Comprehensive Polar Metabolite Analysis in Two Minutes: Rapid CE-MS Separations Combined with Ultrafast, High Resolution Time-of-Flight Mass Spectrometry

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Overview

- This study evaluates ultra-fast CE-MS separations for comprehensive analysis of polar metabolites in minimally treated complex biological matrices (urine and beer).
- Experiments employ a nanospray sheath flow CE-MS interface[1], an in-house built CE instrument accommodating short separation capillaries, and LECO Citius® LC-HRT high resolution TOF mass spectrometer.
- MS and comprehensive CID acquisition were performed at rates of up to 200 spectra per second (100 spectra per second per data channel), and resolution of up to 100,000.
- Narrow separation time windows of fast CE separations can be compensated by high CE separation efficiency combined with high resolution, high mass accuracy, fast acquisition rate, and effective MS and CID deconvolution.
- Two-minute long CE separations can reveal significant differences in an abundance of biologically relevant species.

Introduction

The potential for capillary electrophoresis as the fastest and the most efficient liquid separation is rarely exercised in combination with mass spectrometry detection.

The challenges include the need for robust and sensitive coupling of CE with MS, lack of compact CE autosamplers, and the need for fast and sensitive MS instrumentation.

Recently we demonstrated successful coupling of ultra-fast, high electric field CE separations with high resolution, fast acquisition TOFMS detection[2].

In this work we evaluate the feasibility of fast CE-MS separations for comprehensive analysis of polar metabolites in urine and beer.

Sample complexity in narrow separation time windows is offset by high CE separation efficiency, operation in the nanospray regime, high resolution, high mass accuracy detection, and effective deconvolution software.

Methods

CE-MS experiments employed 20 μm i.d. and 90 μm o.d. 22-24 cm long uncoated capillaries, and 10 μm i.d. glass emitters. CE employed 30 kV separation and 2kV electrospray voltage.

Urine and beer samples were filtered and diluted 1:3 in the sample buffer (200 mM formic acid). Untargeted and targeted peak search was performed with ChromaTOF® software.

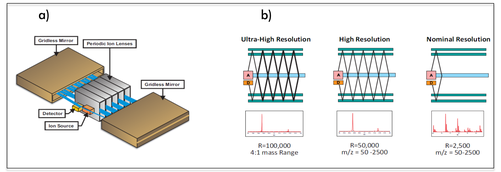
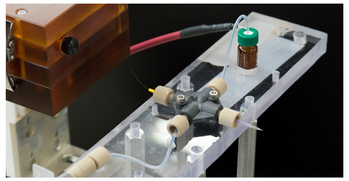


Figure 1. A brief introduction to LECO Citius LC-HRT modes of operation
a) Illustration of the Folded Flight Path® Technology
b) Depiction of the Citius LC-HRT's three modes of operation



Nanospray CE-MS interface based on electrokinetically pumped sheath flow[1]

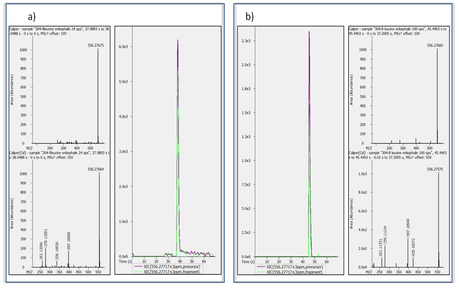


Figure 2. Ultra-high resolution (100,000) CE-MS-CID of Leucine Enkephalin (50 femtomoles). 45V fragmentation offset. Extracted ion electropherogram and a single spectrum at the CE peak apex.
a) 48 spectra per second (24 spectra per second per data channel)
b) 200 spectra per second (100 spectra per second per channel)

