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Metabolomic fingerprinting of serum samples by direct infusion mass spectrometry

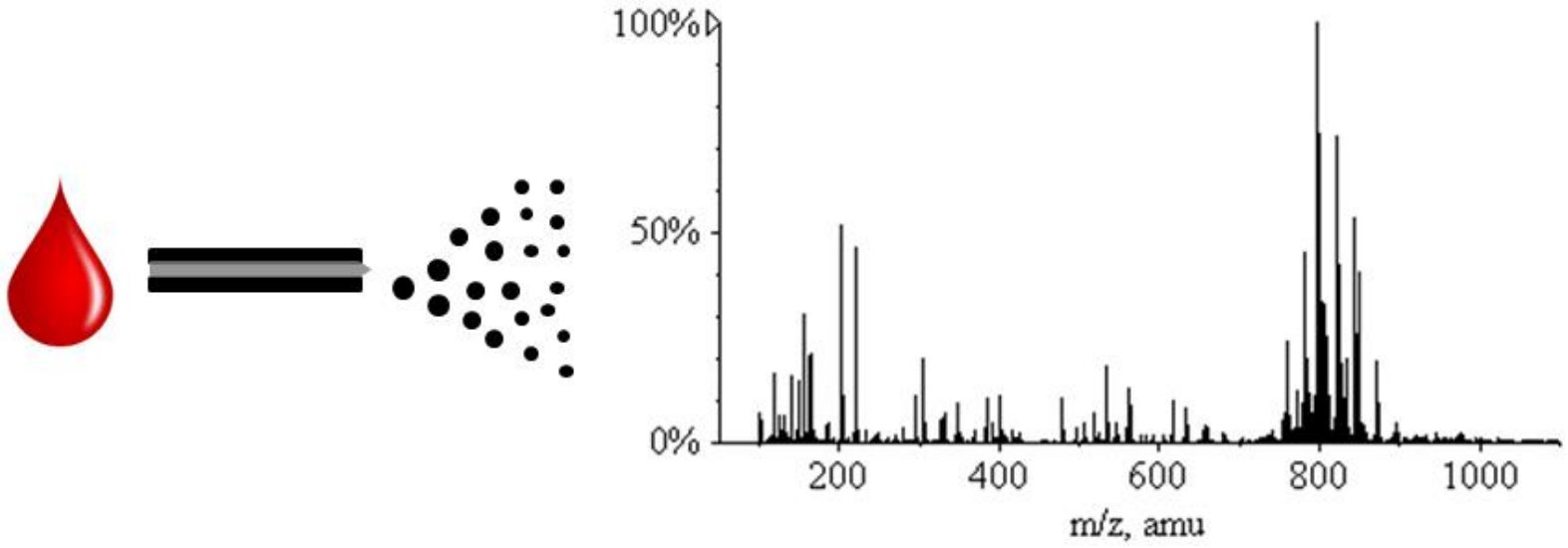
Raúl González-Domínguez *

Department of Chemistry, Faculty of Experimental Sciences. University of Huelva, Spain.

* Corresponding author: raul.gonzalez@dqcm.uhu.es

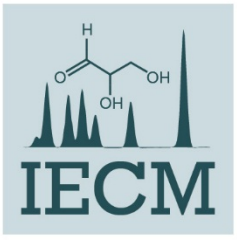
Metabolomic fingerprinting of serum samples by direct infusion mass spectrometry

Graphical Abstract



Abstract:
Metabolomics has demonstrated a great potential in numerous biomedical research fields in the last years, such as the study of the underlying pathology of diseases, discovery of diagnostic biomarkers or drug development. Nowadays, the main challenge in metabolomics is to obtain comprehensive and unbiased metabolomic profiles due to the huge complexity, heterogeneity and dynamism of the metabolome. For this purpose, mass spectrometry represents a very interesting analytical platform, since complexity of metabolome may be overcome through the use of different orthogonal separation techniques, including liquid chromatography, gas chromatography and capillary electrophoresis. Alternatively, direct mass spectrometry analysis, either by direct infusion or flow injection, has been postulated as an alternative in metabolomics, complementing hyphenated approaches. These techniques exhibit several advantages such as the ability for high-throughput screening, fast analysis and wide metabolomic coverage, since there is not exclusion of compounds due to the separation device.
The present work explores the potential of metabolomic platforms based on direct infusion mass spectrometry for metabolic fingerprinting of serum samples. The most important issues to be considered in this type of approach were reviewed, including sample handling, comprehensive analysis, as well as further identification of metabolites and global characterization of metabolomic fingerprints.

