eksigent

application note

high throughput parallel dual vented column nanoLC-MS

The Eksigent NanoLC™-2D system can be configured with dual vented columns to provide higher sample throughput with more thorough washing of the trap and analytical columns to reduce column sample carryover.

Introduction

The trend toward higher throughput analysis of peptide and protein samples in conjunction with a need to decrease column sample carryover has led to the development of hardware configurations and experimental conditions to meet these increasing demands.

The reduction in carryover due to sample sticking on the column (column sample carryover) can be a significant concern in instrument method development for proteomics applications. The detection of peptides from the previous sample can lead to inaccuracy in data reporting. Lengthy washing steps with high organic will reduce the amounts of column sample carryover but also reduce the sample to sample cycle time; thus reducing throughput.

The use of parallel dual vented columns allows for an entire wash cycle to be run on each trap and analytical column while a sample is being analyzed on the opposite trap and analytical column. This is equivalent to running a complete blank in between each sample with no reduction in MS cycle time.

Additionally, a portion of the column wash and re-equilibration can be omitted from the analytical run because these steps are completed during the off-line portion of the method; thus shortening the typical sample to sample cycle time.

experimental

Instrumentation:

- Eksigent NanoLC-2D™ system configured with both channels for nanoLC
- Eksigent AS-1 Autosampler
- Thermo LTQ-FT mass spectrometer

Valves:

- 10-port: Valco Nanovolume CN2 10-port valve with 0.10mm port diameter
- 6-port: Two Valco C2 6-port valves with 0.25mm port diameter

Sample:

- Eksigent peptide test standard

Columns:

- Trap: Waters Symmetry C18 5 µm, 180µm x 20mm
- Analytical: New Objective IntegraFrit 0.075 x 150mm, packed Michrom Magic C18aq

Mobile Phase:

- A: 98/2 water/acetonitrile + 0.1% formic acid
- B: 95/5 water/acetonitrile + 0.1% formic acid

Pump Conditions:

- Sample transport: 2%B at 2µL/min for 5 minutes
- Gradient profile: 2-40%B in 20 minutes; 40-98%B in 0.2 minutes; hold at 98%B for 15 minutes
- Gradient flow rate: 200nL/min
- Wash pump: 2-98%B in 8 minutes, 98-2%B in 4 minutes, 2-98%B in 8 minutes, 98-2%B in 0.2 minutes; hold at 2%B for 15 minutes

Source and Spray Tip Connection:

- Source: Thermo Electron Dynamic Nanospray Source
- Spray Tip: New Objective 360µm OD/ 20 µm ID with 10 µm tip
- Voltage Union: Upchurch U435 with 0.25mm thru hole

The diagram in Figure 1 shows the hardware and plumbing configuration for the dual parallel vented column setup. The channel 1 pump is used for both sample transport and gradient elution while the channel 2 pump is used for washing and re-equilibrating the off-line trap and analytical column.

A sequence begins by positioning the 10-port valve to select either column 1 or column 2. The autosampler then aspirates sample and the sample is transported onto the trap column with the vent valve in open position (flow to waste). The vent valve is then switched to the closed position (vent line blocked), MS data acquisition is initiated, and the gradient is run through the trap and analytical columns. At the same time (in parallel), the off-line trap and analytical columns (the ones not being used for data acquisition) are washed and re-equilibrated using the second binary pump.

results and discussion

Figure 2 shows base peak data for a direct on-column injection and for an injection using the dual vented column setup. The direct on-column data was collected to determine the dispersion effect of adding the 10-port valve post column. The peak width at base for the direct on column injection was between 0.3 and 0.4 minutes while the peak width at base for the dual vented column setup was between 0.4 and 0.6 minutes. The longer gradient delay in the dual vented column setup can be attributed to the additional hardware (tubing, fittings, trap column, and 10-port valve) used in this setup.