Results

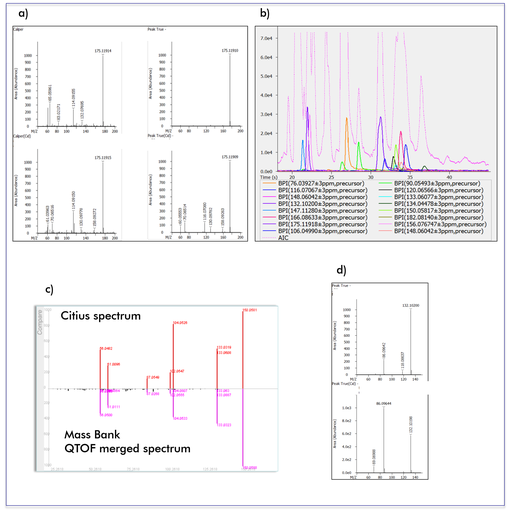


Figure 3. 16 amino acid standards (100 femtomoles each) spiked into a urine sample. 30 spectra per second per channel acquisition, 20V fragmentation offset.
a) Raw and deconvoluted MS and CID spectra of Arginine.
b) Deconvoluted masses corresponding to spiked amino acid standards and total deconvoluted ion current trace (AIC).
c) Deconvoluted spectrum of Methionine evaluated with Metlin database.
d) Deconvoluted CID spectrum of Isoleucine containing its diagnostic ion.

Table 1: Amino acid standards spiked into urine sample, deconvoluted with untargeted and targeted (*) peak search.

name	formula	theoretical $(M+H)^+$	observed $(M+H)^+$	delta ppm
Glycine	$C_2H_5NO_2$	76.03930	76.03927	0.4
Alanine	$C_3H_7NO_2$	90.05495	90.05493	0.2
Serine	$C_3H_7NO_3$	106.04986	106.04990	-0.4
Proline	$C_5H_9NO_2$	116.07060	116.07067	-0.6
Threonine	$C_4H_9NO_3$	120.06552	120.06566	-1.2
Isoleucine	$C_6H_{13}NO_2$	132.10191	132.10200	-0.7
Leucine	$C_6H_{13}NO_2$	132.10191	coeluting	
Asparagine	$C_4H_8N_2O_3$	133.06076	*133.06077	-0.1
Aspartic acid	$C_4H_7NO_4$	134.04478	134.04478	0.0
Lysine	$C_6H_{14}N_2O_2$	147.11280	147.11280	0.0
Glutamic acid	$C_5H_9NO_4$	148.06043	*148.06042	0.1
Methionine	$C_5H_{11}NO_2S$	150.05833	150.05817	1.0
Histidine	$C_6H_9N_3O_2$	156.07675	156.07674	0.1
Phenylalanine	$C_9H_{11}NO_2$	166.08626	*166.08633	-0.5
Arginine	$C_6H_{14}N_4O_2$	175.11895	175.11910	-0.8
Tyrosine	$C_9H_{11}NO_3$	182.08116	*182.08140	-1.3

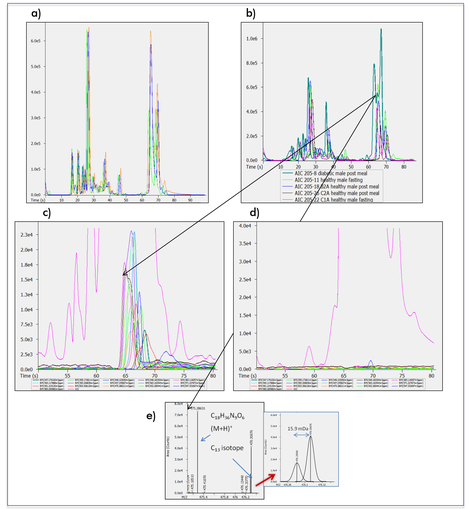


Figure 4. Analysis of urine samples. 30 spectra per second per channel acquisition. m/z range 50-625.
a) Technical replicates of a urine sample. Deconvoluted ion current trace (AIC).
b) AIC's of different urine samples (fasting and post meal from healthy and diabetic males).
c) Selected high molecular weight compounds neutral at pH 2, deconvoluted by untargeted peak search in a diabetic's post meal urine.
d) The same compounds are mostly absent in the healthy man's post-meal urine.
e) Application of high resolution acquisition in spectral separation of compounds. Minimum resolution to separate C_{13} isotope of $C_{18}H_{36}N_9O_6$ from another compound at Full Width Half Height is 30,000.

