Application Note: 537

High-Resolution, Accurate Mass Measurements and Metabolite Identification: An Automated Approach Using Fragment Prediction in Combination with Fragment Ion Search (FISh)

Janine Wank[1], Winfried Wagner-Redeker[1], Anthony Taylor[2], Rose Herbold[3], Paul-Gerhard Lassahn[1]
[1]Swiss BioAnalytics AG, Basel, Switzerland; [2]Thermo Fisher Scientific, Reinach, Switzerland; [3]Thermo Fisher Scientific, San Jose, CA, USA

Key Words

- LTQ Orbitrap Series
- Mass Frontier
- Fragment Ion Search (FISh)
- Metabolite Identification
- Structural Elucidation

Introduction

Structural elucidation of putative metabolites via LC/MS is a time consuming process of manual interpretation of MS^n data and requires a strong background in metabolism pathways as well as gas-phase fragmentation pathways. To evaluate an automated software workflow for metabolite identification and structural elucidation, we processed high-resolution, accurate mass data using the Fragment Ion Search (FISh)™ processing tool in Thermo Scientific Mass Frontier 7.0 software. Data-dependent MS^n data was used for component detection and for the identification of metabolites based on theoretically calculated fragments of the parent structure.

Goal

To evaluate an automated workflow for metabolic profiling and structural elucidation by using Fragment Ion Search (FISh) processing in Mass Frontier™ 7.0 software.

Experimental Conditions

Sample Preparation

The investigated metabolites were derived from ticlopidine, a potent thienopyridine antiplatelet drug known to be extensively metabolized. Ticlopidine was incubated with human liver S9 enzymes. The final experimental concentrations in the 1 mL incubation mixture were 100 mM KPO_4 (pH 7.4), 3 mM NADPH, 3.8 µM ticlopidine, and S9 human liver fraction (BD Bioscience). After the addition of S9 human liver fraction, the incubation mixtures were homogenized gently and placed into a water bath (37 °C). Aliquots of the reaction solution were withdrawn after 0, 30, and 60 minutes, and the reaction was quenched by the addition of acetonitrile. The individual samples were centrifuged and analyzed by LC/MS.

Liquid Chromatography

Separations were performed using a Thermo Scientific Hypersil GOLD column (50 x 2.1 mm, 1.9 µm particle size) with a Thermo Scientific Accela 600 pump and a Thermo Scientific Open Accela autosampler. The chromatographic conditions were as follows:

Mobile phase A:	Water with 0.1% formic acid		
Mobile phase B:	Acetonitrile with 0.1% formic acid		
Flow rate:	150 µL/min		
Gradient:	Time (min)	%A	%B
	0.0	90	10
	2.0	90	10
	16.0	50	50
	18.0	5	95
	23.0	5	95
	23.1	90	10
	28.0	90	10

Mass Spectrometry

MS analysis was carried out with a Thermo Scientific LTQ Orbitrap XL hybrid mass spectrometer in positive ion mode using one high-resolution full MS scan followed by three high-resolution MS^2 scans. Precursor selection was done in the data-dependent operation mode where the most intense ion of the previous scan was selected for fragmentation. The MS conditions were as follows:

MS resolution:	60,000 FWHM at *m/z* 400
MS^2 resolution:	7,500 FWHM at *m/z* 400
Full MS mass range *(m/z)*:	150 - 800
Collision Energy:	CID: 35%

Thermo SCIENTIFIC

In additional experiments, an inclusion list containing the calculated exact masses of putative metabolites was added to the method (Figure 1).

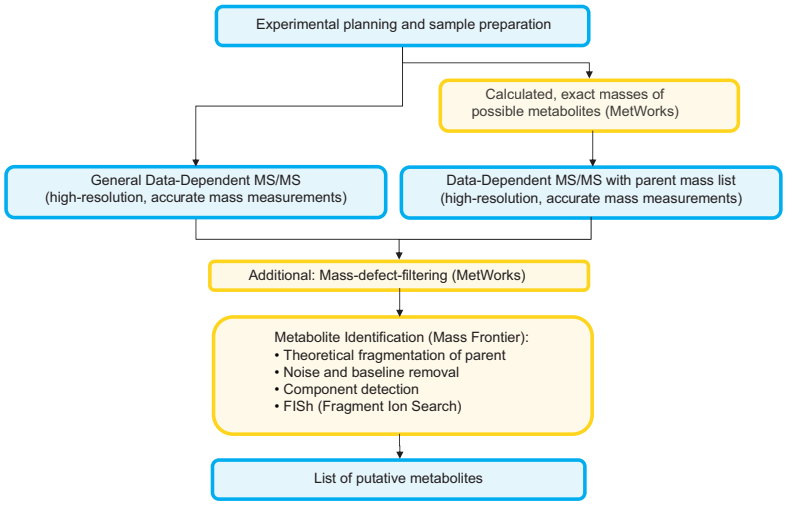

Figure 1. Schematic workflow of the automated data processing for metabolite identification

