

High-throughput non-targeted metabolic profiling with hybrid stationary phase LC column coupled to Q-TOF-MS in cancer research

Hyun-Jin Jung^{1, 2}, Man Ho Choi¹, Kyung Mi Kim¹, Won-Yong Lee², Bong Chul Chung¹

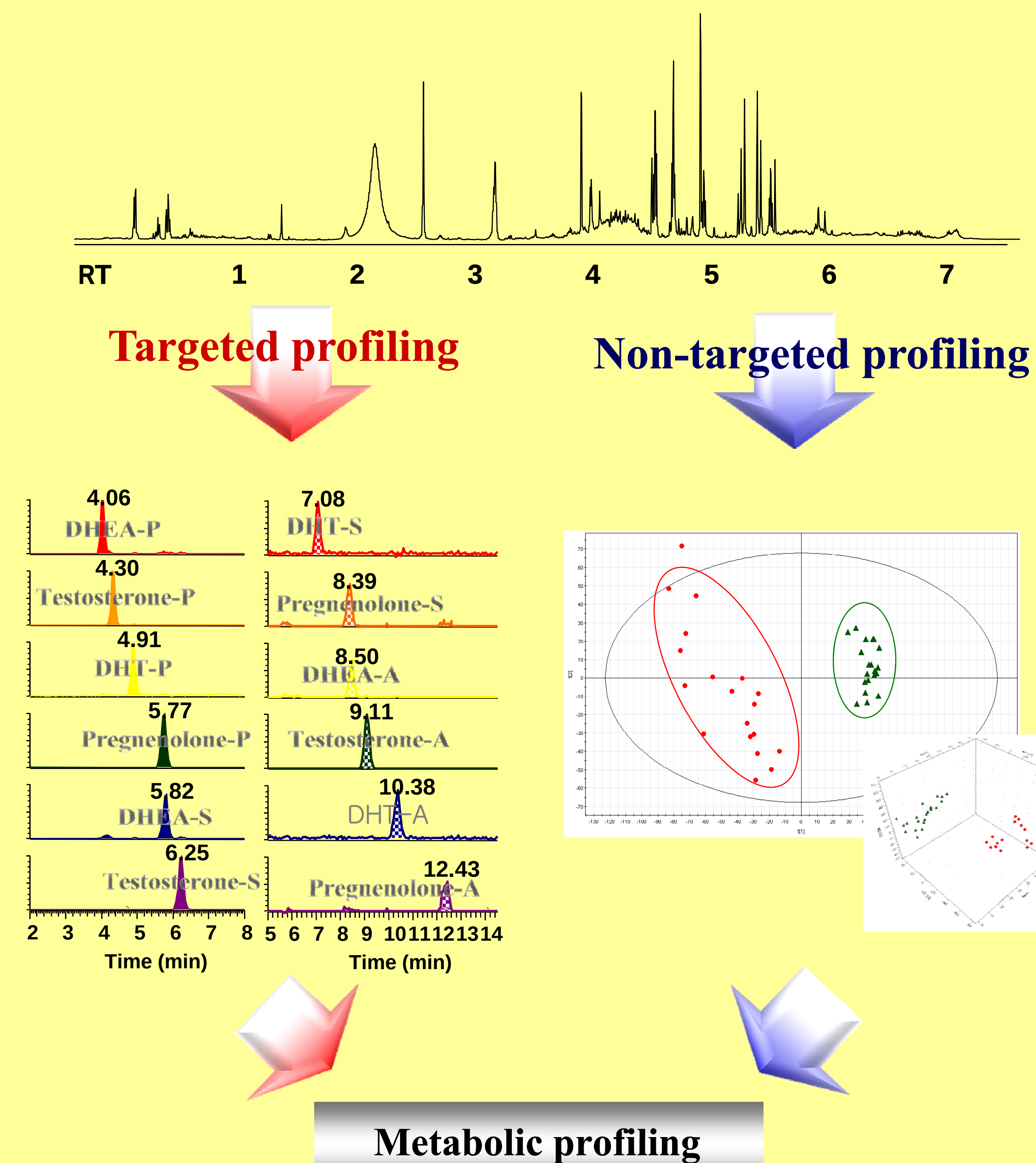
¹ Life Sciences Division, KIST, Seoul 136-791, Korea; ² Department of Chemistry, Yonsei University, Seoul 120-749, Korea



