

Developing Novel Fluidic Solutions for Challenging LC-MS Capillary Separations of Biological and Small Molecules

Graham Shelper Ph.D., Lead Application Scientist
IDEX Health & Science Research & Development

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Executive Overview

IDEX Health & Science, a global leader in fluidics technology, is developing capillary column capability for Mass Spectrometry (MS) centered around a novel tube-in-manifold design. The design comprises of a bioinert, high pressure capillary column module with an integrated rotary shear valve. The design addresses the robustness issues associated with delivering well separated components, at ultra-low flow rates, to MS electrospray emitters and nanoliter volume UV flow cells.

Preface

Mass Spectrometry, in its many forms, has become the gold standard detection method for the rapidly evolving biologics domain as well as for many small molecule separations. As MS systems perform optimally at low flow rates, usually in the $\mu\text{L}/\text{min}$ - nL/min range, there has been a concomitant decrease in LC column dimensions and stationary phase particle sizes being used for these types of separations. These decreased dimensions enable a) reduced sample injection volumes, b) improved compound detection sensitivities and c) significant reductions in mobile phase solvent usage (Table 1), all of which are advantageous as the amounts of available sample continue to shrink while environmental safety concerns steadily increase.

Table 1:

LC Column Parameter Comparison Relative to 4.6mm ID

Column ID(mm)	Column Vol (ul)	Void Vol (ul)	Flow Rate (ul/min)	Sample Load (Relative)	Solvent Saving (%)
4.600	2492	1121	1000	1	0
2.100	519	234	208	0.2084	79.16
1.000	118	53	47	0.0473	95.27
0.200	4.7	2.1	1.89	0.0019	99.81
0.150	2.6	1.2	1.06	0.0011	99.89
0.075	0.7	0.3	0.27	0.0003	99.97

Column Packing density = 0.55, Column Length 150mm

As column dimensions and flow rates decrease, consideration of fluid path geometry, and other physical parameters such as temperature control, become increasingly important: Extra column effects can significantly impact separation efficiency and ultimately analytical performance. LC Systems that work well with "regular" ($>1\text{mm}$) diameter columns, are often not fluidically optimized for the smaller ID capillary columns associated with low-flow applications, making sample injection volume, and overall system dead volume significant contributors to suboptimal chromatography.

The Flow Cell & Scale Factor columns in Table 2 illustrate the importance of using correctly sized UV detector flow cells with $<1\text{mm}$ ID capillary columns. A $200\mu\text{m}$ ID column operating at $2.1\mu\text{L}/\text{min}$ requires a flow cell of only 27nL to be comparable to a 4.6mm column at $1\text{mL}/\text{min}$ connected to a $13\mu\text{L}$ analytical flow cell. This represents a 481 fold reduction in flow cell volume.

Table 2:

Column ID (mm)	Flow Rate (ul/min)	Flow Cell (vol/sec)	Flow Cell (ul)	Scale Factor
4.6	1000	1.282	13	1
2.1	208	1.387	2.5	5
1	47	1.306	0.6	22
0.2	2.1	1.296	0.027	481

Comparison of recommended detector flow cell volume and scale factor vs column ID relative to 4.6mm ID column at $1\text{mL}/\text{min}$. All flow cells shown have been normalized to a flow of 1.3 flow cell volumes/sec for comparative purposes.

A relatively recent innovation aimed at addressing several constraints associated with the movement from regular ID columns to capillary columns for LC-MS separations, has been the development and commercialization of several microfluidic Chip-LC products engineered around fusion bonded, etched channel column technology^(1,2,3). Published data confirmed that these devices were capable of at least equivalent and in some cases, far superior performance when compared with regular LC columns. However, complex designs, sub-optimal channel geometries, and operating pressure limitations, along with large, instrument specific accessory hardware modules required for operation, hampered the widespread adoption of these devices by the LC-MS community.

IDEX Health & Science Capabilities:

IDEX Health & Science, a global leader in fluidics technology, is developing several capabilities around a novel tube-in-manifold capillary column platform concept^(4,5,6), the concept features a cylindrical fluid path with wide radius bends, designed to address the many performance and robustness issues associated with both legacy etched channel Chip-LC, and standard capillary columns currently being used for LC-MS.

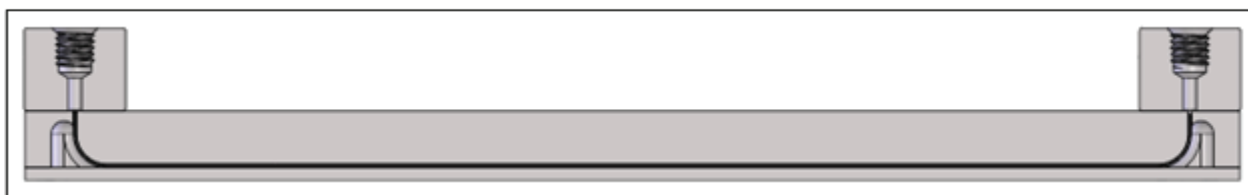


Figure 1. IDEX Health & Science tube-in-manifold high pressure capillary column prototype showing wide radius bends and MarvelX™ face sealing connection ports.

