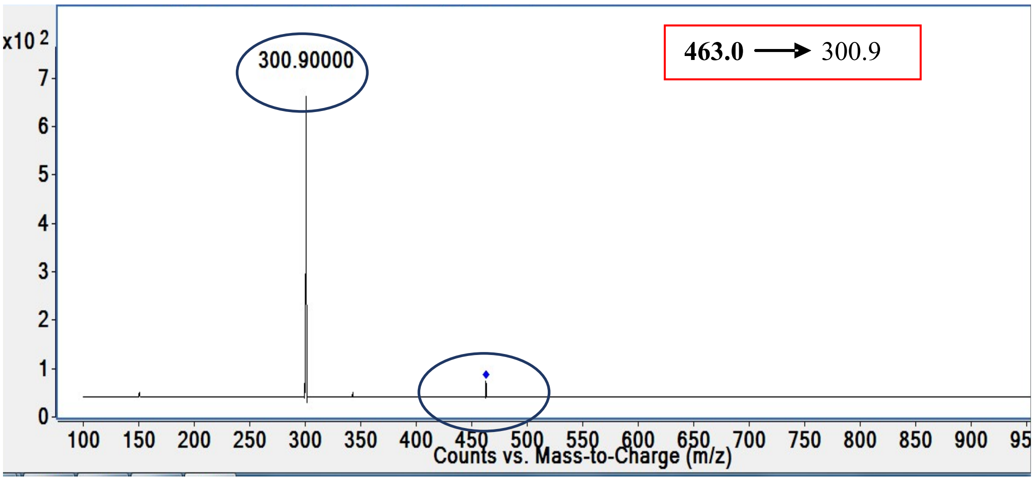
1) UDP glucose and Kaempferol



2) UDP galactose and Quercetin



3) UDP glucose and Quercetin



4) UDP glcnac and Quercetin

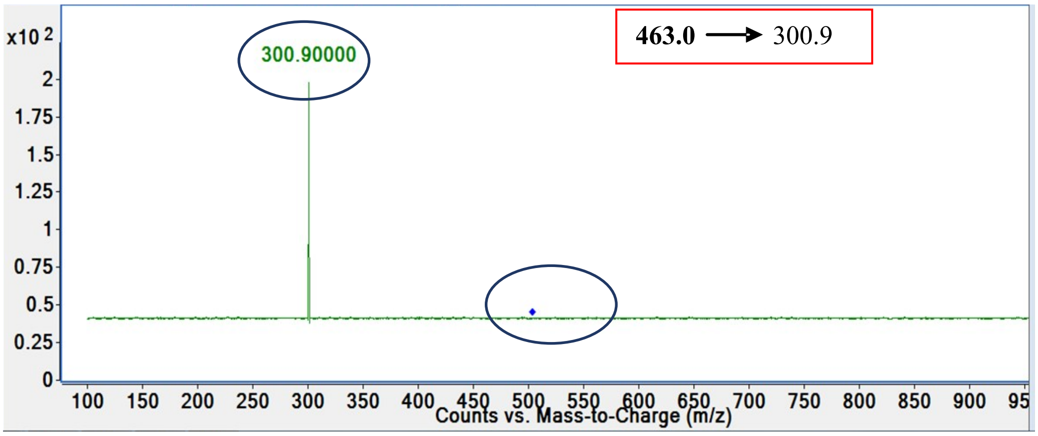


Fig 7: MS spectrum of 76E1 recognition of UDP glc, gal and glcnac

## B) 76E2 Donor screening

### UDP glucose and Quercetin

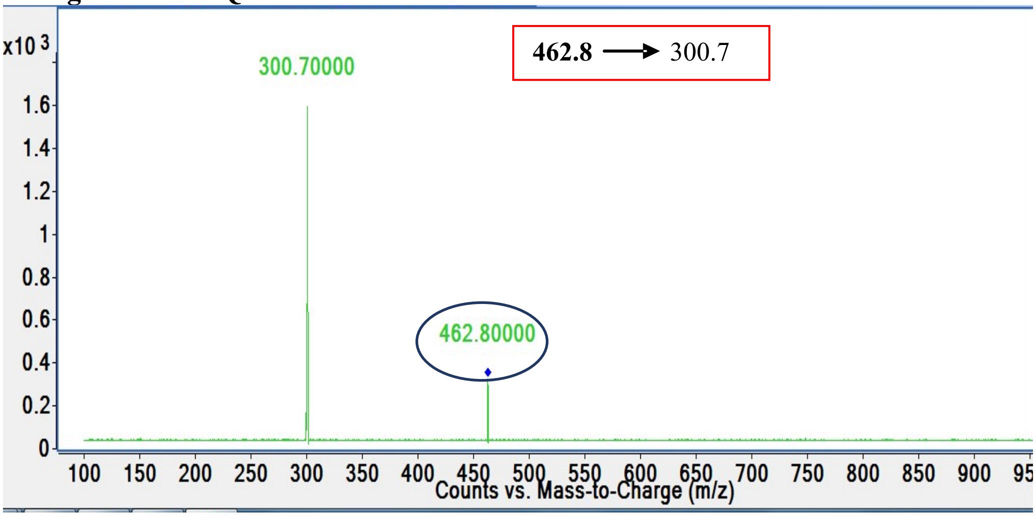


Fig 8: MS spectrum of 76E2 recognition of UDP glc

## C) 76E5 Donor screening

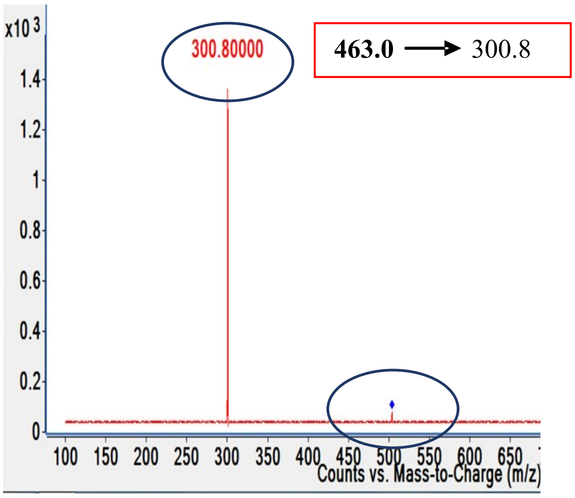
### 1) UDP glucose (Kaempferol and Quercetin)

**447.0** → 284.8

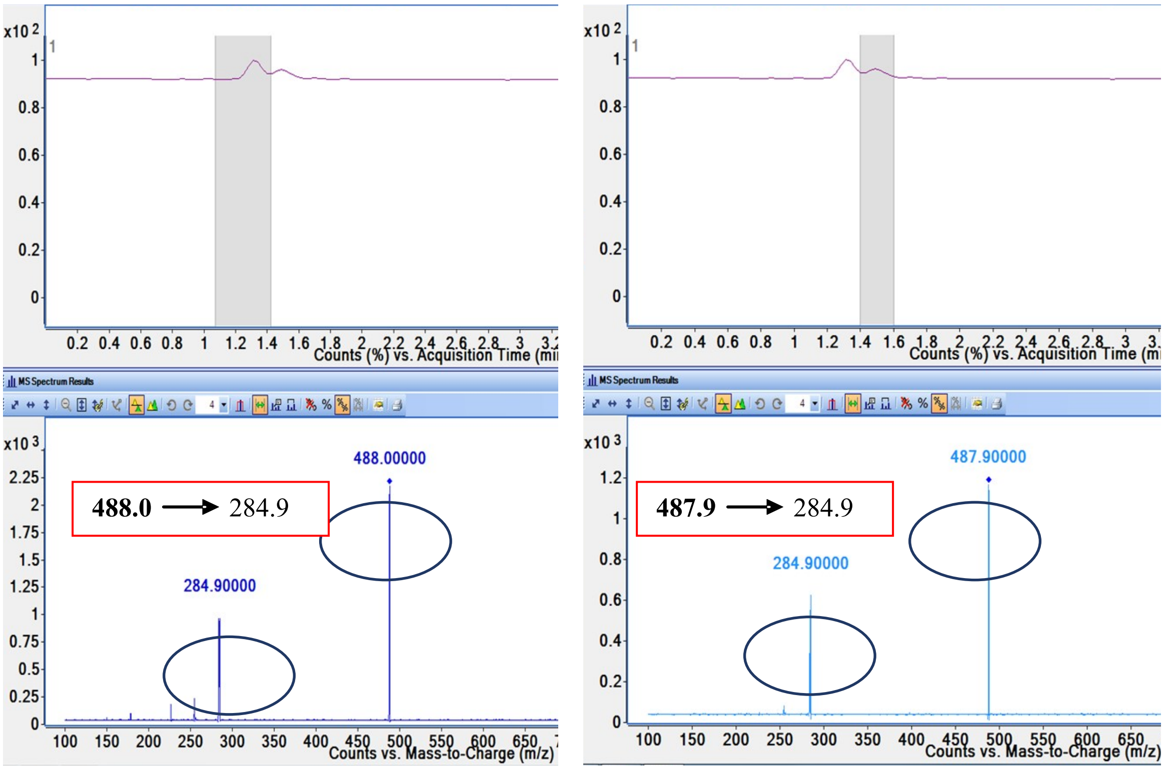
**463.0** → 300.9

### 2) UDP galactose (Kaempferol and Quercetin)

**447.0** → 284.8



### 3) UDP glcnac (Kaempferol and Quercetin)



**463.0** → 300.9

**Fig 9: MS spectrum of 76E5 recognition of UDP glc, gal and glcnac**

### D) 76D1 Donor screening

**i) UDP glucose and Quercetin**

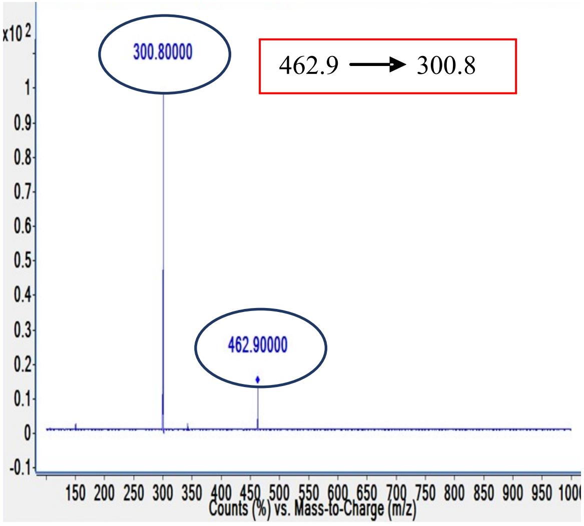


**Fig 10: MS spectrum of 76E5 recognition of UDP glc**

**Table showing m/z of precursor ion and product ion for MS/MS data**

| **Enzyme** | **Reaction** | **Precursor ion (Glycoside peaks)** | **Product ion** |
|---|---|---|---|
| 76E1 | UDP Glc/Gal + Kaempferol<br>UDP Glc + Quercetin<br>UDP GlcNAc + Quercetin | **447**<br>**463**<br>**504** | **285**<br>**301**<br>**301** |
| 76E2 | UDP Glc + Quercetin | **463** | **301** |
| 76D1 | UDP Glc + Quercetin | **463** | **301** |
| 76E5 | UDP Glc/Gal + Kaempferol<br>UDP Glc/Gal + Quercetin<br>UDP GlcNAc + Kaempferol<br>UDP GlcNAc + Quercetin | **447**<br>**463**<br>**488**<br>**504** | **285**<br>**301**<br>**285**<br>**301** |
| 76E2 N320S | UDP Glc/Gal + Kaempferol<br>UDP Glc/Gal + Quercetin<br>UDP GlcNAc + Kaempferol<br>UDP GlcNAc + Quercetin | **447**<br>**463**<br>**488**<br>**504** | **285**<br>**301**<br>**285**<br>**301** |

**Table 2: m/z of precursor and product ions**

## Sanger Sequencing

The sequencing of mutant plasmids was carried out by Source Bioscience to confirm the incorporation of the mutation by template DNAs. The sequence was compared with wild type DNA sequences using NCBI BLAST.

