Urine Organic Acid Analysis by GC-MS: The Need for Methodological Rigor Amidst Diagnostic & Technological Challenges

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Introduction:

Gas chromatography–mass spectrometry (GC-MS) remains the technique of choice for urine organic acid (UOA) analysis, vital in diagnosing organic acidurias, as well as a broader range of inborn errors of metabolism (IEMs), such as mitochondrial and neurological, but also further nutritional and toxicological conditions. Despite its widespread use, recent external quality assessments, including ERNDIM schemes, highlight persistent inter-laboratory variability in the quantification of key metabolites, potentially impacting diagnostic reliability. At the same time, advances in mass spectrometry have promoted a growing interest in both LC-MS/MS and GC-MS/MS methodologies. However, there are significant limiting factors concerning their widespread application in clinical and biochemical laboratories. The sources of result variability as well as the advantages and limitations of the most investigated analytical techniques potentially applicable in UOA analysis are discussed below.

Sources of GC-MS Result Variability

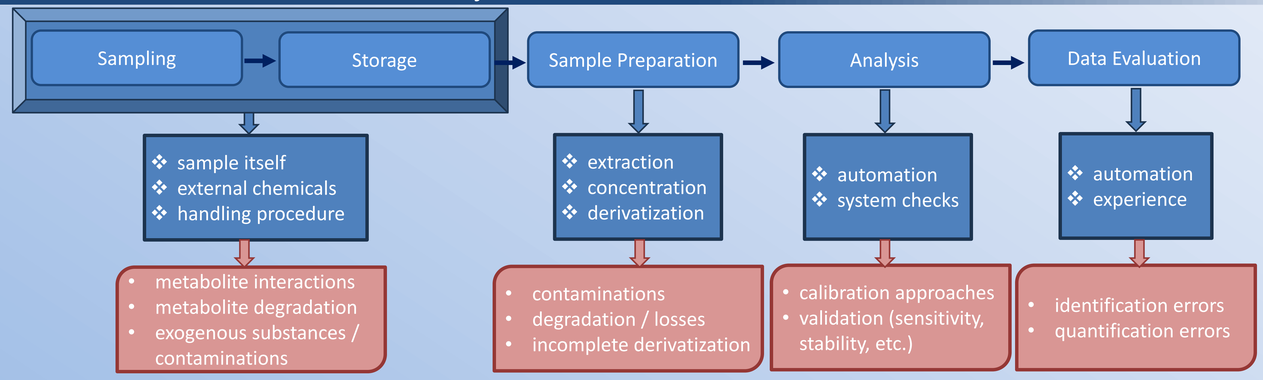

Fig. 1. General workflow of derivatization-based GC-MS metabolomic platforms, accompanied by the major factors in each step that might lead to variations and the corresponding influences and implications [1].

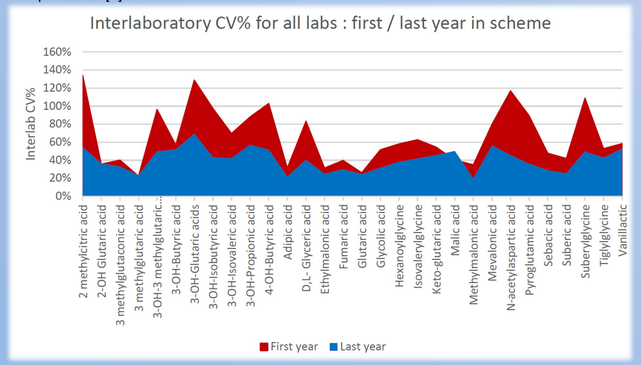

Fig. 2. Difference in Interlaboratory CV% of all labs between the first year and the last year of compound introduction in scheme (15-years interval)[2].

With the sample preparation step introducing most variations, without a harmonized workflow the factors that must be critically evaluated before implementation include, among others, the following [4]:

- selection of internal standard,
- selection of extraction solvent
- extract evaporation temperature
- drying step duration
- application of oximation
- selection of derivatization reagent, time & temperature
- use of catalyst.

On the other hand, method validation is the key factor ensuring both **reproducibility** and **accuracy**.

Conventional & Advanced Analytical Techniques in UOA Analysis

Technique	Use	Targeted Panels	Untargeted Profiling	Discovery / Research Use	Sample Preparation
GC/MS (Q)	✅ Gold standard	⚠ Limited	✅ Yes (Fig. 3)	⚠ Some retrospective use	Extraction (LLE) & chemical derivatization (e.g., oximation + silylation)
GC-MS/MS (QqQ)	✅ Confirmatory tests	✅ Yes	❌ No	⚠ Limited	Extraction & chemical derivatization
GC-HRMS (TOF or Orbitrap)	⚠ Early adoption	✅ Potential	✅ Yes	✅ Yes	Extraction & chemical derivatization
LC-MS/MS (QqQ)	⚠ Limited (NBS)	✅ Yes	❌ No	⚠ Limited	Protein precipitation or dilution; **no derivatization**
LC-HRMS (TOF or Orbitrap)	❌ Not routine (yet)	⚠ Developing	✅ Yes	✅ Yes	Protein precipitation or dilution; **no derivatization**

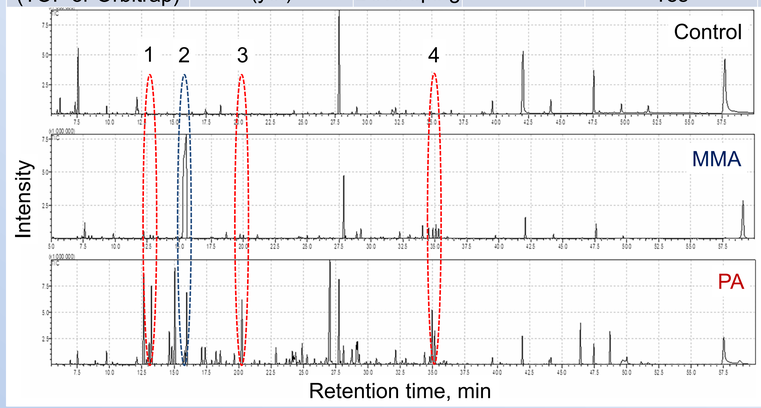

Fig. 3. The typical total ion chromatograms (TICs) of urine metabolic profiling for controls, MMA and PA (from top to bottom), where the most characteristic biomarkers are 3-OH-propionic (1), Methylmalonic (2), Propionylglycine (3) and Methylcitric (4)[3].

Despite the benefit of less demanding sample preparation protocols provided by the liquid chromatographic systems coupled to low and high mass resolution mass spectrometers, **matrix effects** that are common in LC-ESI-MS and the subsequent need for **isotope labeled internal standards** for every investigated analyte renders this approach challenging, when the aim is the accurate determination of multiple analytes, like the analysis of UOAs [5].

Conclusions:

- GC-MS provides broad-spectrum, full-scan metabolite profiling, enabling both qualitative and quantitative evaluation of UOAs, while the results are partially also enabling the retrospective analysis of the obtained data.
- The development of sensitive GC-HRMS methods is of paramount importance in the establishment of new biomarkers, but its cost and the need for specialization demands limit its wider application in clinical and analytical laboratories.
- Result variances can potentially carry significant clinical implications, since incorrect peak identification or missed detection of key metabolites due to inconsistent recovery can compromise diagnostic accuracy.
- Harmonization of the entire analytical workflow applied in UOAs analysis is the key factor in achieving reliable results and eliminating the possibility of misinterpretations.

Reference:

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