Data Analysis

Raw data was processed using Mass Frontier 7.0 software, including the Chromatogram Processor and Database Manager modules. The Fragments and Mechanisms, FISh, FISh Explanation, and Compare Spectra processing tools were used. Multiple Mass Defect Filtering was completed by using Thermo Scientific MetWorks 1.3 software.

Results and Discussion

The structures of ticlopidine and its known metabolites[1] are highlighted in Figure 2. The extracted ion chromatograms of these metabolites (0 min versus 30 min S9 liver fraction incubation; mass accuracy 5 ppm) are displayed and annotated in Figure 3.

The high resolution accurate mass data (data-dependent MS/MS mode) was directly exported to the Mass Frontier 7.0 software program. After the automated removal of noise and baseline signals, the theoretical calculation of possible fragments of ticlopidine was performed. (See Figure 1 for the schematic workflow and Figure 4 for the annotation of all major peaks in the MS/MS spectra of ticlopidine.) Next, a general list of possible Phase I biotransformations was applied, and the LC/MS chromatogram was processed by a component detection algorithm. FISh was then used to screen the detected components and the corresponding spectral ion trees to identify putative metabolites of ticlopidine.

The workflow and FISh data processing results are summarized and illustrated in Figure 5. After the determination of the theoretical fragments of ticlopidine and specification of the Phase l modifications, component detection and FISh processing were used to screen for putative metabolites. The list of predicted fragments was automatically extended by the mass shifts of the modifications through the definition of the expected biotransformations of ticlopidine. The list and the combined ion chromatograms of putative metabolites are displayed after this process. FISh provided an explanation of the MS/MS data including the structures of the fragments and the possible modifications (highlighted in red in Figure 5) for each detected component. For the metabolites M1 and M5, the modifications were further confirmed by a comparison between the observed and theoretical fragments of the putative structures of the metabolites. All fragment ions were explained.

