

An Improved Capillary LC-MS/MS and Its Application to High Sensitivity and High Throughput Small Molecule Quantitation

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ABSTRACT

A novel capillary LC-MS/MS system that combines advances in gradient delivery and flow control, sample injection, ion source and interface design, and mass spectrometric detection is presented. Sensitivity can be increased as much as 6 times compared to conventional LC-MS/MS system, with 3 times faster throughput and a dramatic reduction of solvent consumption. System performance and the figures of merit in terms of reproducibility of retention time and signal intensity, limit of quantitation and linear dynamic range are determined using small molecule compounds-spiked plasma samples. The long-term robustness in various sample matrices has been performed and is reported. The application of the novel system to high sensitivity and high throughput profiling of most common corticosteroids in plasma will be demonstrated.

INTRODUCTION

High sensitivity quantitation of small molecule species present at very low concentrations is of vital clinical importance and requires a LC-MS system with ultimate sensitivity. A low flow LC (nano- or capillary- LC) is often employed for such purposes where the sample volume available for analysis is limited. Although appealingly sensitive, the nanoLC-MS appears less attractive for quantitative studies, mainly because of its low throughput and more sophisticated operation. Common approaches for capillary LC-MS employing a conventional split-flow pumping principle for gradient delivery and a regular interface for conventional flow rates suffer from impaired gradient reproducibility and post-column band broadening and hence reduced separation efficiency and sensitivity. We describe here an improved capillary LC-MS/MS protocol for highly sensitive quantitation of small molecules with comparable robustness to conventional LC-MS/MS with higher throughput and sensitivity.

MATERIALS AND METHODS

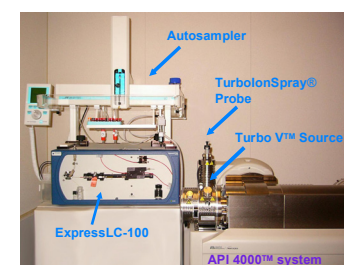
I. An API 4000TM LC-MS/MS system equipped with a Turbo VTM source (Applied Biosystems/MDS SCIEX) was employed in this study. An ExpressLCTM 100 single channel HPLC system (Eksigent) that delivers stable gradient flows at the 0.2-30 μ L/min was used for coupled LC-MS/MS analysis. A 30 micron i.d. fused-silica transfer line after the column was inserted directly to the end of the stainless steel electrospray emitter. This emitter was fully integrated on the standard Turbo V source. For comparison, an Agilent 1100 series conventional LC system was employed for conventional flow rate LC-MS/MS studies.

II. CapLC-MS/MS experiments were carried out using a Zorbax 300SB-C18 column (50 x 0.3 mm, 3.5 μ m, Agilent) and the ExpressLC-100 system. The model compounds - buspirone and reserpine (internal standard) spiked in plasma - were separated with a 20-sec gradient elution by water-acetonitrile-0.1% formic acid at typically 15 μ L/min flow rate. Mobile phase A was 0.1% formic acid in water and B was 0.1% formic acid in acetonitrile. The injection volume was 0.2-5 μ L or as otherwise indicated. The 18 corticosteroids spiked in plasma were separated with a 2.0-min gradient elution by water-acetonitrile-2 mM ammonia acetate. The TurbolonSpray[®] was operated in positive ion mode and was at a source temperature setting of 350°C.

III. Conventional LC-MS/MS experiments at 200-600 μ L/min flow rates were performed using a Luna C18 column (50 x 2.1 mm, 3 μ m, Thermo Electron) and the Agilent 1100 LC system. Mobile phase A was 0.1% formic acid in water and B was 0.1% formic acid in acetonitrile. The injection volume was typically 5 μ L. The TurbolonSpray[®] was operated in positive ion mode and was at a source temperature setting of 700°C.

