Superoxide Radical Anion Oxidative Ionization in Negative LDTD-MS/MS Analysis of Serotonin

MP-332

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OVERVIEW

Purpose

- Understanding of the ionization process for the formation of M-2 primary mass of Serotonin and application of this method for sample analysis.

Method

- Serum protein precipitation.
- Samples dried and analyzed by LDTD-MS/MS.

Quantification

- Linearity: r > 0.995 over the calibration range.
- Between-run accuracy, values between 105.6 and 113.0 are obtained and precision results are lower than 4.6% CV.
- Samples are analyzed with a runtime of 8 seconds using LDTD-MS/MS technique.

INTRODUCTION

Serotonin is an indoleamine neurotransmitter derived from the amino acid tryptophan. Its biological functions are complex, influencing various physiological processes such as mood regulation, memory, and even contributing to undesirable effects like vomiting. The measurement of serotonin concentration in serum is commonly used as a biomarker for diagnosing conditions such as carcinoid syndrome and other related disorders. During method development, the major ion observed is M-2 when using the Laser Diode Thermal Desorption (LDTD) ionization source.

To better understand the ionization process, several experiments are conducted. Ultimately, a reliable method was developed for analyzing serotonin levels in human serum.

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*

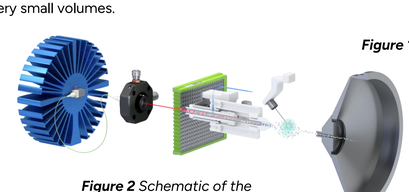
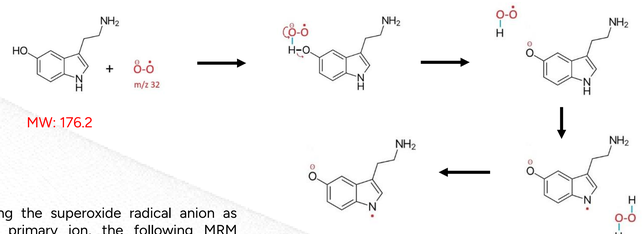


Figure 2 *Schematic of the Luxon Ion Source*

IONIZATION PROCESS

The first step in the oxidative ionization of serotonin by the superoxide radical anion involves the formation of the M+32 (-) species. A signal for this species is observed in the negative Q1 scan experiment for serotonin (208 amu) and serotonin-d_4 (212 amu). The M-H^- species are also observed, but the major ion signal corresponds to the M-$2H^-$ species, with serotonin (174 amu) and serotonin-d_4 (178 amu) being the primary ions detected. Previous reports by Hassan et al. (2017)[1] suggest that this ionization process can be applied to serotonin analogs. Using the superoxide radical anion as the primary ion, the following MRM transitions are used: Serotonin (174 → 144) and Serotonin-d_4 (178 → 146). The following ionization process are suggested:



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METHOD

Extraction

BSA solution (30 mg/mL) and Human serum were spiked to generate calibration curves and QC.

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-d_4, 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 Ion Source S-960
- Mass spectrometer: Sciex, Q-Trap System 5500

Luxon Parameters

- Laser power pattern:
  - Increase laser power to 45% in 3 s
  - 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 (m/z)	Q2 (m/z)	CE (eV)
Serotonin	174.0	144.0	-25
Serotonin-d_4	178.0	146.0	-25

RESULTS

Linearity

Table 2 shows the inter-run correlation coefficients for Serotonin. Values greater than 0.995 are obtained.

Precision and Accuracy

For the accuracy and precision evaluation, the following acceptance criteria are 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 QC is analyzed in triplicate, on five different runs. **Table 3** shows the inter-run precision and accuracy results for Serotonin. The obtained %CV is below 15% and the accuracy is within 15% of the nominal value.

Table 2 *Inter-run calibration curve correlation coefficients (r)*

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

Table 3 *Inter-run precision and accuracy*

Serotonin	QC-L	QC-M	QC-H
Conc (ng/mL)	71.9	120.4	148.1
N	15	15	15
Mean (ng/mL)	81.3	127.2	162.3
%CV	4.3	2.3	4.6
%Nom	113.0	105.6	109.6

Stability

Wet stability of sample extracts:

Following the extraction, the sample extracts are kept at 4°C in closed containers protected from light. 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 on LazWell:

Extracted samples are spotted onto a LazWell™ plate, dried and kept at room temperature for 1 hour before analysis. 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.

Table 4 *Wet and dry stability of Serotonin*

Parameters	Dry stability (1 hour / RT)			Wet stability (1 day / 4°C)		
QC	QCL	QCM	QCH	QCL	QCM	QCH
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 *Cross validation results*

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

- **Ionization process via Superoxide radial anion are obtained.**
- **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**

REFERENCES
1) Hassan I et Al. (2017). Oxidative Ionization under certain negative-ion mass spectrometric conditions, J. Am. Soc. Mass Spectrom.,28:270-277, https://doi.org/10.1007/s13361-016-1527-5.

COI Disclosure: I have a financial relationship with Phytronix Technologies as a salaried employee.