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 quadrupole-time-of-flight (QToF) in technology have enabled the use of high-resolution accurate mass instruments for both discovery and [1]. quantitative analyses 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, HS

A: 2 mM ammonium

B: 2 mM ammonium

Time
C
0.
5.
5.
6.
7.

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

Ionization

Nebulizer gas

Dry gas (L/r

Dry temperatu

Probe gas temper

Probe gas (L

Exhaust

Data Processing: TASQ[®] 2021b

Results

SS T3 (2.1 x 150 mm, 1.8µm)					
acetate in water					
acetate	acetate in acetonitrile/water (95/5)				
nin)	% B at 0.6 mL/min				
	5				
	5				
	30				
	100				
	100				
	5				

	ESI	VIP-HESI
(V)	4500	3000
(Bar)	5	3.5
min)	12	9
re (°C)	250	250
ature (°C)	-	300
/min)	-	5
:	-	On

Derivatized amino acidsFold-increased in Peak area (HESI vs ESI)1-methylhistidine2.43-methylhistidine2.84-hydroxyproline4.1Alanine4.8Alpha-aminobutyric acid3.0Arginine3.8Asparagine3.3Aspartic acid5.0Beta-alanine4.9Beta-alanine5.4Citrulline1.6Ethanolamine2.3Glutamic acid2.3Glutamic acid3.1Isoleucine2.9Glycine2.8Histidine3.1Isoleucine2.8Leucine2.8Lysine2.8Sarcosine6.8Serine5.4Taurine1.2Threonine3.1Tryptophan2.3Tryptophan3.4Sarcosine3.1Serine3.1Serine3.1Serine3.1Sarcosine3.1Sarcosine3.1Serine3.1Sarcosine3.1Sarcosine3.1Sarcosine3.1Serine3.1Serine3.1Sarcosine3.1Serine3.1Sarcosine3.1Serine3.4Sarcosine3.1Sarcosine3.1Sarcosine3.1Sarcosine3.1Sarcosine3.1Sarcosine3.1Sarcosine3.1 <td< th=""><th colspan="5">Α</th></td<>	Α				
1-methylhistidine2.43-methylhistidine2.84-hydroxyproline4.1Alanine4.8Alpha-aminobutyric acid3.0Arginine3.8Asparagine3.3Aspartic acid5.0Beta-alanine4.9Beta-aminobutyric acid5.9Citrulline1.6Ethanolamine5.4Gamma-aminobutyric acid2.3Glutamic acid4.6Glutamine2.9Glycine3.1Isoleucine2.8Leucine2.8Lysine2.8Methionine2.9Phenylalanine2.9Sarcosine6.8Serine5.4Threonine1.2Threonine3.1Threonine3.1Sarcosi	Derivatized amino acids	Fold-increased in Peak area (HESI vs ESI)			
3-methylhistidine2.84-hydroxyproline4.1Alanine4.8Alpha-aminobutyric acid3.0Arginine3.3Asparagine3.3Aspartic acid5.0Beta-alanine4.9Beta-alanine5.9Citrulline1.6Ethanolamine5.4Gamma-aminobutyric acid2.3Glutamic acid4.6Glutamine2.9Glycine5.8Histidine3.1Isoleucine2.9Leucine2.8Lysine2.8Methionine2.9Phenylalanine2.9Froline4.0Sarcosine6.8Serine5.4Taurine1.2Threonine4.9Tryptophan2.3Tyrosine2.3	1-methylhistidine	2.4			
4-hydroxyproline4.1Alanine4.8Alpha-aminobutyric acid3.0Arginine3.8Asparagine3.3Aspartic acid5.0Beta-alanine4.9Beta-alanine5.9Citrulline1.6Ethanolamine2.3Glutamic acid4.6Glutamic acid4.6Glutamine2.9Glycine3.1Isoleucine2.9Leucine2.8Lysine2.8Methionine2.9Phenylalanine2.9Foline4.0Sarcosine6.8Serine5.4Taurine1.2Threonine4.9Tryptophan2.3Tyrosine2.3	3-methylhistidine	2.8			
Alanine4.8Alpha-aminobutyric acid3.0Arginine3.8Asparagine3.3Aspartic acid5.0Beta-alanine4.9Beta-alanine5.9Citrulline1.6Ethanolamine5.4Gamma-aminobutyric acid2.3Glutamic acid4.6Glutamine2.9Glycine3.1Isoleucine2.9Leucine2.8Lysine2.8Methionine2.9Phenylalanine2.9Sarcosine6.8Serine5.4Taurine1.2Threonine4.9Tryptophan2.3Tyrosine2.3	4-hydroxyproline	4.1			
Alpha-aminobutyric acid3.0Arginine3.8Asparagine3.3Asparatic acid5.0Beta-alanine4.9Beta-alanine5.9Citrulline1.6Ethanolamine5.4Gamma-aminobutyric acid2.3Glutamic acid4.6Glutamine2.9Glycine5.8Histidine2.9Leucine2.8Lysine2.8Methionine2.9Phenylalanine2.9Sarcosine6.8Serine5.4Taurine1.2Threonine4.9Tryptophan2.3Tyrosine2.3	Alanine	4.8			
