Quantification of Rivaroxaban in Serum Sample Using LDTD-MS/MS

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Introduction

Rivaroxaban is an anticoagulant used to prevent thromboembolism[1]. Prompt quantification of this drug in a patient's bloodstream is important in order to avoid overdoses and to adjust their medication[2]. Rapid quantification of rivaroxaban in a patient may become critical in some situations. Here are some examples of those situations from Baglin *and al.* 2012[3]:

- Life threatening bleeding
- Before surgery
- Acute renal failure
- Medication overdose

To increase the analysis throughput of the optimization and the follow-up on medications, the Luxon Ion Source® coupled to tandem mass spectrometry (MS/MS) was used.

Our goal for this application note is to present a rapid drug monitoring method in patient serum in 13.2 seconds per sample. To develop this application, we focused on performing a quick and simple sample preparation and analysis.

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 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 during the 9 seconds heating cycle.

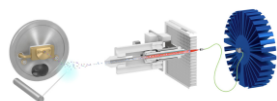**Figure 1 - Luxon Ion Source®**

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 Liquid Handler and were transferred into the system. Each barcoded vial was scanned by the Azeo Liquid Handler (**Figure 3**).

Samples were extracted as follows:
- 150 µL of a serum sample were transferred in a 0.5 mL Eppendorf
- 15 µL internal standard
  - Vortex
- 150 µL NaCl (saturated in water)
- 300 µL Acetonitrile
  - Vortex
  - Centrifuge (5000 rpm / 5 min)
- 4 µL upper-layer phase are spotted into a LazWell™96 plate
- Samples were evaporated to dryness at 40°C for 5 minutes with air flow.

Luxon-MS/MS analysis is done after a complete evaporation.

Figure 3 - Automated Extraction System

Table 1 shows the calibration range used for the quantification of Rivaroxaban in serum.

Table 1 - Calibration Range

Drug	Calibration Range
Rivaroxanban	4 – 500 ng/mL

LDTD®-MS/MS Parameters

LDTD
Model: Luxon SH-960, Phytronix
Carrier gas: 6 L/min (air)
Laser pattern:
- 6-second ramp to 65% power
- 3-second hold at 65 % power

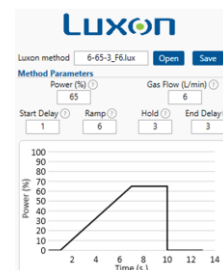**Figure 4** shows the laser pattern used during these analyses.

Figure 4 - Laser pattern

MS/MS
MS model: LC-8060, Shimadzu
Scan Time: 25 msec each transition
Total run time: 13.2 seconds sample to sample
Ionization: APCI (positive)
Analysis Method: MRM mode

Table 2 shows the mass spectrometer transitions in the positive ionization mode for the drug and its internal standard (IS).

Table 2 - Mass spectrometer transitions (Positive)

Drug	Transition	CE
Rivaroxanban	436.1 → 144.9	30
Rivaroxaban-d₄ (IS)	440.1 → 144.9	30

Results and Discussion

Data preparation process
Data acquisition systems of mass spectrometers were not designed to deal with few seconds per sample signal. The synchronization sequence adds 1 to more than 15 seconds between each sample. To bypass this, all samples are acquired in a single file (Error! Reference source not found.). To allow the analysis of such data, Cascade™ software is designed to detect, split, and integrate every sample peak acquired in a single file.
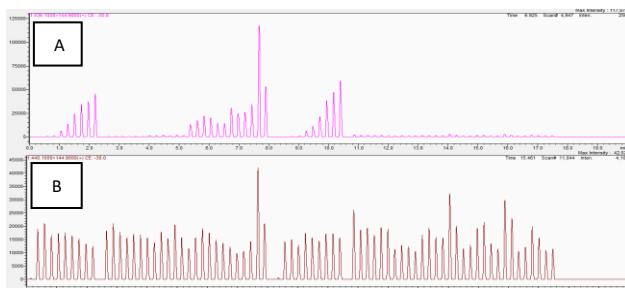
Figure 5 – Single file mass spectrometer data for 80 samples. A) Rivaroxaban transition. B) Internal standard transition.

Desorption peak
Figure 6 shows a zoomed area of the transition of the internal standard in the single-file window. A speed of 13.2 seconds per sample was reached with sufficient separation for adequate integration.
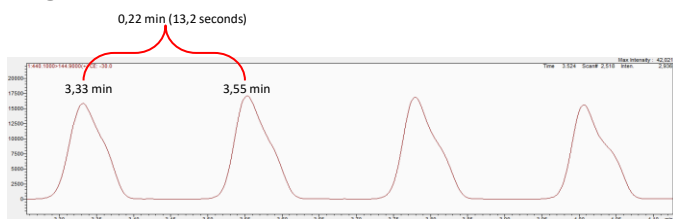
**Figure 6 – Peak to peak timing**

Validation Test
Calibration curves ranging from 4 to 500 ng/mL and QCs (Low, Medium and High) were prepared in serum[1]. 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^2) must be $\geq$ 0.99

Figure 7 shows a typical calibration curve result for Rivaroxaban.

$$Y = 7.5160E-3 * X + 3.8880E-3$$
$$R^2 = 0.99835$$
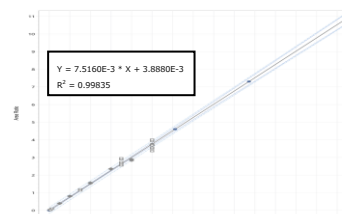
Figure 7 – Rivaroxaban calibration curve

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 intra-run precision and accuracy experiment, each fortified sample set was analyzed in six replicates. **Table 3** shows the intra-run precision and accuracy results for Rivaroxaban. The obtained %CV was below 15% and the accuracy was within 15% of the nominal value.

Table 3 - Intra-Run Precision and Accuracy of Rivaroxaban

Rivaroxaban	LLOQ	QC-L	QC-M	QC-H	ULOQ
Conc (ng/mL)	4	12	150	350	500
N	6	6	6	6	6
Mean (ng/mL)	4.1	12.1	148.5	357.7	488.5
%CV	10.9	7.7	5.0	7.7	7.0
%Nom	102.5	101.1	99.0	102.2	97.7

For the inter-run precision and accuracy, each fortified sample set were analyzed in five different runs. **Table 4** shows the inter-run results. The obtained %CV was below 15% and the accuracy was within 15% of the nominal value.

Table 4 - Inter-Run Precision and Accuracy of Rivaroxaban

Rivaroxaban	QC-L	QC-M	QC-H
Conc (ng/ml)	12	150	350
N	30	30	30
Mean (ng/mL)	12.1	148.5	357.7
SD	0.93	7.44	27.63
%CV	7.7	5.0	7.7
%Nom	101.1	99.0	102.2

Conclusion
Luxon Ion Source® combined to a Shimadzu LC-8060 mass spectrometer system allows fast analysis **(13.2 seconds per sample)** of Rivaroxaban in serum using a simple and automated sample preparation method.

References
1. doi: 10.1371/journal.pone.0171272, PLoS ONE, 2017
2. doi: 10.1371/journal.pone.0145478, PLoS ONE, 2015
3. doi: 10.1111/bjh.12052, British Journal of Haematology, 2012

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