Analysis of Serotonin in Serum as a Biomarker of Clinical Disorders:

Serotonin Quantification in Serum at 8 Seconds per Sample Using LUXON-MS/MS

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Introduction

Serotonin is an indoleamine molecule that is derived from the amino acid tryptophan. Its biological function is complex, and it impacts multiple aspects in 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.

Our goal for this application note is to use an automated sample preparation method for the quantification of serotonin in serum using a single operation in LUXON-MS/MS.

LUXON-MS/MS offers specificity combined with an ultra-fast analysis for an unrivaled quantification method. To develop this application, we focused on performing a quick and simple sample preparation. Serotonin is analyzed and results are obtained in less than 8 seconds per sample.

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. Luxon Ion Source® uses Fiber-Coupled Laser Diode (**Figure 2**) to obtain unmatchable thermal uniformity providing 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 saturation 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 Ionization Source

Sample Preparation Method
Automated Sample Extraction

Serum samples were transferred into barcoded tubes, readable by the Azeo extraction system.

Each barcoded vial was scanned by the Azeo Liquid Handler and an automatic batch file was created. The Azeo extraction system (**Figure 3**) is used to extract the samples using the following conditions:

- 100 µL of serum sample were transferred from the vials to a deep-well plate placed in the Lumo Vortexer.
- 100 µL of Internal standard (Serotonin-d$_4$, 100 ng/mL in water) were added to each sample.
    - Mix
- 200 µL of extraction buffer and 300 µL acetonitrile were added into a deep-well plate.
    - Mix
    - Centrifuge 2 minutes/5000 rpm
- Spot 4 µL Butylated Hydroxytoluene (BHT, 100 µg/mL) followed by 4 µL upper layer phase onto a LazWell™ 96 plate.
    - Dry 4 minutes at 40°C in the Aura LazWell Dryer.

Figure 3 - Automated extraction system

LDTD®-MS/MS Parameters
LDTD
Model: Luxon S-960, Phytronix
Carrier gas: 3 L/min (air)
Laser pattern:
3-second ramp to 45% power
MS/MS
MS model: Q-Trap System® 5500, Sciex
Scan Time: 50 msec
Total run time: 8 seconds per sample
Ionization: APCI
Analysis Method: Negative MRM mode

Table 1 - MRM transitions for Luxon-MS/MS

	Transition	CE
Serotonin	174 → 144	-25
Serotonin-d$_4$	178 → 146	-25

Results and Discussion

Validation Test

Calibration curves ranging from 10 to 500 ng/mL were prepared in a 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.
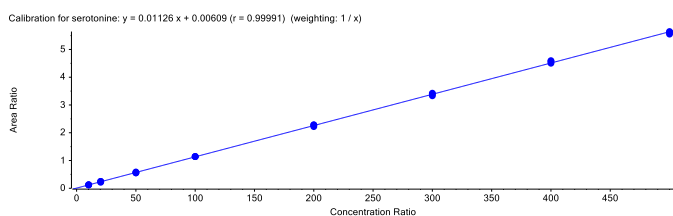

Calibration for serotonine: y = 0.01126 x + 0.00609 (r = 0.99991) (weighting: 1 / x)

Figure 4 – Serotonin calibration curve

Table 2 – Inter-day calibration curve correlation coefficients

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

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 of Serotonin

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

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

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

The Luxon Ion Source® combined with Sciex Q-Trap 5500 mass spectrometer system allows ultra-fast (**8 seconds per sample**) analysis of serotonin in serum.