

Higher Sensitivity and Improved Resolution Microflow UHPLC with Small Diameter Turbo V™ Source Electrodes and Hardware for use with the ExpressHT™-Ultra System

Improved Chromatography and Faster Separations for High Throughput Bioanalytical LC/MS

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Microflow UHPLC /MS with column diameters ≤ 1 mm is an emerging approach for high-throughput bioanalytical LC/MS. High-throughput LC/MS workflows require fast, high performance separations, and microflow UHPLC offers these capabilities in addition to other known benefits, including improved sensitivity for a given injection volume, reduced solvent consumption, and reduced contamination of the mass spectrometer.

The ExpressHT™-Ultra system is a microflow UHPLC system designed for applications that benefit from fast separations (1 to 1.5 minute cycle times). The system can deliver rapid and highly accurate gradients at flow rates from 5 -200 μ L/min with very low gradient delays and fast re-equilibration times. To realize the high performance of microflow separations one must control post-column dispersion, including that generated by the electrospray ionization (ESI) source.

Two new small diameter electrospray ionization electrodes (25 μ m ID and 50 μ m ID) for the AB SCIEX Turbo V™ and DuoSpray™ ion sources have been developed. These electrodes minimize post-column dispersion, and when used with the ExpressHT™-Ultra system, enable fast, high performance



separations for 0.3 mm and 0.5 mm ID columns. The new hybrid electrodes are made of PEEKsil tubing with a short (65 μ m ID x 1 cm) stainless steel tip. The short stainless steel tip provides minimal residence time, compared to an entirely stainless steel electrode, to reduce any possible surface interactions of analytes that can cause signal variability. The new electrodes can be inserted into a standard Turbo V™ source probe and do not require any additional parts or fittings.

In addition, new ExpressHT-Ultra column heater mounting hardware has been designed to allow very close coupling of the heated column to the Turbo V or DuoSpray source on all of the AB SCIEX MS systems, to further minimize post-column dispersion. When combined with the smaller diameter electrodes, these improvements provide improved chromatographic resolution resulting in narrower peaks with increased peak heights.

The new hardware and small diameter electrodes were designed exclusively for the ExpressHT™-Ultra System to extend its capability by improving the speed, resolution and sensitivity for a variety of bioanalytical micro LC/MS/MS applications.

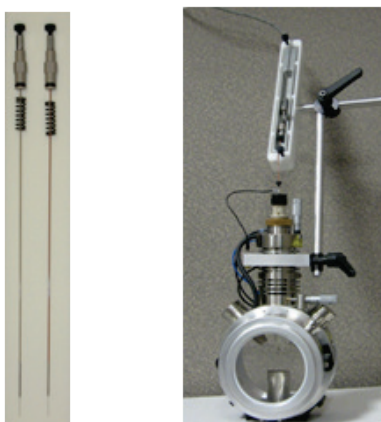


Figure 1. Small Diameter Electrodes for the Turbo V™ Source. Two new hybrid ESI electrodes (25 μ m and 50 μ m ID), made of PEEKsil with a short, stainless steel tip, have been developed for microflow LC. New small ID electrodes combined with new grounding and column heater mounting hardware used with the Turbo V source enable improved chromatographic separations at flow rates from 5–200 μ L/min.

Experimental

HPLC system: An Eksigent ExpressHT™-Ultra binary gradient HPLC system was used at flow rates between 5 and 50 $\mu\text{L}/\text{min}$ for 0.3 mm ID and 0.5 mm ID columns. The system uses Eksigent's proprietary Microfluidic Flow Control™ (MFC) technology, which maintains highly reproducible flow rates by continuously measuring the flow rate of each mobile phase. An embedded processor uses the flow rate measurements to control a rapid, electronically adjusted pressure source. This approach allows the generation of very rapid gradients with excellent accuracy and reproducibility.

Autosampler: A CTC HTC-autosampler was used as part of the ExpressHT-Ultra system. Injections were made using the metered injection mode.

MS/MS Conditions: An AB SCIEX QTRAP® 5500 system with Turbo V™ Source with standard probe was used. The new small diameter electrodes were used in the probe, replacing the standard 100 μm ID electrode for the TurboV probe, and performance was compared to the current smaller bore 65 μm ID electrode that has been used for microflow LC. The mass spectrometer was used in positive ion mode with data collected by MRM analysis.