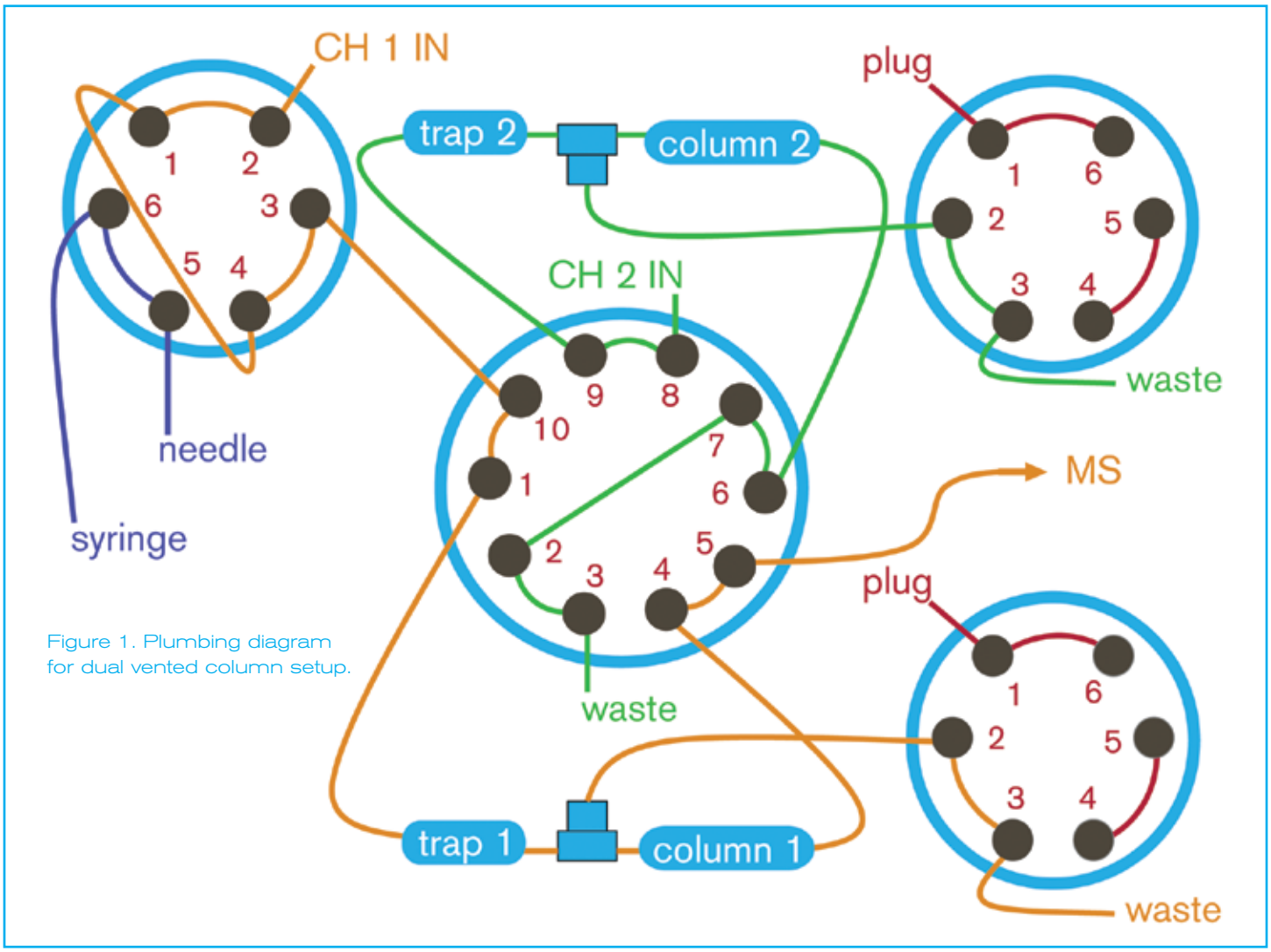

Figure 1. Plumbing diagram for dual vented column setup.

