

step versus linear gradient elution for on-line 2D nano LC/MS/MS

The Eksigent NanoLC™-2D system enables the user to choose step or linear gradient elution mode as part of an on-line 2D LC/MS/MS analysis with both modes being equally successful in identifying peptides that represent the proteins in the original sample.



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introduction

On-line two-dimensional (2D) chromatography is often used to increase peak capacity for the analysis of complex peptide samples by LC/MS/MS. Increased separation of the peptides typically results in a higher number of identified peptides in a “shotgun” approach proteomics experiment. A Strong Cation Exchange (SCX) column is commonly used for the first dimension separation in combination with a Reversed Phase (RP) column in the second dimension.

Peptides can be eluted by stepwise changing the ionic strength of the eluent in the first dimension, collecting peptides on a trap and then subjecting them to reversed phase chromatography in the second dimension. Alternatively, peptides can be eluted from the ion exchange column with a continuous or discontinuous linear gradient. Studies in the literature have reported broad peaks [1,2] or fewer identified peptides [3] with step gradient elution in the SCX dimension. However, these studies often overlooked other contributing factors such as elution time which also plays a critical role in on-line SCX-RP analyses [4].

The ability of the Eksigent NanoLC™-2D to operate in a variety of modes without requiring changes to the hardware configuration has allowed us to investigate the effect of elution mode on peak shape and peptide identification while keeping other parameters constant. Step and linear gradient SCX elution modes are compared for an on-line 2D nano LC/MS/MS analysis using a five-protein digest.

experimental

- Instruments: Eksigent NanoLC-2D, New Objective PicoView™ nanospray source, Thermo LTQ™ mass spectrometer
- Sample: Five Yeast Protein digest purchased from Michrom Bioresource
- Experimental conditions: See Table 1 for experimental conditions

The diagram in Figure 1 shows the hardware configuration for the on-line 2D experiments. Initially, peptides are injected onto the SCX column in 10 mM ammonium formate. After injection, the first five-minute ion exchange elution segment begins. Peptides not retained on the SCX column are trapped on one of the two enrichment columns connected to the 10-port valve. Channel 1 flow through the SCX column is stopped after the five-minute segment and the 10-port valve is switched to place the enrichment column in line with the C18 analytical column and mass spectrometer. The reversed phase gradient is delivered to the analytical column (by Channel 2) to elute the trapped peptides. After the first reversed phase separation is complete, the 10-port valve is switched back to the initial position. Flow from Channel 1 resumes for the second elution segment during which the salt buffer is increased in concentration either stepwise or linearly to elute the next fraction of peptides. The second fraction of peptides is trapped on the enrichment column and then eluted by the reversed phase gradient. This process is repeated until all the peptides have eluted from the columns. A total of five elution segments were used for each injection in this application. Diagrams illustrating the timing of the ion exchange and reversed phase elution segments are shown in Figures 2a and 2b.

Table 1. Experimental conditions.

	Ion Exchange	Reversed Phase
column	SCX: 0.3 x 50 mm, 5 µm, PolySULFOETHYL Aspartamide™ (PolyLC) 2 pmol sample injected in 2 µL	trap: 0.3 x 50 mm, 5 µm, C18 analytical: 0.075 x 150 mm, 3.5 µm, C18
mobile phase	A: 8 mM ammonium formate (NH ₄ CO ₂ H) + 150 mM formic acid + 2% acetonitrile (pH = 2.9) B: 150 mM ammonium formate + 25 mM formic acid + 2% acetonitrile (pH = 4.9)	A: 98% water + 2% acetonitrile + 0.1% formic acid B: 10% water + 90% acetonitrile + 0.1% formic acid
flow rate	4 µL/min, Eksigent Channel 1	250 nL/min, Eksigent Channel 2
elution profile	step elution: five 5-min steps at 10, 20, 40, 80, 150 mM NH ₄ CO ₂ H linear gradient: five 5-min ramps: 10-20, 20-40, 40-65, 65-105 and 105-150 mM NH ₄ CO ₂ H	initial hold at 5%B (5 min), 5-45%B (60 min), 85%B wash (10 min), re-equilibration at 5%B (20 min)

Figure 1. Diagram of 2D configuration for on-line 2D experiments.