Results

The coupling of the ExpressHT-Ultra system with the mass spectrometer requires an optimized ESI electrode to minimize dispersion and achieve optimal peak widths and chromatographic resolution (equivalent to high-flow UHPLC). Figure 1 shows the new hybrid electrodes, as well as an

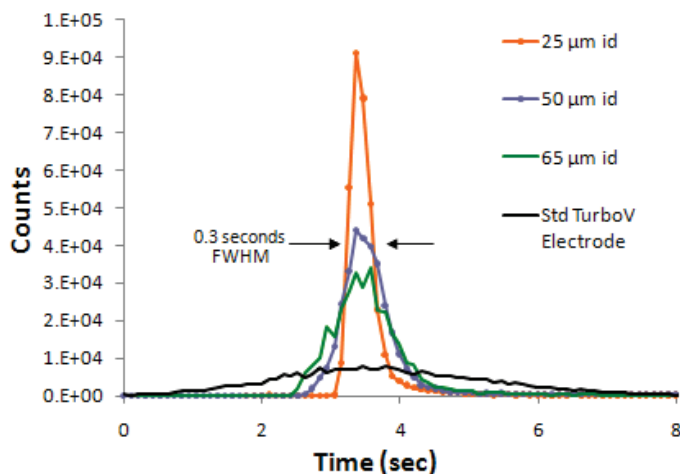


Figure 2. Electrode Dispersion at 10 $\mu\text{L}/\text{min}$. Peak dispersion observed in a flow injection experiment at 10 $\mu\text{L}/\text{min}$ is shown for four ESI electrodes with different internal diameter sizes. The orange and blue lines illustrate the peak dispersion improvement utilizing the new smaller ID hybrid electrodes.

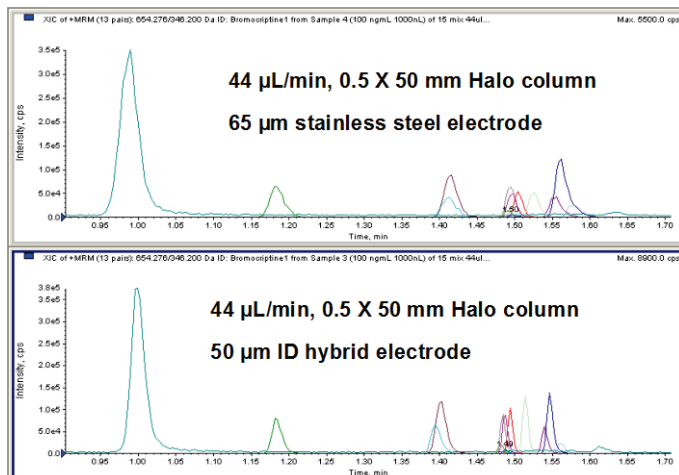


Figure 3. Comparing LC Peak Widths for Smaller Diameter Electrodes for Small Molecules. Comparison of peak widths obtained on an 11 compound drug mixture with the 65 μm ID stainless steel electrode vs. the new 50 μm ID hybrid electrode is shown.

electrode inserted into the Turbo V source, combined with the new grounding and mounting hardware for the column heater.

The smaller diameter electrodes consist of either 25 or 50 μm ID PEEKsil with a 1 cm (65 μm ID) stainless steel tip. Figure 2 shows examples of the peak broadening that can occur due to post-column dispersion. In this experiment, flow injection of a 10 ng/mL peptide sample was performed using 10 $\mu\text{L}/\text{min}$ and the injection volume was 30 nL, using metered injection mode. The 25 μm ID electrode shows the least broadening and greatest peak height versus the larger ID electrodes at the 10 $\mu\text{L}/\text{min}$ flow rate. The results shows that incorporating narrow ID PEEKsil into the electrode limits dispersion and, therefore, peak broadening, which can result in significantly improved chromatography.