Acquiring a thorough understanding of the physical and chemical properties associated with high pressure, low flow capillary fluid paths and the chromatographic performance envelope of these, has been an essential element of this work to this end, some of the capabilities being evaluated at IDEX Health & Science as part of this initiative include:

- **Extensive use of Computational Fluid Dynamic (CFD) Modelling** to evaluate and optimize fluidic geometries when operating within LC-MS flow rate, and system pressure ranges.
- **Minimizing System Dead Volume** by positioning the sample introduction valve, binary gradient mixing, capillary column, any post column modules, and connection fittings as close together as physically possible as a single, tightly integrated unit.
- **Ensuring a Bioinert Fluid Path** from the point of sample introduction, with capability options that include sample infusion, trapping, desalting, and subsequent nanoliter sample aliquot injection directly onto an immediately adjacent column and out through a closely interfaced emitter or flow cell.
- **Accurately Controlling Temperature** of the mobile phase and column while samples pass through the system to improve component separation efficiency and injection-to-injection reproducibility.
- **Designing Post Column Modules** that include isolated ion source emitter connections, high pressure, low volume UV/Vis flow cells, or 2D-LC valves that connect directly to the column outlet port.
- **Developing Robust Tools and Techniques** for straightforward and repeatable high pressure packing of, and inlet/outlet frit insertion into, tube-in-manifold capillary columns.

Selected aspects of this IDEX Health & Science tube-in-manifold capillary column platform concept R&D initiative are detailed on the next page.

Testing

CFD modelling of the IDEX Health & Science wide radius tube-in-manifold capillary column platform concept (Figure 2a), against similarly dimensioned packed fluid paths having cross-sectional geometries commonly associated with etched channel devices (Figure 2c-d), showed that these etched channel designs produce larger flow gradients at transition points that could negatively impact packing and subsequent chromatographic performance. Calculated peak retention time, resolution, asymmetry, and theoretical plate count data (Figure 3) supported these findings and showed that the IDEX Health & Science wide radius tube-in-manifold design would produce improved chromatographic performance with significantly higher separation efficiencies.

It is worth noting that, while developing CFD models for low-flow capillary LC requires significant up-front input with regard to the parameterization and optimization of many variables, once established, running comparative simulations with alternative dimensions and geometries becomes relatively straightforward and can realize significant cost and engineering effort savings prior to any actual mechanical prototyping.

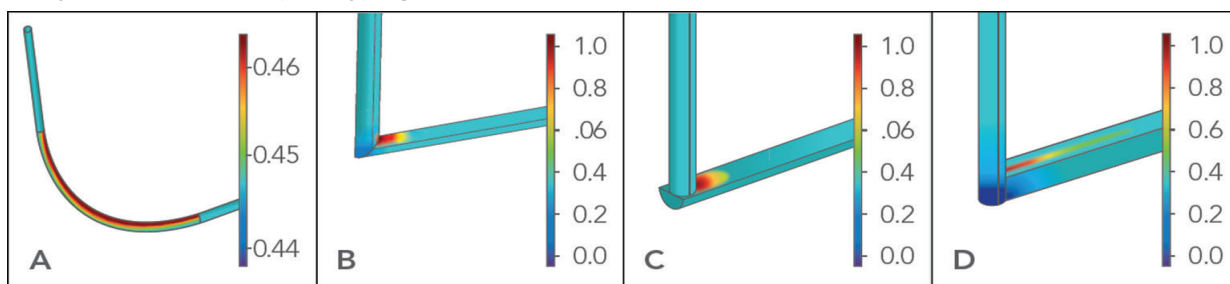


Figure 2. CFD modelling of flow velocities within A) IDEX Health & Science's junctionless wide radius fluidpath vs B) cylindrical, C) D shaped and , D) square with right angle junctions associated with legacy etched channel designs.