Keywords: metabolomics; direct infusion mass spectrometry; serum



Introduction

Mass spectrometry – based metabolomics

- High sensitivity and selectivity
- Qualitative and quantitative analysis
- Analysis of complex samples
- **Versatility**
  - *Ionization mode* (**ESI**, APCI, APPI)
  - *Sample introduction* (chromatography, capillary electrophoresis, **direct infusion**)

Introduction

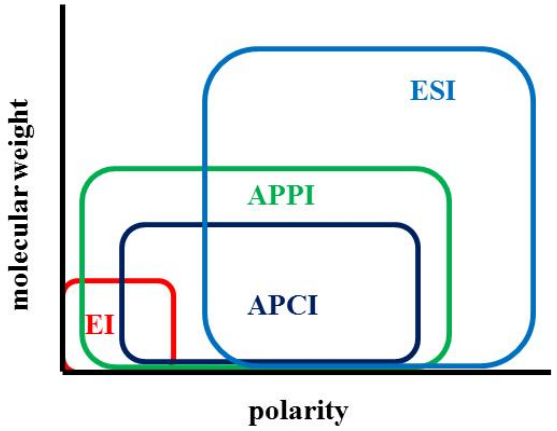
IONIZATION MODE

• The huge complexity and heterogeneity of metabolome make necessary the use of complementary ionization modes

Electrospray (ESI): ionizes compounds over a large mass range (largely used in metabolomics)

Atmospheric pressure chemical ionization (APCI): analysis of less polar compounds

Atmospheric pressure photoionization (APPI): non-polar compounds, low susceptibility to matrix effects



Introduction

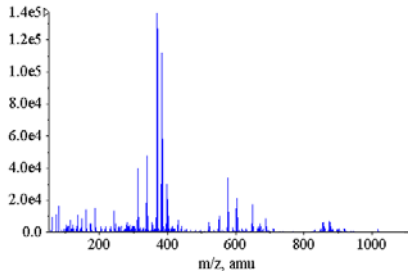
SAMPLE INTRODUCTION

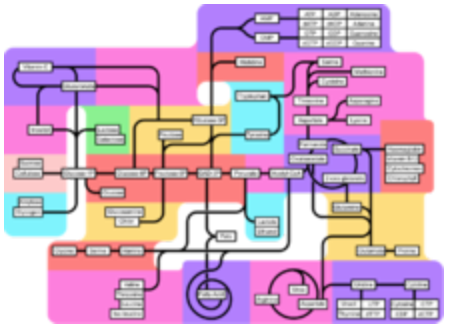
i. Combination with **separation techniques** (LC, GC, CE): reduction of mass spectra complexity

ii. **Shotgun analysis** (direct infusion or flow injection)

- Short analysis time
- High sensitivity
- Instrumental reproducibility
- Non-discriminant analysis
- Simpler data pre-processing

High-throughput metabolomic fingerprinting





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Objectives

Development of a metabolomic approach based on **direct infusion mass spectrometry** (DIMS) for high-throughput fingerprinting of serum samples

- Protocol for exhaustive extraction of metabolites from serum
- Analysis by direct infusion - electrospray - high resolution mass spectrometry (DI-ESI-QTOF-MS)
- Characterization of metabolomic fingerprints in order to assess the metabolome coverage

