Ultra-fast LCMS analysis of Antiarrhythmic drugs in plasma

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1. Introduction

The need for high throughput liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis for drug quantitation in biological fluids is becoming more and more important in modern clinical/pharmaceutical laboratories. In this poster, we describe the use of a novel LC-MS/MS configuration for the ultra-fast and precise quantitation of drugs in plasma samples within less than 50 sec. With the purpose to assess the reliability of the novel LC configuration, we evaluated the quantification of spiked human plasma samples for several antiarrhythmic drugs. Antiarrhythmic agents are usually monitored in plasma, due to their narrow therapeutic window and inter individual variability. They belong to different classes and with different chemical properties: Verapamil (Class IV, Ca^{2+} channel blocker), Nor-Verapamil (main active metabolite of Verapamil), Propranolol and Atenolol and Metoprolol (Class II, β -adrenergic blocker), Sotalol (Class III, affect potassium K+ efflux). Linearity over clinical-relevant concentration ranges, intra-day and inter-day assay variability of QC samples, were always within CLSI validation guidelines.

Overall, the obtained results are a proof of concept of the effectiveness of a novel solution for ultra-fast LC-MS/MS drugs quantitation in biological fluids. This study could, therefore, have broad application in clinical research, analytical toxicology, and many other areas.

2. Materials and Methods

The instrumental configuration includes a Nexera™ XR LC system with LCMS-8050 (Shimadzu Corp.) (Figure 1). The newly designed autosampler SIL-40C XR was used in a single-channel configuration using the ultrafast injection cycle time (<7 sec).

2.1 Reagents

Analytical standards of Verapamil, Nor-Verapamil, Atenolol, Propranolol, Metoprolol , Sotalol and deuterated Verapamil (D_6) were purchased from Wako Chemicals. Individual stock solutions at 100 mg/mL were prepared in Methanol and further diluted in blank plasma to make calibration standards (5 levels) and QC (2 levels). The calibration range was from 0.5 to 1000 µg/L.

Figure 1. Instrument configuration (representative scheme).

2.2 Sample preparation

Spiked plasma samples (100 µL) were diluted 1:3 with precipitant solution (Acetonitrile + Formic Acid 0.1%) containing labelled internal standard. After stirring for 1 min the samples were incubated at room temperature for 20 min. They were were centrifuged and supernatant was transferred into vial, and 1 µL was injected on the analytical column (Shim-pack™ Velox EXP guard column Biphenyl 5.0 x 3.0 mm 2.7 µm), (Figure 2).

2.3 Analytical conditions

Isocratic elution mode and gradient elution mode were both applied to short analytical column (guard column), acquisition was performed in MRM using positive ESI ionization.

Table 1 Analytical conditions

System	: Nexera XR
Analytical column	: Shim-pack™ Velox EXP guard column Biphenyl (5.0 x 3.0 mm 2.7 µm)
Temperature	: Room Temperature
Mobile Phases	: A: Water +0.1% formic acid B: Acetonitrile:Methanol 50:50 + formic acid 0.1%
Flow Rate	: 1000 µL/min
Rinse needle	: Flow Rinse + Dip Rinse (2 seconds) , using Acetonitrile
Injection Volume	: 1 µL
Elution mode	: Isocratic 40%B / Gradient 5%B 1 sec, 5% B -> 95% B 25 sec
Total Run Time	: < 25 sec - < 50 sec (sample to sample)

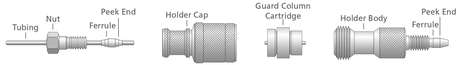

Figure 2. Shim-pack Velox EXP column.

3. Results

3.1 Method evaluation: Isocratic elution mode

Isocratic elution was the fastest method, with an analytical cycle time of <25 sec (sample to sample). In these conditions, three molecules (Verapamil, Nor-Verapamil and Propranolol) were within the CLSI validation guidelines [1] for linearity , accuracy and precision.

Table 2 Isocratic elution mode. Method evaluation [1]

Drug	Range (ng/mL)	r	Accuracy	Pecision RSD% Intra-day (LLMI - MQC - ULMI)	Pecision RSD% Inter-day (LLMI - MQC - ULMI)	Carry over (% of LOQ)
Verapamil	1 - 400	0.999	89% - 111%	7.3% - 3.5% - 1.7%	6.7% - 3% - 2.5%	0.00%
Nor-Verapamil	1 - 500	0.998	94% - 112%	9.8% - 9.4% - 1.5%	9.2% - 9.1% - 1.8%	0.20%
Propranolol	2 - 450	0.997	94% - 114%	12.4% - 4.9% - 2.6%	8.8% - 5.2% - 3.1%	0.00%
Atenolol	5 - 1000	/	/	/	/	/
Metoprolol	1 - 500	/	/	/	/	/
Sotalol	1.5 - 1000	/	/	/	/	/
Verapamil D6	/	/	/	3.70%	4%	0.00%

3.2 Method evaluation: Gradient elution mode

In order to overcome limitations of the isocratic method for the accurate quantitation of all the selected molecules, a 20-second gradient separation was implemented. Gradient elution was shown to be beneficial in obtaining the complete separation of Atenolol and Metoprolol. Moreover, a strong reduction in the ion suppression was reported also for early eluting molecules as shown by the scan experiments (Figure 4). The column seems to be cleaned after each sample by some components of the matrix. This could help to maintain the analytical performances over a long time.