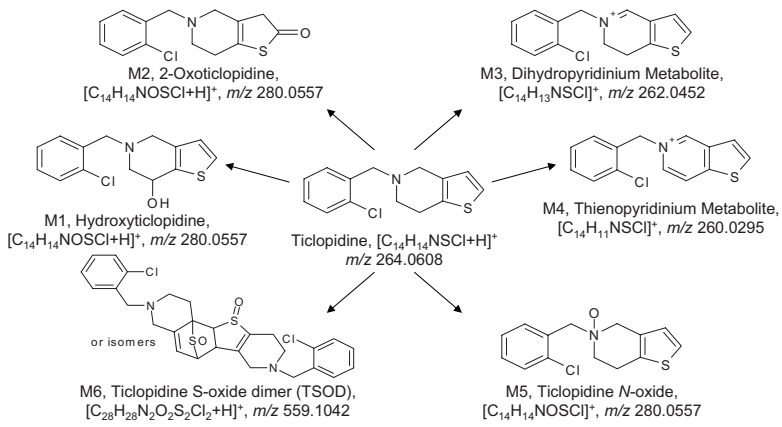

Figure 2. Ticlopidine and its known metabolites

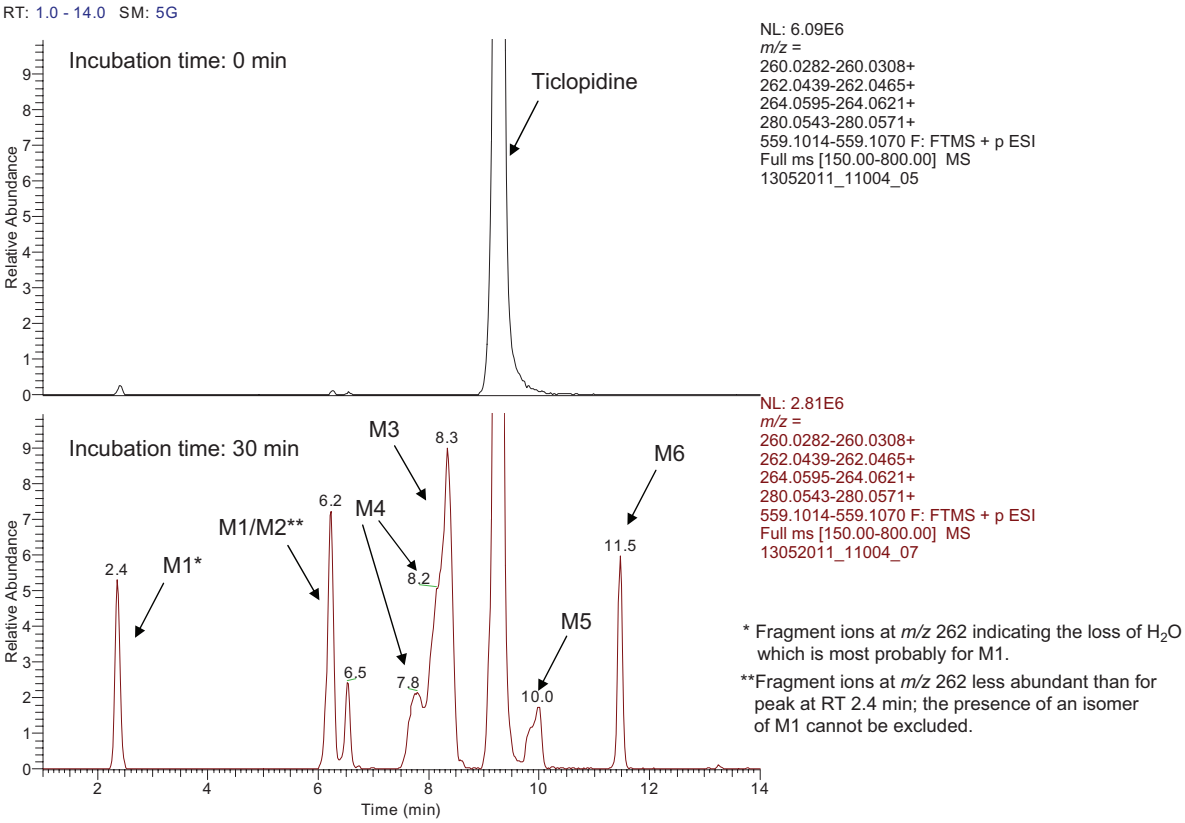

Figure 3. Extracted ion chromatograms of ticlopidine and its known metabolites M1-M6 (mass accuracy 5 ppm, retention-time 1.0-14.0 min)

