Phytronix Breakaway Technologies

Sciex

Clinical

Application Note : 2415

Rapid Assessment of Cortisol Levels cerebrospinal fluid:

Hormones Analysis in cerebrospinal fluid at 10 seconds per sample using LDTD-MS/MS.

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Introduction

The level of cortisol in the CSF can guide the diagnosis in a patient with meningitis. Bacterial meningitis and aseptic (viral) meningitis are not treated the same way. Bacterial meningitis will be treated with antibiotics, while the same antibiotics will have no effect in aseptic meningitis. Untreated, meningitis can damage the brain and nerves and can be life-threatening[1]. Rapid diagnosis of the type of meningitis can allow rapid treatment and thus increase the cure rate.

The objective of this application note is to develop a method for ultra-rapid quantification of cortisol in cerebrospinal fluid (CSF) using LUXON-MS/MS technology. The LUXON-MS/MS system offers specificity combined with ultra-rapid analysis for the analysis of cortisol in cerebrospinal fluid. To develop this application, we focused on making sample preparation simple and quick. LDTD-MS/MS analysis is performed in less than 10 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 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 Ionization Source

Sample Preparation Method

Sample Collection

Cerebrospinal fluid samples are collected from a lumbar puncture (spinal tap). For calibration curves and quality control samples, the matrix used is artificial cerebrospinal fluid (aCSF).

Table 1. Artificial cerebrospinal liquid composition

Compounds	Concentration
NaCl	126 mM
KCl	3 mM
$CaCl_2 \cdot H_2O$	2 mM
NaH_2PO_4	2 mM
$MgSO_4$	2 mM
$NaHCO_3$	26 mM
D-glucose	10 mM
BSA	0.4 mg/mL

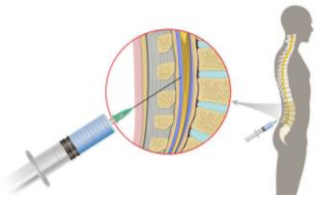

Figure 3. Cerebrospinal fluid collection

Sample Extraction

- In a 1.5 mL centrifugation tube, 15 µL of Internal standard in methanol is added
- Add 152 µL of CSF samples or spiked aCSF (for calibration curves and QCs)
  - Vortex (1000 rpm/30 seconds)
- Add 200 µL of extraction buffer (KH_2PO_4, 1M)
  - Vortex (1000 rpm/30 seconds)
- Add 500 µL of methyl tert-butyl ether (MTBE)
  - Vortex (1000 rpm/30 seconds)
  - Centrifuge (14000 rpm/4 min)
- In a second borosilicate tube (10X75 mm) transfer 400 µL of the upperlayer from 1.5 mL centrifugation tube
  - Evaporate until dryness with gentle airflow at room temperature (RT)
- Add 80 µL of methanol to reconstitute samples.
  - Vortex (1000 rpm/30 seconds)
- Add 80 µL of water.
  - Vortex (1000 rpm/30 seconds)
- Spot 6 µL of mixture on a LazWell™96 CORT plate
  - Dry 6 minutes at 40°C.

LDTD®-MS/MS Parameters

LDTD

Model: Luxon S-960, Phytronix
Carrier gas: 6 L/min (air)
Infusion: 1 % Formic acid in water at 20 µL/min
Laser pattern:

- 1-second start delay
- 6-second ramp to 75% power
- 2-seconds hold

MS/MS

MS model: QTRAP 550, Sciex
Scan Time: 50 msec
Ionization: APCI
Analysis Method: Negative ionization mode

Table 2 – MRM transitions for Luxon-MS/MS

Compound	Transition	CE	DP
Cortisol	407.0 → 331.1	-24	-80
Cortisol-d_6	413.0 → 335.0	-24	-80

Results and Discussion

Linearity

Sample were spiked at 5 ng/mL to 100 ng/mL in artificial cerebrospinal fluid. Standard working solutions (10X) were prepared in methanol.

The following acceptance criterion was used:

- Each calibration curve, for each run, must have a coefficient of correlation (r) higher than 0.99.

Table 3. Coefficient of correlation obtain for 6 runs

Run	Equation (Y = a X +b)	Coefficient of correlation (r)
1	Y= 0.00722 X - $1.06061 \cdot 10^{-4}$	0.99551
2	Y= 0.00528 X + 0.00232	0.99202
3	Y= 0.00578 X + 0.00721	0.99200
4	Y= 0.01351 X - 0.00355	0.99522
5	Y= 0.00906 X + 0.01528	0.99446
6	Y= 0.00923 X + 0.00891	0.99648

Precision and Accuracy

Each quality controls were used to validation the precision and the accuracy of the method. Quality controls concentrations were at 15 ng/mL for QCL (low), at 50 ng/mL for QCM (medium) and at 75 ng/mL for QCH (high). The peak area against the internal standard (IS) ratio was used to normalize the signal. Replicate extractions are deposited on a LazWell™CORT plate and dried before analysis.