Table 2: Selected compounds across mass range with significantly overexpressed abundance.

theoretical $(M+H)^+$	observed $(M+H)^+$	Formula	delta ppm	fasting urine	urine after meal	diabetic fasting urine	diabetic urine after meal
393.21057	393.21029	$C_{16}H_{32}N_4O_6$	0.7	no	yes	no	no
180.06551	180.06557	$C_6H_9NO_3$	-0.3	yes	yes	no	no
138.05495	138.05504	$C_7H_7NO_2$	-0.7	no	yes	no	yes
583.29468	583.29552	$C_{28}H_{38}N_{10}O_6$	-1.4	no	yes	no	no
437.23678	437.23669	$C_{18}H_{32}N_6O_6$	0.2	yes	yes	no	no
160.13320	160.13318	$C_8H_{17}NO_2$	0.1	no	yes	no	yes
175.07133	175.07116	$C_6H_{10}N_2O_4$	1.0	no	yes	no	no
363.16227	363.16322	$C_{13}H_{22}N_6O_5$	-2.6	yes	yes	no	no
475.28613	475.28730	$C_{18}H_{38}N_8O_6$	-2.5	no	yes	no	no
146.08100	146.08120	$C_6H_{11}NO_3$	-1.4	no	no	yes	no

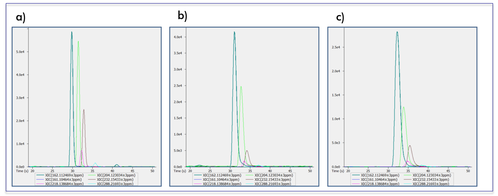


Figure 5. Carnitine species detected in post-meal urine samples: **a)** diabetic male, **b)** healthy male, **c)** healthy male after energy drink.

peak	formula	compound(s)
1	C_5H_5NS	pyridine thiolate
2	C_5H_9NO2	proline
3	$C_5H_{11}NO_2$	valine
4	$C_{18}H_{32}O_{16}$	tri –saccharide(s)
	$C_{24}H_{42}O_{21}$	tetra-saccharide(s)
	$C_{12}H_{22}O_{11}$	di-saccharide(s)

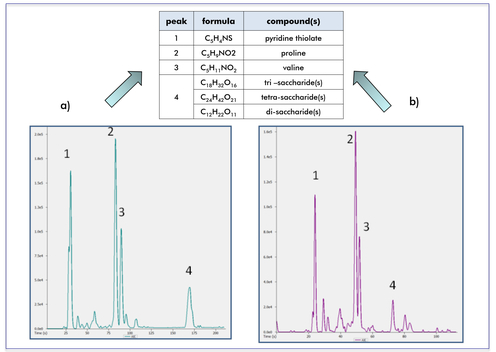


Figure 6. AIC of microbrewed beer samples with selected common compounds. 30 spectra per second per channel acquisition rate. 55-700 m/z range.
a) Stout.
b) Amber ale.

Conclusions

Comprehensive metabolite analysis typically requires large separation time windows for exhaustive sample characterization. Nevertheless, fast and efficient separations coupled with fast, high resolution, high mass accuracy MS detection and effective untargeted peak deconvolution, can detect significant differences between the samples.

Effective untargeted deconvolution can aid in detection of coeluting, poorly separated species, such as neutral compounds in capillary zone electrophoresis.

References

[1] Wojcik *et al RCMS*. **2010**; 24: 2554–2560.
[2] Wojcik *et al*, manuscript in submission.