Comparison of three platforms for absolute quantitation of oxysterols: LC-MS/MS, GC-MS/MS, GC-MS

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Overview

- This work compares the performance of three analytical platforms – LC-MS/MS, GC-MS/MS and GC-MS – for the absolute quantitation of oxysterols.
- Each of the tested techniques, with its advantages and disadvantages, proves to be suitable for the quantitative analysis of oxysterols in biological matrix.

Introduction

Historically, GC-MS has been the most common technique for analysis of steroids, but it is increasingly being replaced by LC-MS/MS. Now with GC-MS/MS emerging on the market, researchers have even more analytical choices with which to perform analysis of steroids. All these approaches come with inherent advantages and disadvantages, making selection of the most suitable method a complex matter (when not simply limited to instrument availability).

This study compares three different analytical platforms – single quad GC-MS, triple quad GC-MS/MS, and triple quad LC-MS/MS – for the quantitative analysis of sterols, cholesterol and its derivatives in particular. Criteria include sensitivity, selectivity, chromatographic resolution, speed of analysis and sample preparation; these are evaluated by obtaining standard calibration curves with isotope dilution in biological matrix.

Instrument platforms

LC-MS/MS

LC system: Thermo Fisher Scientific Accela 1250 LC
Injection volume: 5 µL
Solvent A: 50% MeOH, 10% ACN, 1% AcOH
Solvent B: MeOH
Column: Waters Acquity UPLC BEH C18, 1.7 µm, 2.1 x 100 mm
Flow rate: 350 µL/min
MS detection: Thermo Fisher Scientific
TSQ Vantage triple quadrupole MS
Ionization mode: APCI
Scanning mode: Selected Reaction Monitoring (SRM)
Analysis time: 22 min

GC-MS/MS

GC system: Bruker 450-GC
Injection: 1µL, pulsed splitless
Inlet temperature: 250°C
Column: BR-5ms, 30 m x 0.25 mm ID, 0.25 µm film thickness
Column He flow: 1:1/min
Oven temperature program: 180°C(3min) → 250°C → 300°C(2min) → 310°C(3min)
Transfer line temp: 280°C
MS detection: Bruker Scion triple quadrupole MS
Ionization mode: EI, 70eV, 250°C
Scanning mode: Selected Reaction Monitoring (SRM)
Analysis time: 27.50 min

Results

1. Overview

2. Introduction

3. Instrument platforms

4. Sample preparation

5. Extraction

6. Conclusion

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References

TABLE 2. Calibration curve range and linearity. Solvent vs. biological matrix. Calibration samples were analyzed and observed to give linear responses from 10 fmol to 50 nM LLOQs. Data as signal to noise (SN) ratio of 10 to 1, were measured in both matrix free solvent and plasma (bold).

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FIGURE 3. Total Ion Chromatograms (TIC). LC-MS/MS (A), GC-MS/MS (B) and GC-MS (C) chromatograms whose very good base peak separation of all tested analytes. All methods allow successful chromatographic resolution of epimers.

TABLE 3. Comparison of technical features. Sensitivity, selectivity and matrix interference are all highly analyte dependant. Sensitivity of the method varies depending on the matrix. While it is possible to chromatographically resolve and quantify oxysterols, identification of endogenous isomers or other isotopic coelutes of the analyte should be considered.

Conclusions

- Each of the tested platforms proved to be suitable for absolute quantification of numerous oxysterols – however, none of the methods worked equally well for all of the analytes as a whole.
- The choice of the most suitable technique should be made on a case by case basis, with the following primary considerations: LC-MS/MS seems to be applicable to a wider range of analytes, but is not as sensitive as GC based techniques for most of the analytes.

GC-MS/MS provides the best overall sensitivity but requires more laborious sample preparation and has limited analyte resolution, which makes it less suitable for analyzing large batches of samples.
- GC-MS, even though highly sensitive, lacks the selectivity necessary for analysis of biological samples and suffers from significant matrix interferences.

In the GC methods, EI provides a unique fragmentation profile of molecules allowing greater selection of quantifiers. All techniques achieve epimer separation, but have limited high throughput capabilities.

All techniques – LC-MS/MS, GC-MS/MS and GC-MS – should be considered complementary rather than competing methods.

References