### 76E1 S318N

```
Score              Expect   Identities         Gaps          Strand       Frame
2002 bits(1084)    0.0()    1092/1098(99%)     0/1098(0%)    Plus/Minus
Features:
Query  72    CTACATGAAATTCATCATTTTTAAGGAATTGACAAAGTTGTCTAATGAGCTGAATGAGGA  131
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Sbjct  1362  CTACATGAAATTCATCATTTTTAAGGAATTGACAAAGTTGTCTAATGAGCTGAATGAGGA  1303
Query  132   ACCTCTACTCTTGACAGAGGCTTGAAGCTTCTCTTTCAAGTTGATAACTCTCTTCCTCAT  191
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1302  ACCTCTACTCTTGACAGAGGCTTGAAGCTTCTCTTTCAAGTTGATAACTCTCTTCCTCAT  1243
Query  192   TTCTGCTCCTTCTTCATCCATAATCAATCTCTCTACAGCTCTCTCCACTGTTCCTTTATC  251
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Sbjct  1242  TTCTGCTCCTTCTTCATCCATAATCAATCTCTCTACAGCTCTCTCCACTGTTCCTTTATC  1183
Query  252   CAGCTCTCCTTCCAATTGAACCCCAATTCTCCAAACTCTCTCTAAGTACCTCGCATTGAC  311
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Sbjct  1182  CAGCTCTCCTTCCAATTGAACCCCAATTCTCCAAACTCTCTCTAAGTACCTCGCATTGAC  1123
Query  312   TTTCTGATCTCCCGTAAAAGGCCTACAGATCATCGGAACTCCTTCCCCGATGCTCTCTAG  371
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Sbjct  1122  TTTCTGATCTCCCGTAAAAGGCCTACAGATCATCGGAACTCCTTCCCCGATGCTCTCTAG  1063
Query  372   GGTCGAGTTCCATCCGCAGTGACTCCAAAACCCTCCCACTGCAGGATGTCTGAGAACTTC  431
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Sbjct  1062  GGTCGAGTTCCATCCGCAGTGACTCCAAAACCCTCCCACTGCAGGATGTCTGAGAACTTC  1003
Query  432   TATCTGTGGTGCCCATTTCACAATGTAACCTCTTTCTGAAACCAACCCATTCAATTCCTC  491
             ||||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||
Sbjct  1002  TATCTGTGGTGCCCATTTCACAATGTAACCTCTTTCTGAAACCAACCCACTCAATTCCTC  943
Query  492   CGGTAAAGACTCTGTCCATTCCGAGCCGGGAATAGAACCCGGTCGGATCACCCATAAGAA  551
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  942   CGGTAAAGACTCTGTCCATTCCGAGCCGGGAATAGAACCCGGTCGGATCACCCATAAGAA  883
Query  552   AGGTTGGTTGCTATTACGTAAACCCCAAGCCATCTCCAACATGTCTTTAGTTTCCATTAG  611
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  882   AGGTTGGTTGCTATTACGTAAACCCCAAGCCATCTCCAACATGTCTTTAGTTTCCATTAG  823
Query  612   AGCCAAGCTTCCCAAACTTATGTAAATCACTGAGCCTATTTTTTGCTTGTTCAACCACTC  671
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  822   AGCCAAGCTTCCCAAACTTATGTAAATCACTGAGCCTATTTTTTGCTTGTTCAACCACTC  763
Query  672   AAGGCAACTCCTGTCCTCTTCAAGTAAACTAGAAGGCGCTGAAGCTGCAATGTGAAGTGG  731
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Sbjct  762   AAGGCAACTCCTGTCCTCTTCAAGTAAACTAGAAGGCGCTGAAGCTGCAATGTGAAGTGG  703
Query  732   GCCTATAGGATACACTGGAACTTGCAGTTGTTTTTGTAACCATGCCAAAGATGAGCTCTC  791
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Sbjct  702   GCCTATAGGATACACTGGAACTTGCAGTTGTTTTTGTAACCATGCCAAAGATGAGCTCTC  643
Query  792   TAGACAGCTTGTTGAGTTGATGATAACTGCCGAAGCTGTTCGAATGTTGACAGTCTCACT  851
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Sbjct  642   TAGACAGCTTGTTGAGTTGATGATAACTGCCGAAGCTGTTCGAATGTTGACAGTCTCACT  583
Query  852   GTAAACCTTGAGTATACTCTCTAATGGCCCAAATGCTGAAGTTGGCAGGTCCTTGTACCT  911
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  582   GTAAACCTTGAGTATACTCTCTAATGGCCCAAATGCTGAAGTTGGCAGGTCCTTGTACCT  523
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**Fig 11: Query and subject sequence; 76E1 S318N and 76E1 wild type respectively. Codon ATT (reverse AAT) in query is asparagine while ACT (reverse AGT) in subject is serine.**

## 76E5 S311N

| Score | Expect | Identities | Gaps | Strand | Frame |
|---|---|---|---|---|---|
| 2002 bits(1084) | 0.0() | 1092/1098(99%) | 0/1098(0%) | Plus/Minus | |

Features:

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Query  72    CTACATGAAATTCATCATTTTTAAGGAATTGACAAAGTTGTCTAATGAGCTGAATGAGGA  131
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Sbjct  1362  CTACATGAAATTCATCATTTTTAAGGAATTGACAAAGTTGTCTAATGAGCTGAATGAGGA  1303

Query  132   ACCTCTACTCTTGACAGAGGCTTGAAGCTTCTCTTTCAAGTTGATAACTCTCTTCCTCAT  191
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1302  ACCTCTACTCTTGACAGAGGCTTGAAGCTTCTCTTTCAAGTTGATAACTCTCTTCCTCAT  1243

Query  192   TTCTGCTCCTTCTTCATCCATAATCAATCTCTCTACAGCTCTCTCCACTGTTCCTTTATC  251
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1242  TTCTGCTCCTTCTTCATCCATAATCAATCTCTCTACAGCTCTCTCCACTGTTCCTTTATC  1183

Query  252   CAGCTCTCCTTCCAATTGAACCCCAATTCTCCAAACTCTCTCTAAGTACCTCGCATTGAC  311
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1182  CAGCTCTCCTTCCAATTGAACCCCAATTCTCCAAACTCTCTCTAAGTACCTCGCATTGAC  1123

Query  312   TTTCTGATCTCCCGTAAAAGGCCTACAGATCATCGGAACTCCTTCCCCGATGCTCTCTAG  371
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Sbjct  1122  TTTCTGATCTCCCGTAAAAGGCCTACAGATCATCGGAACTCCTTCCCCGATGCTCTCTAG  1063

Query  372   GGTCGAGTTCCATCCGCAGTGACTCCAAAACCCTCCCACTGCAGGATGTCTGAGAACTTC  431
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Sbjct  1062  GGTCGAGTTCCATCCGCAGTGACTCCAAAACCCTCCCACTGCAGGATGTCTGAGAACTTC  1003

Query  432   TATCTGTGGTGCCCATTTCACAATGTAACCTCTTTCTGAAACCAACCCATTCAATTCCTC  491
             ||||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||
Sbjct  1002  TATCTGTGGTGCCCATTTCACAATGTAACCTCTTTCTGAAACCAACCCACTCAATTCCTC  943

Query  492   CGGTAAAGACTCTGTCCATTCCGAGCCGGGAATAGAACCCGGTCGGATCACCCATAAGAA  551
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  942   CGGTAAAGACTCTGTCCATTCCGAGCCGGGAATAGAACCCGGTCGGATCACCCATAAGAA  883

Query  552   AGGTTGGTTGCTATTACGTAAACCCCAAGCCATCTCCAACATGTCTTTAGTTTCCATTAG  611
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Sbjct  882   AGGTTGGTTGCTATTACGTAAACCCCAAGCCATCTCCAACATGTCTTTAGTTTCCATTAG  823

Query  612   AGCCAAGCTTCCCAAACTTATGTAAATCACTGAGCCTATTTTTTGCTTGTTCAACCACTC  671
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Sbjct  822   AGCCAAGCTTCCCAAACTTATGTAAATCACTGAGCCTATTTTTTGCTTGTTCAACCACTC  763

Query  672   AAGGCAACTCCTGTCCTCTTCAAGTAAACTAGAAGGCGCTGAAGCTGCAATGTGAAGTGG  731
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Sbjct  762   AAGGCAACTCCTGTCCTCTTCAAGTAAACTAGAAGGCGCTGAAGCTGCAATGTGAAGTGG  703

Query  732   GCCTATAGGATACACTGGAACTTGCAGTTGTTTTTGTAACCATGCCAAAGATGAGCTCTC  791
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Sbjct  702   GCCTATAGGATACACTGGAACTTGCAGTTGTTTTTGTAACCATGCCAAAGATGAGCTCTC  643
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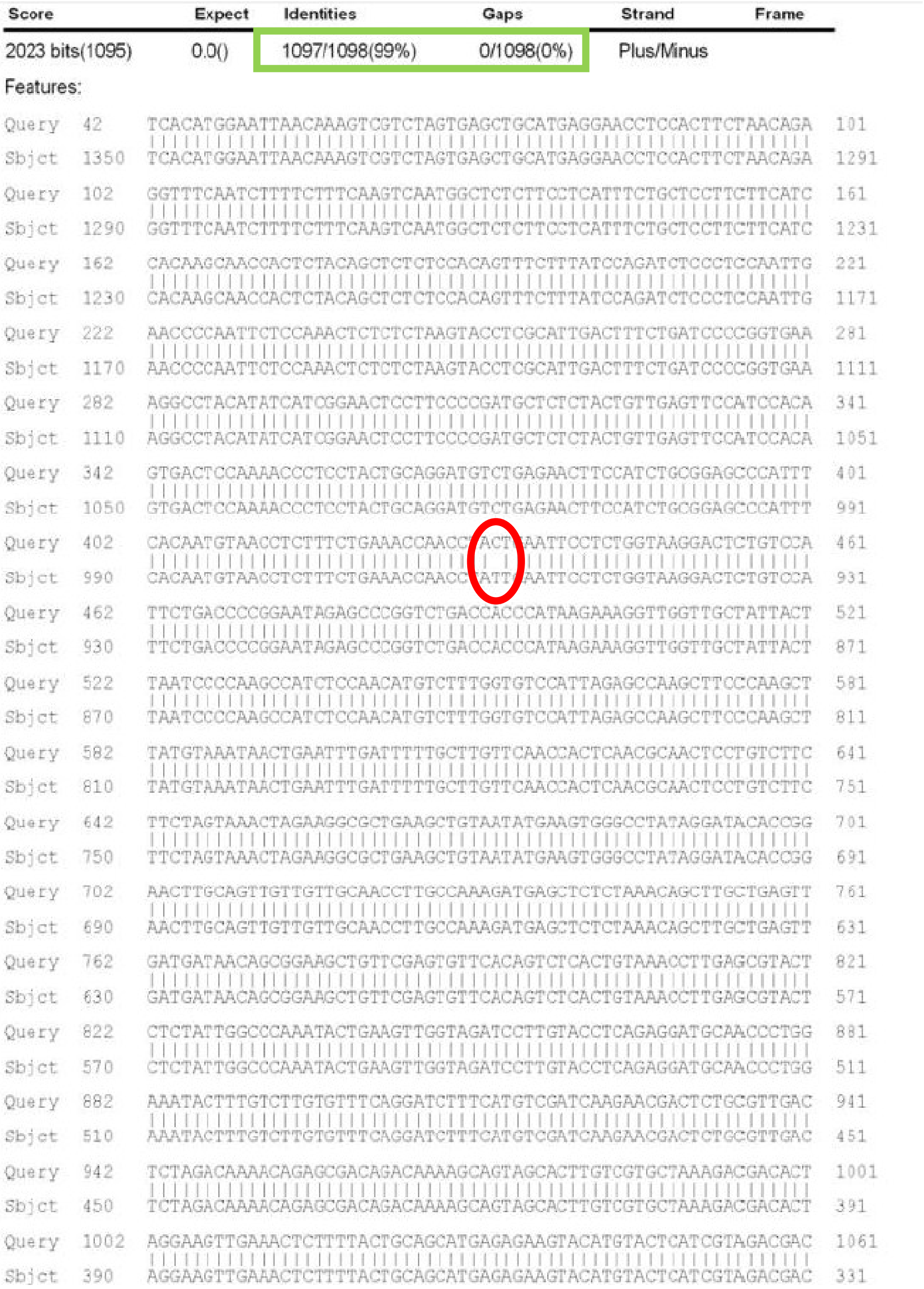
**Fig 12: Query and subject sequence; 76E5 S311N and 76E5 wild type respectively. Codon ATT (reverse AAT) in query is asparagine while ACT (reverse AGT)in subject is serine.**

### 76E2 N320S



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Score            Expect   Identities          Gaps          Strand      Frame
2023 bits(1095)  0.0()    1097/1098(99%)      0/1098(0%)    Plus/Minus
Features:

Query  42    TCACATGGAATTAACAAAGTCGTCTAGTGAGCTGCATGAGGAACCTCCACTTCTAACAGA  101
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Sbjct  1350  TCACATGGAATTAACAAAGTCGTCTAGTGAGCTGCATGAGGAACCTCCACTTCTAACAGA  1291

Query  102   GGTTTCAATCTTTTCTTTCAAGTCAATGGCTCTCTTCCTCATTTCTGCTCCTTCTTCATC  161
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Sbjct  1290  GGTTTCAATCTTTTCTTTCAAGTCAATGGCTCTCTTCCTCATTTCTGCTCCTTCTTCATC  1231

Query  162   CACAAGCAACCACTCTACAGCTCTCTCCACAGTTTCTTTATCCAGATCTCCCTCCAATTG  221
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Sbjct  1230  CACAAGCAACCACTCTACAGCTCTCTCCACAGTTTCTTTATCCAGATCTCCCTCCAATTG  1171

Query  222   AACCCCAATTCTCCAAACTCTCTCTAAGTACCTCGCATTGACTTTCTGATCCCCGGTGAA  281
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Sbjct  1170  AACCCCAATTCTCCAAACTCTCTCTAAGTACCTCGCATTGACTTTCTGATCCCCGGTGAA  1111

Query  282   AGGCCTACATATCATCGGAACTCCTTCCCCGATGCTCTCTACTGTTGAGTTCCATCCACA  341
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Sbjct  1110  AGGCCTACATATCATCGGAACTCCTTCCCCGATGCTCTCTACTGTTGAGTTCCATCCACA  1051

Query  342   GTGACTCCAAAACCCTCCTACTGCAGGATGTCTGAGAACTTCCATCTGCGGAGCCCATTT  401
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Sbjct  1050  GTGACTCCAAAACCCTCCTACTGCAGGATGTCTGAGAACTTCCATCTGCGGAGCCCATTT  991

Query  402   CACAATGTAACCTCTTTCTGAAACCAACCCACT[illegible]AATTCCTCTGGTAAGGACTCTGTCCA  461
             ||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||
Sbjct  990   CACAATGTAACCTCTTTCTGAAACCAACCCATT[illegible]AATTCCTCTGGTAAGGACTCTGTCCA  931

Query  462   TTCTGACCCCGGAATAGAGCCCGGTCTGACCACCCATAAGAAAGGTTGGTTGCTATTACT  521
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Sbjct  930   TTCTGACCCCGGAATAGAGCCCGGTCTGACCACCCATAAGAAAGGTTGGTTGCTATTACT  871

Query  522   TAATCCCCAAGCCATCTCCAACATGTCTTTGGTGTCCATTAGAGCCAAGCTTCCCAAGCT  581
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Sbjct  870   TAATCCCCAAGCCATCTCCAACATGTCTTTGGTGTCCATTAGAGCCAAGCTTCCCAAGCT  811

Query  582   TATGTAAATAACTGAATTTGATTTTTGCTTGTTCAACCACTCAACGCAACTCCTGTCTTC  641
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  810   TATGTAAATAACTGAATTTGATTTTTGCTTGTTCAACCACTCAACGCAACTCCTGTCTTC  751

Query  642   TTCTAGTAAACTAGAAGGCGCTGAAGCTGTAATATGAAGTGGGCCTATAGGATACACCGG  701
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Sbjct  750   TTCTAGTAAACTAGAAGGCGCTGAAGCTGTAATATGAAGTGGGCCTATAGGATACACCGG  691

Query  702   AACTTGCAGTTGTTGTTGCAACCTTGCCAAAGATGAGCTCTCTAAACAGCTTGCTGAGTT  761
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Sbjct  690   AACTTGCAGTTGTTGTTGCAACCTTGCCAAAGATGAGCTCTCTAAACAGCTTGCTGAGTT  631

Query  762   GATGATAACAGCGGAAGCTGTTCGAGTGTTCACAGTCTCACTGTAAACCTTGAGCGTACT  821
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Sbjct  630   GATGATAACAGCGGAAGCTGTTCGAGTGTTCACAGTCTCACTGTAAACCTTGAGCGTACT  571

Query  822   CTCTATTGGCCCAAATACTGAAGTTGGTAGATCCTTGTACCTCAGAGGATGCAACCCTGG  881
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Sbjct  570   CTCTATTGGCCCAAATACTGAAGTTGGTAGATCCTTGTACCTCAGAGGATGCAACCCTGG  511

Query  882   AAATACTTTGTCTTGTGTTTCAGGATCTTTCATGTCGATCAAGAACGACTCTGCGTTGAC  941
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Sbjct  510   AAATACTTTGTCTTGTGTTTCAGGATCTTTCATGTCGATCAAGAACGACTCTGCGTTGAC  451

Query  942   TCTAGACAAAACAGAGCGACAGACAAAAGCAGTAGCACTTGTCGTGCTAAAGACGACACT  1001
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Sbjct  450   TCTAGACAAAACAGAGCGACAGACAAAAGCAGTAGCACTTGTCGTGCTAAAGACGACACT  391

Query  1002  AGGAAGTTGAAACTCTTTTACTGCAGCATGAGAGAAGTACATGTACTCATCGTAGACGAC  1061
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Sbjct  390   AGGAAGTTGAAACTCTTTTACTGCAGCATGAGAGAAGTACATGTACTCATCGTAGACGAC  331
```
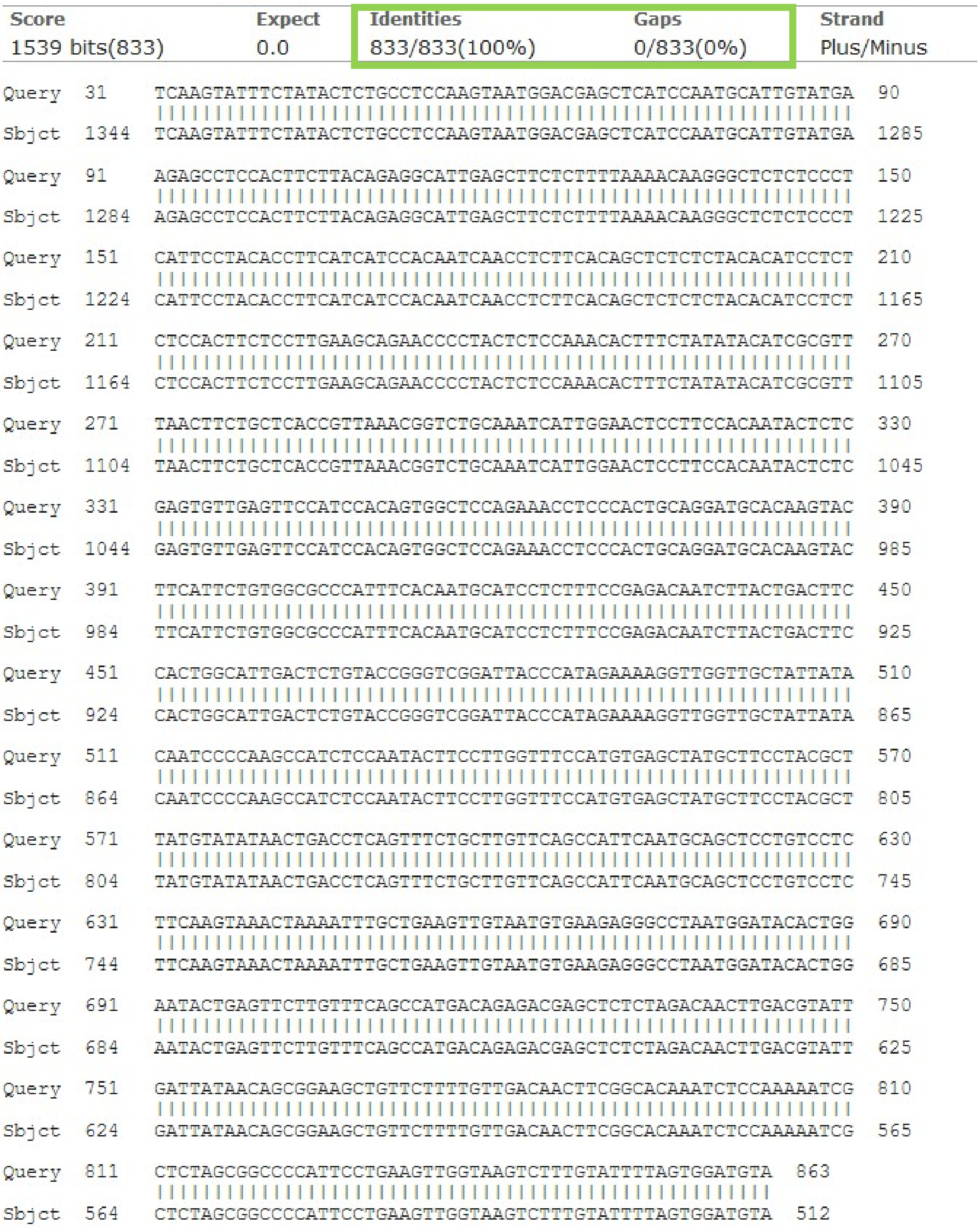