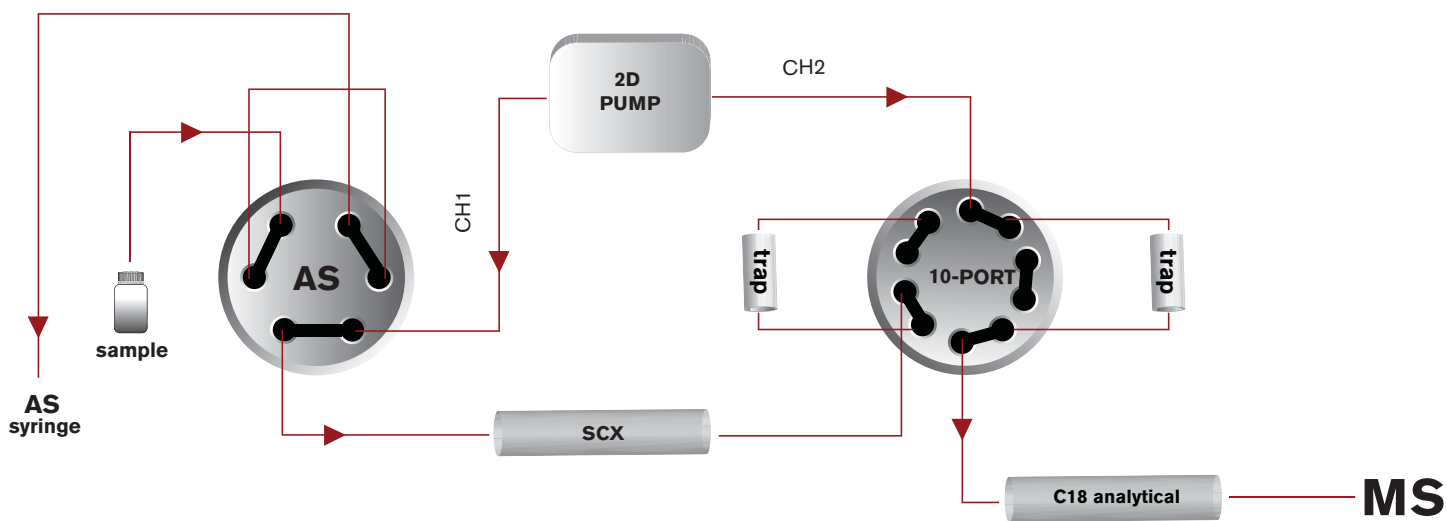
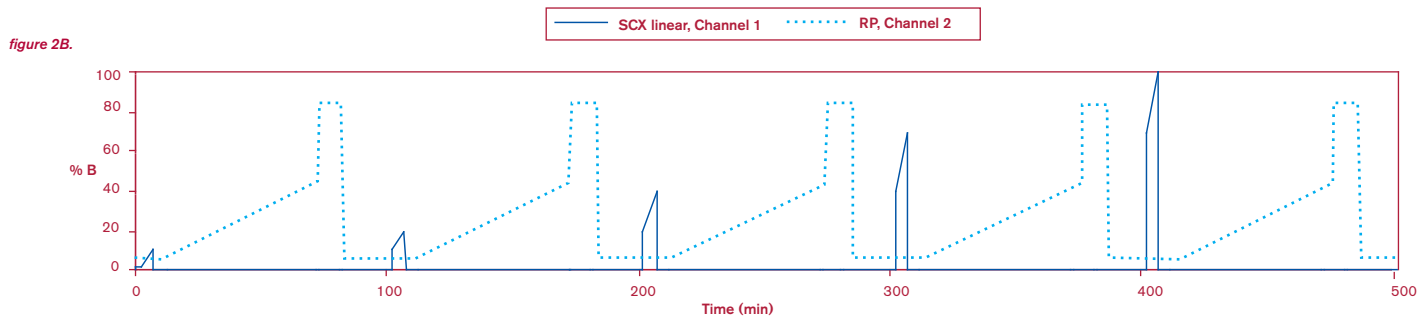
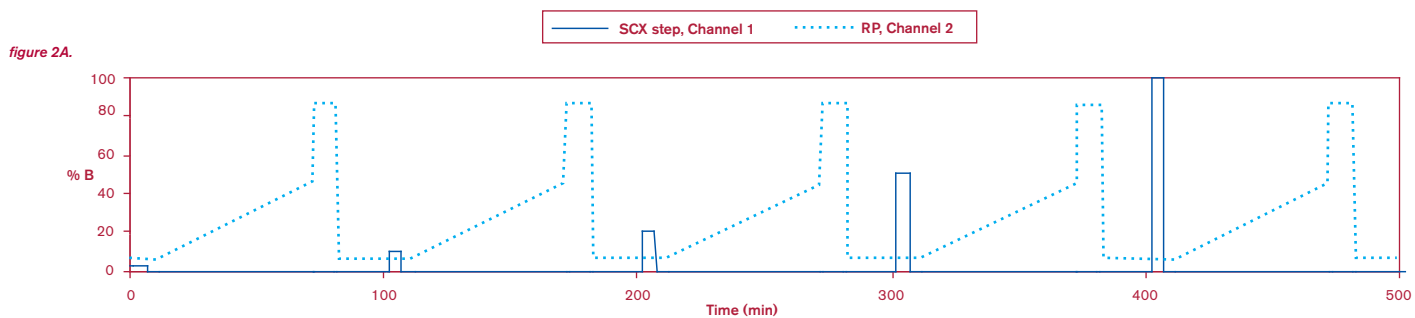