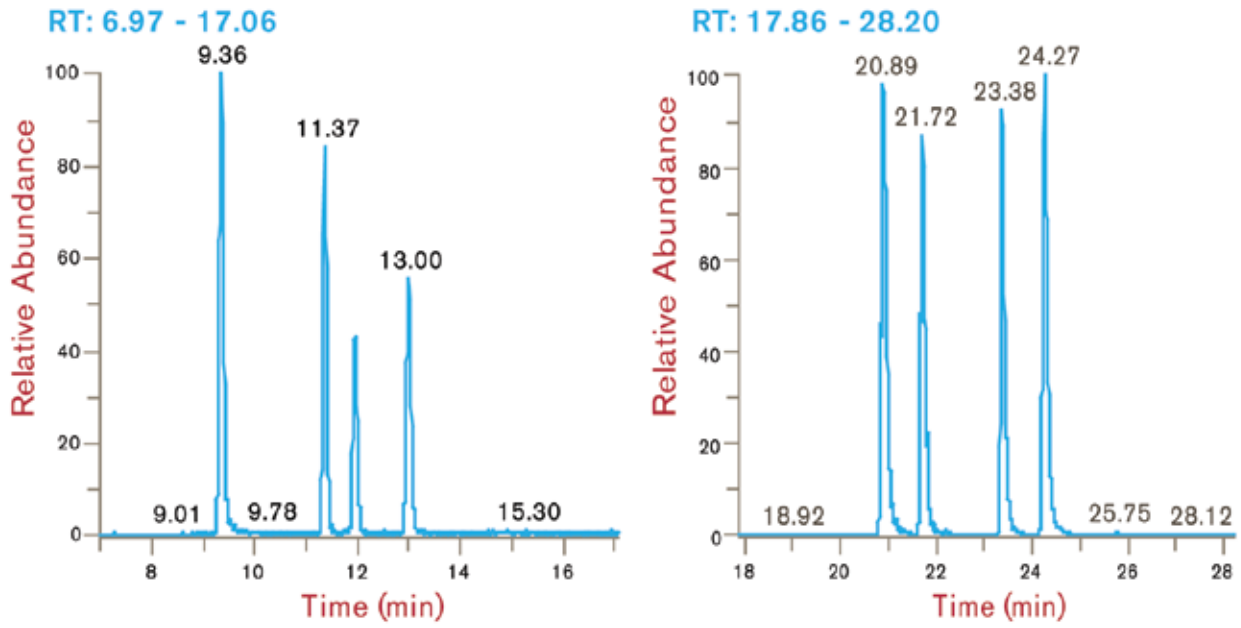

Figure 2: Direct on-column and vented Injections. Base peak chromatograms for direct on-column injection (left) and for an injection with the dual vented column setup (right).