**Fig 13: Query and subject sequence; 76E2 N320S and 76E2 wild type respectively. Codon ACT (reverse AGT) in query is serine while ATT (reverse AAT) in subject is asparagine.**

## WILD TYPE SEQUENCING

## 76E5



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Sbjct  1344  TCAAGTATTTCTATACTCTGCCTCCAAGTAATGGACGAGCTCATCCAATGCATTGTATGA  1285

Query  91    AGAGCCTCCACTTCTTACAGAGGCATTGAGCTTCTCTTTTAAAACAAGGGCTCTCTCCCT  150
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Sbjct  1284  AGAGCCTCCACTTCTTACAGAGGCATTGAGCTTCTCTTTTAAAACAAGGGCTCTCTCCCT  1225

Query  151   CATTCCTACACCTTCATCATCCACAATCAACCTCTTCACAGCTCTCTCTACACATCCTCT  210
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Sbjct  1224  CATTCCTACACCTTCATCATCCACAATCAACCTCTTCACAGCTCTCTCTACACATCCTCT  1165

Query  211   CTCCACTTCTCCTTGAAGCAGAACCCCTACTCTCCAAACACTTTCTATATACATCGCGTT  270
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Sbjct  1164  CTCCACTTCTCCTTGAAGCAGAACCCCTACTCTCCAAACACTTTCTATATACATCGCGTT  1105

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Sbjct  1104  TAACTTCTGCTCACCGTTAAACGGTCTGCAAATCATTGGAACTCCTTCCACAATACTCTC  1045

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Sbjct  864   CAATCCCCAAGCCATCTCCAATACTTCCTTGGTTTCCATGTGAGCTATGCTTCCTACGCT  805

Query  571   TATGTATATAACTGACCTCAGTTTCTGCTTGTTCAGCCATTCAATGCAGCTCCTGTCCTC  630
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Sbjct  804   TATGTATATAACTGACCTCAGTTTCTGCTTGTTCAGCCATTCAATGCAGCTCCTGTCCTC  745

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Sbjct  684   AATACTGAGTTCTTGTTTCAGCCATGACAGAGACGAGCTCTCTAGACAACTTGACGTATT  625

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Sbjct  624   GATTATAACAGCGGAAGCTGTTCTTTTGTTGACAACTTCGGCACAAATCTCCAAAAATCG  565

Query  811   CTCTAGCGGCCCCATTCCTGAAGTTGGTAAGTCTTTGTATTTTAGTGGATGTA  863
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Sbjct  564   CTCTAGCGGCCCCATTCCTGAAGTTGGTAAGTCTTTGTATTTTAGTGGATGTA  512
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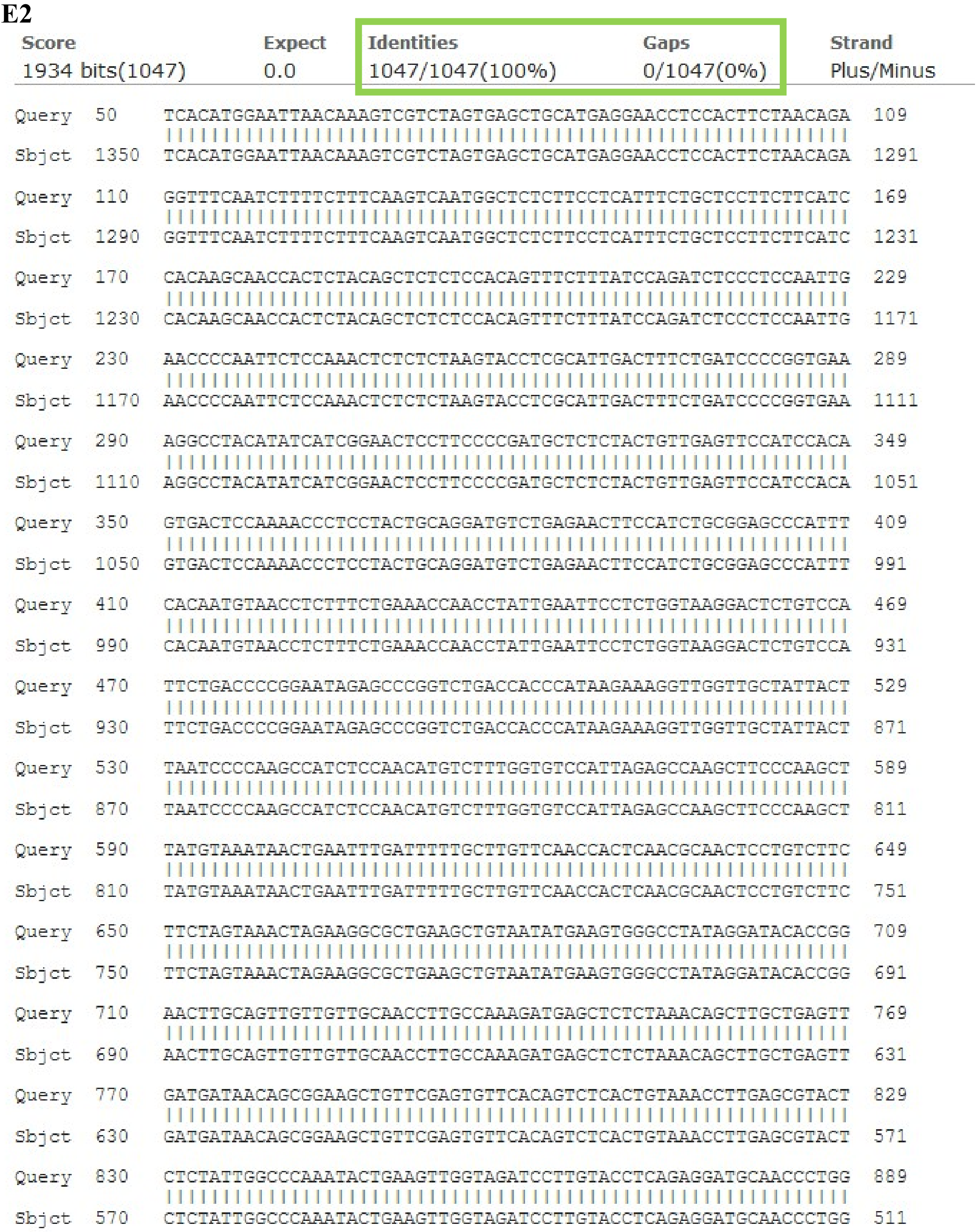
**Fig 14: Query and subject sequence; 76E5 recombinant plasmid and 76E5 sequence on database respectively.**

**76E2**



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Query  110   GGTTTCAATCTTTTCTTTCAAGTCAATGGCTCTCTTCCTCATTTCTGCTCCTTCTTCATC  169
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Sbjct  1230  CACAAGCAACCACTCTACAGCTCTCTCCACAGTTTCTTTATCCAGATCTCCCTCCAATTG  1171

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Sbjct  1170  AACCCCAATTCTCCAAACTCTCTCTAAGTACCTCGCATTGACTTTCTGATCCCCGGTGAA  1111

