Quantification of Beta-Hydroxybutyric acid and Tryptophan in plasma as metabolic biomarkers of cancer using the LDTD-MS/MS technique

THP-032

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OVERVIEW

Purpose

- Automated extraction process and LDTD-MS/MS quantification of Tryptophan and Beta-Hydroxybutyric acid in plasma

Method

- Automated Assisted Liquid-Liquid Extraction
- Samples dried and analyzed by LDTD-MS/MS

Quantification

- Linearity: $r^2 > 0.99$ over the calibration range
- Between-run accuracy, values between 86,0% and 107,6% were obtained and the precision results were lower than 15% CV.
- Samples analyzed with a runtime of 8 seconds using LDTD-MS/MS technique

INTRODUCTION

Metabolomics has the potential to map early biochemical changes in cancer cells and hence provides an opportunity to quantify cancer biomarkers in blood that can trigger earlier diagnosis. Imbalances in tryptophan (TRP) metabolism have been linked to cancer-related immune escape and implicated in several cancers, including lung cancer. Accumulated metabolites of β-hydroxybutyric acid (β-HBA) from higher energy production by cancer cells is considered to be one of the hallmarks of cancer development. Therefore, the rapid and accurate determination of Tryptophan and β-HBA concentration in plasma would be useful for the development of an early cancer diagnosis tool.

For this project, an automated extraction method is developed. TRP and β-HBA drugs in plasma are extracted and quantification using Laser Diode Thermal Desorption and tandem mass spectrometry (LDTD-MS/MS) is chosen as a fast-analytical technique.

LUXON Ionization Source:

The Luxon Ion Source (Figure 1) is the second-generation sample introduction and ionization source based on the LDTD technology for mass spectrometry. The Luxon Ion Source uses a Fiber-Coupled Laser Diode (Figure 2) to obtain unmatchable thermal uniformity giving more precision, accuracy and speed. The process begins with dry samples which are rapidly evaporated using indirect heat. The thermally desorbed neutral molecules are carried into a corona discharge region. High-efficiency protonation and strong resistance to ionic suppression characterize this type of ionization and is the result of the absence of solvent and mobile phase. This thermal desorption process yields high-intensity molecular ion signal in less than 1 second sample-to-sample and allows working with very small volumes.

Figure 1 Luxon Ion Source

Figure 2 Schematic of the Luxon Ion Source

METHOD

Automated Assisted Liquid-Liquid extraction

45 µL of plasma are mixed with 45 µL of the internal standard solution (TRP-d5 and β-HBA-d4, 30 µg/ml and 20 µg/ml in methanol:water 25:75). 5 µL are transferred into a deep-well extraction plate on a vortexer system and mixed with 250 µL of methanol:water (1:1). Finally, 5 µL of the extraction and then 5 µL of desorption solution were spotted onto LazWell™ 96 plates and evaporated to complete dryness before analysis.

Figure 3 Azeo: Automated extraction system

Instrumentation

- Ion source: Phytronix, Luxon S-960 Ion Source
- Mass spectrometer: Sciex, Q-Trap System 6500+

Luxon Parameters

- Laser power pattern:
  - Increase laser power to 65% in 6 sec
  - Hold 2 seconds
  - Decrease laser power to 0%
- Carrier gas flow: 6 L/min (Air)

Table 1 MS parameters

Compound	TRP	β-HBA
APCI mode	+	-
Curtain	20	20
CAD	7	7
Dwell Time	30	25

Table 2 MRM transitions parameters

Compound	Q1 (Da)	Q3 (Da)	CE (V)
TRP	205	118	35
TRP-d5	210	122	35
β-HBA	103	59	-15
β-HBA-d4	107	59	-15

RESULTS

Validation test

Quality Control (QC) plasma samples were prepared using the SeraCon™ I Negative Diluent and the endogenic concentration values were determined by extrapolation of the calibration curves, a correction of the standards solutions was then applied and used as a target value. Repeated extractions were deposited onto a LazWell™ plate and dried before analysis. The peak area against the internal standard (IS) ratio was used to normalize the signal.