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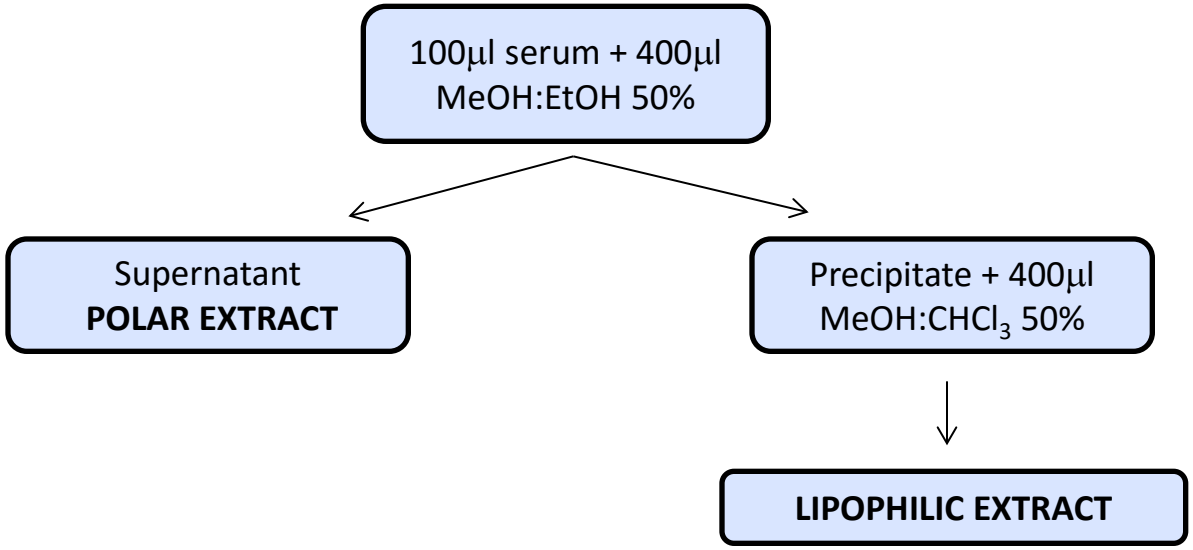
Results and discussion

1. METABOLITES EXTRACTION

• Common procedures based on protein precipitation with organic solvents fails to extract lipophilic components, which may remain adsorbed to protein precipitate

• Optimization of **a two-step serum extraction** method



Results and discussion

2. METABOLOMIC ANALYSIS BY DI-ESI-MS

• Analysis in positive and negative modes (wider metabolome coverage)

ESI-QTOF-MS
(QSTAR XL, Applied Biosystems)

	ESI(+)	ESI(-)
ion spray voltage	3300 V	-4000 V
declustering potential	60 V	-100 V
focusing potential	250 V	-250 V
curtain gas (N_2)	1.13 L/min	
nebulizer gas (N_2)	1.56 L/min	
heater gas (N_2)	0	
source temperature	60ºC	
m/z range	50-1100	
flow rate	5 μL/min	

