Determination of sterols in liver organoids using liquid chromatography-mass spectrometry (LC-MS)

Henriette Nordli, Hanne Røberg-Larsen, Elsa Lundanes and Steven Ray Wilson
Department of Chemistry, University of Oslo

Introduction

Non-alcoholic fatty liver disease (NAFLD) has become the most common cause of chronic liver disease, estimated to affect 25% of the adult population in developed countries. To investigate disease development, research models resembling the complexity of human biology is essential. Organoids is a research model predicted to surpass current well-established models like cell lines and animal models, as organoids has a self-organizing cell nature creating an organ cell structure mimicking the complexity of human biology more accurately [1]. The only diagnostic tool for NAFLD is liver biopsy. Hence, a less invasive method is needed using e.g., biomarkers. Oxysterols (OHCs) (endogenous metabolites of cholesterol), see **Figure 1**, are biologically active molecules proposed to be a potential biomarker for NAFLD as they are involved in some of the same biological pathways as seen in NAFLD [2]. Oxysterols are of low abundance in biological samples, and in combination with limited sample amount (only about 80 000 cells in 50 liver organoids) the quantification of oxysterols requires a highly sensitive nanoLC-MS system. 2.5 μm core-shell super phenyl hexyl particles have been proven to separate the oxysterol-isomers well [3], but nanoLC columns with these particles are not commercially available.

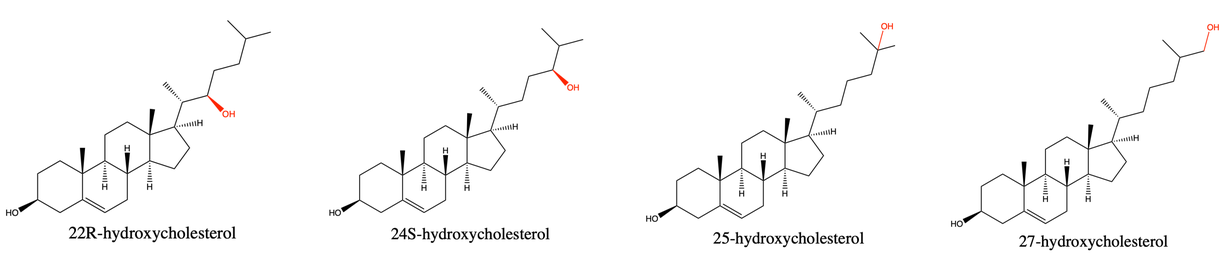

Figure 1. The structure of the oxysterols 22R-OHC, 24S-OHC, 25-OHC and 27-OHC.

Aim of study

The aim of study was to prepare trap and analytical nanoLC columns for the development of a highly sensitive nanoLC-MS system for quantification of 22R-, 24S-, 25- and 27-OHC in both healthy and diseased liver organoids to reveal if the oxysterols could be biomarkers for non-alcoholic fatty liver disease.

Chromatographic method

Due to the neutral origin of the oxysterols, derivatization with a Girard T reagent was performed to enable ionization with the electrospray ionization source. The chromatographic system includes an automatic filtration and filter backflushing (AFFL) system possessing an on-line sample preparation and clean up using a trap column and a filter, prior to the analytical column, as illustrated in **Figure 2**. Isocratic elution with 57/33/10 water/ACN/MeOH and 0.1% formic acid was used. The loading of the sample onto the trap column was done with an 100% aqueous MP to avoid oxysterol elution from the trap column. Detection was done with a TSQ Quantiva triple quadrupole MS operated in MRM mode.