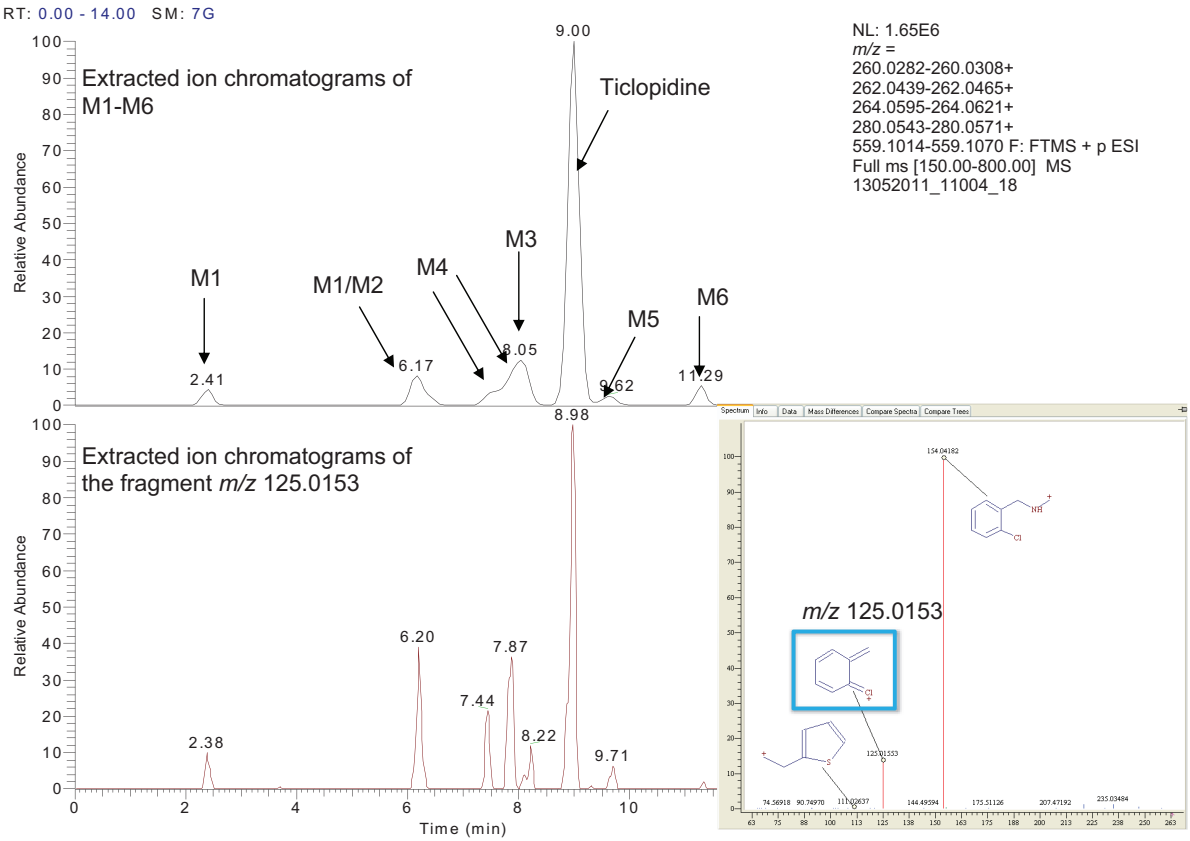

Figure 4. Extracted ion chromatograms of the ticlopidine metabolites M1-M6 and of the fragment *m/z* 125.0153 (C_7H_6Cl, mass accuracy 5 ppm, incubation time 30 min)

