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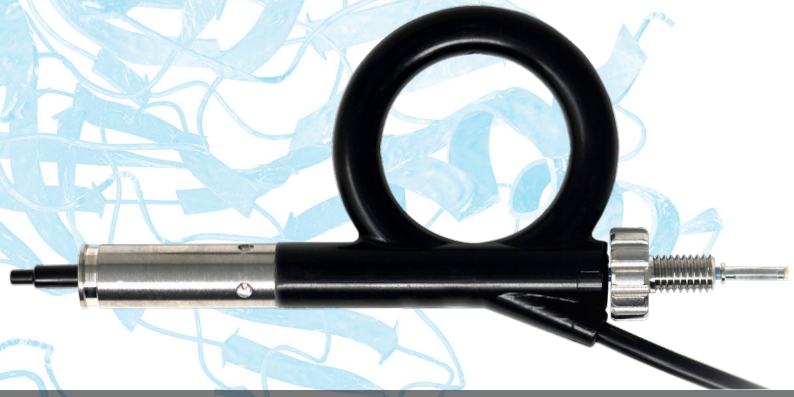

Diagnosing EASY-Spray chromatographic problems

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Introduction

Chromatographic problems with Thermo Scientific™ EASY-Spray™ columns are best evaluated using a simple diagnostic standard such as Thermo Scientific™ Pierce™ Peptide Retention Time Calibration mixture (PRTC), Thermo Scientific™ Pierce™ BSA (bovine serum albumin) Protein Digest Standard, or Thermo Scientific™ Dionex™ Cytochrome C digest.

This document describes the steps to collect and analyze the appropriate data for diagnosing issues with EASY-Spray columns to facilitate in the identification of system contamination, sample quality issues and overall column performance.

Use of diagnostic standards for EASY-Spray columns

1. Diagnostic standards

- Use a diagnostic standard that allows the extraction of a known peptide *m/z*
- The standard used in EASY-Spray quality testing is Thermo Scientific™ Pierce™ Bovine Serum Albumin – Tryptic Digest, MS grade (PN 88341)
- Alternate standards that may be used include Piece PRTC mixture (PN 88320) and Cytochrome C digest (PN 161089)

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2. Instrument Settings

- If using the BSA standard, use the LC-MS Analysis Parameters on the Quality Assurance Report shipped with the column as your reference
- For example, column ES804A LC settings:

Mobile phase A:	100% Water, 0.1% Formic acid	Mobile phase B:	100% Acetonitrile, 0.1% Formic acid
Flow rate:	300 nL/min	Column temperature:	35 °C
Gradient:	5–40% B in 15 min 40–95% B in 5 min 95% B for 5 min	Run time:	25 min
Equilibration:	5% B for 20 min		

- MS settings

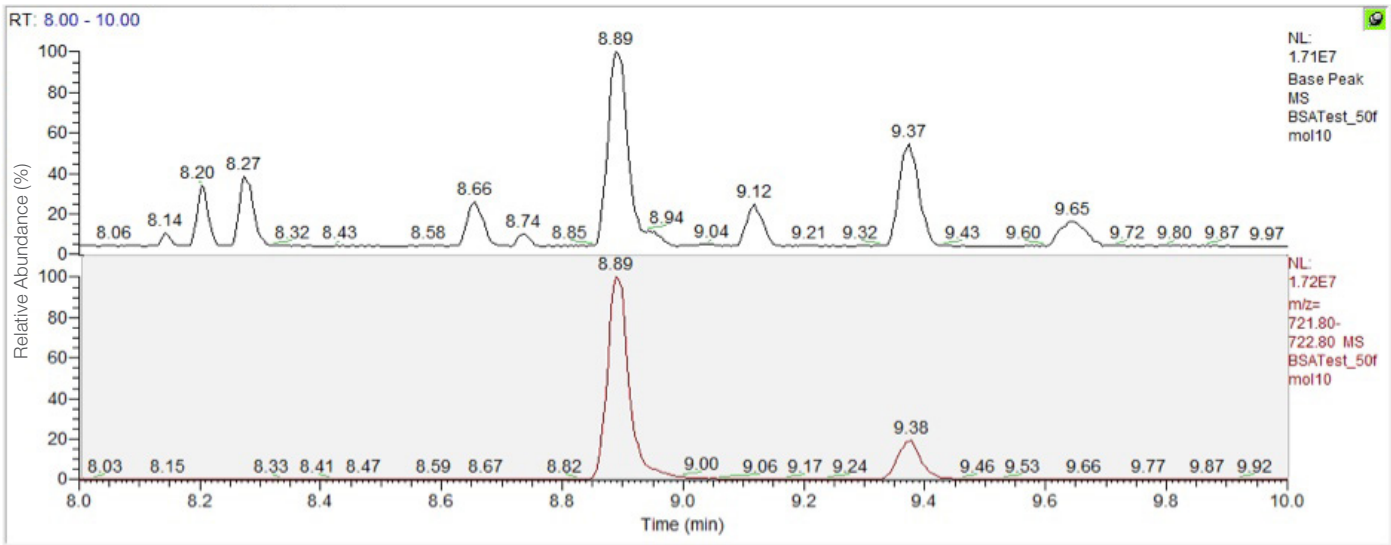
Acquisition range:	400–2000 *m/z*	Spray voltage:	1.9 kV
Scan rate:	Normal	Polarity:	Positive
Scan type:	Full	Data type:	Centroid

3. Review of chromatography

- Are the hydrophilic peptides retaining as well as the hydrophobic peptides?
- What is the peak width and symmetry?
- Are all expected ions present?
- Is the intensity appropriate to amount injected?

Extraction of known peptide

- Extract a known peptide from the chromatogram, for example *m/z* 722.3 in BSA Digest (YIC*DNQDTISSK)
- Evaluate the peak width and symmetry
  Specification for *m/z* 722.3:
  PWHH (sec): 1.5–3.5
  Asymmetry (EP): 0.9–1.7



4. Post-run cleaning gradients

- After the gradient, add three fast “seesaw” cycles of organic to aqueous
- Review the chromatography – If impurities can be seen eluting during the high organic areas of the “seesaw” gradient, the column is contaminated
- Seesaw gradients can be used to clean the column

Related Thermo Scientific products

88320	**Thermo Scientific™ Pierce™ Peptide Retention Time Calibration Mixture**
88341	**Thermo Scientific™ Pierce™ BSA Protein Digest, MS grade**
161089	**Thermo Scientific™ Dionex™ Cytochrome C Digest**

Current versions of product instructions are available at separatedbyexperience.com/chromexpert.

Learn more about EASY-Spray columns at
thermofisher.com/EASYspray

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