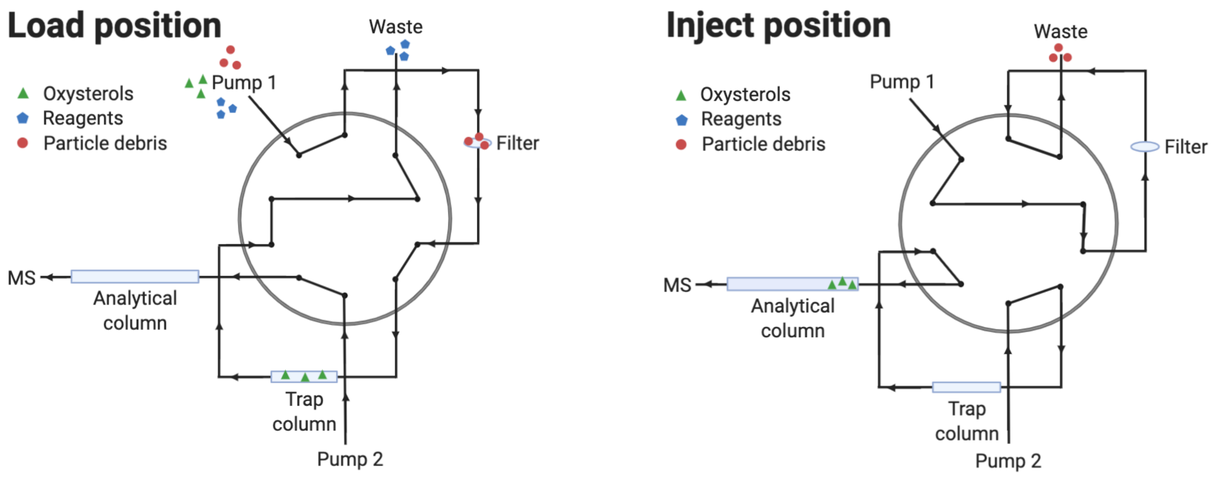

Figure 2. Illustration of the automatic filtration and filter backflushing system performing on-line sample preparation and clean up prior to separation of the oxysterols on the analytical column.

[1] M. Eisenstein, *Organoids: the body builders*, Nature methods, (**2018**). https://www.nature.com/articles/nmeth.4538
[2] T. Raselli, T. Hearn, A. Wyss, K. Atrott, A. Peter, I. Frey-Wagner, M.R. Spalinger, E.M. Maggio, A.W. Sailer, J. Schmitt, P. Schreiner, A. Moncsek, J. Mertens, M. Scharl, W.J. Griffiths, M. Bueter, A. Geier, G. Rogler, Y. Wang, B. Misselwitz, *Elevated oxysterol levels in human and mouse livers reflect nonalcoholic steatohepatitis*, J Lipid Research, 60 (**2019**) 1270-1283. https://pubmed.ncbi.nlm.nih.gov/31113816/
[3] S. Solheim, S.A. Hutchinson, E. Lundanes, S.R. Wilson, J.L. Thorne, H. Roberg-Larsen, *Fast liquid chromatography-mass spectrometry reveals side chain oxysterol heterogeneity in breast cancer tumour samples*, The Journal of Steroid Biochemistry and Molecular Biology, 192 (**2019**) 105309. https://pubmed.ncbi.nlm.nih.gov/30779932/
[4] H. Roberg-Larsen, K. Lund, T. Vehus, N. Solberg, C. Vesterdal, D. Misaghian, P.A. Olsen, S. Krauss, S.R. Wilson, E. Lundanes, *Highly automated nano-LC/MS-based approach for thousand cell-scale quantification of side chain-hydroxylated oxysterols*, Journal of lipid research, 55 (**2014**) 1531-1536. https://pubmed.ncbi.nlm.nih.gov/24792927/

Results

NanoLC columns (75 and 100 μm ID) have been packed with 2.5 μm core-shell super phenyl hexyl particles suspended in various slurry solvents (80% and 90% ACN and 100% acetone) to investigate the performance. 100% acetone was found to give the best column performance regarding plate height and asymmetry, as shown in **Figure 3.** Testing of the column performance was done using 0.1% toluene as test compound, and an LC-UV system for simplicity.