Introduction

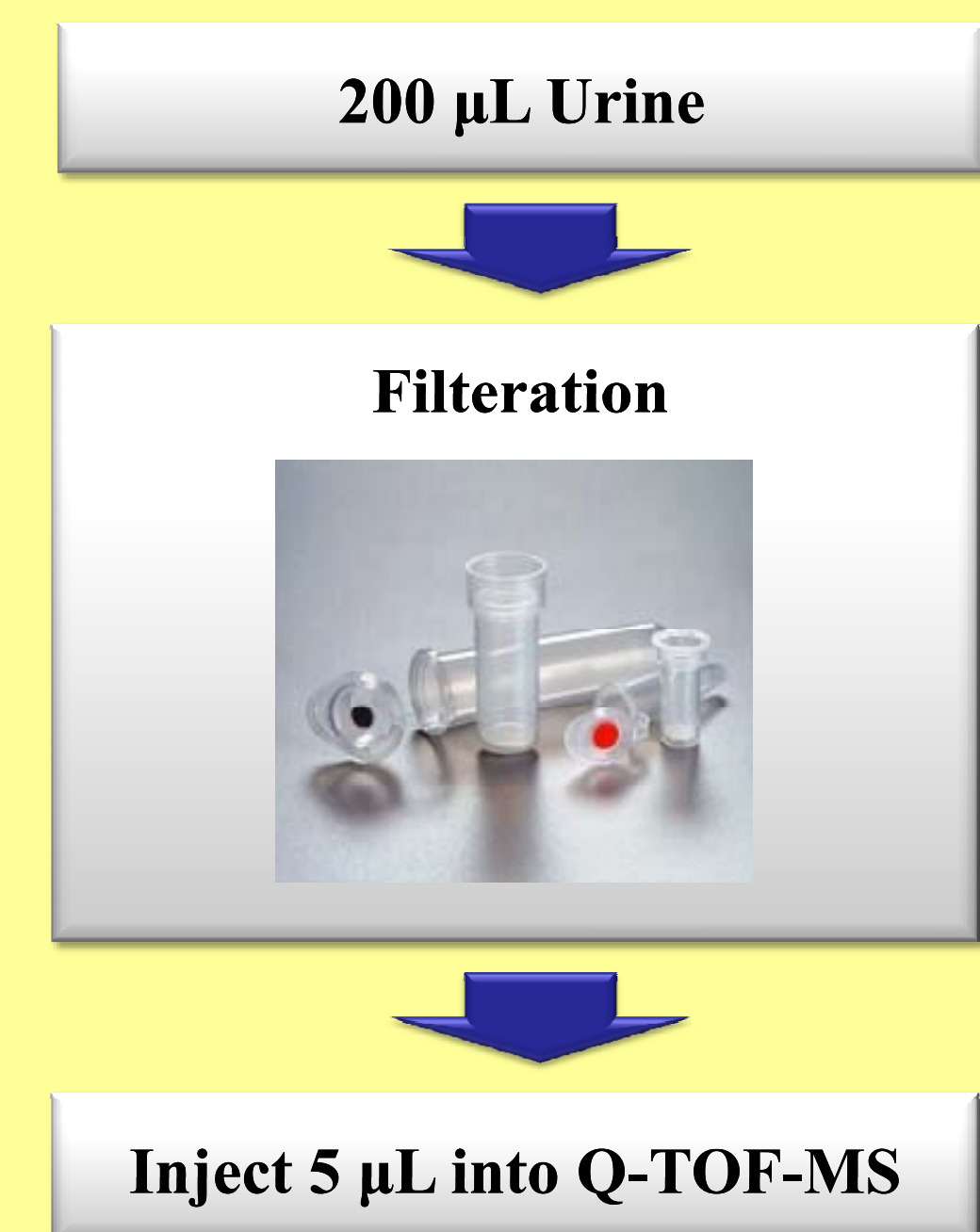
Metabolomics is a suitable approach in monitoring early changes of metabolic pathways and these metabolic perturbations represent a biomarker of cancer, which is one of highly complex diseases. Although gas chromatography-mass spectrometry based approaches have been primarily used in metabolomics, difficulties in detection of polar compounds with high molecular weight or hydrophilicity limit this technique. In contrast targeted analysis, non-targeted metabolic profiling involves a large number of different metabolites with the objective of identifying a specific metabolite responsible for biological changes. A rapid and reproducible sample preparation is necessary in non-targeted metabolomics because it may affect reproducibility as a result of the heterogeneity of metabolites derived from cell populations. Here, we introduce a non-targeted metabolic profiling technique using hybrid stationary phase LC column coupled to a quadrupole-time-of-flight mass spectrometer (Q-TOF-MS) to identify altered metabolites and to find potential biomarkers, which may indicate progress of cervical and prostate cancers.