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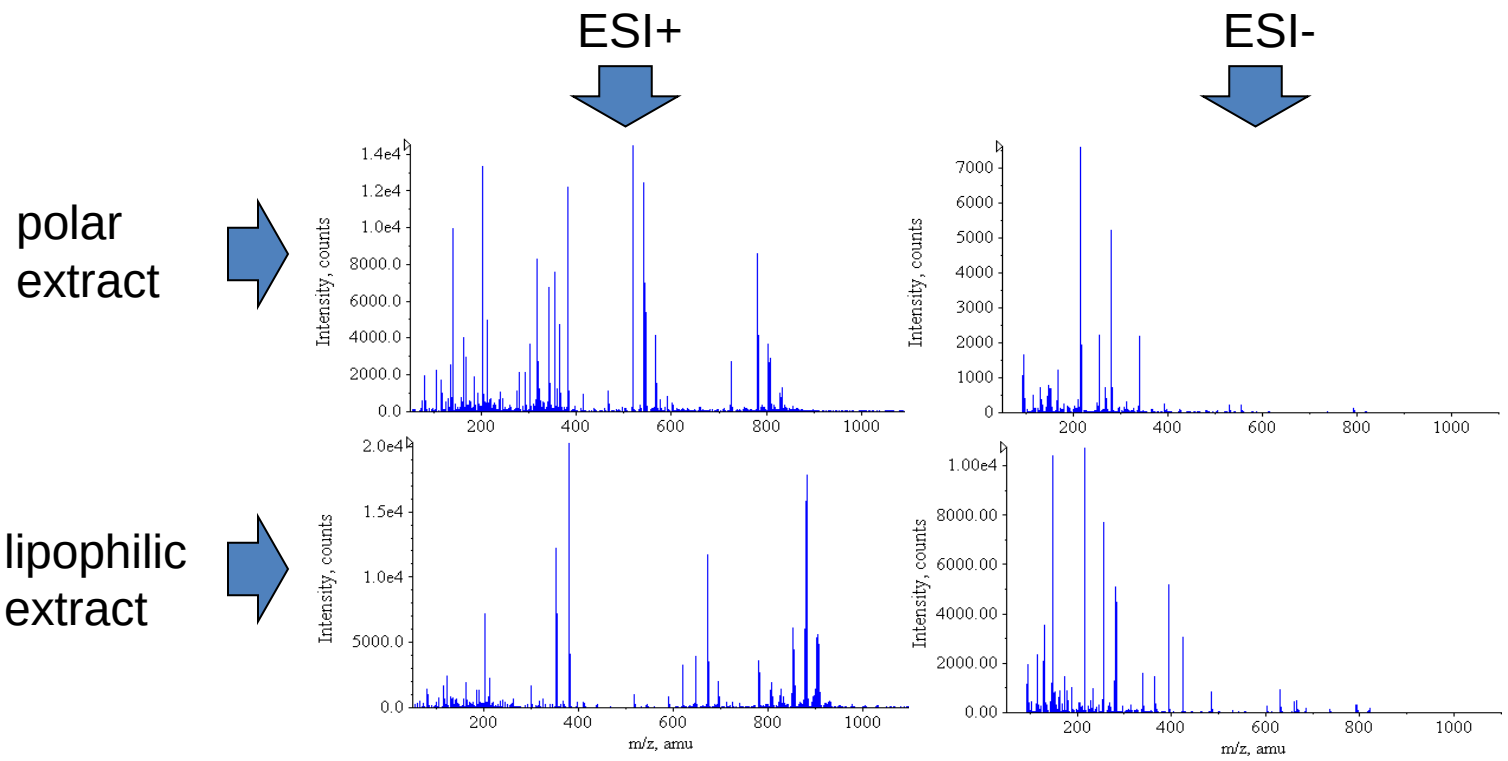
Results and discussion