Figure 3 shows an example that demonstrates the improvement in peak widths that can be obtained using the new 50 μm ID hybrid electrode compared to the 65 μm ID stainless steel electrode. In this example, an 11 compound drug mixture was tested, with the same volume and concentration sample injected in both experiments. Mobile phase A was water with 0.1% formic acid and mobile phase B was acetonitrile with 0.1% formic acid. The gradient was 10% to 90% in B in 1 minute. The top panel in Figure 3 shows the separation of the 11 compound drug mixture using an Eksigent HALO fused core C18, 2.7 μm , 0.5 X 50 mm column, at a flow rate of 44 $\mu\text{L}/\text{min}$. The peak widths at base for the chromatogram shown in the top panel, where the 65 μm ID stainless steel electrode was used, are about 3 seconds, whereas the peak widths at base for the chromatogram shown in the bottom panel, where the 50 μm ID hybrid electrode was used, are 2 seconds or less.

Figure 4 shows an example with peptides that demonstrates the quality of data that can be obtained using the new 25 µm ID hybrid electrode vs. the current 65 µm ID stainless steel electrode. The peptide VDEDQPFPAVPK from a tryptic digest of Beta Galactosidase (P00722) was analyzed in MRM mode using an Eksigent HALO C18, 2.7 µm, 0.5 x 100 mm column at a flow rate of 10 µL/min. All conditions were kept constant except the exchange of the standard 100 µm ID Turbo V source electrode vs. the new 25 µm ID electrode. Note the increased peak width for the 100 µm electrode of 1.2 min, in comparison to the 25 µm electrode, which shows only a 12 sec peak width. While the peak area was constant at 2×10^5 counts, the signal response (peak intensity) increased dramatically by a factor of 4 from 8.7×10^3 cps to 3.3×10^4 cps.

Table 1 below summarizes the recommended electrode sizes to be used with different diameter columns and flow rate ranges.

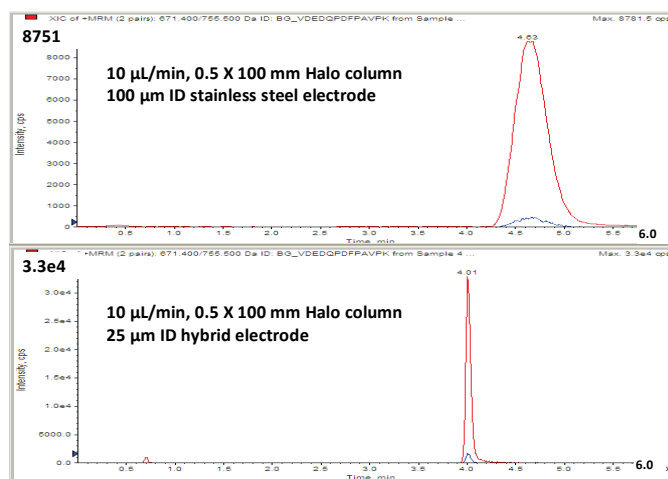


Figure 4. Comparing LC Peak Widths for Smaller Diameter Electrodes for Peptides. Comparison of peak widths obtained from MRM analysis of the peptide VDEDQPFPAVPK from a tryptic digest of Beta Galactosidase with the 100 µm ID stainless steel electrode vs. the new 25 µm ID hybrid electrode. Peak height increased by 4 fold which will result in better quantitative results.