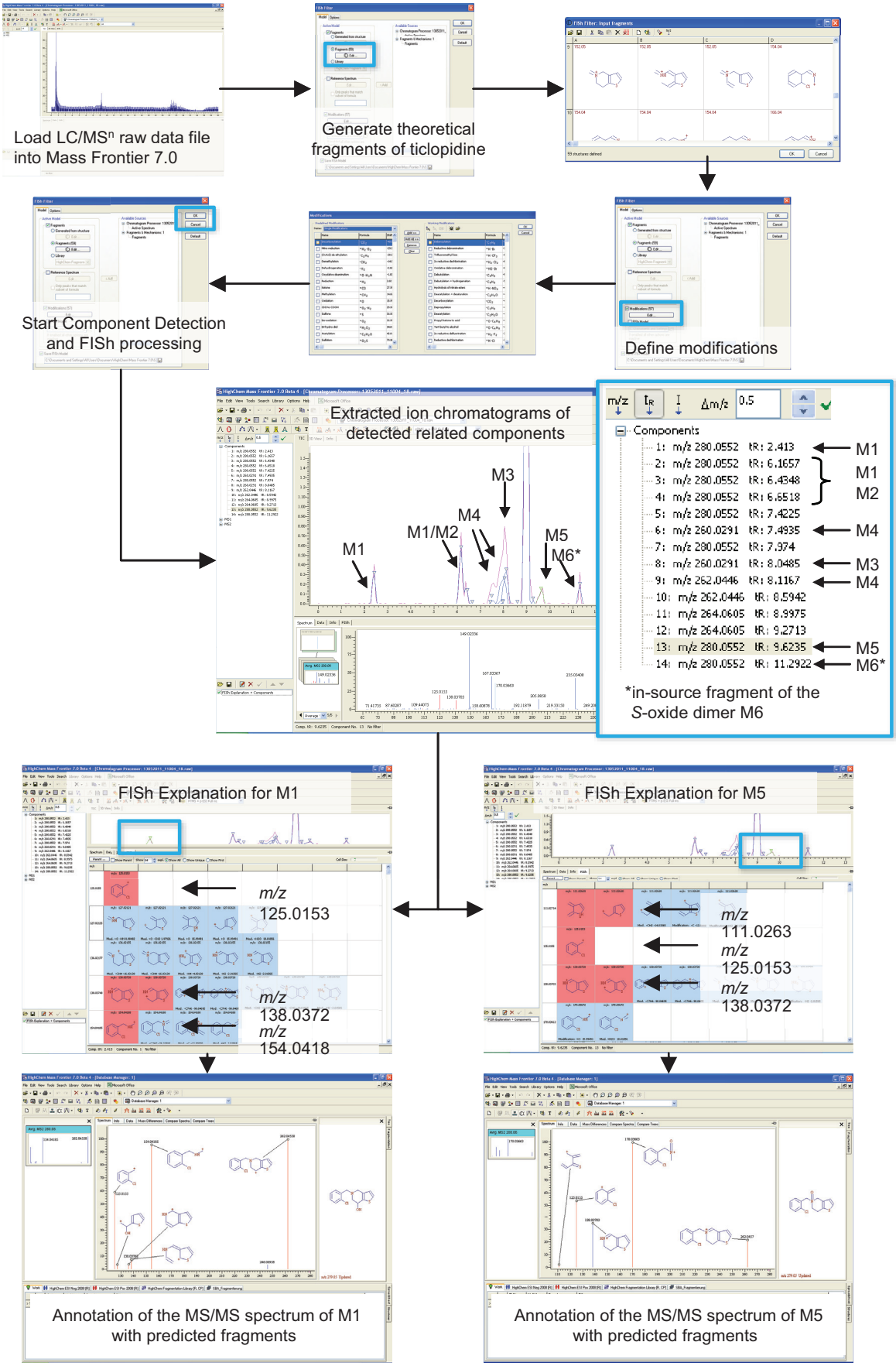

Figure 5. Explanation of the automated approach for metabolite identification using fragment prediction in combination with Fragment Ion Search (FISh)

The results of the FISh processing and a comparison between expected and detected metabolites[1] are displayed in Table 1. All known metabolites, including the unusual S-oxide dimer metabolite M6, were detected and identified by the automated FISh processing approach of the original raw data file. The unusual S-oxide dimer metabolite M6 was detected by FISh through the identification of an in-source fragment (Figure 6).

Table 1. Summary of the Fragment Ion Search (FISh) processing results

Expected Metabolite	FISh	FISh with mass defect filtered data
M1	✓	✓
M2	✓	✓
M3	✓	✓
M4	✓	✓
M5	✓	✓
M6*	✓	✓

* M6 was detected through the identification of an in-source fragment

An additional comparison was performed to assess if the automated FISh processing workflow could be combined with the Multiple Mass Defect Filtering (MMDF) tool within MetWorksTM 1.3 software. MMDF is a popular data processing tool used in metabolite identification to remove endogenous background ions.[2] The same FISh processing workflow was repeated on a MMDF preprocessed raw data file. The results showed no differences in the detected metabolites in comparison to without MMDF preprocessing (Table 1). However, the FISh processing time for the MMDF preprocessed file was shorter. This demonstrates that MMDF and FISh can be effectively combined for fast and automated metabolite identification without incurring false negatives.

To demonstrate the benefit of high-resolution, accurate mass data, the measured and simulated Full MS data of the co-eluting metabolites M3 and M4 are displayed in Figure 6. The high-resolution, accurate mass measurement of the isotopic pattern provided verification of the putative identification of the two metabolites.