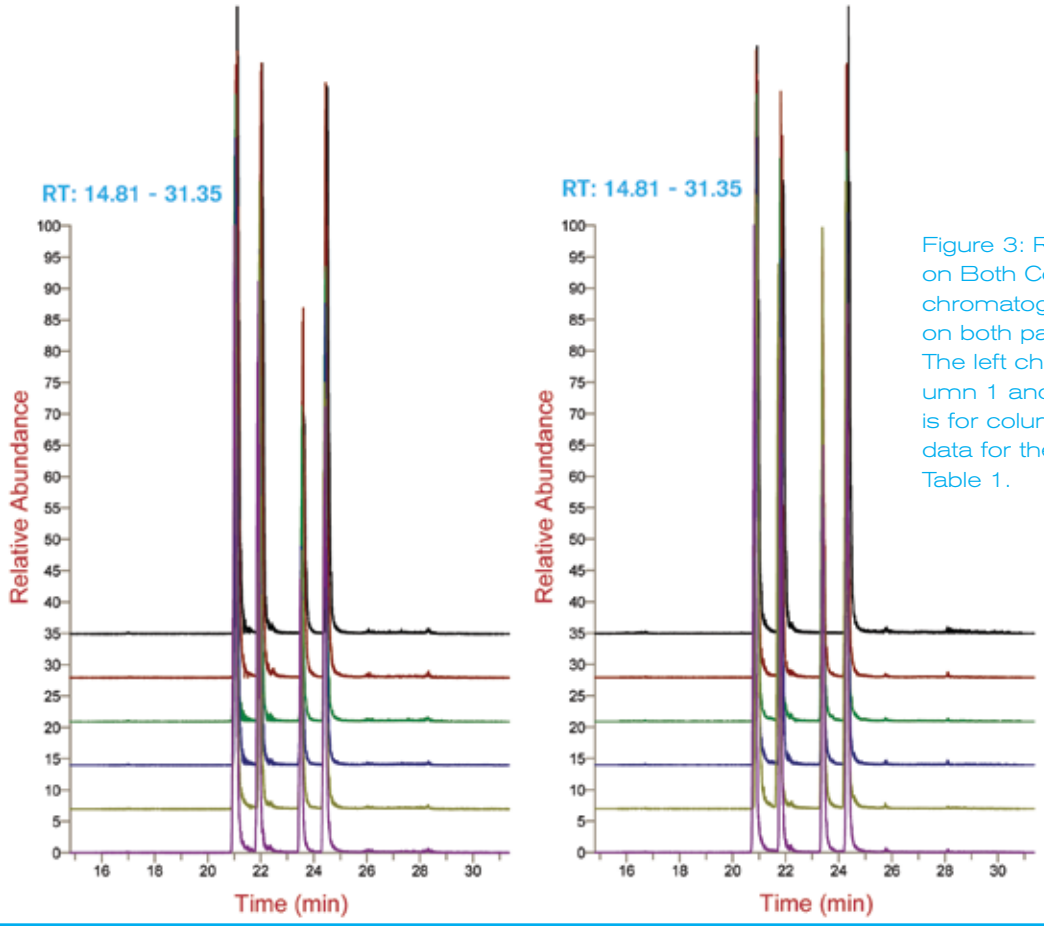

Figure 3: Replicate Injections on Both Columns. Base peak chromatograms for 6 injections on both parallel vented columns. The left chromatogram is for column 1 and the right chromatogram is for column 2. The retention time data for these runs is shown in Table 1.

Column/Trap 1				
Injection	Peak 1	Peak 2	Peak 3	Peak 4
1	21.12	22.04	23.6	24.51
2	21.12	22.02	23.61	24.45
3	21.05	21.96	23.56	24.45
4	21.06	21.95	23.51	24.42
5	21.04	21.96	23.54	24.4
6	20.99	21.9	23.49	24.42
Average	21.0633	21.9717	23.5517	24.4417
SD	0.0501	0.0508	0.0479	0.0387
%RSD	0.2377	0.2310	0.2035	0.1583

Column/Trap 2				
Injection	Peak 1	Peak 2	Peak 3	Peak 4
1	20.92	21.9	23.43	24.37
2	20.89	21.81	23.38	24.3
3	20.9	21.78	23.38	24.29
4	20.89	21.83	23.37	24.32
5	20.89	21.72	23.38	24.27
6	20.81	21.8	23.41	24.31
Average	20.8833	21.8067	23.3917	24.3100
SD	0.0378	0.0592	0.0232	0.0341
%RSD	0.1809	0.2716	0.0990	0.1401

Table 1: Retention times, standard deviations, and relative standard deviations (%RSD) for four peptides across six injections, on both columns.

conclusions

The Eksigent NanoLC-2D system configured with dual vented columns increases sample throughput up to 100% by performing the wash and re-equilibration steps off-line, while collecting data in parallel with the opposite column. The increase in throughput is accomplished without a significant dispersion effect from the addition of a post column valve (an increase of 0.1 to 0.2 minutes to the peak width at base).

The excellent reproducibility in retention time (<0.3% RSD) shows that equilibrating the trap/column 'off-line' does not affect the subsequent injection.

This hardware configuration reduces column sample carryover by allowing the user to program longer wash steps (up to the full gradient length, less the re-equilibration time) for the trap and analytical columns between each sample analysis cycle.

about Eksigent Technologies

Eksigent is creating new possibilities for life science research, drug discovery & development, and medical devices with its innovative MicroFlow™ and NanoFlow™ fluid delivery systems.

Eksigent's LC systems deliver dramatic increases in analysis speed, throughput, and sensitivity. Eksigent's drug delivery systems bring new levels of precision to portable drug delivery.

Today, leading research, pharmaceutical, and biotechnology firms around the world use Eksigent's innovative solutions.

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