Metabolomic approaches



Experimental procedures

Sample pretreatment steps



Instrumental conditions

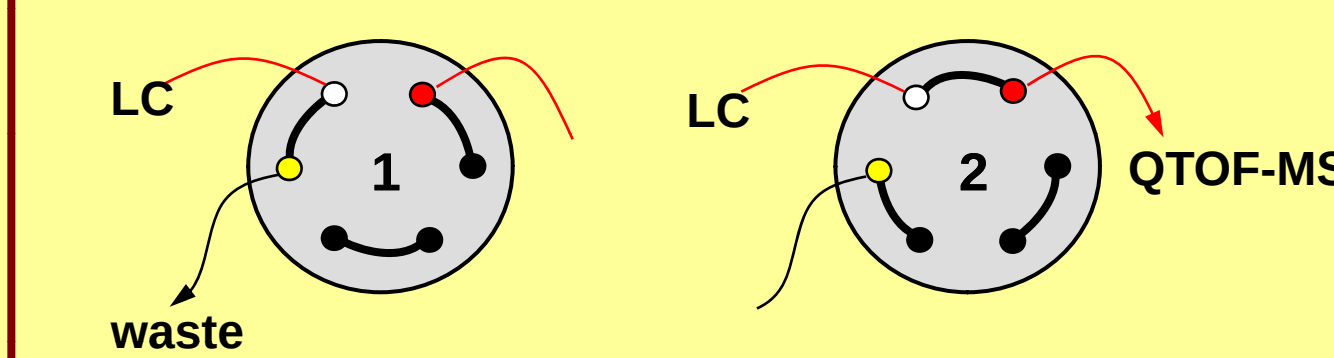
LC parameters

Instrument: ACQUITYTM Ultra Performance Liquid Chromatography system
Column: Cadenza HS-C18 (2.0 × 100 mm, 3 µm)
Flow rate: 0.4 mL/min
Injection volume: 5 µL
Column temperature: 40 °C
Mobile phase:
A: 0.1 % formic acid in 5 % ACN
B: 0.1 % formic acid in 95 % ACN
Gradient:
100 % A (4 min) → 10 % A → 100 % A