Query  290   AGGCCTACATATCATCGGAACTCCTTCCCCGATGCTCTCTACTGTTGAGTTCCATCCACA  349
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Sbjct  1110  AGGCCTACATATCATCGGAACTCCTTCCCCGATGCTCTCTACTGTTGAGTTCCATCCACA  1051

Query  350   GTGACTCCAAAACCCTCCTACTGCAGGATGTCTGAGAACTTCCATCTGCGGAGCCCATTT  409
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Sbjct  1050  GTGACTCCAAAACCCTCCTACTGCAGGATGTCTGAGAACTTCCATCTGCGGAGCCCATTT  991

Query  410   CACAATGTAACCTCTTTCTGAAACCAACCTATTGAATTCCTCTGGTAAGGACTCTGTCCA  469
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Sbjct  990   CACAATGTAACCTCTTTCTGAAACCAACCTATTGAATTCCTCTGGTAAGGACTCTGTCCA  931

Query  470   TTCTGACCCCGGAATAGAGCCCGGTCTGACCACCCATAAGAAAGGTTGGTTGCTATTACT  529
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Sbjct  930   TTCTGACCCCGGAATAGAGCCCGGTCTGACCACCCATAAGAAAGGTTGGTTGCTATTACT  871

Query  530   TAATCCCCAAGCCATCTCCAACATGTCTTTGGTGTCCATTAGAGCCAAGCTTCCCAAGCT  589
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Sbjct  810   TATGTAAATAACTGAATTTGATTTTTGCTTGTTCAACCACTCAACGCAACTCCTGTCTTC  751

Query  650   TTCTAGTAAACTAGAAGGCGCTGAAGCTGTAATATGAAGTGGGCCTATAGGATACACCGG  709
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Sbjct  690   AACTTGCAGTTGTTGTTGCAACCTTGCCAAAGATGAGCTCTCTAAACAGCTTGCTGAGTT  631

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Sbjct  570   CTCTATTGGCCCAAATACTGAAGTTGGTAGATCCTTGTACCTCAGAGGATGCAACCCTGG  511
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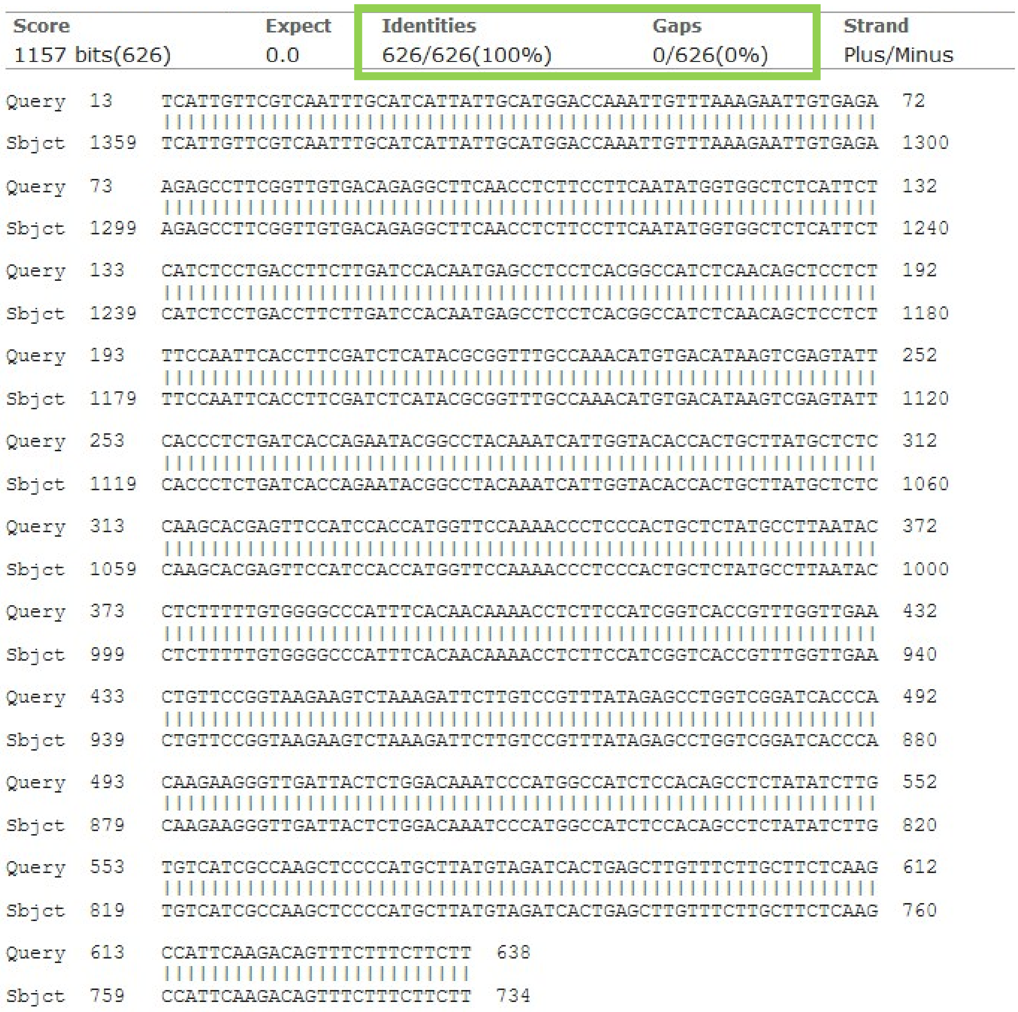
**Fig 15: Query and subject sequence; 76E2 recombinant plasmid and 76E2 sequence on database respectively.**

## 76D1



| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 1157 bits(626) | 0.0 | 626/626(100%) | 0/626(0%) | Plus/Minus |

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Sbjct  1359  TCATTGTTCGTCAATTTGCATCATTATTGCATGGACCAAATTGTTTAAAGAATTGTGAGA  1300

Query  73    AGAGCCTTCGGTTGTGACAGAGGCTTCAACCTCTTCCTTCAATATGGTGGCTCTCATTCT  132
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Sbjct  1299  AGAGCCTTCGGTTGTGACAGAGGCTTCAACCTCTTCCTTCAATATGGTGGCTCTCATTCT  1240

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Sbjct  1239  CATCTCCTGACCTTCTTGATCCACAATGAGCCTCCTCACGGCCATCTCAACAGCTCCTCT  1180

Query  193   TTCCAATTCACCTTCGATCTCATACGCGGTTTGCCAAACATGTGACATAAGTCGAGTATT  252
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Sbjct  1179  TTCCAATTCACCTTCGATCTCATACGCGGTTTGCCAAACATGTGACATAAGTCGAGTATT  1120

Query  253   CACCCTCTGATCACCAGAATACGGCCTACAAATCATTGGTACACCACTGCTTATGCTCTC  312
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Sbjct  1119  CACCCTCTGATCACCAGAATACGGCCTACAAATCATTGGTACACCACTGCTTATGCTCTC  1060

Query  313   CAAGCACGAGTTCCATCCACCATGGTTCCAAAACCCTCCCACTGCTCTATGCCTTAATAC  372
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Sbjct  1059  CAAGCACGAGTTCCATCCACCATGGTTCCAAAACCCTCCCACTGCTCTATGCCTTAATAC  1000

Query  373   CTCTTTTTGTGGGGCCCATTTCACAACAAAACCTCTTCCATCGGTCACCGTTTGGTTGAA  432
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Sbjct  999   CTCTTTTTGTGGGGCCCATTTCACAACAAAACCTCTTCCATCGGTCACCGTTTGGTTGAA  940

Query  433   CTGTTCCGGTAAGAAGTCTAAAGATTCTTGTCCGTTTATAGAGCCTGGTCGGATCACCCA  492
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Sbjct  939   CTGTTCCGGTAAGAAGTCTAAAGATTCTTGTCCGTTTATAGAGCCTGGTCGGATCACCCA  880

Query  493   CAAGAAGGGTTGATTACTCTGGACAAATCCCATGGCCATCTCCACAGCCTCTATATCTTG  552
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Sbjct  879   CAAGAAGGGTTGATTACTCTGGACAAATCCCATGGCCATCTCCACAGCCTCTATATCTTG  820

Query  553   TGTCATCGCCAAGCTCCCCATGCTTATGTAGATCACTGAGCTTGTTTCTTGCTTCTCAAG  612
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  819   TGTCATCGCCAAGCTCCCCATGCTTATGTAGATCACTGAGCTTGTTTCTTGCTTCTCAAG  760

Query  613   CCATTCAAGACAGTTTCTTTCTTCTT  638
             ||||||||||||||||||||||||||
Sbjct  759   CCATTCAAGACAGTTTCTTTCTTCTT  734
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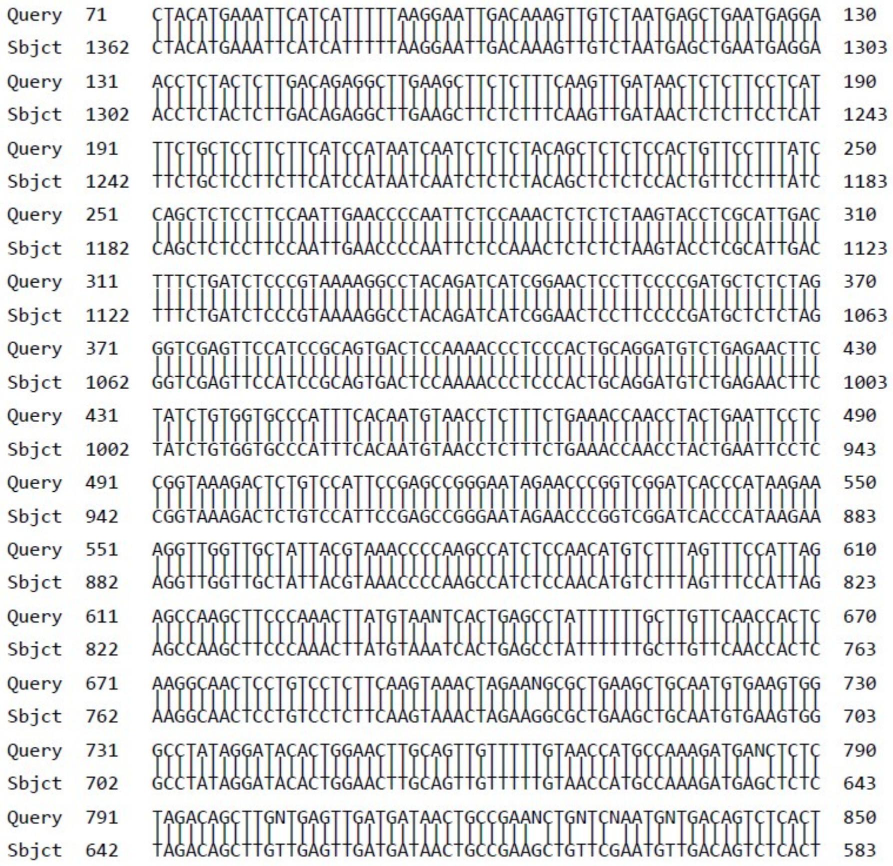
Fig 16: Query and subject sequence; 76D1 recombinant plasmid and 76D1 sequence on database respectively.

## 76E1



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Query  131   ACCTCTACTCTTGACAGAGGCTTGAAGCTTCTCTTTCAAGTTGATAACTCTCTTCCTCAT  190
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Sbjct  1302  ACCTCTACTCTTGACAGAGGCTTGAAGCTTCTCTTTCAAGTTGATAACTCTCTTCCTCAT  1243

Query  191   TTCTGCTCCTTCTTCATCCATAATCAATCTCTCTACAGCTCTCTCCACTGTTCCTTTATC  250
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Sbjct  1242  TTCTGCTCCTTCTTCATCCATAATCAATCTCTCTACAGCTCTCTCCACTGTTCCTTTATC  1183

Query  251   CAGCTCTCCTTCCAATTGAACCCCAATTCTCCAAACTCTCTCTAAGTACCTCGCATTGAC  310
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Sbjct  1182  CAGCTCTCCTTCCAATTGAACCCCAATTCTCCAAACTCTCTCTAAGTACCTCGCATTGAC  1123

Query  311   TTTCTGATCTCCCGTAAAAGGCCTACAGATCATCGGAACTCCTTCCCCGATGCTCTCTAG  370
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Sbjct  1122  TTTCTGATCTCCCGTAAAAGGCCTACAGATCATCGGAACTCCTTCCCCGATGCTCTCTAG  1063

Query  371   GGTCGAGTTCCATCCGCAGTGACTCCAAAACCCTCCCACTGCAGGATGTCTGAGAACTTC  430
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Sbjct  1062  GGTCGAGTTCCATCCGCAGTGACTCCAAAACCCTCCCACTGCAGGATGTCTGAGAACTTC  1003

Query  431   TATCTGTGGTGCCCATTTCACAATGTAACCTCTTTCTGAAACCAACCTACTGAATTCCTC  490
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Sbjct  1002  TATCTGTGGTGCCCATTTCACAATGTAACCTCTTTCTGAAACCAACCTACTGAATTCCTC  943

Query  491   CGGTAAAGACTCTGTCCATTCCGAGCCGGGAATAGAACCCGGTCGGATCACCCATAAGAA  550
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Sbjct  942   CGGTAAAGACTCTGTCCATTCCGAGCCGGGAATAGAACCCGGTCGGATCACCCATAAGAA  883

Query  551   AGGTTGGTTGCTATTACGTAAACCCCAAGCCATCTCCAACATGTCTTTAGTTTCCATTAG  610
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Sbjct  882   AGGTTGGTTGCTATTACGTAAACCCCAAGCCATCTCCAACATGTCTTTAGTTTCCATTAG  823

