# Development of a new clinical diagnostic tool for cardiovascular risk assessment by LC-MS/MS

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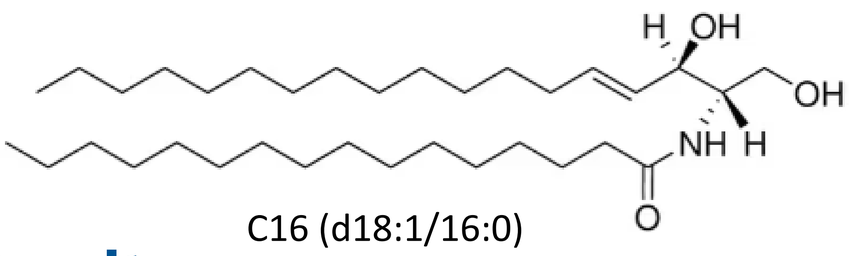
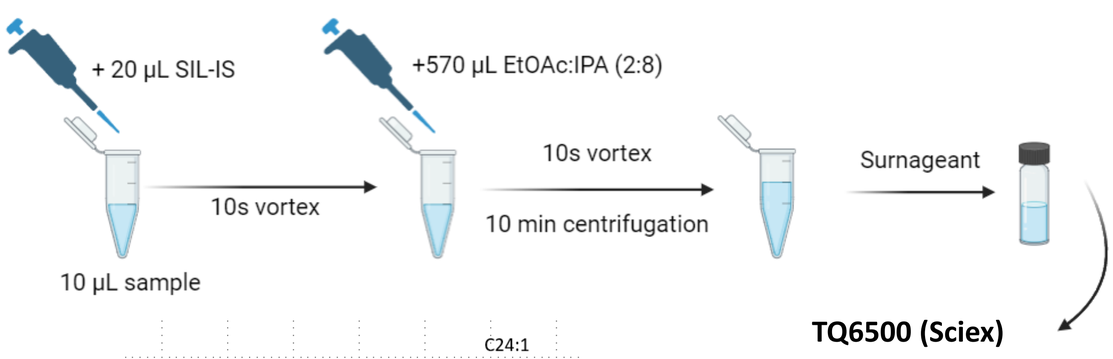
## Introduction:

Cardiovascular diseases (CVD) are the leading cause of mortality in Belgium, accounting for 31 000 deaths per year. Ceramides, which are bioactive lipids, are strongly associated with cardiometabolic conditions and play a crucial role in cellular processes such as apoptosis, inflammation, and atherosclerosis. Clinical data have revealed that ceramides are not only of significant biochemical interest but may also serve as diagnostic biomarkers and prognostic markers for cardiovascular risk assessment. Our study focuses on four ceramides, including the C16 form represented below, which research has shown could be reliable indicators of cardiovascular risk. Our goal is to provide clinicians with new emerging markers to support existing parameters and scores, enhacing diagnosis and improving prevention.




C16 (d18:1/16:0)

## Materials and Methods:





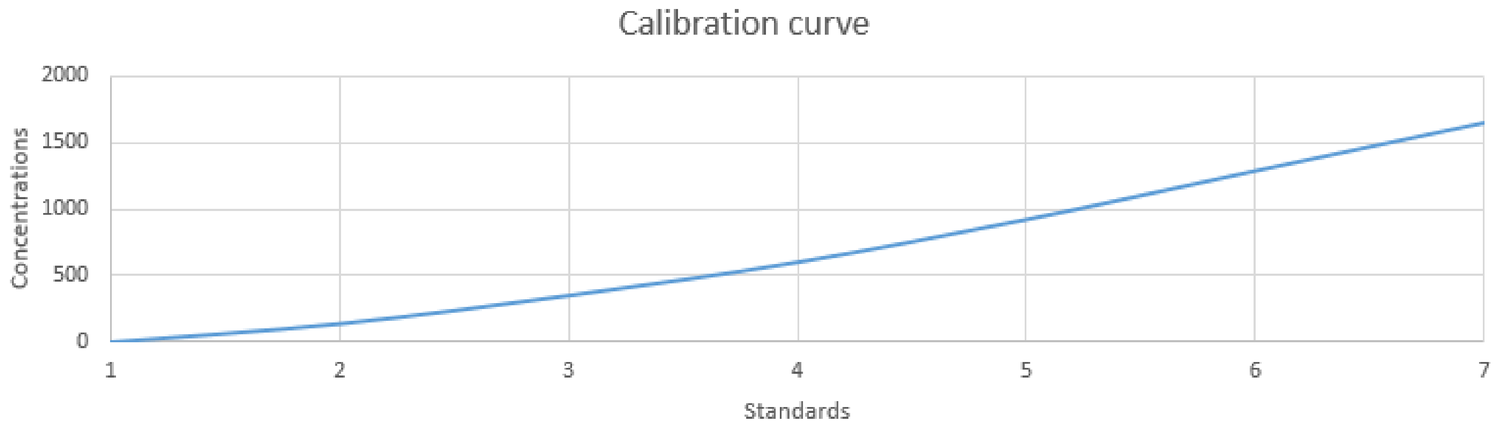
The sample preparation involves precipitating protein with a mixture of ethyl acetate and isopropanol.

## Results:

### Q1 Scan – MRM – Post column flow injection

| | Cer d18:1/16:0 | Cer d18:1/18:0 | Cer d18:1/24:0 | Cer d18:1/24:1 |
|---|---|---|---|---|
| **Transitions** | 538,4 > 264,3/520,5 | 566,4 > 264,3/548,5 | 650,5 > 264,4/632,6 | 648,4 > 264,3/630,5 |
| **Compounds parameters** | DP: 55<br>EP: 4,5<br>CXP: 4/6<br>CE: 31/21 | DP: 51<br>EP: 5<br>CXP: 4/8<br>CE: 33/19 | DP: 41<br>EP: 10<br>CXP: 4/6<br>CE: 39/27 | DP: 61<br>EP: 4<br>CXP: 4/8<br>CE: 41/24 |
| **Source parameters** | CUR: 25,0<br>CAD: Medium | IS: 5000<br>TEM: 300 | GS1: 40,0<br>GS2: 30,0 | |

### Calibration curve




A calibration curve was generated by spiking a surrogate matrix at seven levels, with the first level corresponding to an unspiked blank. The figure shows the curve obtained for the C16 form (d18:1/16:0), with concentrations expressed in µg/mL.

## Conclusion and perspective:

The method optimization is nearly complete and will soon be analytically validated according to the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI) (C62A) for use in the quantification of various ceramides.

The project's perspective is to adapt the method to sample collection using "Dried Blood Spot" (DBS). Blood micro-sampling is a minimally invasive technique compared to standard venipuncture and offers logistical ease for transport and storage. DBS collection is particularly suitable for sampling from, among other, newborns, the elderly, and patients requiring repeated and frequent sampling.