MS parameters

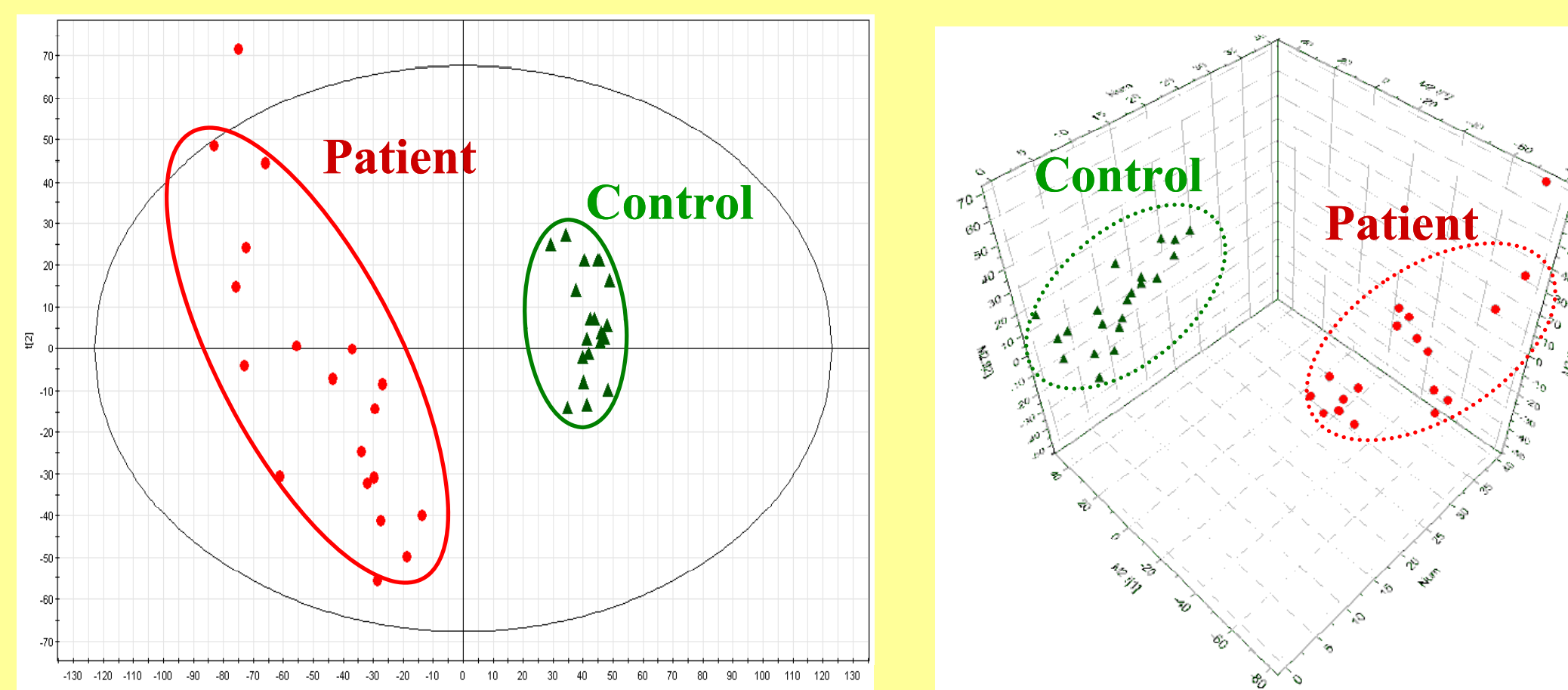
Instrument: Waters Q-TOF micro MS
Ionization: ESI⁺ ion modes
Acquisition mode: Scan (m/z 50-1,200)
Nebulization gas: 600 L/h
Cone gas: 60 L/h
Source temperature: 105 °C
Capillary voltage: 3,100
Cone voltage: 40 V

