APPLICATION BRIEF 21955

Best practices for EASY-Spray columns

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Introduction

This document describes best practices for use of the Thermo Scientific™ EASY-Spray™ column and describes the steps to be taken to confirm that a new column is ready for use. These processes and procedures have been optimized to ensure optimal column performance and lifetime.

Best practices checklist

System readiness

If your LC supports evaluation scripts, run them to verify that the system is ready for use.

Solvents

- Use high-purity freshly made and degassed solvents. Recommended solvents include Fisher Chemical™ Optima™ LC/MS Grade Acetonitrile (P/N A955-500), Fisher Chemical™ Optima™ LC/MS Grade Water (P/N W6500) and Thermo Scientific™ Pierce™ LC-MS Grade Formic Acid (P/N PI128905).
- Use of premixed solvents is not recommended. If premixed solvents have been used on your system, replace the solvent reservoirs and sample loop followed by a full system flush before installing your EASY-Spray column.
- Replace solvents regularly and do not add solvents to solvent bottles already in use.

Positioning

- Use the EASY-Spray emitter positioning tool each time the column is installed to avoid breaking the tip and voltage-induced damage. The minimum distance to the source is 3 mm.
- **Apply a spray voltage of approximately 1800 V, the most common usage setting, to establish spray (recommended usage range is 1200-2500 V).**

Conditioning

- All columns need to be conditioned any time flow is introduced into a column that was at rest. Poor column conditioning will present as sputtering and elevated operating pressure.
- Thermo Scientific™ EASY-nLC™ 1200 UHPLC systems have scripts for column conditioning.
- Manual conditioning should be performed with other LCs, starting with a flow rate of 50 nL/min and increasing stepwise in 2 minute intervals, by 50 nL/min steps, until the maximum column pressure or system pressure is reached.

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Column	Total duration (min)	Max pressure (bar)	No. of steps
ES900	20	500	10
ES901	30	800	15
ES902	40	1000	20
ES903	40	1000	20
ES904	30	800	15
ES905	45	1200	22
ES911	30	800	15
ES912	20	500	10

Column	Equilibration volume (µL)
ES900	3.5
ES901	1.5
ES902	5.5
ES903	11.0
ES904	3.5
ES905	16.5
ES911	3.5
ES912	3.5

Monitor performance

- Qualify new columns using a standard protein digest and a standard gradient.
- Use a diagnostic standard (such as Thermo Scientific™ Pierce™ Peptide Retention Time Calibration mixture, BSA digest, or Cytochrome C digest) that allows the extraction of known peptide *m/z*.
- Save and log chromatograms before and after sample sets to see what changes occurred as a result of exposure to different experiments.

Equilibration

- Column equilibration has a dramatic effect on run to run reproducibility.
- As a guide use 5x column volume, but more may be used as needed.
- The table to the right is a guide to the volume required at the start (or end) of a gradient to equilibrate the column for the next injection.

Related Thermo Scientific products

Part Number	Description
ES232	Thermo Scientific™ EASY-Spray™ Emitter Positioning Tool
A955-500	Acetonitrile, Optima LC/MS Grade, Fisher Chemical
W6500	Water, Optima LC/MS Grade, Fisher Chemical
PI128905	Thermo Scientific™ Pierce™ LC-MS Grade Formic Acid
MB124-X	Thermo Scientific™ ChromaCare™, LC-MS Biologics Flush Solution, 45% 2-Propanol: 45% Acetonitrile: 10% Acetone
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.

Find out more at **thermofisher.com/EASYspray**

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