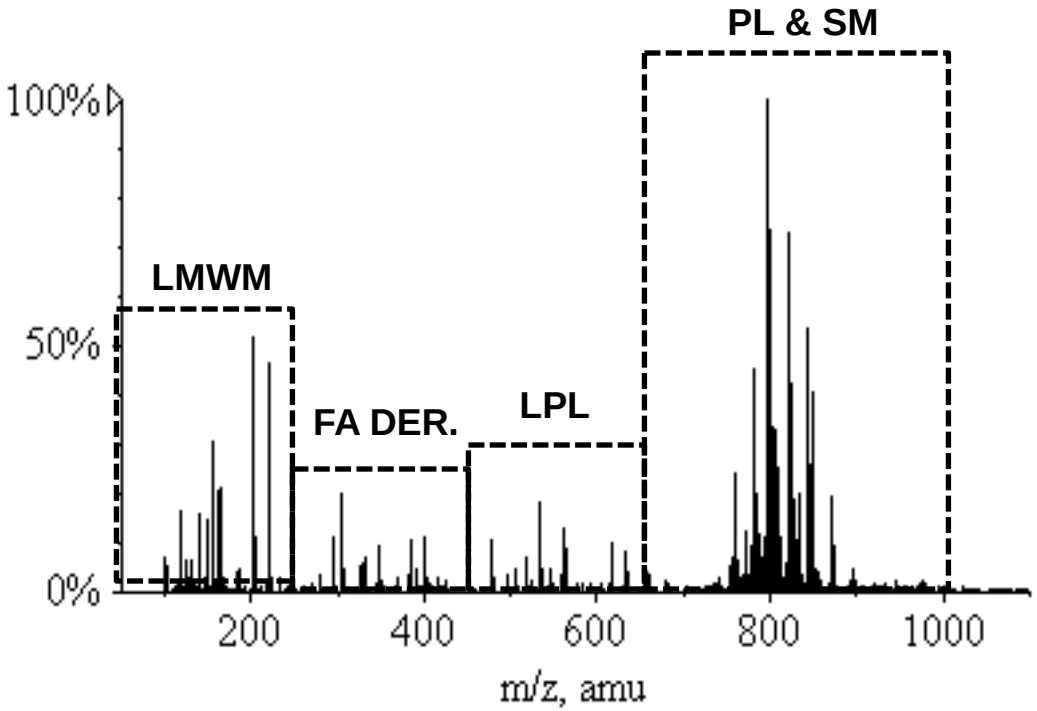
2. METABOLOMIC ANALYSIS BY DI-ESI-MS

- Detection of numerous metabolites in a wide range of molecular weights



Results and discussion

3A. CHARACTERIZATION OF METABOLOMIC FINGERPRINTS: ESI+ (Polar extracts)



LMWM: low molecular weight metabolites
FA DER: fatty acid derivatives; acyl-carnitines (ACAR), eicosanoids.
LPL: lyso-phospholipids
PL: phospholipids
SM: sphingomyelins

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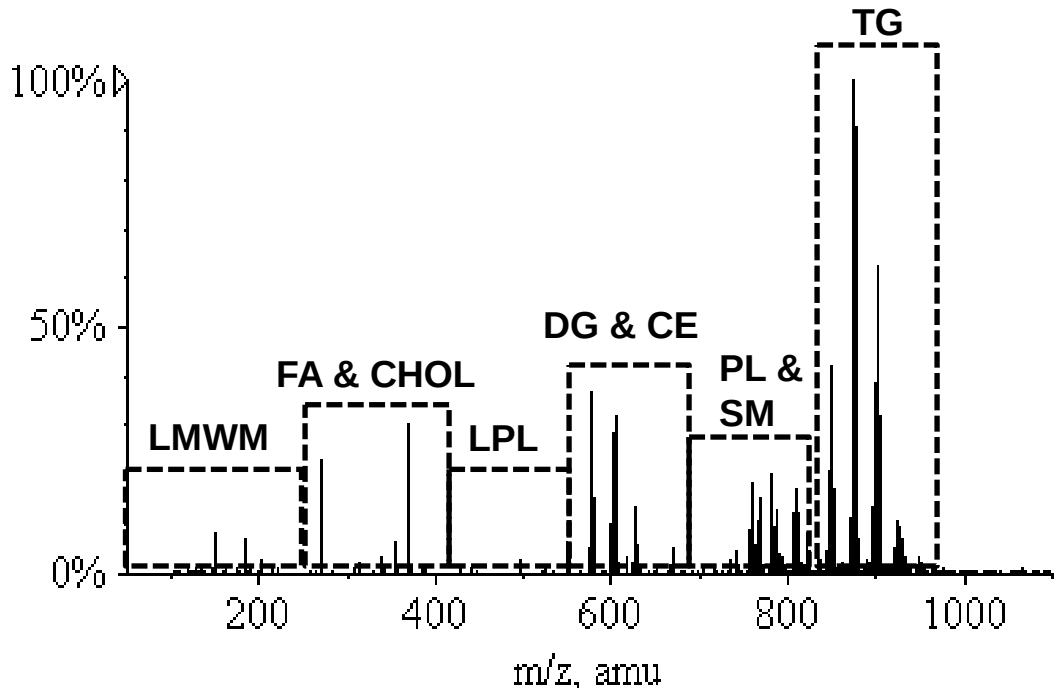
Results and discussion

3B. CHARACTERIZATION OF METABOLOMIC FINGERPRINTS: ESI+ (Lipophilic extracts)



LMWM: low molecular weight metabolites
FA: fatty acid derivatives; acyl-carnitines (ACAR), eicosanoids.
CHOL: cholesterol derivatives
LPL: lyso-phospholipids
DG: diglycerides
CE: cholesteryl esters
PL: phospholipids
SM: sphingomyelins
TG: triglycerides