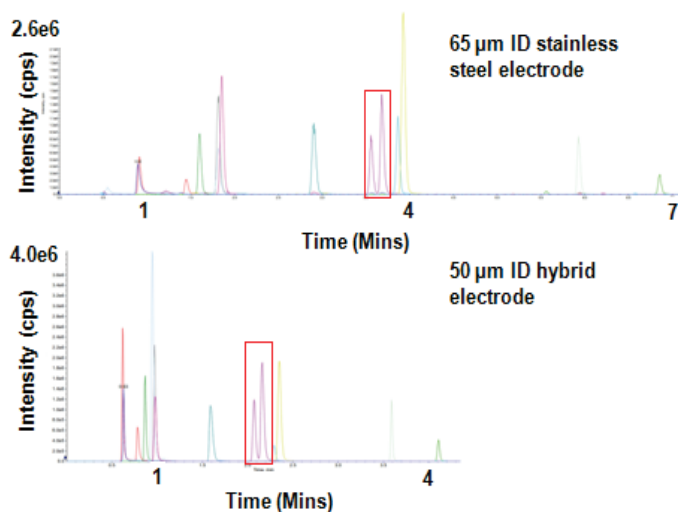


Figure 5. Higher Peak Resolution Enables Faster Chromatography. Comparison of chromatographic resolution obtained on a high throughput analysis of Fmoc-amino acids from plasma extracts, with the 65 µm ID stainless steel electrode vs. the new 50 µm ID hybrid electrode is shown. The gradient length could be decreased from 7 minutes to 4.3 minutes when using the smaller diameter electrode, resulting in a dramatic 35% increase in throughput, while still enabling separation of the critical isoleucine/leucine pair (highlighted in the red box).

Table 1. Recommended Column Diameters and Flow Rate Ranges for Electrodes.

Column Diameter	Flow Rate Range	Electrode Size
0.3 mm ID	5 – 25 µL/min	25 µm ID
0.5 mm ID	15 – 60 µL/min	25 or 50 µm ID
1.0 mm ID	50 – 200 µL/min	50 or 65 µm ID

The last example shown in Figure 5 illustrates how the improvement in resolution can enable significantly improved throughput for a complex separation of Fmoc-amino acids in clinical research samples (from deproteinized plasma extracts). For this separation, isoleucine and leucine must be chromatographically separated, since they are isobaric and cannot be differentiated by traditional quantitative electrospray ionization mass spectrometry. The sample was analyzed using a 0.5 x 100 mm Eksigent HALO C18 column. Mobile phase A was 15 mM ammonium acetate, 5% acetonitrile, 5% isopropanol. Mobile phase B was acetonitrile. The flowrate used was 28-35 µL/min, and the equivalent of 70 nL of deproteinized plasma was injected on column. The top panel shows the 7 minute analysis using the 65 µm ID stainless steel electrode, and the bottom panel shows the faster, 4.3 minute analysis obtained (while still separating isoleucine and leucine as shown in the red box) using the new 50 µm ID hybrid electrode. The improved chromatographic resolution enabled a dramatic 35% increase in throughput. In addition, the signal response (peak intensities) increased by a factor of 1.5.

Summary

Two new smaller diameter electrospray ionization hybrid electrodes (25 μm ID and 50 μm ID) have been developed to minimize post-column dispersion, enabling fast, high performance separations at 5 – 200 $\mu\text{L}/\text{min}$ with sub-2 second peak widths when utilized with the Turbo V™ Source on AB SCIEX mass spectrometry systems. The new hybrid electrodes are made up of PEEKsil tubing, with a short (65 μm ID x 1 cm) stainless steel tip. The short stainless steel tip provides minimal residence time, compared to an entirely stainless steel electrode, to reduce any possible surface interactions of analytes that can cause signal variability.

When combined with new column heater mounting hardware that allows placement of the column as close as possible to the Turbo V Source, the small diameter electrodes enable improved chromatographic resolution resulting in narrower peaks with increased peak heights at flow rates from 5-200 $\mu\text{L}/\text{min}$ —all optimized specifically for the ExpressHT™-Ultra system. Several examples, including quantitative LC/MS separations of drug compounds, peptides and amino acids included all demonstrate the advantages of using these new electrodes with the ExpressHT™-Ultra system.

Acknowledgements

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Table 2. Part Numbers for Electrodes, Hardware Kits and Columns.

Part Number	Description
5016411	50 μm ID Electrode for AB SCIEX Turbo V™ Source, <i>requires grounding kit (5016941)</i>
5016874	25 μm ID Electrode for AB SCIEX Turbo V™ Source, <i>requires grounding kit (5016941)</i>
5016941	Grounding Kit for Turbo V™ Source Electrodes 5016411 & 5016874
5015996	Clamp and Rod Assembly, Mounting for HT column oven
801-00084	Kit, ExpressHT, AB SCIEX bundle <i>Includes 25 μm ID electrode, 50 μm ID electrode, column oven clamp (5015996), grounding kit (5016941) and MS interface cable (700-00049)</i>
805-10100	HALO Fused-Core C18 column, 2.7 μm , 0.5 x 50 mm
805-10101	HALO Fused-Core C18 column, 2.7 μm , 0.5 x 100 mm

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