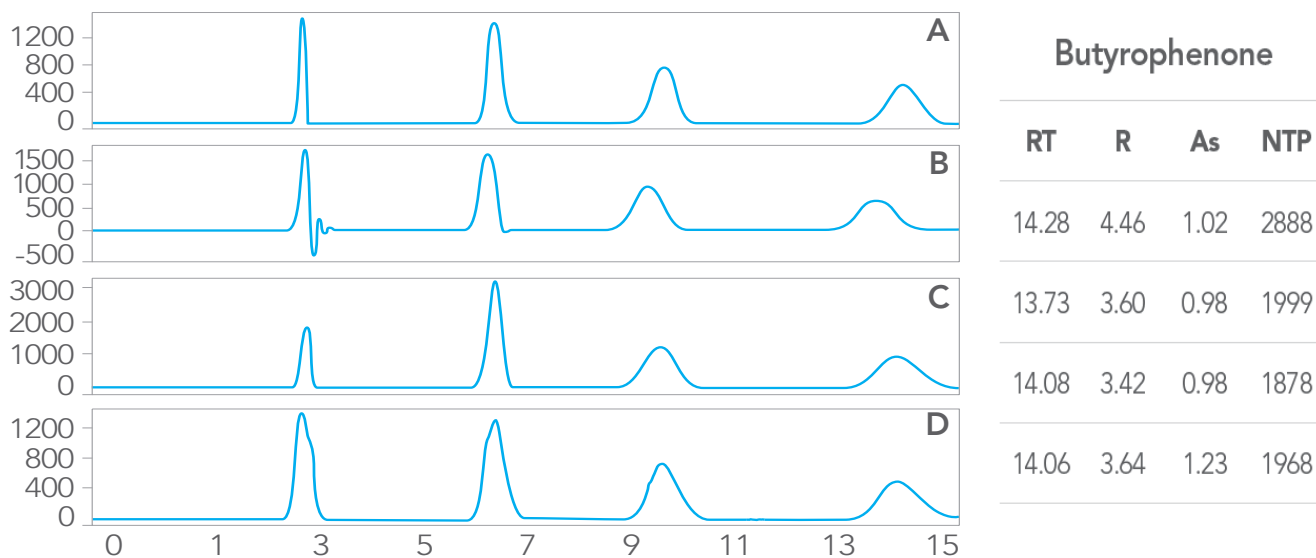


Figure 3. Predicted chromatograms and Butyrophene (4th) peak data derived for IDEX Health & Science's junctionless wide radius fluid path geometry (A) versus right angle tube (B), D form (C) and square form (D) packed fluidic channel geometries.

Integrating a sampling valve with the IDEX Health & Science tube-in-manifold capillary column, eliminates the requirement for any external transfer lines, and interfacing post column modules such as ion source emitters, UV/Vis flow cells or additional valves, directly with the column outlet, reduces the overall system dead volume to the absolute minimum. Adding binary gradient mixing directly into the sampling valve, which is already located at the head of the column, also effectively eliminates any gradient delay which can be significant at low $\mu\text{L}/\text{min}$ flow rates in non-optimized LC systems.

Complete Solution

As a leading OEM solution provider, IDEX Health & Science considers the development of robust tools and techniques associated with packing capillary columns to be an integral part of any technology evaluation initiative. Tooling for the precise cutting, placing and insertion of column inlet and outlet frits into tube-in-manifold prototypes, has been developed and successfully tested. This tool can be used to cut and insert polymeric or metallic frits manufactured from a selection of sintered or fibrous materials.

An IDEX Health & Science column packing station (Fig. 4), designed for safe operation at pressures up to 20kpsi has also been manufactured and repetitively used to pack 150mm x 200µm ID tube-in-manifold prototypes with sub 3µm particles. This module uses standard high pressure connections and a single locking nut simplifies routine servicing.

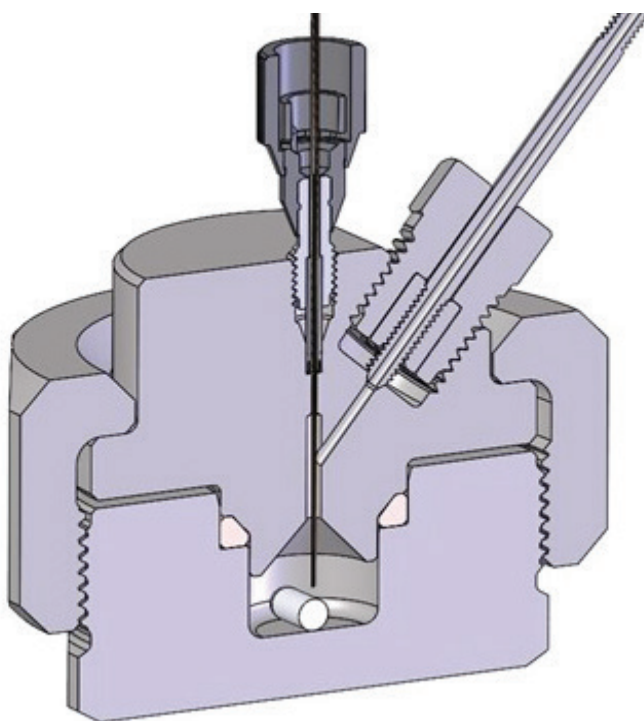


Figure 4: Capillary column packing station capable of operating at 20kpsi and outlet frits.

Conclusion

IDEX Health & Science's modular tube-in-manifold capillary column platform could be installed onto many existing LC systems as an adjunct subsystem, bringing the benefits associated with separating suitably prepared biological samples and small molecules, in a minimal dead volume through a fluidically optimized flowpath, at LC-MS compatible flow rates and delivering these as close to the ionization or UV/Vis detection source as possible. This compact integration would also bring the added advantage of being able to multiplex by using more than one module per analyzer to increase sample throughput, and at the same time, decrease solvent usage by up to 1000 fold.

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