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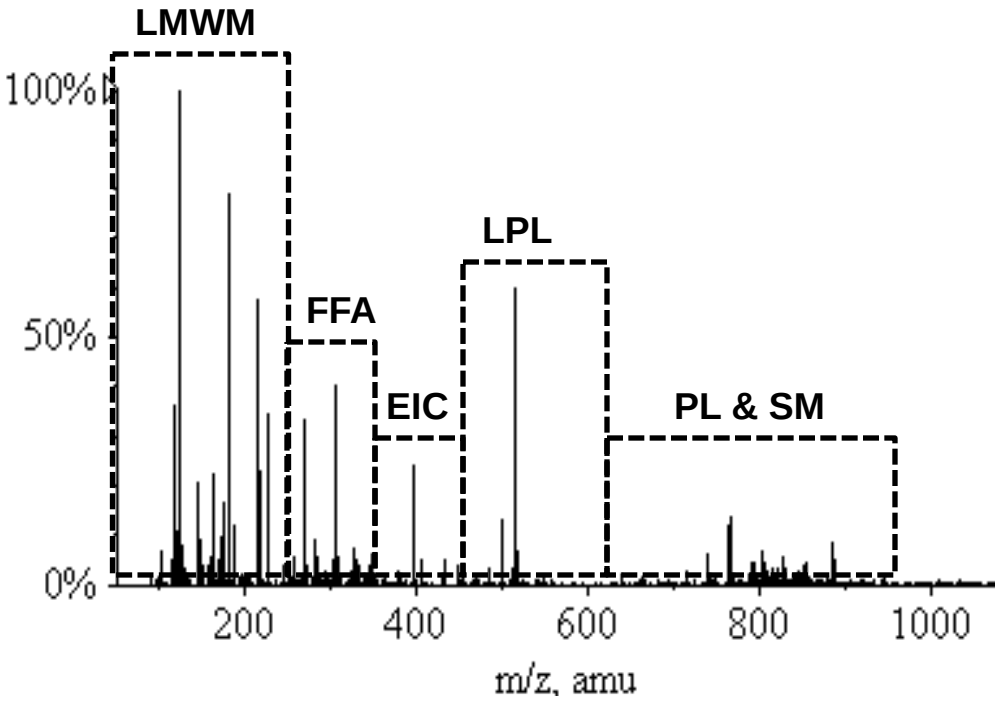
Results and discussion

3C. CHARACTERIZATION OF METABOLOMIC FINGERPRINTS: ESI- (Polar & Lipophilic extracts)



LMWM: low molecular weight metabolites
FFA: free fatty acids
EIC: eicosanoids.
LPL: lyso-phospholipids
PL: phospholipids
SM: sphingomyelins

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Results and discussion

COMPARISON OF DIMS WITH CONVENTIONAL MS-BASED APPROACHES

	advantages	disadvantages
GC-MS	- reproducibility - high sensitivity - good separation resolution - availability of mass spectral libraries	- limited to low molecular weight metabolites - derivatization step needed - time consuming separation step
LC-MS	- wide range of applicability - high sensitivity	- ion suppression - time consuming separation step
CE-MS	- good separation resolution - need of small amount of sample	- low robustness and reproducibility - time consuming separation step
DIMS	- high throughput analysis - reduced analysis time - instrumental simplicity - data processing simplicity - wide metabolome coverage	- ion suppression - differentiation of isobars - mass spectra complexity

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Conclusions

- Direct infusion mass spectrometry exhibits a great potential for high throughput metabolomic analysis because of its reduced analysis time and wide metabolome coverage

- The use of a two-step extraction procedure allows recovering non-polar metabolites, which usually remain adsorbed into the protein precipitate when conventional metabolomic protocols are employed

- This metabolomic approach enabled the identification of multiple classes of metabolites ranging very diverse physicochemical properties, from low molecular weight metabolites, such as amino acids, carbohydrates or nucleotides; to different lipid classes, including phospholipids, glycerolipids, fatty acids and derivatives, among others

- Accordingly, DI-ESI-MS stands out as a suitable analytical tool for fast and comprehensive “first pass” metabolomic screening

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Acknowledgments

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