Gradient elution was a widely applied method, with an analysis cycle time of < 50 s (sampling). In these conditions, all molecules were within CLSI guidelines [1] for linearity, accuracy and precision (Table 2). The gradient elution mode resulted in providing good chromatographic separation of all molecules (Figure 3) and the ion suppression from the residual matrix was strongly reduced (Figure 4).

Table 2 Gradient elution mode. Method evaluation [1]

Drug	Range (ng/mL)	r	Accuracy	Pecision RSD% Intra-day (LLMI - MQC - ULMI)	Pecision RSD% Inter-day (LLMI - MQC - ULMI)	Carry over (% of LOQ)
Verapamil	0.5 - 400	0.998	90% - 114%	7.4% - 1.9% - 2.0%	9.1% - 5.5% - 3.5%	0.00%
Nor-Verapamil	0.75 - 500	0.999	95% - 112%	12% - 1.3% - 2.2%	11% - 4.4% -6%	0.20%
Propranolol	2 - 450	0.999	93% - 109%	11% - 4.7% - 6.4%	12.6% - 4.8% 6%	0.00%
Atenolol	5 - 1000	0.999	92% - 112%	7.3% - 4.7% - 5.1%	9.1% - 4.7% - 3.4%	0.00%
Metoprolol	1 - 500	0.998	95% - 107%	13% - 1.9% - 1.9%	15% - 3.4% - 2.1%	0.00%
Sotalol	1.5 - 1000	0.999	91% - 107%	8.5% - 2.7% - 3.3%	7.1% -2.2% - 3.5%	0.00%
Verapamil D6	/	/	/	3.80%	3.90%	

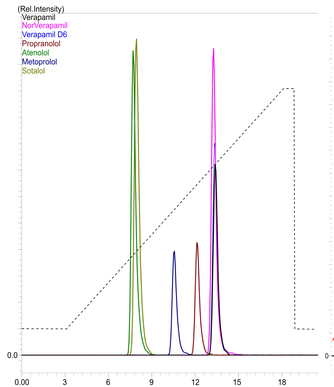

Figure 3 Gradient profile

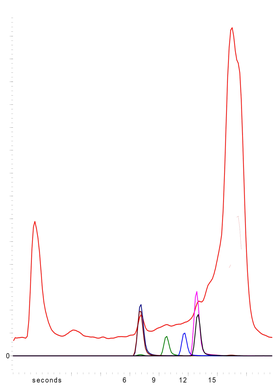

Figure 4 MRM and scan experiment

3.3 Column durability

Column deterioration (in terms of retention time, peak width, backpressure) over 2000 injections was not observed. For both the first eluting molecule and the last eluting molecule, RSD% for rt and peak width were acceptable (Figures 5 and 6).

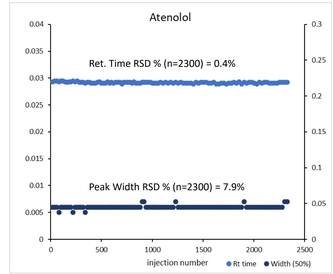

Figure 5 Retention time and peak width for the first eluting molecule over 2300 injections range.

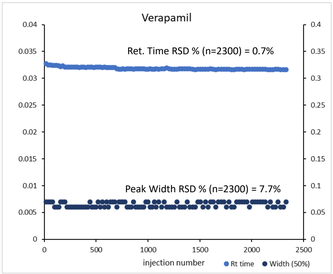

Figure 6 Retention time and peak width for the last eluting molecule over 2300 injections range.

4. Conclusion

- The results provided a proof of concept that the combination of a Nexera UHPLC instrument with UFMS technology can be suitable for analysis of small molecules in plasma with “tens of seconds” throughput.
- The use of a newly designed Autosampler (SIL-40C XR, Shimadzu) with ultrafast injection cycle time was suitable for high-throughput analysis even in single channel mode.
- The use of a Shim-pack Velox EXP guard column allowed keeping the chromatographic separation in the range of “tens of seconds” (sample to sample) and was beneficial in reducing matrix effects with only little sacrifice with regards to throughput.
- The method was effective for accurate quantitation of small drug panels (Antiarrhythmics) with overall cycle time (sample to sample) <50 seconds (<25 seconds for Isocratic separation).
- This approach can be beneficial in achieving high-throughput analysis for quantitation of drugs in biological samples and could find further applicability in clinical research, analytical toxicology, and many other areas.

5. References

1. CLSI C62-A Liquid Chromatography-Mass Spectrometry Methods; Approved Guidelines