Figure 2: Timing diagrams for ion exchange and reversed phase elution profiles for step (A) and linear (B) SCX elution modes.

In step mode, the salt concentration is held constant within each 5-minute elution segment whereas in linear mode the salt concentration increases within each elution segment. The initial and final buffer concentrations were identical for step and linear elution modes. Reversed phase solvent (Channel 2) flows continuously but the ion exchange buffer (Channel 1) stops flowing between elution segments.



results and discussion

On-line 2D nanoLC/MS chromatograms are shown for step (Fig. 3) and linear (Fig. 4) gradient elution modes. The five RP chromatograms shown in each figure correspond to the five peptide fractions obtained from the ion exchange column. A selected peptide (m/z 734) ion chromatogram is extracted from the data to demonstrate the ability of the ion exchange separation to adequately retain peptides and isolate peptides in single salt segments (Fig. 5). Although shown for the step elution mode, linear gradient segments are found to isolate this peptide equally well. Minor differences in peptide distribution are observed between the step and linear elution modes, as expected, but the overall chromatographic appearance is similar.

A MASCOT search was performed on combined MS/MS data sets from the five step elution chromatograms and again for the combined data sets from the five linear elution chromatograms. A greater percentage coverage and greater number of peptides are identified from the step elution data than from the linear elution data for four of the five proteins in the sample (Table 2). However, further optimization of the peptide separation by adjusting the salt concentrations in each of the step and linear segments might minimize the observed differences between the step and linear modes. The numbers of identified peptides are adequate in all cases to represent the five proteins known to exist in the sample.

Figure 3: Step SCX Elution.

Base peak chromatograms from analysis of a yeast protein digest (step mode).

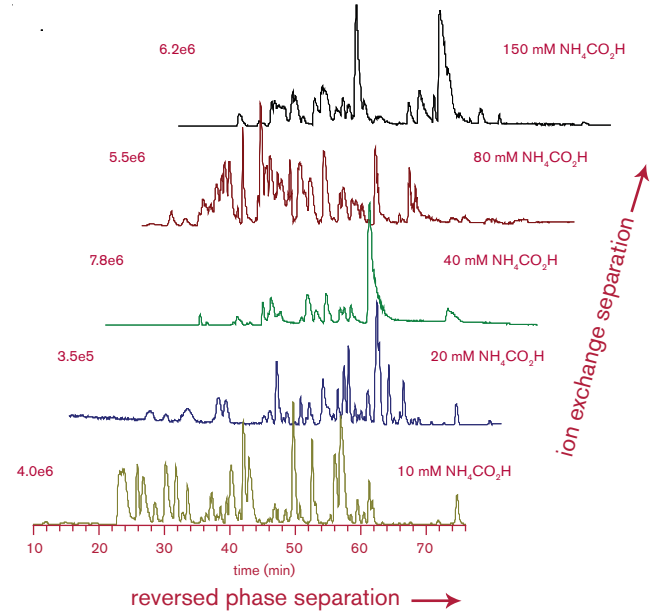


Figure 4: Linear SCX Elution.