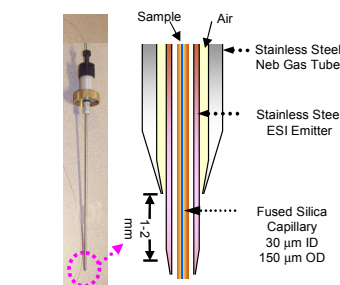
RESULTS

Figure 1. Integrated capillary LC-MS/MS



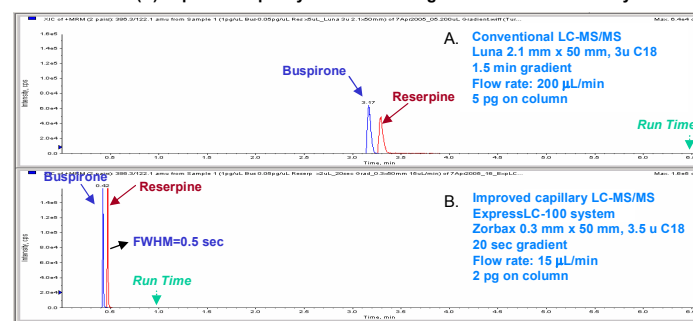
A 30-micron fused-silica transfer line is used to deliver the low microliter to nanoliter per minute flow-rates from the capillary LC system to the LC-MS/MS system via the TurbolonSpray[®] emitter on the Turbo VTM source.

Figure 2. A Schematic diagram of the "Plug and Play" TurbolonSpray[®] probe used in this study



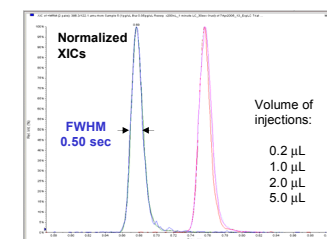
The fused silica transfer line was inserted to the end of the stainless steel emitter, with slight protrusion. The positioning of the capillary and the emitter in relative to the nebulizing gas tube is adjustable.

Figure 3. Analysis of buspirone and reserpine spiked in plasma by: (A) conventional LC-MS/MS and (B) improved capillary LC-MS/MS using API 4000TM LC-MS/MS system



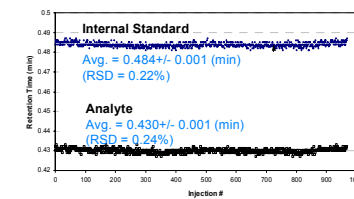
Although the initial desire to interface capillary HPLC with MS for increasingly small sample quantities available for analysis and the increased separation efficiencies provided by the capillary columns are most relevant to their coupling with MS, the continued interest arose because of their increased throughput for sample analysis. In Figure 3, it is clear that, in addition to the increased sensitivity (6-times) and narrower peak shape, for a batch of 55 sample analysis, the acquisition time using the capillary LC-MS/MS is 3-times shorter thus achieving a 3-times higher throughput, with a 140-times less consumption of organic solvent.

Figure 4. Overlay of XICs with various injection volume by capillary LC-MS/MS



The peak shape and the separation efficiency are maintained with the increased volume of sample injection.

Figure 5. Reproducibility of retention time over 1000 injections



With a 20-sec gradient elution and a 1.5 min total cycle time, the retention of the analyte and internal standard are very reproducible, with a relative standard deviation of 0.22%, and 0.24%, respectively, demonstrating the system's flow precision, gradient mixing capabilities, and interfacing efficiencies.

