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 drugdevelopment 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 mentioned 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, Ca2+ channel blocker), Nor-Verapamil (main active metabolite of Verapamil), Propranolol and Atenolol and Metoprolol (Class II, beta-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.

2. Materials and Methods

2.1 Reagents

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 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.

2.2 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 centrifuged for 5 min. The supernatant was transferred into vial, and 1 µL was injected on the analytical column (Figure 1). 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).

3. Results

3.1 Method evaluation: Isocratic elution mode

Isocratic elution mode and gradient elution mode were both applied to short analytical column (100 mm). The gradient elution mode was implemented with an analysis cycle time of <50 s (sampling). In these conditions, all molecules were within CLSI guidelines 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).

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 columns seem to be cleaned after each sample by some components of the matrix. This could help to maintain the analytical performances over a longer time.

4. Conclusion

• 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).

• 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” 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

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