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Asparagine3.3Aspartic acid5.0Beta-alanine4.9Beta-alanine5.9Citrulline1.6Ethanolamine5.4Gamma-aminobutyric acid2.3Glutamic acid4.6Glutamine2.9Glycine5.8Histidine3.1Isoleucine2.9Leucine2.8Lysine2.8Methionine2.2Phenylalanine2.9Sarcosine6.8Serine5.4Threonine1.2Threonine3.1Jisoleucine2.9Agentine3.1Sarcosine6.8Serine3.4Threonine3.4Jisoleucine3.4Sarcosine3.8Serine3.4Serine3.4Jisoleucine3.4Serine3.4Sarcosine3.8Serine3.4Jisoleucine3.4Serine3.4Sarcosine3.8Serine3.4Jisoleucine3.4Serine3.4Serine3.4Serine3.4Serine3.4Serine3.9Serine3.9Serine3.9Serine3.9Serine3.9Serine3.9Serine3.9Serine3.9Serine3.9Serine3.9Serine3.9 <td>Arginine</td> <td>3.8</td>	Arginine	3.8			
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Beta-alanine4.9Beta-aminoisobutyric acid5.9Citrulline1.6Ethanolamine5.4Gamma-aminobutyric acid2.3Glutamic acid4.6Glutamine2.9Glycine5.8Histidine3.1Isoleucine2.9Leucine2.8Lysine2.8Phenylalanine2.9Sarcosine6.8Serine5.4Taurine5.4Threonine4.9Tryptophan2.3Tyrosine2.3	Aspartic acid	5.0			
Beta-aminoisobutyric acid5.9Citrulline1.6Ethanolamine5.4Gamma-aminobutyric acid2.3Glutamic acid4.6Glutamine2.9Glycine5.8Histidine3.1Isoleucine2.9Leucine2.8Lysine2.8Methionine2.2Phenylalanine2.9Sarcosine6.8Serine5.4Taurine1.2Threonine4.9Tryptophan2.3Tyrosine2.0	Beta-alanine	4.9			
Citrulline1.6Ethanolamine5.4Gamma-aminobutyric acid2.3Glutamic acid4.6Glutamine2.9Glycine5.8Histidine3.1Isoleucine2.9Leucine2.8Lysine2.8Methionine2.2Phenylalanine2.9Sarcosine6.8Serine5.4Taurine1.2Threonine4.9Tryptophan2.3Tyrosine2.0	Beta-aminoisobutyric acid	5.9			
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Lysine2.8Methionine2.2Phenylalanine2.9Proline4.0Sarcosine6.8Serine5.4Taurine1.2Threonine4.9Tryptophan2.3Tyrosine2.0	Leucine	2.8			
Methionine2.2Phenylalanine2.9Proline4.0Sarcosine6.8Serine5.4Taurine1.2Threonine4.9Tryptophan2.3Tyrosine2.0	Lysine	2.8			
Phenylalanine2.9Proline4.0Sarcosine6.8Serine5.4Taurine1.2Threonine4.9Tryptophan2.3Tyrosine2.0	Methionine	2.2			
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Sarcosine6.8Serine5.4Taurine1.2Threonine4.9Tryptophan2.3Tyrosine2.0	Proline	4.0			
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Threonine4.9Tryptophan2.3Tyrosine2.0	Taurine	1.2			
Tryptophan2.3Tyrosine2.0	Threonine	4.9			
Tyrosine 2.0	Tryptophan	2.3			
	Tyrosine	2.0			
Valine 3.0	Valine	3.0			

- when comparing VIP-HESI to the standard ESI source.



A) Overall increases in sensitivity were observed in the 30 physiological biogenic amines (range: 1.2-6.8 fold),

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

- samples (n=7)
- sources (n=41).

References

Conclusions

- an average of 3.6-fold.
- in urine samples.
- HESI sources.

VIP-HESI Metabolomics



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

D. Box and Whiskers plots of both ESI and VIP-HESI produced equivalent concentration measurements $(\pm 3.2\%)$ in human urine samples

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

The new VIP-HESI source improved sensitivity of the current validated 30 physiological biogenic amines assay by

Increased sensitivity improved peak reproducibility with 93% of the investigated amines having <15% RSD

Similar levels of endogenous biogenic amines measurements were observed between both Apollo II ESI and VIP-

 VIP-HESI improved detection limits of biogenic amines and retained the excellent robustness and quantitative accuracy observed in standard ESI.