Metabolomics

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1 Introduction

Lung cancer cells (A549) are treated with TNF-α and compared to a control group. The harvested cells are lysed and incubated with methanol, so the metabolites in the supernatant can be extracted. After drying, the analytes are derivatized and diluted in hexane and measured with a GC-EI-MS setup.

2 Results

Three metabolites were chosen among the analyzed metabolome and presented in more detail. For three additional compounds a computational assignment to the biochemical pathways has been executed.

2.1 Phenylalanine

MW: 309 Da RT: 7,51 - 7,53

$MW_{measured} - (n \times (m_{Trimethylsilyl} - m_H)) + n \times m_H$

$309Da - (2 \times 73Da) + 2Da = 165Da$

Literature: 165,08 Da

Figure 1 shows the obtained fragmentation plot of phenylalanine on the top and below the mass spectrum of the library. Single fragments are identified via their characteristic m/z values. The personal predictions shown in Figure 2b are revised with a competitive fragmentation model, which also provides the literature molecular masses. [1]

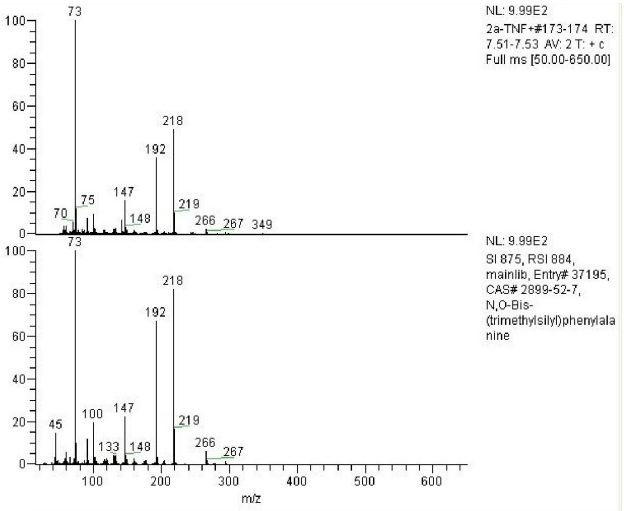
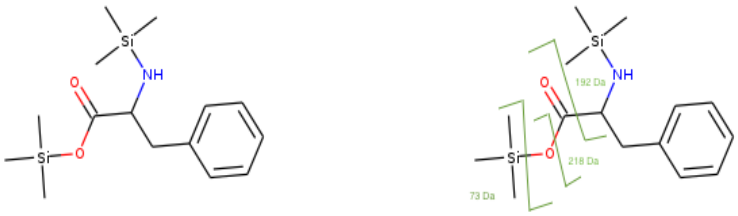


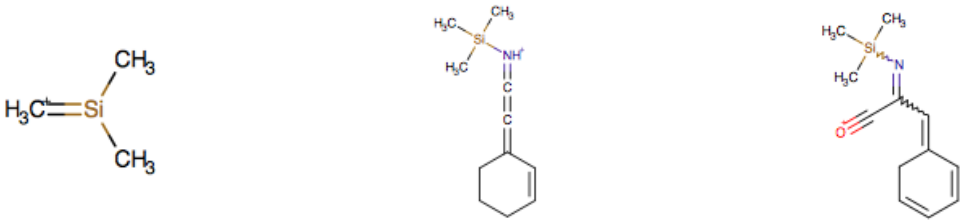
Figure 1: Fragmentaion Derivatized Phenylalanine



(a) Trimethylsilylphenylalanine **(b)** Predicted Fragmentation

Figure 2: Molecular structure Phenylalanine

Possible Fragmentation Molecules:

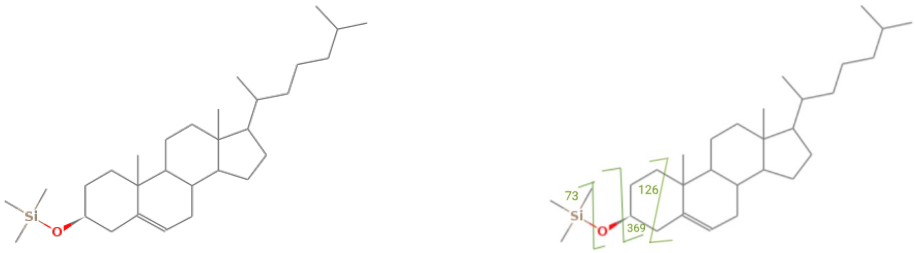


(a) m/z 73 **(b)** m/z 218 **(c)** m/z 192

Figure 3: Phenylalanine fragmentation

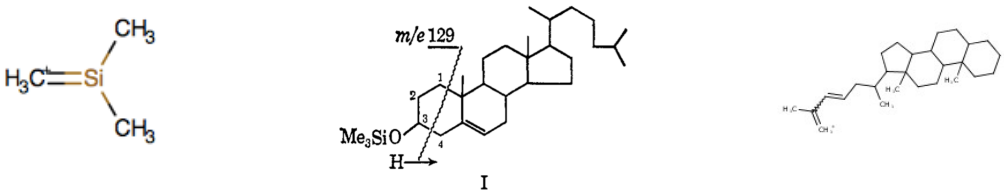
2.2 Cholesterol

MW: 458 Da RT: 15,75 - 15,83
$MW_{measured} - (n \times (m_{Trimethylsilyl} - m_H)) + n \times m_H$
$458Da - (1 \times 73Da) + 1Da = 386Da$
Literature: 386,35 Da



(a) Trimethylsilylcholesterol **(b)** Predicted Fragmentation

Figure 4: Molecular structure Cholesterol



(a) m/z 73 **(b)** m/z 126 [2] **(c)** m/z 369

Figure 6: Cholesterol fragmentation

Figure 5 shows the obtained fragmentation plot of cholesterol on the top and below the mass-spectrum of the library. Single fragments are identified via their characteristic m/z values. The personal predictions shown in Figure 4b are revised with a competitive fragmentation model, which also provides the literature molecular masses. While Figure 4 shows the generated fragments of cholesterol.

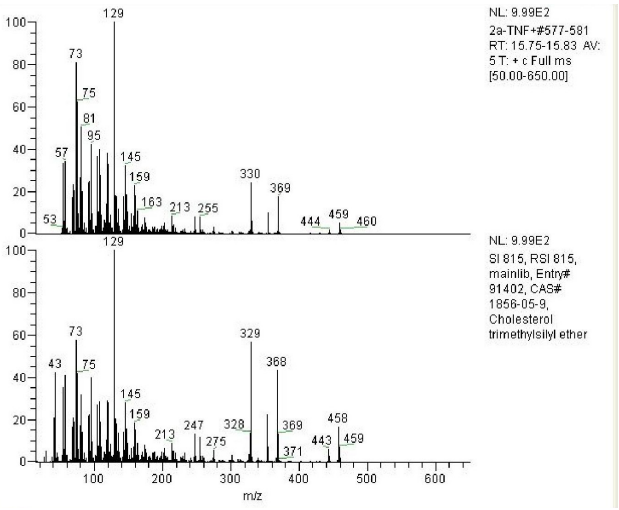


Figure 5: Fragmentation derivatized cholesterol

2.3 Serine

MW: 321 Da RT: 5,49 - 5,51

$MW_{measured} - (n \times (m_{Trimethylsilyl} - m_H)) + n \times m_H$

$321Da - (3 \times 73Da) + 3Da = 105Da$

Literature: 105,093 Da

Figure 7 shows the obtained fragmentation plot of serine on the top and below the mass spectrum of the library. Single fragments are identified via their characteristic m/z values. The personal predictions shown in Figure 8b are revised with a competitive fragmentation model, which also provides the literature molecular masses. Figure 9 shows the calculated fragments.