Column switching

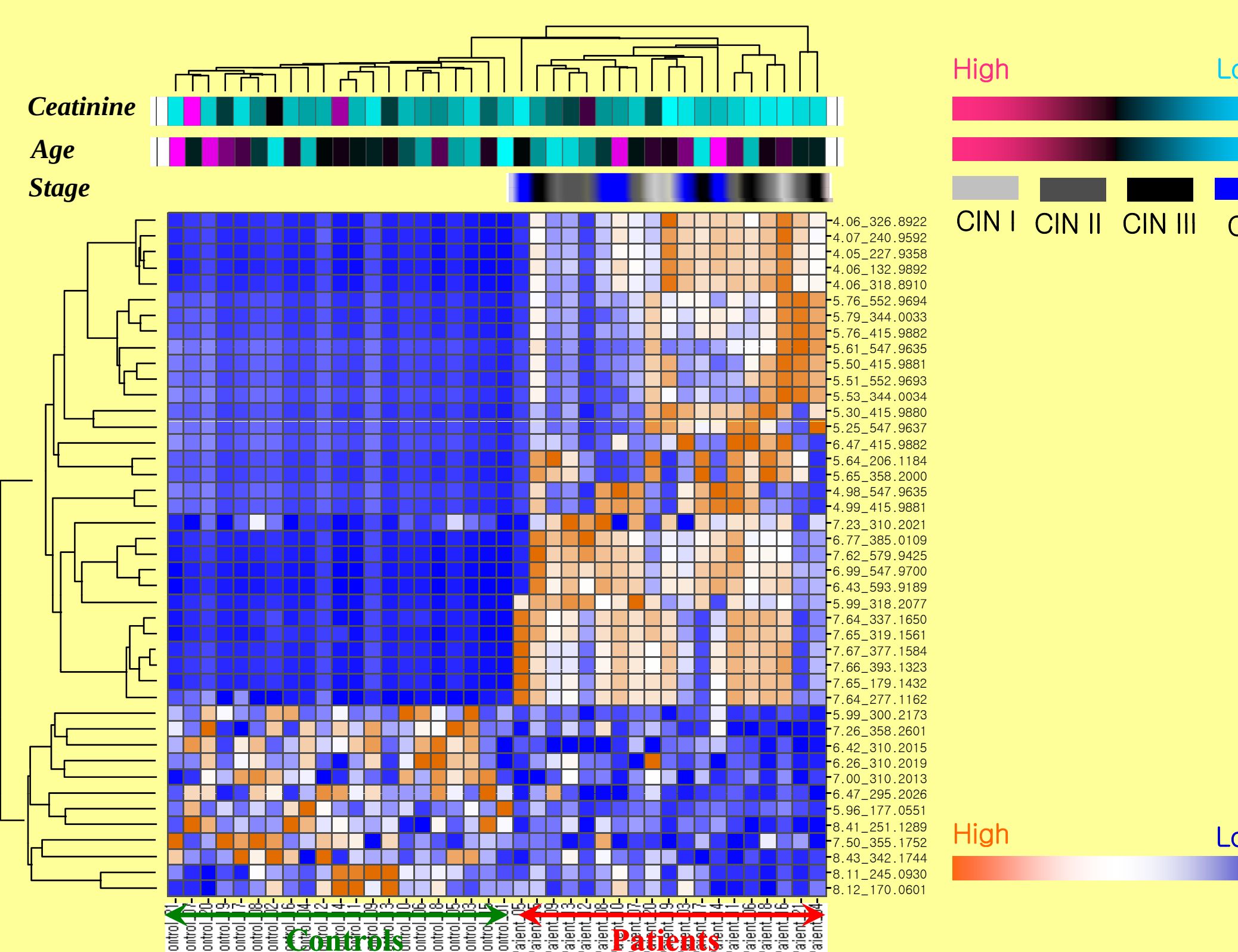


Statistical analysis

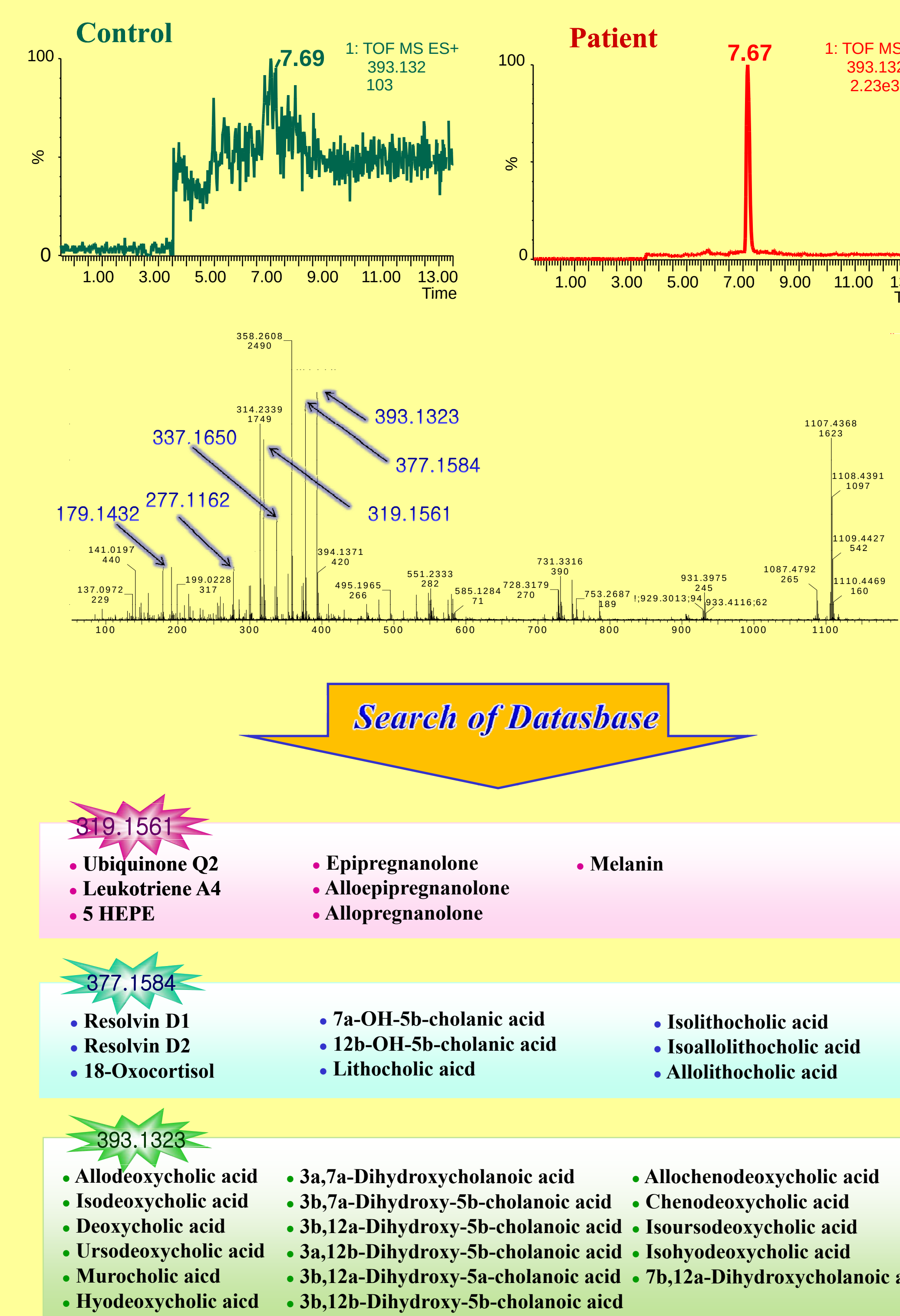
PLS-DA score plot



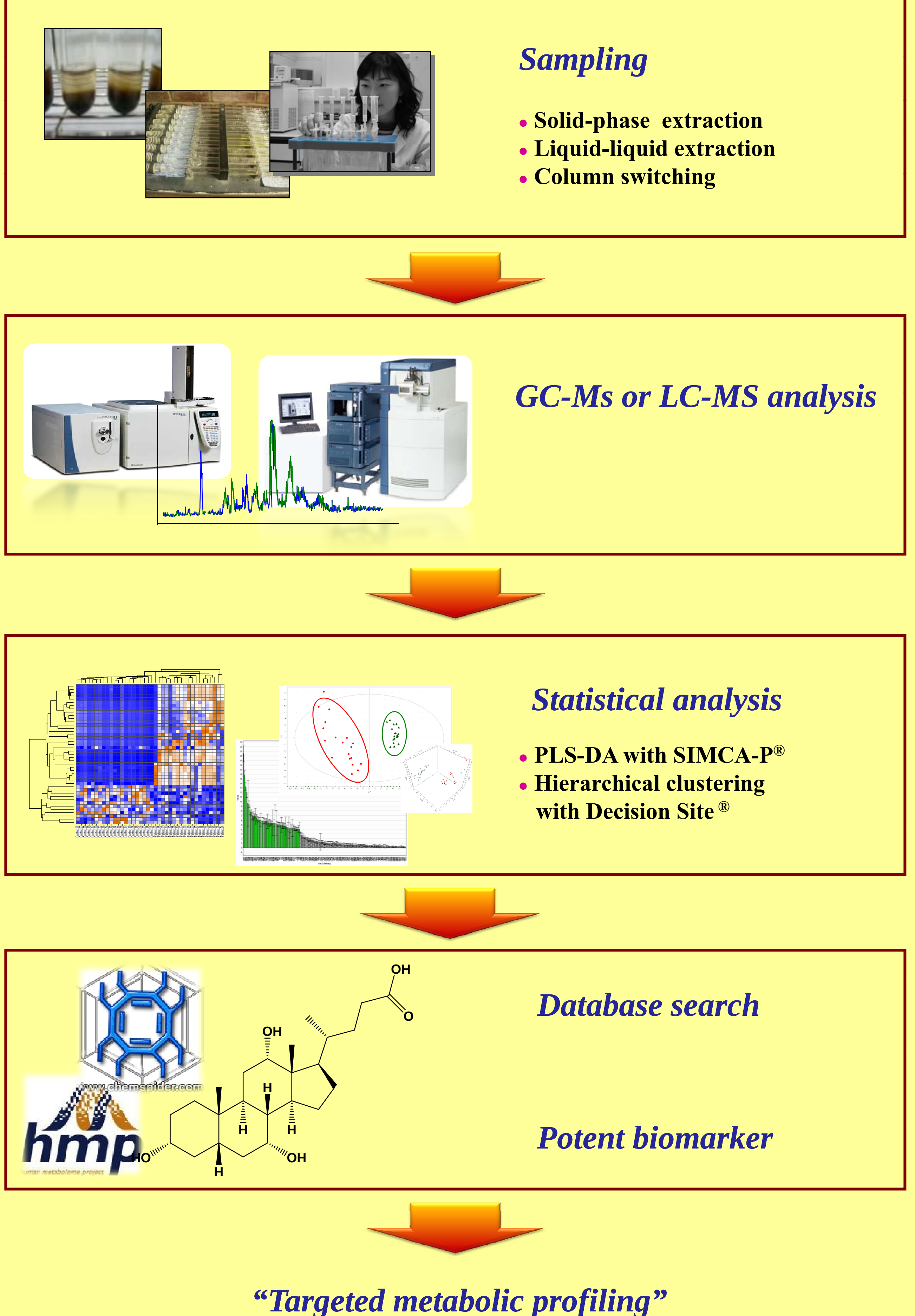
Hierarchical clustering analysis



Identification of potent biomarker



Procedure for Non-targeted profiling



Conclusion

Using the present non-targeted metabolic profiling technique combined with hybrid stationary phase LC column, two potential biomarkers which may indicate progress of cervical dysplasia was identified and they might be correlated with lipid or energy metabolism.