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Query  731   GCCTATAGGATACACTGGAACTTGCAGTTGTTTTTGTAACCATGCCAAAGATGANCTCTC  790
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Query  791   TAGACAGCTTGNTGAGTTGATGATAACTGCCGAANCTGNTCNAATGNTGACAGTCTCACT  850
             ||||||||||| |||||||||||||||||||||| ||| || |||| |||||||||||||
Sbjct  642   TAGACAGCTTGTTGAGTTGATGATAACTGCCGAAGCTGTTCGAATGTTGACAGTCTCACT  583
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**Fig 17: Query and subject sequence; 76E1 recombinant plasmid and 76E1 sequence on database respectively.**

# MUTAGENESIS EXPERIMENTS - MASS SPECTRA

## 76E2 N320S DONOR SCREEN (New activity)

### UDP Galactose + Kaempferol

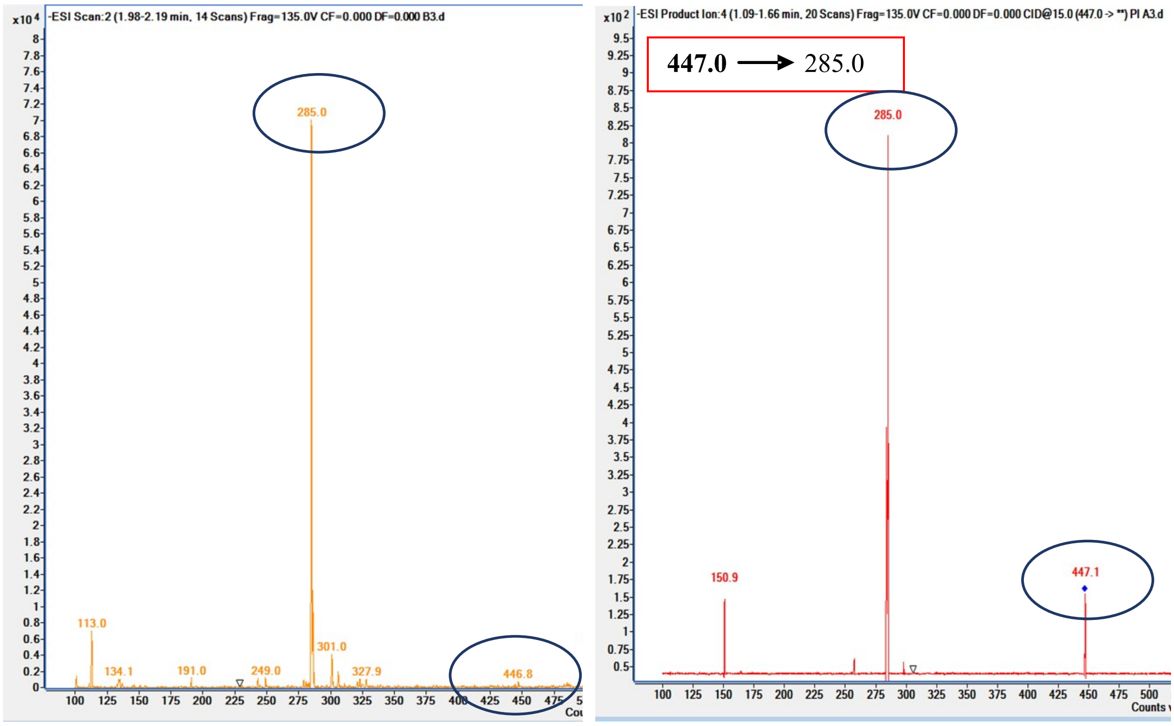


Fig 18a: MS spectrum of 76E2 N320S glycosylation of kaempferol (Full scan) 18b: MS spectrum showing fragmentation of kaempferol galactoside (Product ion scan)

### UDP Galactose + Quercetin

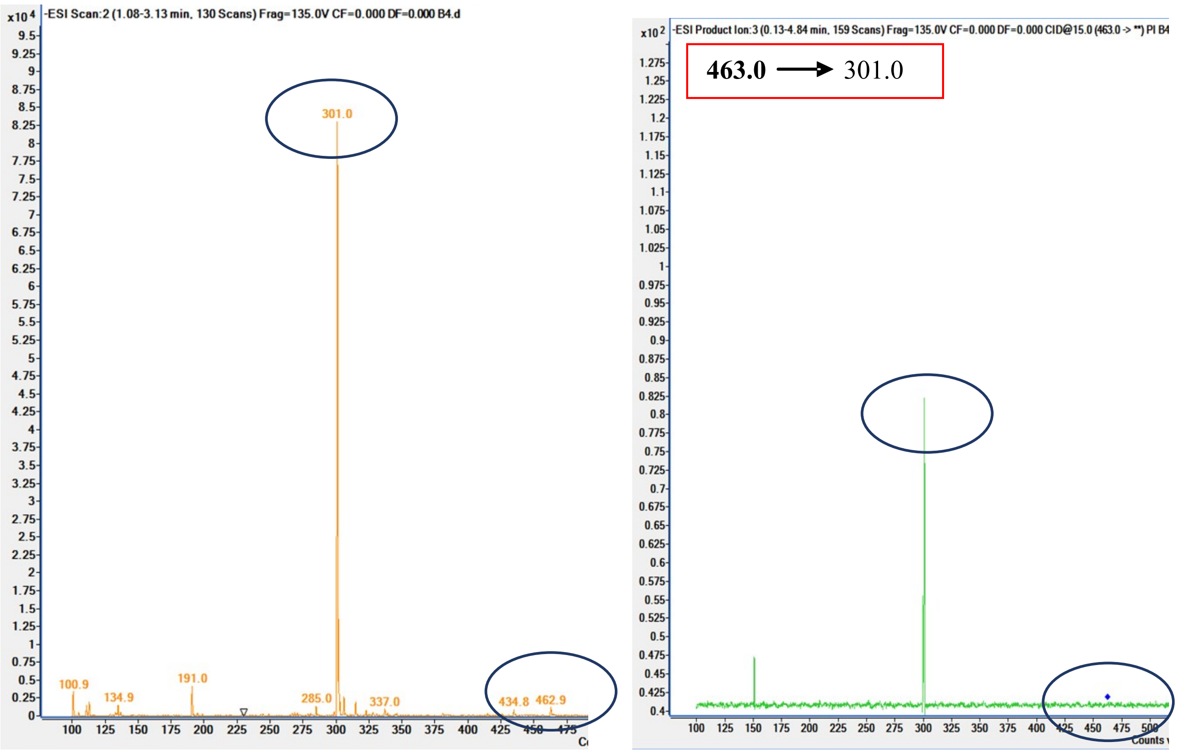


Fig 19a: MS spectrum of 76E2 N320S glycosylation of quercetin (Full scan) 19b: MS spectrum showing fragmentation of quercetin galactoside (Product ion scan)

### UDP GlcNAc + Kaempferol

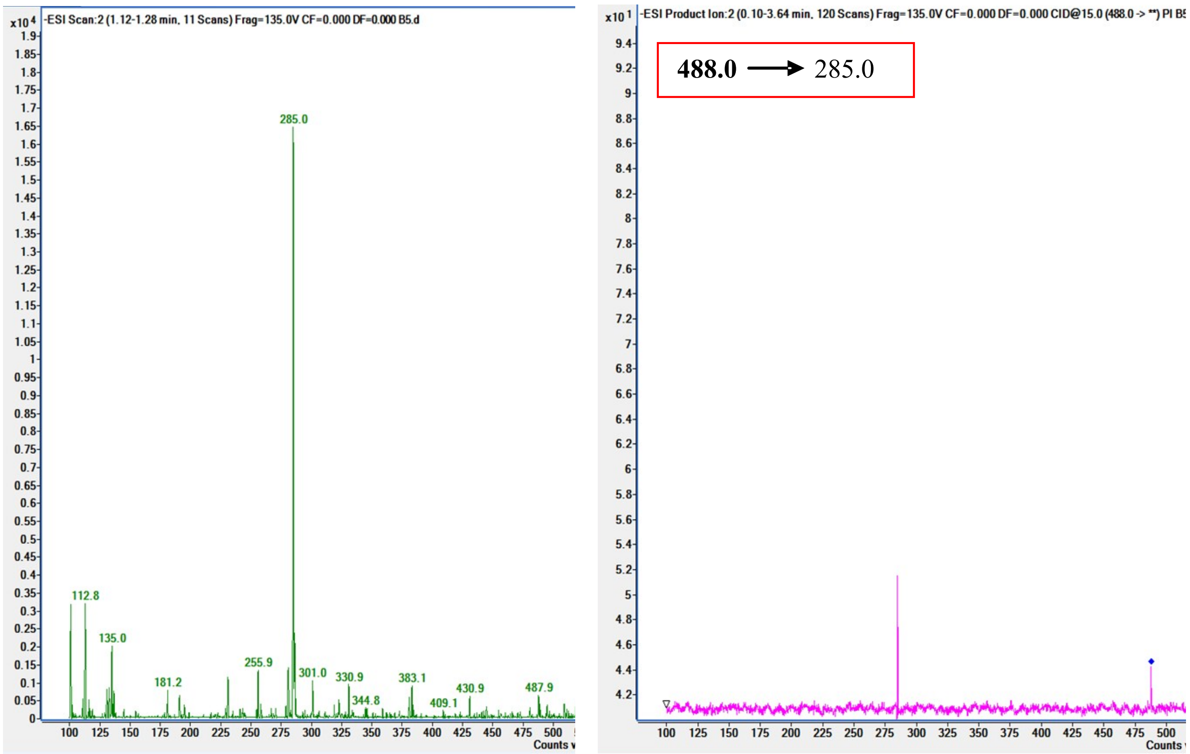


**Fig 20a: MS spectrum of 76E2 N320S glycosylation of kaempferol (Full scan) 20b: MS spectrum showing fragmentation of kaempferol glycoside (Product ion scan)**

### UDP GlcNAc + Quercetin

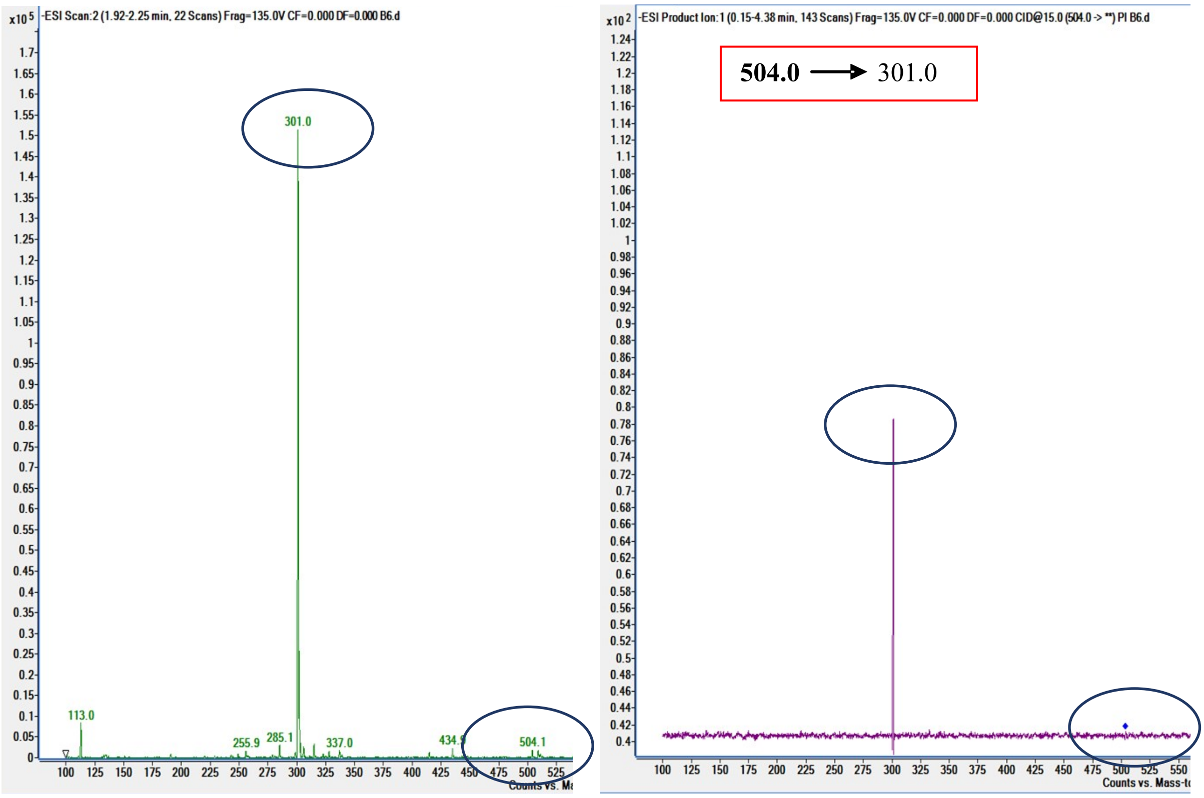


**Fig 21a: MS spectrum of 76E2 N320S glycosylation of quercetin (Full scan) 21b: MS spectrum showing fragmentation of quercetin glycoside (Product ion scan)**