The following acceptance criteria were used:

- Each quality control must not exceed 15%CV for at least 66.6% of samples.
- Each quality control must not exceed 15% Bias for at least 66.6% of samples.

For intra-run precision and accuracy experiment, a fortified quality control set in aCSF was extracted and analyzed in sextuplicate. **Table 4** shows intra-run results, %CV and % Bias obtained were lower that 15 % for each concentration analyzed. For the inter-run precision experiment, each fortified sample set is analyzed in sextuplicate on six different runs. **Table 5** shows the inter-run precision and accuracy results. Accuracy and precision were below for 15%.

Table 4 - Intra-Run Precision and Accuracy Results

Cortisol	QCL	QCM	QCH
Conc (µg/mL)	15	50	75
N	6	6	6
Mean (µg/mL)	15.2	49.3	81.0
%CV	8.3	12.9	7.6
%Bias	1.4	1.5	8.0

Table 5 - Inter-Run Precision and Accuracy Results

Cortisol	QCL	QCM	QCH
Conc (µg/mL)	15	50	75
N	30	30	30
Mean (µg/mL)	15.4	52.1	75.8
%CV	12.2	10.1	10.7
%Bias	2.8	4.3	1.1

Wet Stability of Sample Extracts

Following the extraction, the extracted sample was transferred into a closed container kept at 4°C. After 1 day, extracted samples are spotted on a LazWell™CORT plate, dried and analyzed. Samples precision and accuracy are reported in **Figure 4A** and **Figure 4B.** 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™CORT plate, dried and kept at room temperature for 3 hours before analysis. The precision and accuracy results are reported in **Figure 4A** and **Figure 4B** respectively. All the results are within the acceptable criteria range for 3 hours at room temperature.

The following acceptance criteria were used for wet and dry stability:

- Each quality control must not exceed 15%CV for at least 66.6% of samples.
- Each quality control must not exceed 15% Bias for at least 66.6% of samples.

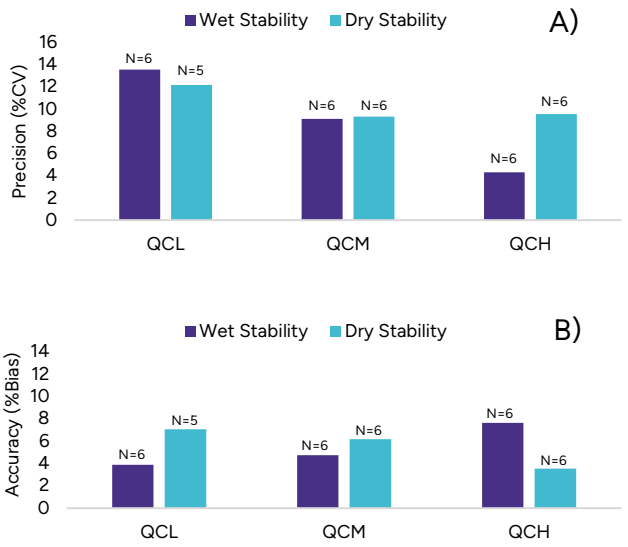

Figure 4 – Wet and Dry stability evaluation: A) Precision results B) Accuracy results

Multi-Matrix validation

Six different cerebrospinal fluid matrices analyzed by LDTD-MS/MS and by LC-MS/MS. Sample were extracted using the method previously mentioned. Multi-matrix validation results are shown in **Table 5**.

The following acceptance criterion was used:

- The difference between LC-MS/MS results and LUXON-MS/MS results must be lower than 20% difference.

The difference (in %) between the two instrumental methods were lower than 10 % for all six matrices.

Table 5 - Multi-matrix validation for cholesterol (n=1)

	LUXON-MS/MS	LC-MS/MS	% Difference
Matrix 1	9.7	9.7	0
Matrix 2	7.7	8.0	3
Matrix 3	18.1	17.5	3
Matrix 4	8.8	8.6	2
Matrix 5	12.8	12.1	5
Matrix 6	12.2	11.6	5

Conclusion

Luxon Ion Source® combined to a Sciex QTRAP 5500 mass spectrometer system allows ultra-fast (**10 seconds per sample**) for the quantification of cortisol in cerebrospinal fluid using a simple sample preparation method for diagnostics of bacterial or aseptic meningitis.

Reference

1. National Health Service (NHS). Overview: Meningitis. [On line] URL: https://www.nhs.uk/conditions/meningitis/#:~:text=It%20can%20affect%20anyone%2C%20but,to%20the%20brain%20or%20nerves.