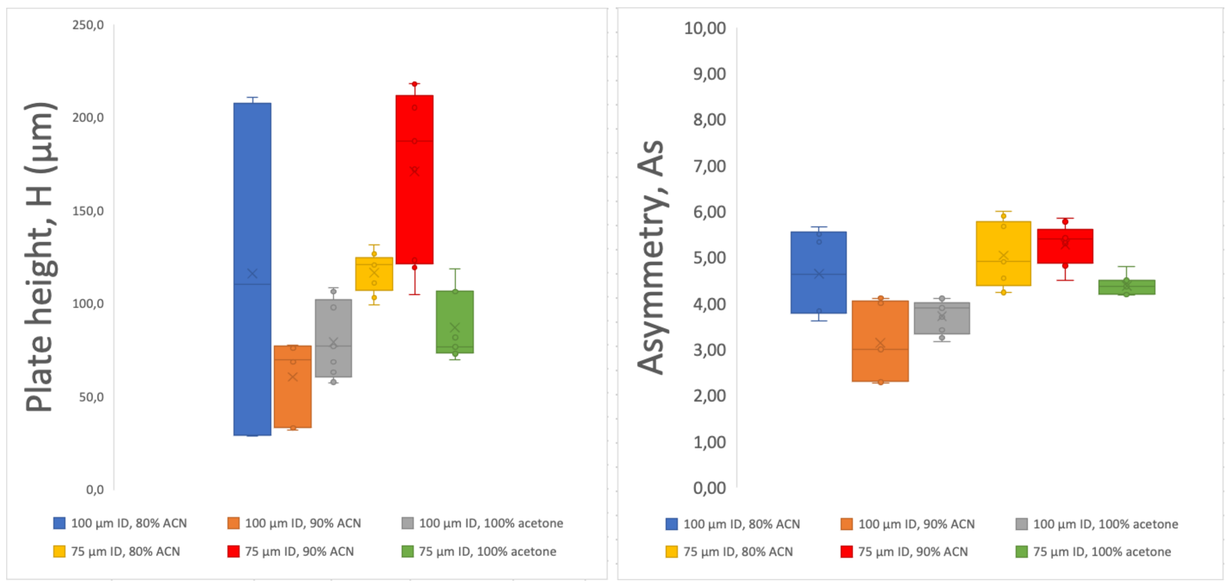

Figure 3. The plate height and asymmetry of in-house packed 75 and 100 μm ID columns packed with either 80% ACN, 90% ACN or 100% acetone as slurry solvent. X represents the average value over all data points, the bar represents the median value, and the dispersion represents the variations.

Columns were also packed with 5 μm phenyl hexyl particles (180 μm column ID), to investigate whether the 5 μm particles can be suitable as a trap column. The retention factor showed to be smaller for the 5 μm particles than for the 2.5 μm particles (1.1 vs. 4.1, respectively) for the derivatized oxysterols, suggesting that the 5 μm particles may be well suited in a trap column. Additionally, optimized MS-transitions and collision energies (CE) has been found for the oxysterols and the respective internal standards (IS), summarized in **Table 1**. See **Figure 4** for the MS-fragmentation of the 27-OHC Girard T derivative.

Table 1. Summary of the optimized MS transitions of the oxysterols and deuterated oxysterols used as internal standards with the respective collision energies.

Compound	Transition type	MS transition	CE
Oxysterol	Quantifier	514.5 → 455.410	35
	Qualifier	514.5 → 437.398	40
Deuterated oxysterol (d6) as IS	Quantifier	520.5 → 461.445	35
	Qualifier	520.5 → 443.398	39
Deuterated oxysterol (d7) as IS	Quantifier	521.5 → 462.418	37
	Qualifier	521.5 → 434.443	35

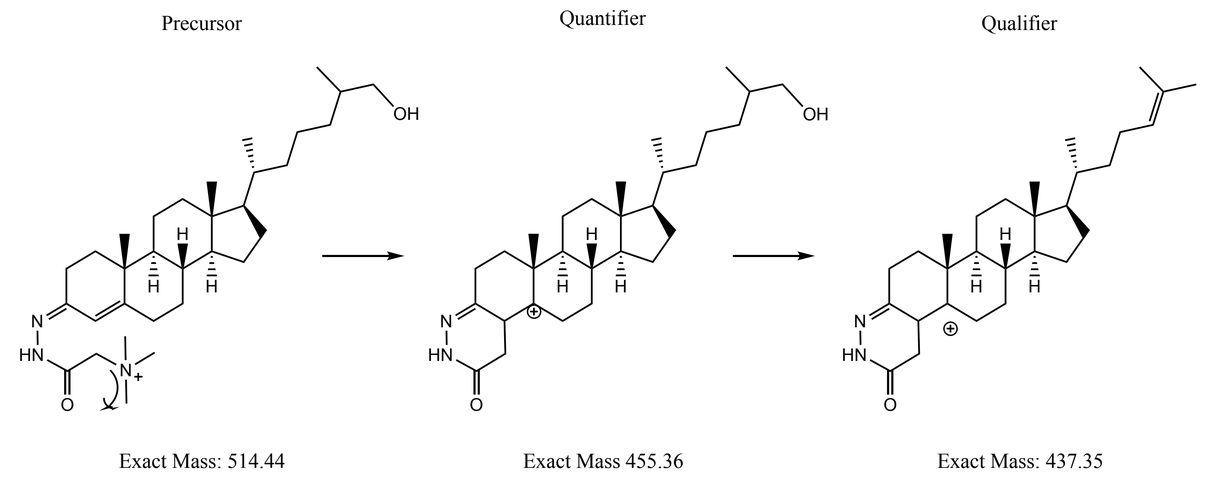

Figure 4. The fragmentation for the quantifier and qualifier ion of the 27-OHC Girard T derivative. All oxysterols and internal standards will fragment in the same way [4].

Conclusion and further work

75 μm ID nanoLC columns for oxysterol quantification have been packed in-house and suited particles for a trap column have been found for derivatized oxysterols. Further work includes completion of the full nanoLC-MS system set up in order to quantify 22R-, 24S-, 25- and 27-OHC in healthy liver organoids, and organoids induced with the disease to map the potential change in oxysterol-levels.

UiO : Life Science
University of Oslo

UiO : Hybrid Technology Hub
Faculty of Medicine