Enhancing metabolic phenotyping by increased sensitivity provided by a vacuum insulated probe heated ESI (VIP-HESI) source

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Overview

Metabolic phenotyping is a highly informative approach in systems biology research that enriches the understanding of the metabolic mechanisms of infectious disease pathogenesis.
Advancements in quadrupole-time-of-flight (QToF) technology have enabled the use of high-resolution accurate mass instruments for both discovery and quantitative analyses [1]. However, metabolite concentrations can vary drastically between individuals and if sample volumes are limited, the dynamic range and sensitivity of the instrument become critical. Of significant influence to metabolite sensitivity is the design of the ionization source and efficient generation of charged ions.

Here, we determine if improvements in sensitivity and reproducibility occur in the newly developed ion source vacuum-insulated-probe heated electrospray ionization source (VIP-HESI) and compare the results to a previously validated method for detection 30 physiological biogenic amines on an Apollo II ESI source coupled to a timsTOF Pro.

Methods

Sample: 41 human Urine samples (10µL) were centrifuged and transferred to a 96-well plate. Internal standards were added to each of the samples before derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate to transform primary and secondary amines into highly stable derivatives. The subsequent derivatized samples were diluted 1:4 with water for analysis. A series of calibrators between the range (1 to 400 µmol/L) were used for quantification.

LC: Acquity UPLC, HSS T3 (2.1 x 150 mm, 1.8µm)

A: 2 mM ammonium acetate in water

B: 2 mM ammonium acetate in acetonitrile/water (95/5)

Time (min)	% B at 0.6 mL/min
0	5
0.2	5
5.0	30
5.1	100
6.1	100
7.5	5

Injection volume: 2 µL

MS: timsTOF Pro (Bruker)

Acquisition: Broadband CID (bbCID) positive mode (8 Hz)

Collision energy: 6eV (MS); 20 and 50 eV (MS/MS)

Mass range: 30 – 1000 *m/z*

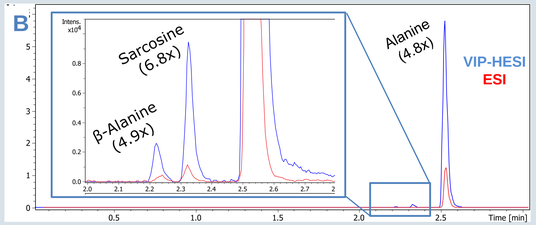
Source	ESI	VIP-HESI
Ionization (V)	4500	3000
Nebulizer gas (Bar)	5	3.5
Dry gas (L/min)	12	9
Dry temperature (°C)	250	250
Probe gas temperature (°C)	-	300
Probe gas (L/min)	-	5
Exhaust	-	On

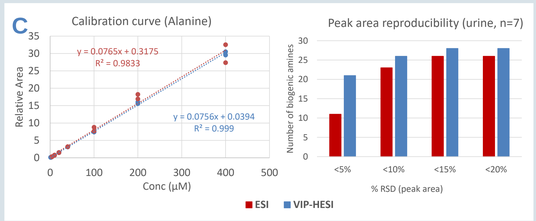
Data Processing: TASQ® 2021b

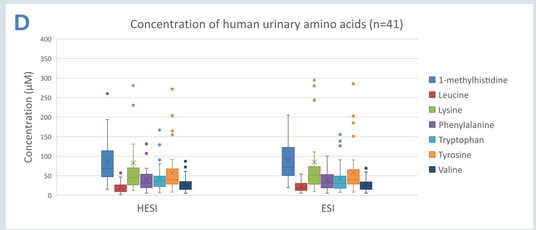
Results

A

Derivatized amino acids	Fold-increased in Peak area (HESI vs ESI)
1-methylhistidine	2.4
3-methylhistidine	2.8
4-hydroxyproline	4.1
Alanine	4.8
Alpha-aminobutyric acid	3.0
Arginine	3.8
Asparagine	3.3
Aspartic acid	5.0
Beta-alanine	4.9
Beta-aminoisobutyric acid	5.9
Citrulline	1.6
Ethanolamine	5.4
Gamma-aminobutyric acid	2.3
Glutamic acid	4.6
Glutamine	2.9
Glycine	5.8
Histidine	3.1
Isoleucine	2.9
Leucine	2.8
Lysine	2.8
Methionine	2.2
Phenylalanine	2.9
Proline	4.0
Sarcosine	6.8
Serine	5.4
Taurine	1.2
Threonine	4.9
Tryptophan	2.3
Tyrosine	2.0
Valine	3.0







A) Overall increases in sensitivity were observed in the 30 physiological biogenic amines (range: 1.2-6.8 fold), when comparing VIP-HESI to the standard ESI source.

B) VIP-HESI improve sensitivity and peak shape of typical low level isomers β-alanine, sarcosine of alanine.

C. Similar calibration curves (i.e. alanine) are observed between the 2 sources. VIP-HESI also showed better reproducibility (peak area) with 87% of the biogenic amines exhibiting <10% RSD (peak area) with repeated injection of urine samples (n=7)

D. Box and Whiskers plots of both ESI and VIP-HESI sources produced equivalent concentration measurements (± 3.2%) in human urine samples (n=41).

References

[1] https://doi.org/10.1016/j.talanta.2020.121872

Conclusions

- The new VIP-HESI source improved sensitivity of the current validated 30 physiological biogenic amines assay by an average of 3.6-fold.
- Increased sensitivity improved peak reproducibility with 93% of the investigated amines having <15% RSD in urine samples.
- Similar levels of endogenous biogenic amines measurements were observed between both Apollo II ESI and VIP-HESI sources.
- VIP-HESI improved detection limits of biogenic amines and retained the excellent robustness and quantitative accuracy observed in standard ESI.

VIP-HESI Metabolomics