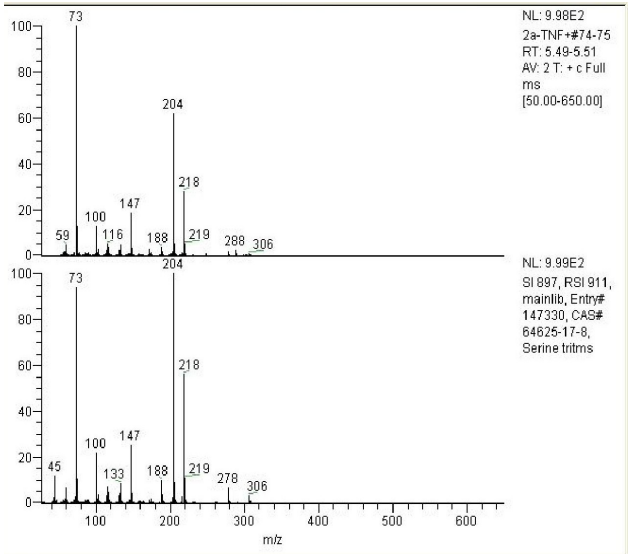
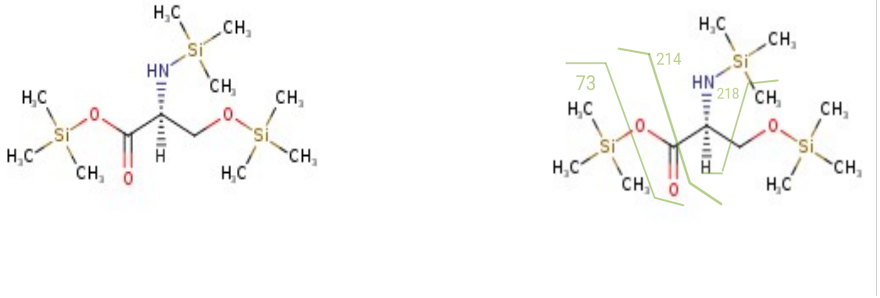


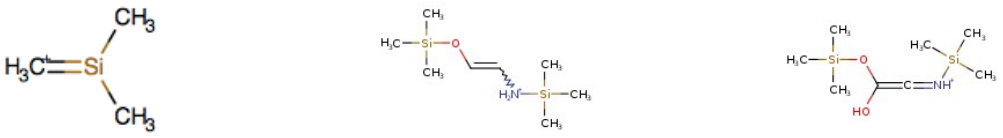
Figure 7: Fragmentaion derivatized serine



(a) Serine fully derivatized with trimethylsilyl

(b) Predicted Fragmentation

Figure 8: Trimethylsilylserine



(a) m/z 73

(b) m/z 204

(c) m/z 218

Figure 9: Serine fragmentation

2.4 GC-Chromatogram

Figure 10 displays the obtained chromatographic plot and highlights retention time of the described sample metabolites.(Serine at 5,50 minutes/ Phenylalanine at 7,52 minutes/ Cholesterol at 18,80 minutes) Figure 11 shows the chromatogram of the untreated sample.

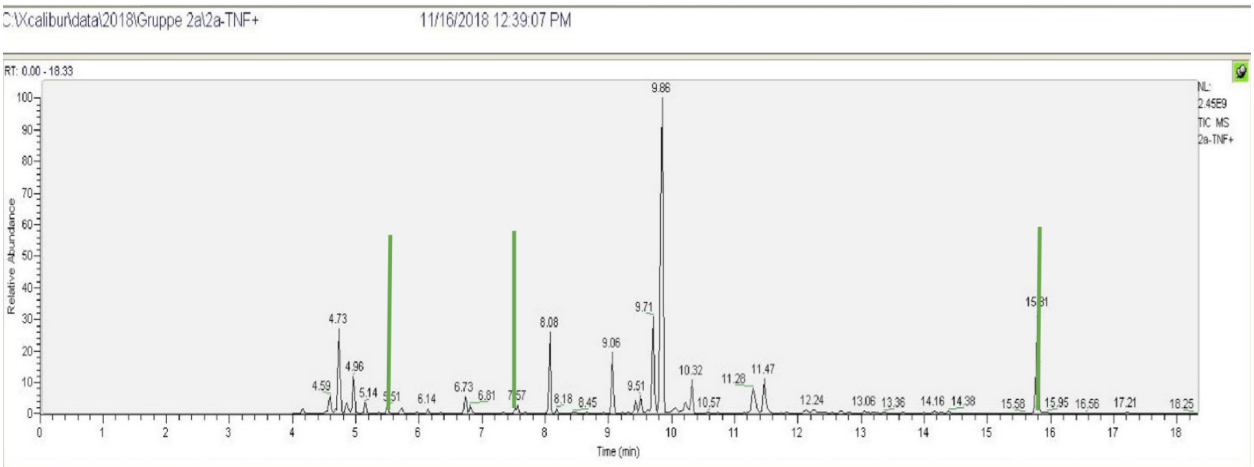


Figure 10: Chromatogram of the treated sample.