Base peak chromatograms from analysis of a yeast protein digest (linear mode).

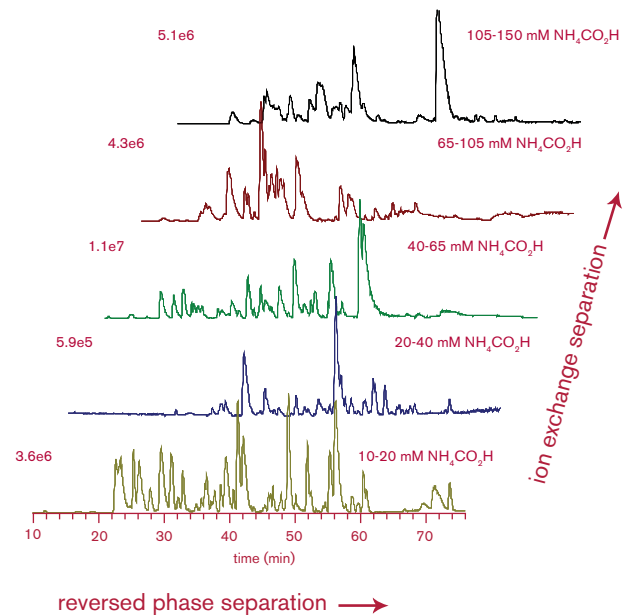
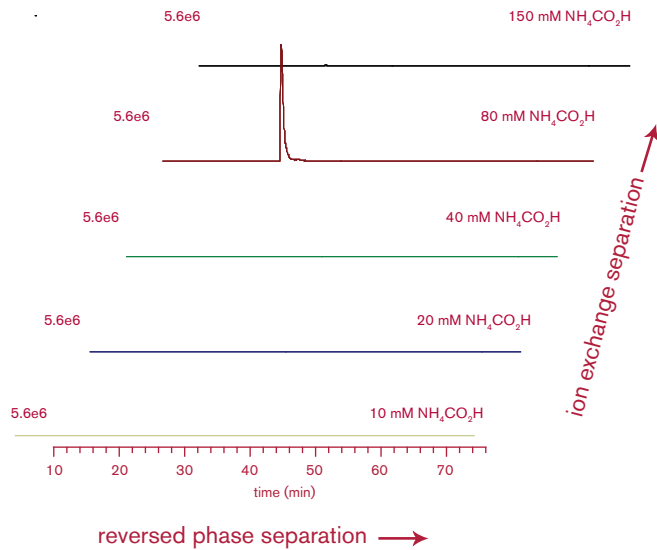


Figure 5: m/z Extracted.

Extracted ion chromatograms (m/z 734) show the isolation of a peptide in one salt fraction.



conclusions

The Eksigent NanoLC-2D system allows flexibility for the user to choose from a variety of possible ion exchange elution modes as part of an on-line 2D LC/MS/MS analysis. Contrary to past reports, step elution mode performed at least as well as and possibly better than linear elution mode for the identification of peptides in this application. Step and linear gradient elution modes were equally successful in identifying an adequate number of peptides to represent the proteins in the original sample.

references

- [1] Nägele, E., Vollmer, M., Hörth, P. J. Chromatogr. A. 2003, 1009, 197-205.
- [2] Winnik, W. M. Anal. Chem. 2005, 77, 4991-4998.
- [3] Nägele, E., Vollmer, M., Hörth, P. J. Biomol. Tech. 2004, 15, 134-143.
- [4] Le Bihan, T., Duewel, H. S., Figeys, D. J. Am. Soc. Mass Spectrom. 2003, 14, 719-727.

Table 2: Results of MASCOT database search for the two 5-min elution modes.

Protein	Step Elution			Linear Elution		
	Score	SC(%)	pept#	Score	SC(%)	pept#
AD	605	56	22	608	46	16
E	287	30	18	250	39	18
GPD	364	44	25	890	40	21
H	440	25	16	140	20	12
PI	372	48	27	471	37	21

AD: Alcohol Dehydrogenase

E: Enolase

GPD: Glucose-6-Phosphate Dehydrogenase

H: Hexokinase

PI: Phosphoglucose Isomerase



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