## UDP Glucose + Kaempferol

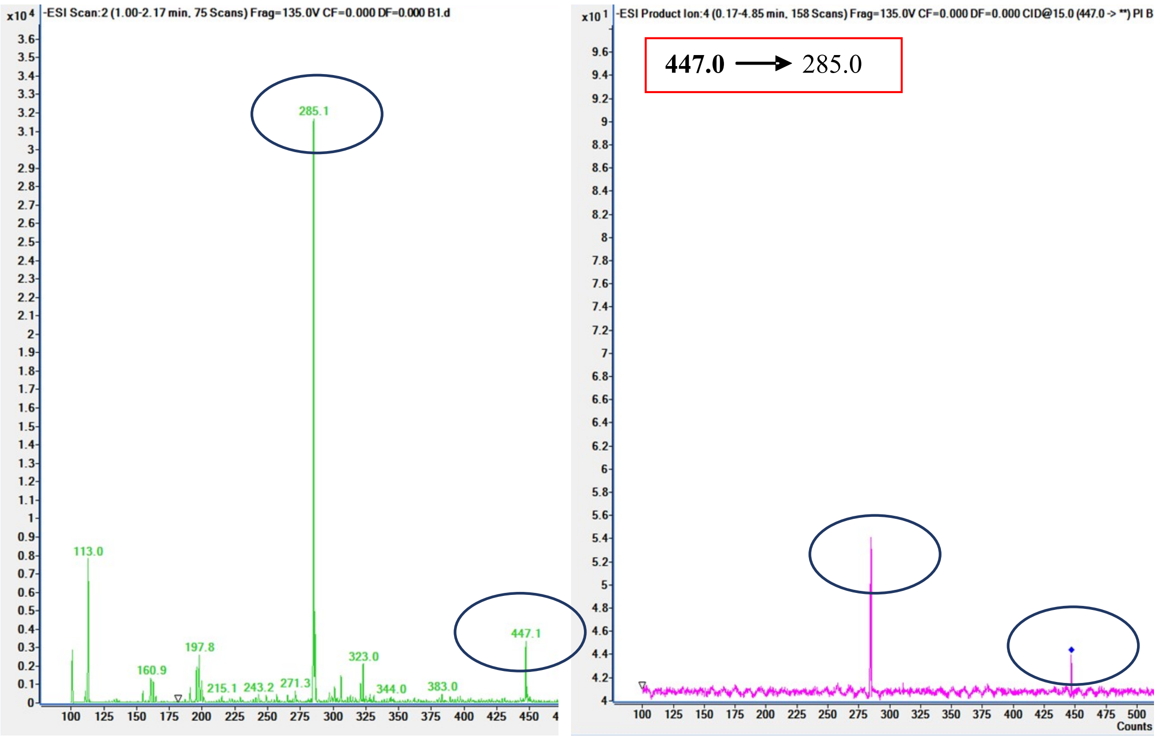


**Fig 22a: MS spectrum of 76E2 N320S glycosylation of kaempferol (Full scan) 22b: MS spectrum showing fragmentation of kaempferol glucoside (Product ion scan)**

## UDP Glucose + Quercetin

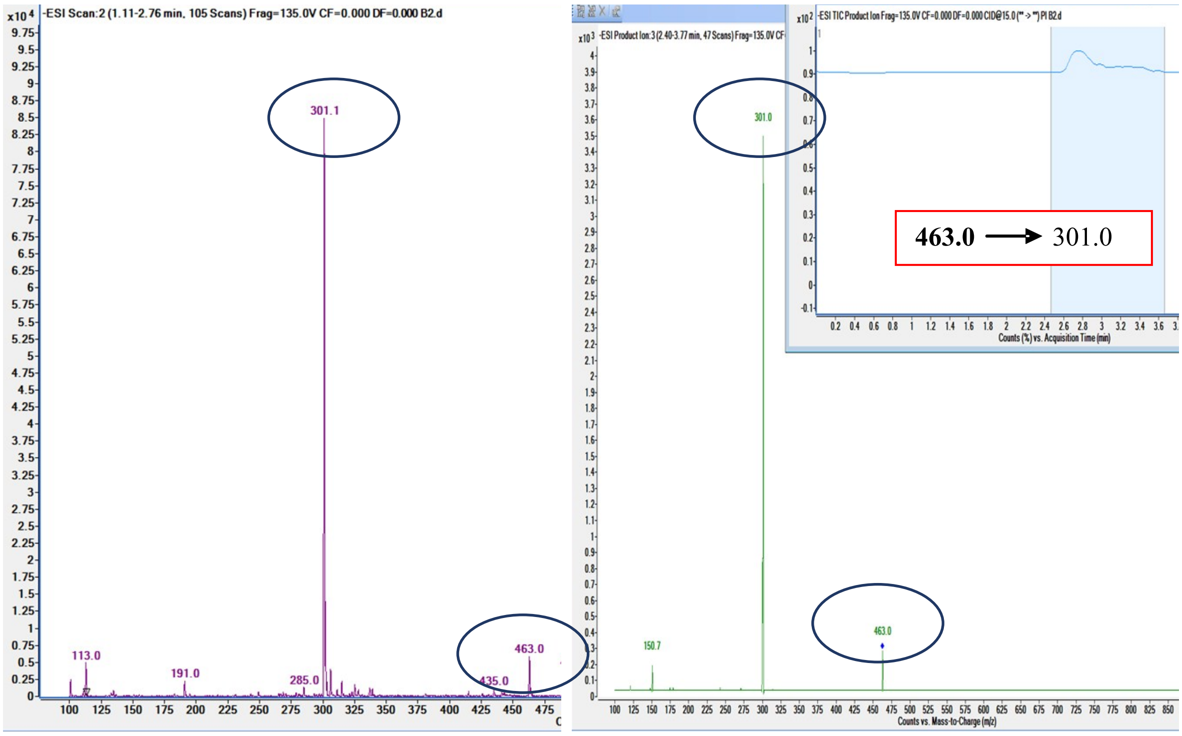
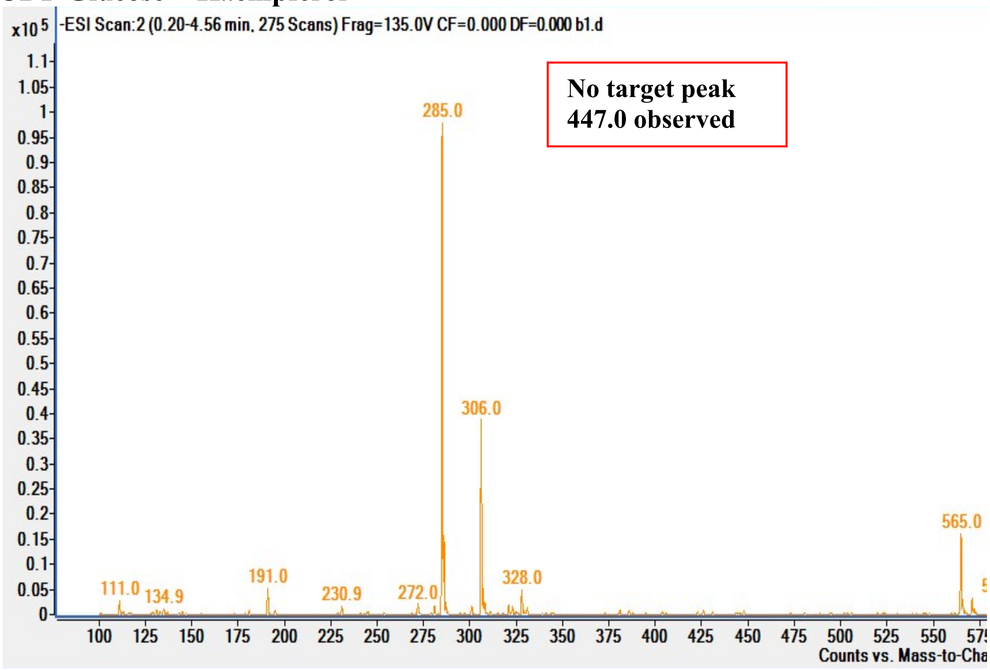


**Fig 23a: MS spectrum of 76E2 N320S glycosylation of quercetin (Full scan) 23b: MS spectrum showing fragmentation of quercetin glucoside (Product ion scan)**

## 76E1 S318N and 76E5 S311N DONOR SCREEN (Loss of activity)

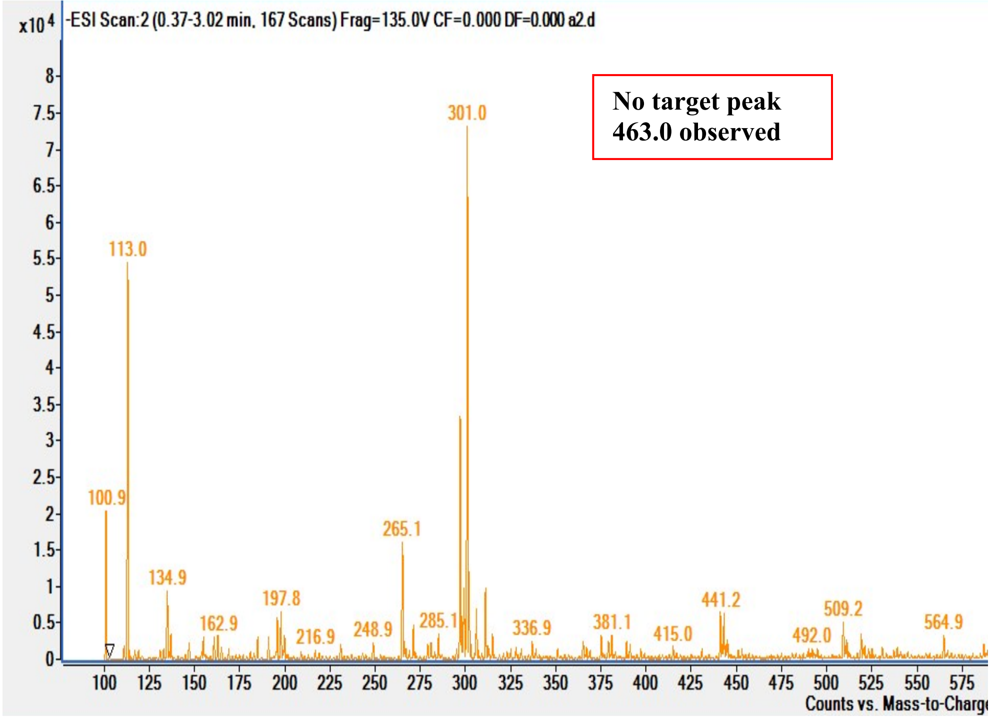
### UDP Glucose + Kaempferol



No target peak
447.0 observed

Fig 24a: MS spectrum of asparagine mutants showing no glycosylation with kaempferol (Product ion scan)

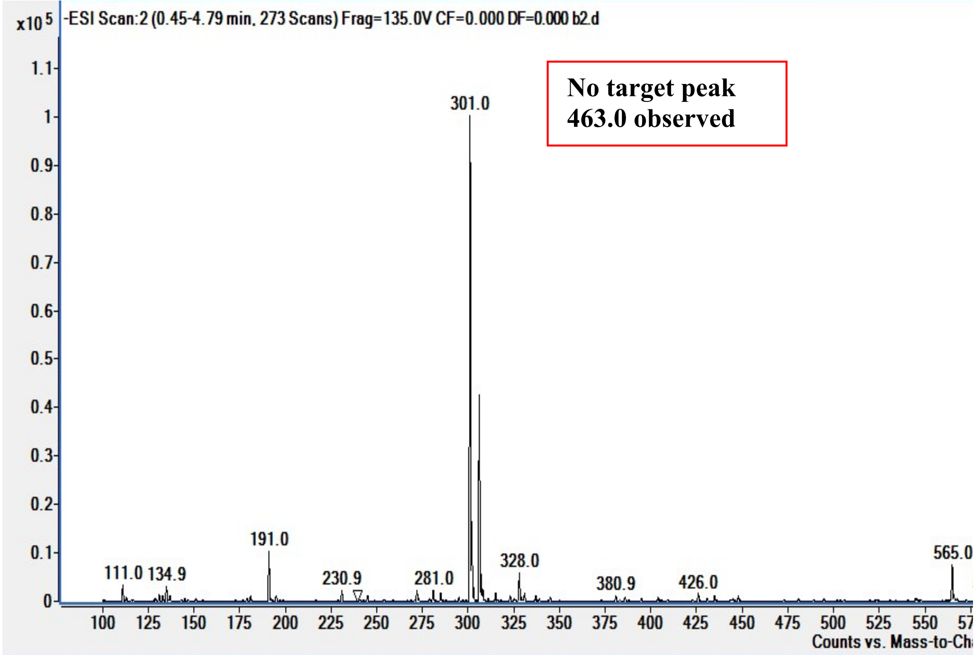
### UDP Glucose + Quercetin



No target peak
463.0 observed

Fig 24b: MS spectrum of asparagine mutants showing no glycosylation with quercetin (Product ion scan)