Linearity

The calibration curves were plotted using the peak area ratio and the nominal concentration of standards. For the linearity test, the following acceptance criteria was used:

- Linear regression (r) must be ≥ 0.995

Table 3 shows the inter-day correlation coefficients for TRP and β-HBA. Values greater than 0.995 are obtained. **Figure 4** shows a typical calibration curve result for β-HBA.

RESULTS

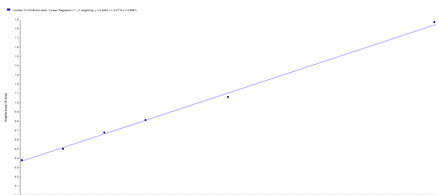

Figure 4 β-HBA calibration curve

Stability

Wet stability of Sample Extracts:

Following the extraction, sample extracts are kept at 4°C in closed containers. After 1 day, sample extracts are spotted on a LazWell™ plate, dried and analyzed. Precision and accuracy of QC samples are reported in **Table 5.** All the results are within the acceptable criteria range for 1 day at 4°C.

Dry stability of Samples spotted in LazWell:

Extracted samples are spotted onto a LazWell™ plate, dried and kept at room temperature for 2 hour before analysis. The precision and accuracy results of QC samples are reported in **Table 5.** All the results are within the acceptable criteria range for 2 hour at room temperature.

Table 5 Wet and Dry stability of TRP and β-HBA

Parameters	Dry stability (2 hour / RT)				Wet stability (1 day / 4°C)			
molecule	TRP		β-HBA		TRP		β-HBA	
QC	QC1	QC2	QC1	QC2	QC1	QC2	QC1	QC2
Conc. (µg/mL)	17,8	62,8	17,0	62,0	15,8	60,8	16,2	61,2
N	3	3	3	3	3	3	3	3
Mean (µg/mL)	15,9	60,4	16,4	62,7	17,2	55,43	16,5	54,4
%CV	4,18	0,34	3,91	0,82	3,44	2,35	2,47	2,86
%Target	93,5	96,1	96,7	101,3	108,7	91,1	101,8	88,8

Table 3 Inter-day calibration curve correlation

	TRP	β-HBA
Curve 1	0,9986	0,9998
Curve 2	0,9979	0,9996
Curve 3	0,9986	0,9987
Curve 4	0,9978	0,9967
Curve 5	0,9979	0,9996

Precision and Accuracy

For the accuracy and precision evaluation, the following acceptance criteria were used:

- Each concentration must not exceed 20% CV
- Each concentration must be within ±3 SD of the target value

For the inter-run precision and accuracy experiment, each standard was analyzed in triplicate, on five different days. **Tables 4A and 4B** show the inter-run precision and accuracy results for TRP and β-HBA . The obtained %CV was below 10% and the accuracy was within ±3 SD of the target value.

Table 4A Inter-run precision and accuracy for TRP

TRP	QC1	QC2
Conc (ng/ml)	15,2	60,2
N	15	15
Mean (ng/mL)	16,4	51,8
SD	1,4	4,0
%CV	8,6	7,7
%Target	107,6	86,0

Table 4B Inter-run precision and accuracy for β-HBA

β-HBA	QC1	QC2
Conc (ng/ml)	17,6	62,6
N	15	15
Mean (ng/mL)	17,5	60,9
SD	1,6	3,4
%CV	9,2	6,5
%Target	101,1	97,6

CONCLUSION

- **Efficient Automated Assisted Liquid-Liquid Extraction is used to extract Tryptophan and β-Hydroxybutyric acid in plasma**
- **High-throughput analysis using LDTD-MS/MS**
- **Linearity, accuracy, precision and stability within the acceptance criteria**
- **Sample-to-sample analysis of 8 seconds**
- **System can be customized for molecular detection of cancer**

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