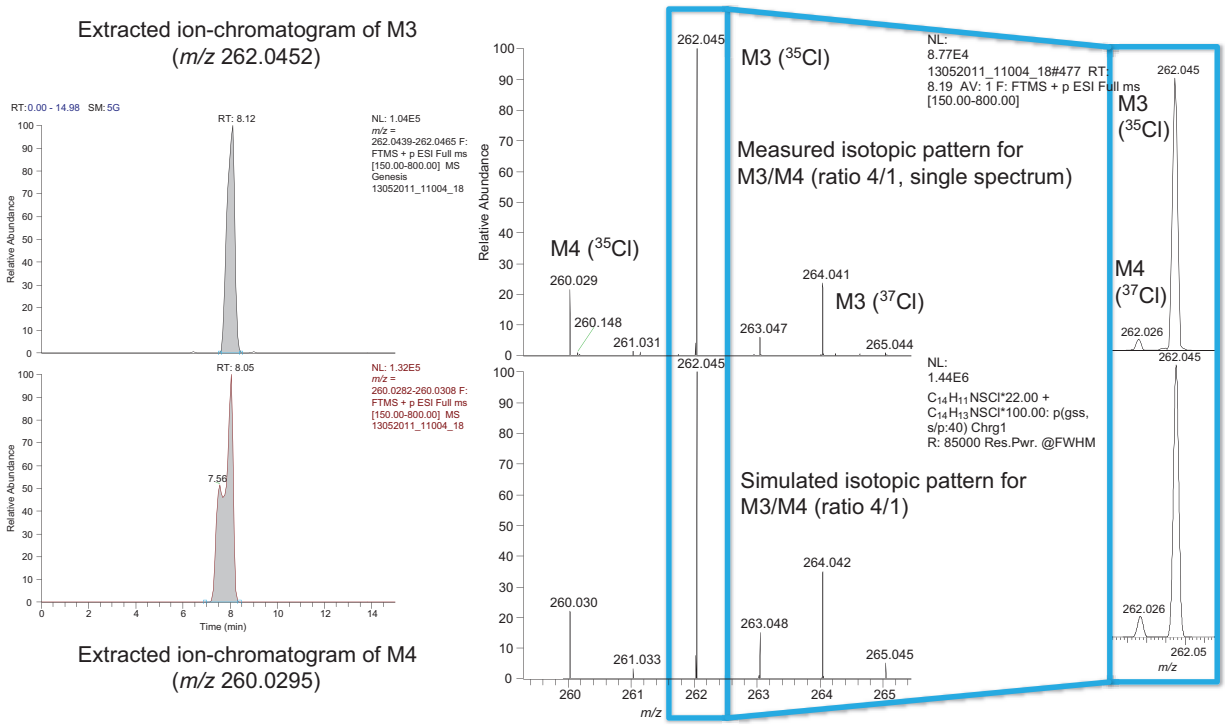

Figure 6. Co-elution of the metabolites M3/M4 and the benefit of high-resolution, accurate mass data

Conclusion

Metabolic profiling and structural elucidation of possible metabolites via LC/MS/MS was simplified and accelerated by Fragment Ion Search (FISh) processing in Mass Frontier 7.0 software. This workflow can be combined with Multiple Mass Defect Filtering (MMDF) in MetWorks 1.3 software for reduced processing times without incurring false negatives. The availability of high-resolution, accurate mass LC/MS/MS data minimizes the possibility of false positives in the generated list of related components. All known metabolites of ticlopidine in a S9 human liver fraction incubation were detected and identified using this automated processing workflow, including the unusual S-oxide dimer metabolite. This is interesting to note because FISh was able to provide an indication for the presence of this metabolite just through the identification of an in-source fragment.

References

1. Talakad, J.C.; Shah, M.B.; Walker, G. S.; Xiang, C.; Halpert, J.R.; Dalvie, D. Comparison of In Vitro Metabolism of Ticlopidine by Human Cytochrome P450 2B6 and Rabbit Cytochrome P450 2B4 *Drug Metab. Dispos.* **2011**, 39(3), 539-550.
2. Huang, Y.; Liu, S.; Miao, S.; Jeanville, P.M. *Using Multiple Mass Defect Filters and Higher Energy Collisional Dissociation on an LTQ Orbitrap XL for Fast, Sensitive and Accurate Metabolite ID*; Thermo Fisher Scientific Application Note 417, 2008.

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

Africa-Other
+27 11 570 1840
Australia
+61 3 9757 4300
Austria
+43 1 333 50 34 0
Belgium
+32 53 73 42 41
Canada
+1 800 530 8447
China
+86 10 8419 3588
Denmark
+45 70 23 62 60
Europe-Other
+43 1 333 50 34 0
Finland/Norway/ Sweden
+46 8 556 468 00
France
+33 1 60 92 48 00
Germany
+49 6103 408 1014
India
+91 22 6742 9434
Italy
+39 02 950 591
Japan
+81 45 453 9100
Latin America
+1 561 688 8700
Middle East
+43 1 333 50 34 0
Netherlands
+31 76 579 55 55
New Zealand
+64 9 980 6700
Russia/CIS
+43 1 333 50 34 0
South Africa
+27 11 570 1840
Spain
+34 914 845 965
Switzerland
+41 61 716 77 00
UK
+44 1442 233555
USA
+1 800 532 4752

www.thermofisher.com

AN63435_E 08/16S