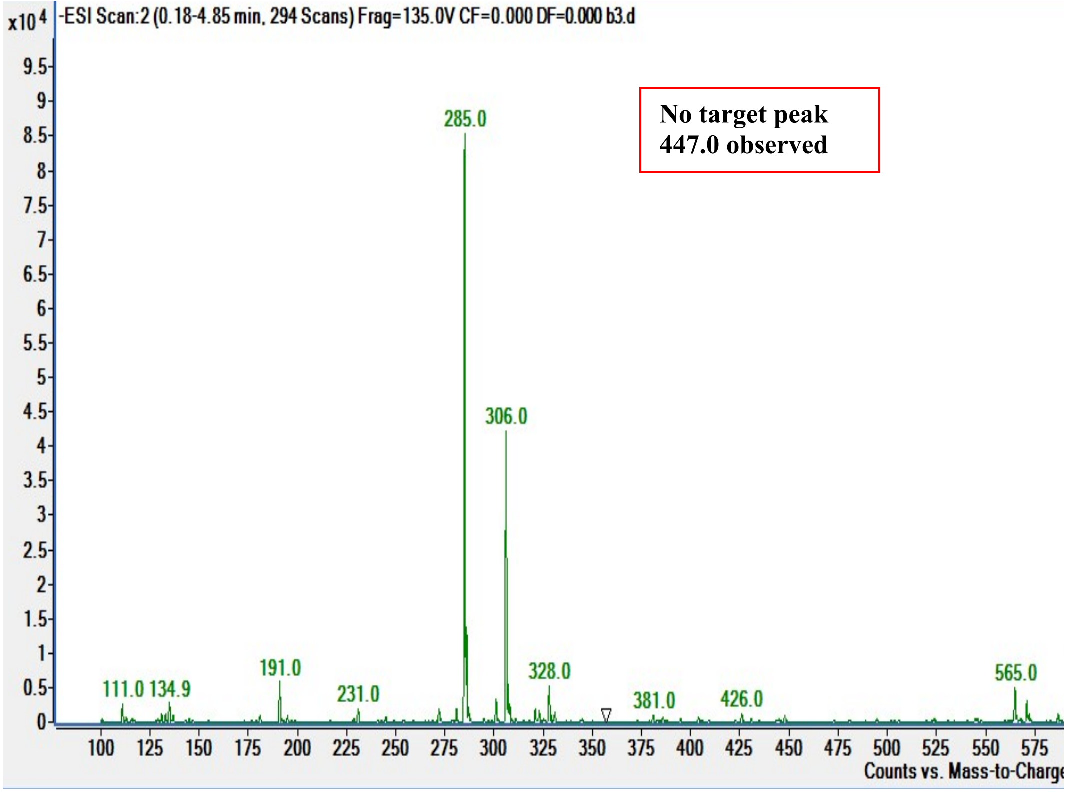
**UDP Galactose + Quercetin**



No target peak
463.0 observed

Fig 24c: MS spectrum of asparagine mutants showing no glycosylation with quercetin (Product ion scan)

**UDP Galactose + Kaempferol**



No target peak
447.0 observed

Fig 24d: MS spectrum of asparagine mutants showing no glycosylation with kaempferol (Product ion scan)

## UDP GlcNAc + Kaempferol

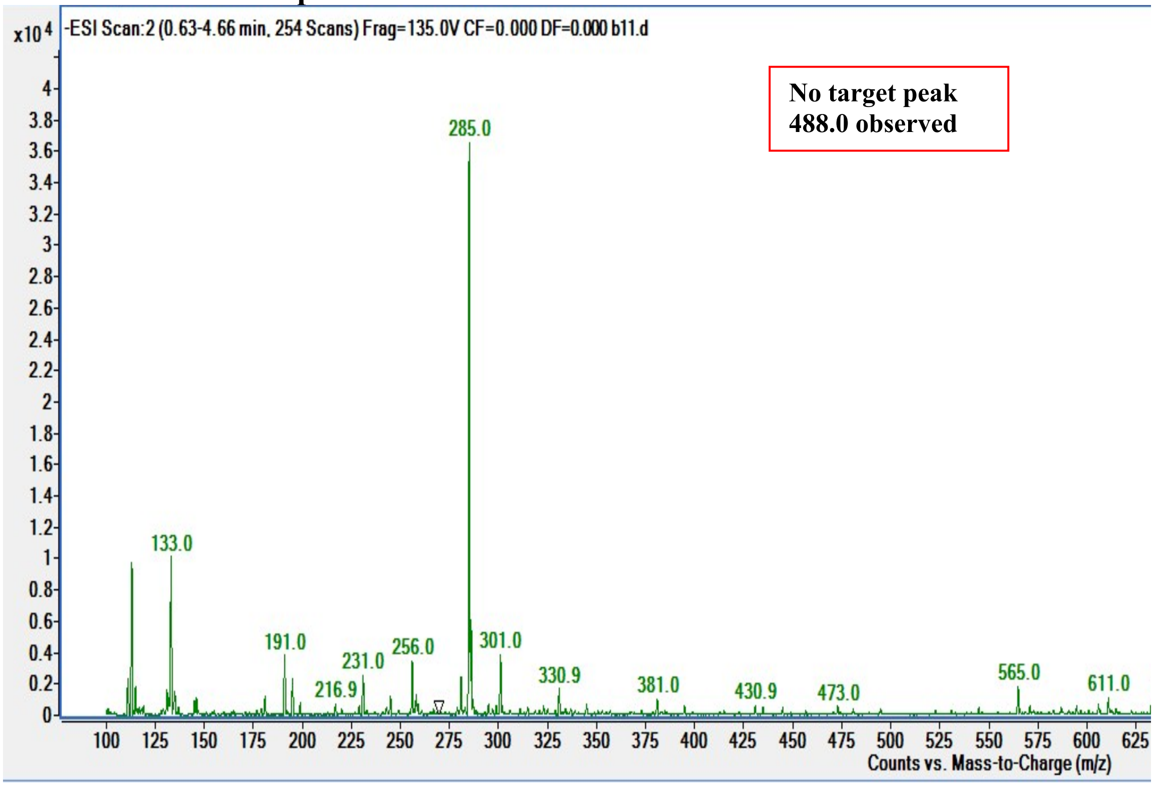


**Fig 24e: MS spectrum of asparagine mutants showing no glycosylation with kaempferol (Product ion scan)**

## UDP GlcNAc + Quercetin

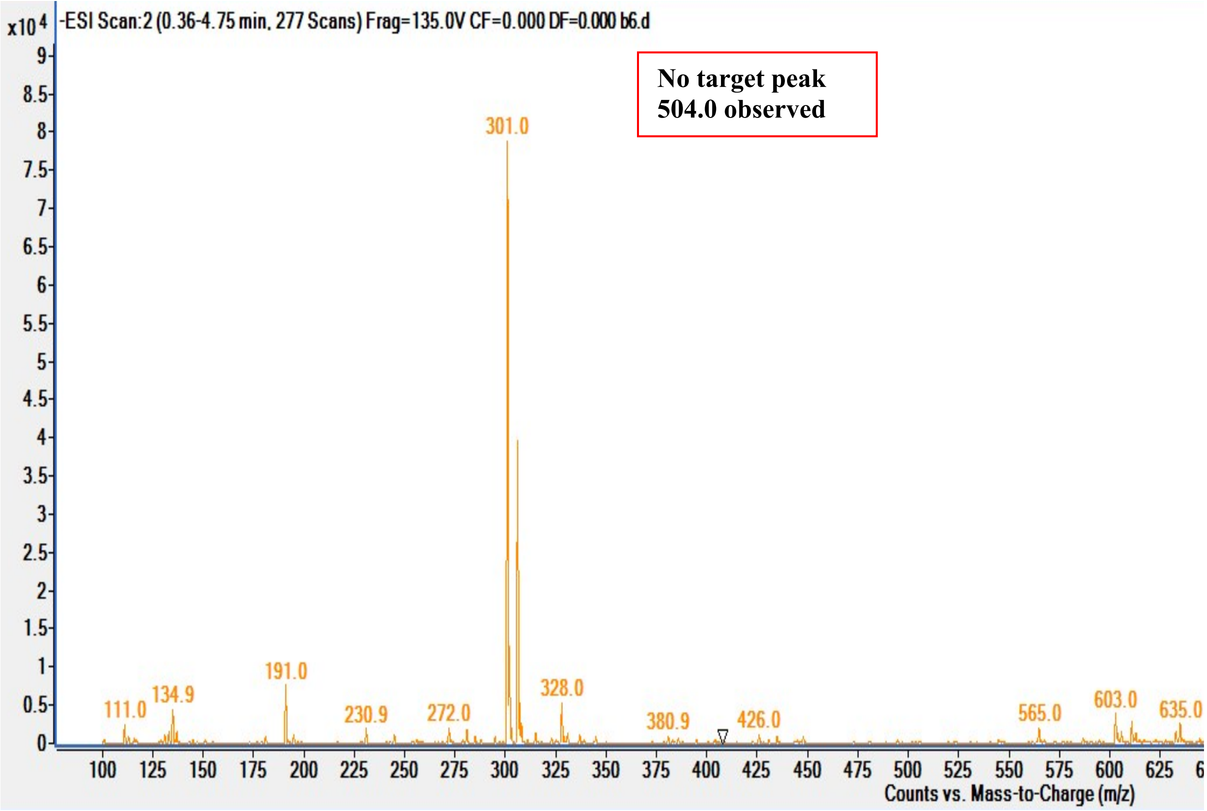
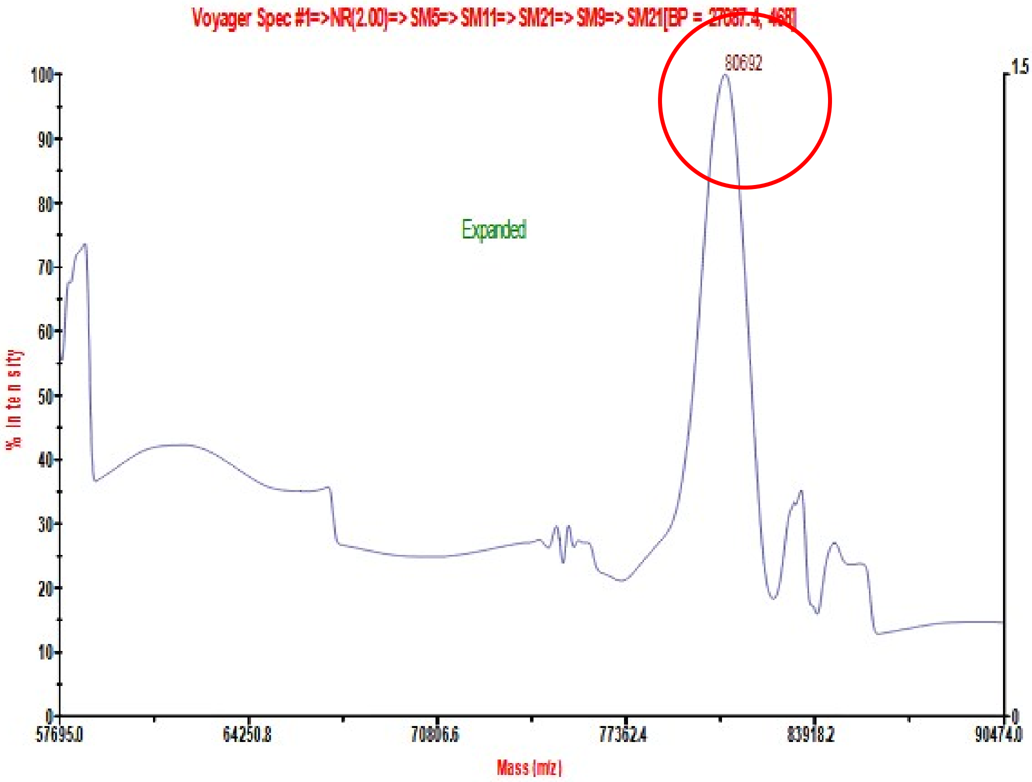
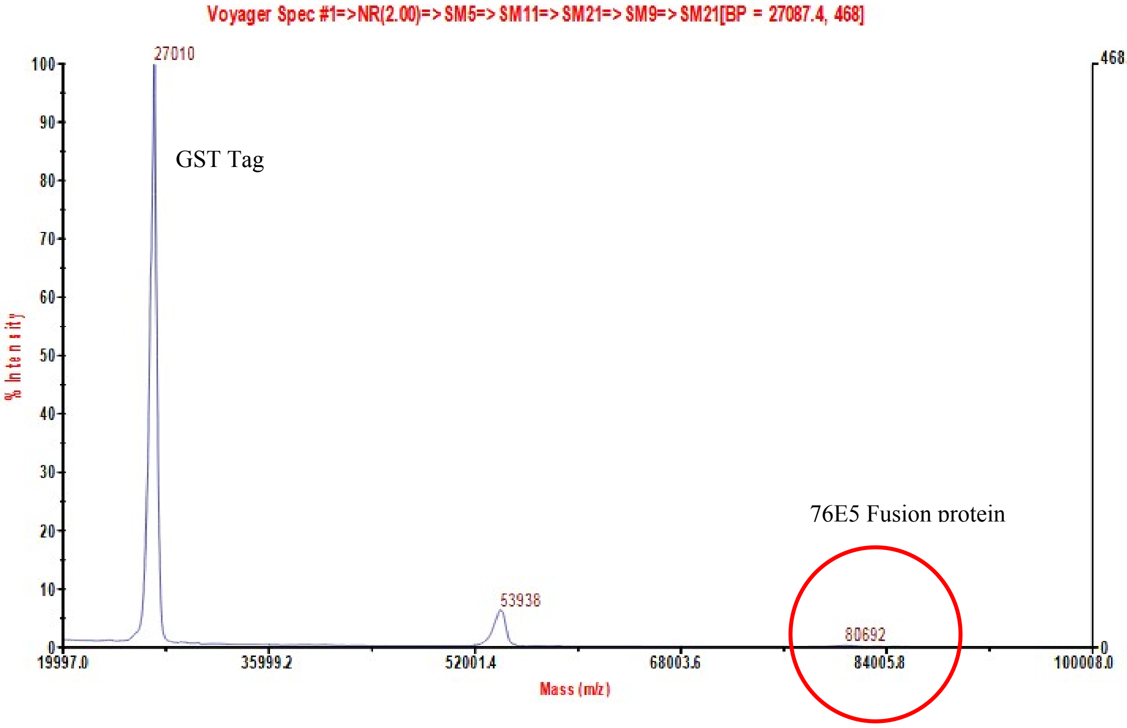


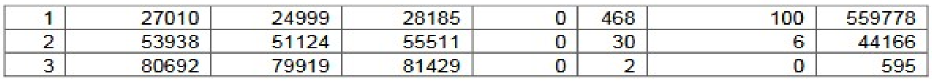
**Fig 24f: MS spectrum of asparagine mutants showing no glycosylation with quercetin (Product ion scan)**

## MALDI-TOF CONFIRMATION OF PROTEIN EXPRESSION

### 1. 76E5







| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | 27010 | 24999 | 28185 | 0 | 468 | 100 | 559778 |
| 2 | 53938 | 51124 | 55511 | 0 | 30 | 6 | 44166 |
| 3 | 80692 | 79919 | 81429 | 0 | 2 | 0 | 595 |

**Fig 25: MALDI spectrum to confirm protein expression**

The empirical determination of 76E5 molecular weight (using our sequenced DNA data, was converted into protein sequence and lastly used to calculate the weight) was determined. This was confirmed by the MALDI-TOF data, 80.69kda.

