MP-042

Analysis of Serotonin in Serum as Biomarker of Clinical Disorders at 8 seconds per Sample using the LDTD-MS/MS Technique

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OVERVIEW

Purpose

- Optimization of an automated extraction process and LDTD-MS/MS analysis of Serotonin in serum

Method

- Automated Salt Assisted Liquid-Liquid Extraction (SALLE)
- Samples dried and analyzed by LDTD-MS/MS

Quantification

- Linearity: r^2> 0.99 over the calibration range
- Between-run accuracy, values between 105.6 and 113.0 were obtained and the precision results were lower than 4.6% CV.
- Samples analyzed with a runtime of 8 seconds using LDTD-MS/MS technique

INTRODUCTION

Serotonin is an indoleamine molecule that is derived from the amino acid tryptophan. Its biological function is complex, and it impacts multiple aspects of our lives, such as our mood, our memory, and even undesirable effects such as vomiting. The determination of the serotonin concentration in a serum sample is used as a biomarker for the diagnosis of carcinoid syndrome and other clinical disorders.

For this project, an automated extraction method is developed. Serotonin drugs in serum 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 SALLE extraction

100 µL of serum are transferred into a deep-well extraction plate on a vortexer system and mixed with 100 µL of the internal standard solution (Serotonin-d4, 100 ng/mL in water). Then, an extraction buffer (500 µM K_2HPO_4 and 50 mM NaOH in saturated solution of NaCl) and 300 µL of acetonitrile were added, mixed, and centrifuged. Finally, 4 µL Butylated hydroxytoluene (BHT) at 1 mg/mL in acetonitrile, followed by 4 µL of the upper-layer were spotted onto LazWell96 plates and evaporated to complete dryness before analysis by LDTD-MS/MS.

Figure 3 Azeo: Automated extraction system

Instrumentation

- Ion source: Phytronix Luxon S-960 Ion Source
- Mass spectrometer: Sciex, Q-Trap System 5500

Luxon Parameters

- Laser power pattern:
  - Increase laser power to 45% in 3 sec
  - Hold 2 seconds
  - Decrease laser power to 0%
- Carrier gas flow: 3 L/min (Air)

MS Parameters

- APCI (-)
- Curtain: 10
- CAD: 8
- Time: 50 msec
- MRM mode

Table 1 MRM transitions parameters

Compound	Q1 (Da)	Q3 (Da)	CE (V)
Serotonin	174	144	-25
Serotonin-d_4	178	146	-25

RESULTS

Validation test

BSA solution (30 mg/mL) and three human serum samples were used as QC (endogenic concentration values were evaluated with a reference method and used as a nominal value). Replicate 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 2 shows the inter-day correlation coefficients for serotonin. Values greater than 0.999 are obtained. **Figure 4** shows a typical calibration curve result for serotonin.

Table 2 Inter-day calibration curve correlation

	Serotonin (r)
Curve 1	0.99991
Curve 2	0.99988
Curve 3	0.99969
Curve 4	0.99917
Curve 5	0.99973

RESULTS

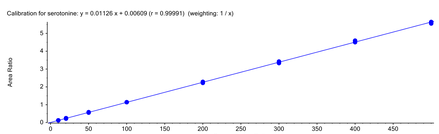

Figure 4 Serotonin calibration curve

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 4.** 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 1 hour before analysis. Serotonin must be stabilized with BHT to avoid drug degradation on the LazWell™ plate. The precision and accuracy results of QC samples are reported in **Table 4.** All the results are within the acceptable criteria range for 1 hour at room temperature. An important signal lost is observed after 1 hour. Analysis within 30 minutes after sample evaporation is strongly recommended.

Table 4 Wet and Dry stability of Serotonin

Parameters	Dry stability (1 hour / RT)			Wet stability (1 day / 4°C)		
QC	QC1	QC2	QC3	QC1	QC2	QC3
Conc. (ng/mL)	71.9	120.4	148.1	71.9	120.4	148.1
N	3	3	3	3	3	3
Mean (ng/mL)	82.3	135.4	168.4	71.7	121.5	149.6
%CV	0.9	3.1	1.4	2.1	1.4	1.0
%Nom	114.4	112.5	113.7	99.8	100.9	101.0

Precision and Accuracy

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

- Each concentration must not exceed 15% CV
- Each concentration must be within 100 ± 15% of the nominal concentration

For the inter-run precision and accuracy experiment, each standard was analyzed in triplicate, on five different days. **Table 3** shows the inter-run precision and accuracy results for serotonin. The obtained %CV was below 15% and the accuracy was within 15% of the nominal value.

Table 3 Inter-run precision and accuracy

Serotonin	QC1	QC2	QC3
Conc (ng/ml)	71.9	120.4	148.1
N	15	15	15
Mean (ng/mL)	81.3	127.2	162.3
SD	3.50	2.98	7.42
%CV	4.3	2.3	4.6
%Nom	113.0	105.6	109.6

Cross validation study

Real patients' serum samples (N=12) have been tested with this method to correlate with results obtained by traditional LC-MS/MS. The percentage of difference between the values are evaluated. A difference below 15% is obtained. Results are reported in **Table 5.**

Table 5 Comparison between serotonin concentration values

Serotonin	LC (ng/mL)	Luxon (ng/mL)	%Diff (%)
M1	148.1	157.0	-5.8%
M2	71.9	82.2	-13.3%
M3	120.4	125.6	-4.2%
M4	197.6	205.3	-3.8%
M5	119.6	138.1	-14.3%
M6	169.3	180.4	-6.4%
M7	49.2	55.6	-12.3%
M8	80.4	85.2	-5.8%
M9	64.3	71.9	-11.1%
M10	93.8	99.3	-5.7%
M11	80.2	85.5	-6.4%
M12	107.1	117.1	-8.9%

CONCLUSION

- Efficient Automated Salt Assisted Liquid-Liquid Extraction (SALLE) is used to extract Serotonin
- High-throughput analysis using LDTD-MS/MS
- Linearity, accuracy, precision and stability within the acceptance criteria.
- Sample-to-sample analysis of 8 seconds