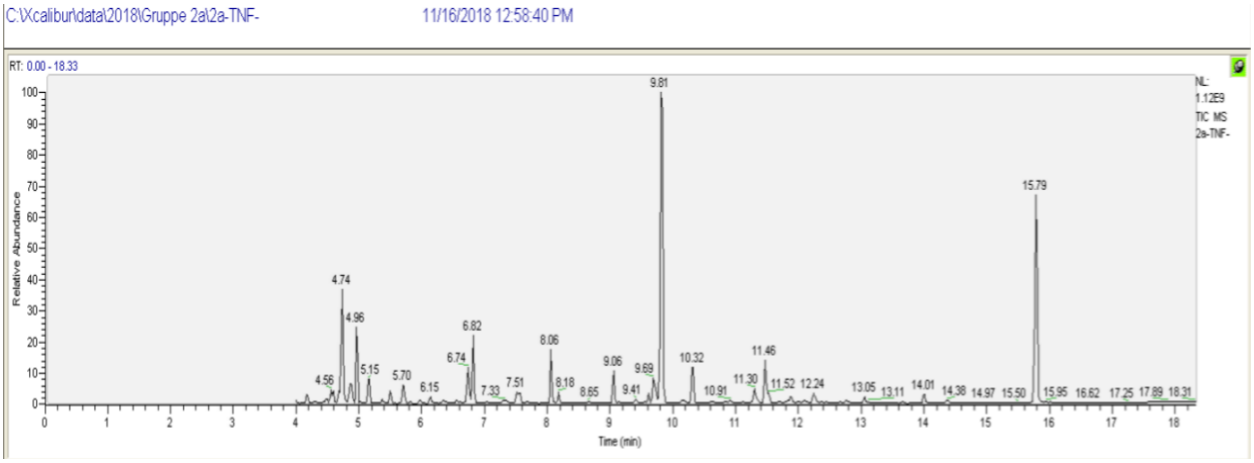


Figure 11: Chromatogram of the untreated sample

2.5 Metabolic Pathway map:

Cholesterol is found in the steroid metabolism, whereas compounds like Leucine or Threonine are found in amino acid metabolism. Ribitol is found in the pentose phosphate cycle pathway within the carbohydrate metabolism. [3]

3 Discussion

Several metabolites have been successfully identified with the procedure described in the lab journal. Quantitative statements can not be made as well as enrichment claims due to the operating method and evaluation separation. The presented metabolites are confidently identified by the Xcalibur fragmentation library and literature comparison of the resulting molecular masses. The personal predictions of metabolite fragmentation have been backed up by a computational fragmentation model but can not be confirmed by literature, due to a lack of publications. The comparison between the two different samples is very limited, since the specimen have been analyzed by different groups. The two chromatograms have a high degree of congruence with some variations in the relative abundance. The peak at $\approx$ 15,8 minutes has a relative abundance of around 30%, in contrast to roughly 70% in the untreated group. Also at $\approx$ 6,74-6,82, the peaks in the control spike at roughly 20%, while the treated peaks only reach about 5%. These metabolites could indeed be interesting for further research. We would recommend a quantitative approach so statistical enrichment can be validated. This experimental confirmation could then lead to a deeper pathway analysis and open up possible treatment approaches.

List of Figures

References

[1] Felicity Allen, Allison Pon, Russ Greiner, and David Wishart. Computational prediction of electron ionization mass spectra to assist in gc/ms compound identification. *Analytical chemistry*, 88(15):7689–7697, 2016.

[2] John Diekman, James B Thomson, and Carl Djerassi. Mass spectrometry in structural and stereochemical problems. cxli. electron-impact induced fragmentations and rearrangements of trimethylsilyl ethers, amines and sulfides. *The Journal of organic chemistry*, 32(12):3904–3919, 1967.

[3] F. Hoffmann-La Roche Ltd. Biochemical pathways. http://biochemical-pathways.com/#/map/1, November 2018.