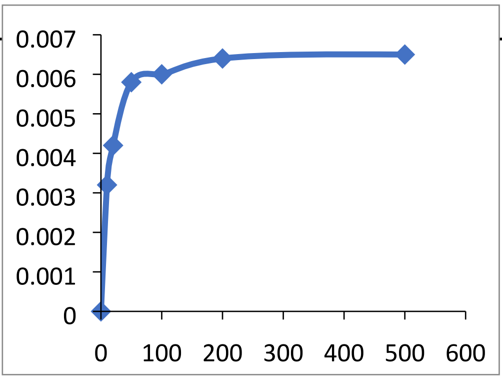
https://web.expasy.org/translate/ (DNA sequence conversion to protein sequence)
https://www.bioinformatics.org/sms/prot_mw.html (protein sequence changed to molecular weight)

## KINETICS DATA

### 1) 76E1 UDP GLUCOSE

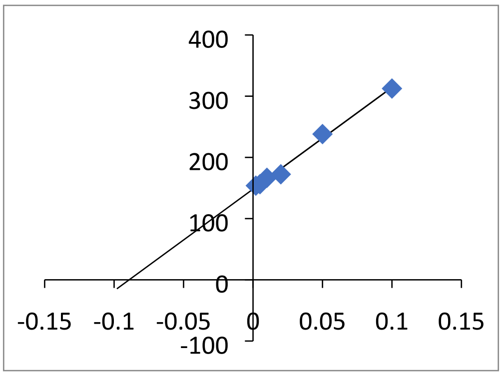
**Michaelis menten plot**

| S(uM) | V (uM/min) |
|---|---|
| 0 | 0 |
| 10 | 0.0032 |
| 20 | 0.0042 |
| 50 | 0.0058 |
| 100 | 0.006 |
| 200 | 0.0064 |
| 500 | 0.0065 |



**Lineweaver Burk**

| 1/S | 1/V |
|---|---|
| 0.1 | 312.5 |
| 0.05 | 238.0952 |
| 0.02 | 172.4138 |
| 0.01 | 166.6667 |
| 0.005 | 156.25 |
| 0.002 | 153.8462 |



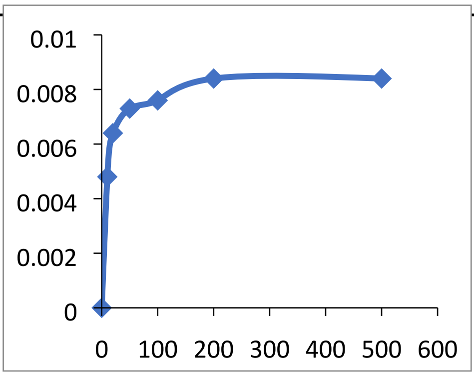
| | |
|---|---|
| **Km** | 10.5± 1.38 µM |
| **$k_{cat}/K_M$** | 0.79 $s^{-1}mM^{-1}$ |
| **kcat** | 0.00832 $s^{-1}$ |

**Fig 26: Michaelis menten and Lineweaver-Burk plots for 76E1**

## 2) 76E2 UDP GLUCOSE

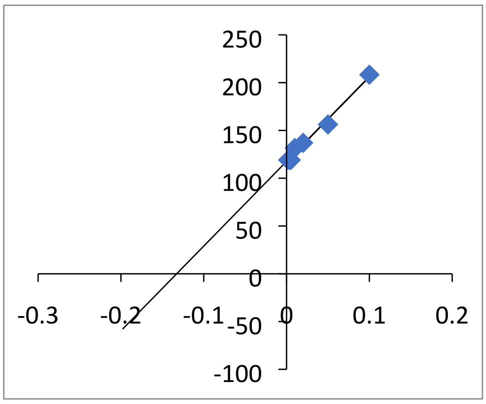
**Michaelis menten plot**

| S (uM) | V (uM/min) |
|---|---|
| **0** | 0 |
| **10** | 0.0048 |
| **20** | 0.0064 |
| **50** | 0.0073 |
| **100** | 0.0076 |
| **200** | 0.0084 |
| **500** | 0.0084 |



**Lineweaver Burk**

| 1/S | 1/V |
|---|---|
| **0.1** | 208.3333 |
| **0.05** | 156.25 |
| **0.02** | 136.9863 |
| **0.01** | 131.5789 |
| **0.005** | 119.0476 |
| **0.002** | 119.0476 |



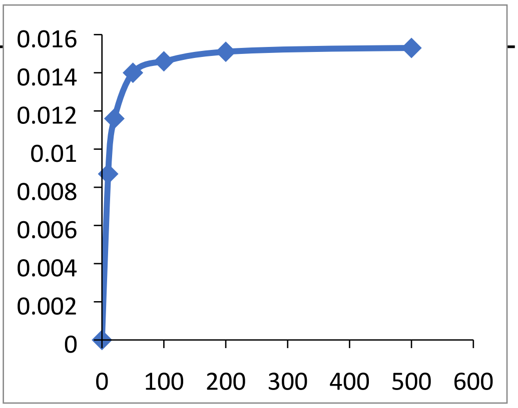
| | |
|---|---|
| Km | 8.75 ± 1.38μM |
| $k_{cat}/K_M$ | 1.23 $s^{-1}mM^{-1}$ |
| kcat | 0.0107 $s^{-1}$ |

**Fig 27: Michaelis menten and Lineweaver-Burk plots for 76E2**

## 3) a) 76E5 UDP Glucose

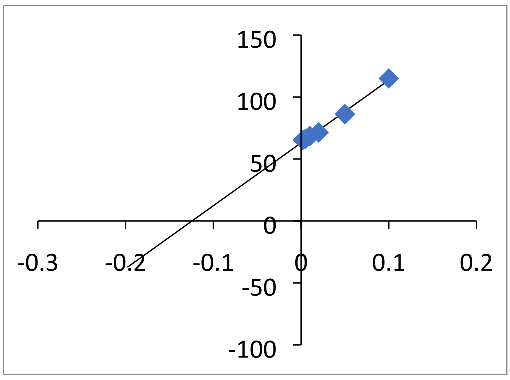
### Michaelis menten plot

| S (uM) | V (uM/min) |
|---|---|
| 0 | 0 |
| 10 | 0.0087 |
| 20 | 0.0116 |
| 50 | 0.014 |
| 100 | 0.0146 |
| 200 | 0.0151 |
| 500 | 0.0153 |



### Lineweaver Burk

| 1/S | 1/V |
|---|---|
| 0.1 | 114.9425 |
| 0.05 | 86.2069 |
| 0.02 | 71.42857 |
| 0.01 | 68.49315 |
| 0.005 | 66.22517 |
| 0.002 | 65.35948 |



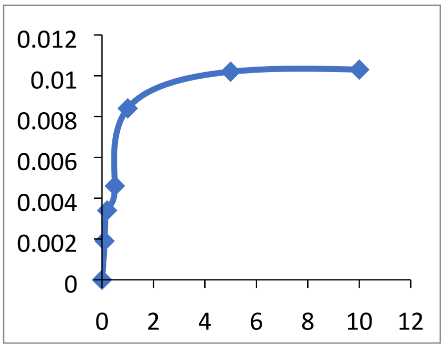
| | |
|---|---|
| **Km** | 8.79± 1.64 µM |
| **$k_{cat}/K_M$** | 2.21 $s^{-1}mM^{-1}$ |
| **kcat** | 0.0194 $s^{-1}$ |

**Fig 28a: Michaelis menten and Lineweaver-Burk plots for 76E5**

## b) 76E5 UDP Galactose

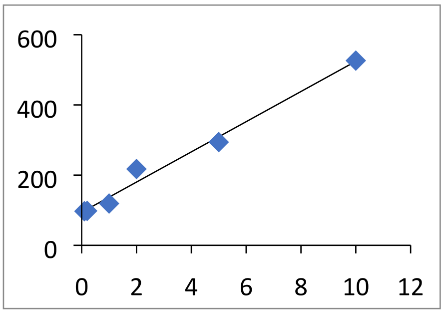
**Michaelis menten plot**

| S (uM) | V (uM/min) |
|---|---|
| 0 | 0 |
| 0.1 | 0.0019 |
| 0.2 | 0.0034 |
| 0.5 | 0.0046 |
| 1 | 0.0084 |
| 5 | 0.0102 |
| 10 | 0.0103 |



**Lineweaver Burk**

| 1/S | 1/V |
|---|---|
| 10 | 526.3158 |
| 5 | 294.1176 |
| 2 | 217.3913 |
| 1 | 119.0476 |
| 0.2 | 98.03922 |
| 0.1 | 97.08738 |



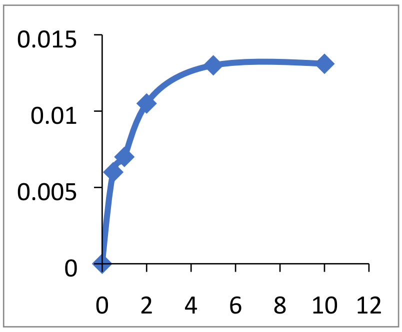
| | |
|---|---|
| **Km** | 0.57± 0.19 µM |
| **$k_{cat}/K_M$** | 22.8 $s^{-1}mM^{-1}$ |
| **kcat** | 0.0130 $s^{-1}$ |

**Fig 28b: Michaelis menten and Lineweaver-Burk plots for 76E5**

## c) 76E5 UDP N-acetyl glucosamine

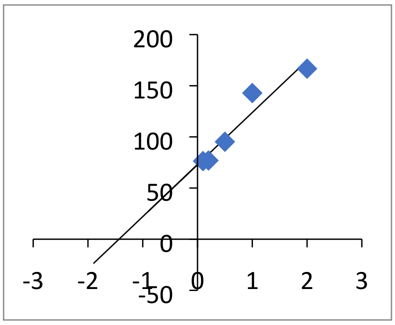
**Michaelis menten plot**

| S (uM) | V (uM/min) |
|---|---|
| 0 | 0 |
| 0.5 | 0.006 |
| 1 | 0.007 |
| 2 | 0.0105 |
| 5 | 0.013 |
| 10 | 0.0131 |



**Lineweaver Burk**

| 1/S | 1/V |
|---|---|
| 2 | 166.6667 |
| 1 | 142.8571 |
| 0.5 | 95.2381 |
| 0.2 | 76.92308 |
| 0.1 | 76.33588 |



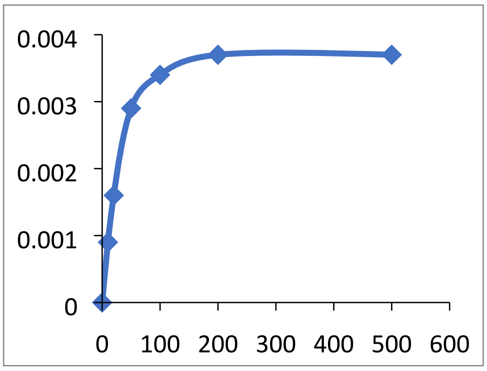
| | |
|---|---|
| **Km** | 0.78 ± 0.28μM |
| **$k_{cat}/K_M$** | 21.3 $s^{-1}mM^{-1}$ |
| **kcat** | 0.0166 $s^{-1}$ |

**Fig 28c: Michaelis menten and Lineweaver-Burk plots for 76E5**

## 4) 76D1 UDP GLUCOSE

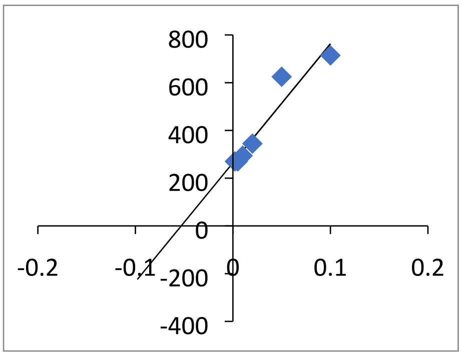
### Michaelis menten plot

| S (uM) | V (uM/min) |
|---|---|
| 0 | 0 |
| 10 | 0.0009 |
| 20 | 0.0016 |
| 50 | 0.0029 |
| 100 | 0.0034 |
| 200 | 0.0037 |
| 500 | 0.0037 |



### Lineweaver Burk

| 1/S | 1/V |
|---|---|
| 0.1 | 714.2857 |
| 0.05 | 625 |
| 0.02 | 344.8276 |
| 0.01 | 294.1176 |
| 0.005 | 270.2703 |
| 0.002 | 270.2703 |



| | |
|---|---|
| **Km** | 25.77 ± 8.82 μM |
| **$k_{cat}/K_M$** | 0.185 $s^{-1}mM^{-1}$ |
| **kcat** | 0.00476 $s^{-1}$ |

**Fig 29: Michaelis menten and Lineweaver-Burk plots for 76D1**