Figure 6. Reproducibility of signal intensities over 1000 injections of plasma sample

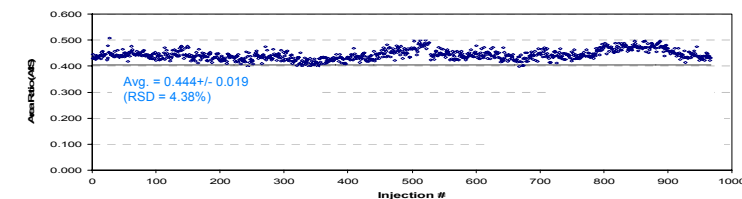
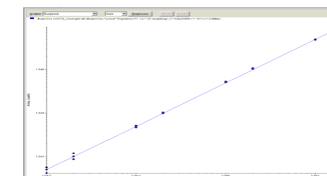


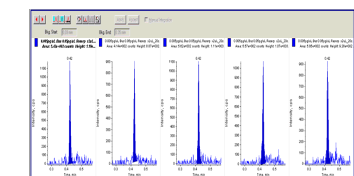
Figure 6 illustrates signal reproducibility of continuous injections of the analyte-spiked plasma samples. The relative standard deviation of the peak area is less than 5%, showing the system's robust performance over large number of biological sample analysis.

Figure 7. Calibration curve of buspirone in plasma by capillary LC-MS/MS



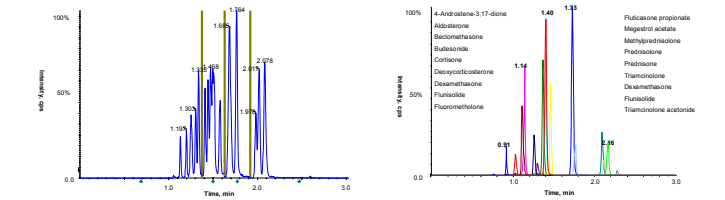
The calibration curve demonstrates a 3.5-4.0 orders of magnitude of linearity ($r^2 > 0.999$) with a limit of quantitation in the 5-10 fg range.

Figure 8. Injection of a LLOQ level sample showing the reproducibility



At concentrations of 0.050 μ g/ μ L and above, the relative standard deviation of peak area of 5 replicates is less than 5%. At lower concentrations, it is less than 14%.

Figure 9. Capillary LC-MS/MS profiling of 18 corticosteroids in plasma: (A) 3-period experiment using 40-ms dwell time, and (B) 1-period experiment using 5-ms dwell time for each MRM transition



The most common corticosteroids are good representation of a group of compounds with similar structures and chromatographic characteristics. The excellent reproducibility of analyte retention owing to precise control of gradient and flow rate makes it possible to design an experiment with multiple periods for screening of as many as hundreds of analytes in a single run, even though two adjacent groups of analytes are separated by no more than 0.1 min.

CONCLUSIONS

Unlike conventional LC-MS, where hundreds of micro-liter/min flow rates are typically employed with ease, crucial to capillary LC-MS/MS protocol for high throughput and high sensitivity quantitation is the ability to generate stable and reproducible gradient flows at low micro-liter/min with short cycle time. It is also crucial to use minimal transfer lines and connections to reduce dead volumes. These parameters have to be considered to perform successful chromatographic separation of analytes from matrices on a capillary column within minutes along with proper coupling to a rugged and robust interface design that provides efficient desolvation, ionization and ion transmission.

The figures of merit of the improved capillary LC-MS/MS system presented here in terms of throughput, reproducibility, sensitivity, and linear dynamic range were determined using small molecule compounds-spiked plasma samples. The replicate analysis indicated that this system provided rapid and precise quantitative profiling of small molecule compounds with a relative standard deviation of retention time and peak intensity of less than 0.3% and 5.0%, respectively. An increase of 6 times more sensitivity and 3 times more throughput has been demonstrated with a dramatic reduction of solvent consumption compared to conventional LC-MS/MS. Sensitivity measurements provided 3.5-4 orders of magnitude of linear dynamic range and a limit of quantitation at 5-10 fg on column. The integration of the precise micro flow gradient delivery system, the robust interface, and the high sensitivity mass spectrometry delivers truly unachievable LC-MS/MS performance using conventional LC-MS/MS formats, and has been successfully applied to rapid and sensitive profiling of 18 corticosteroids in plasma.

ACKNOWLEDGEMENTS

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