

# APPLICATIONS

## Column Reproducibility and Robustness using the bioZen™ Nano 2.6 µm XB-C18 LC Column

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### Overview

Column to column and batch to batch reproducibility is an important performance attribute in nano LC. Without reproducible columns, it could become necessary to repeat all of the injections in the batch of runs in order for retention times to be compared if a single column fails. Furthermore, robust columns that are able to perform hundreds of injections without any degradation in chromatography is also of key importance. In this application we show reproducible results across multiple columns as well as the robust ability of the bioZen 2.6 µm XB-C18 column after 100 injections.

### MS/MS Conditions

**Ion Source:** Positive  
**CUR:** 30  
**CAD:** Low  
**GS1:** 35  
**GS2:** 0  
**IS:** 2500  
**IHT:** 150  
**DP:** 80  
**EP:** 10  
**CXP:** 15

### LC-MS/MS Conditions

**Column:** bioZen 2.6 µm XB-C18

**Dimension:** 150 x 0.075 mm

**Part No.:** [00F-4768-AW-21](#)

**Pressure (bar):** 200 bar

**Mobile Phase:** A: 0.1 % Formic Acid in Water  
B: 0.1 % Formic Acid in Acetonitrile

Gradient:	Time (min)	%A	%B
	0	97	3
	15	70	30
	18	20	80
	24	20	80
	25	97	3
	35	97	3

**Flow Rate:** 350 µL/min

**Temperature:** Ambient

**LC System:** NanoLC™ 425 (SCIEX®)

**Detection:** MS/MS

**Detector:** 6500 QTRAP® (SCIEX)

**Injection Volume:** 1 µL

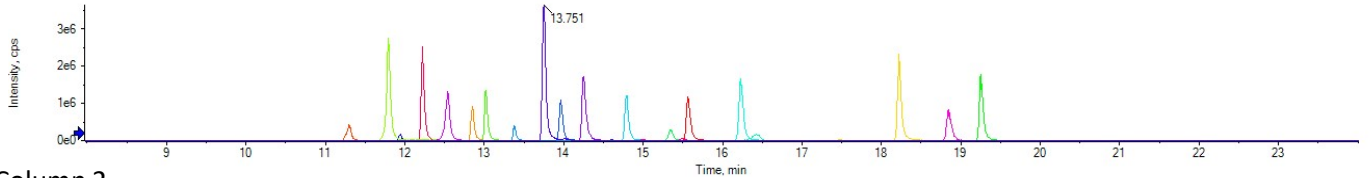
### MRM Transitions

ID	Q1 M/Z	Q3 M/Z	CE
PEPCALMIX.AETSELHTSLK.+3Y5.HEAVY	408.55	593.35	24.3
PEPCALMIX.AGLIVAEGVTK.+2Y7.HEAVY	533.32	711.41	26.5
PEPCALMIX.ALENDIGVPSDATVK.+2Y7.HEAVY	768.9	725.39	35.6
PEPCALMIX.AVGANPEQLTR.+2Y6.HEAVY	583.31	753.41	31.5
PEPCALMIX.AVIFYAPQIPLYANK.+2Y6.HEAVY	883.47	713.41	47.9
PEPCALMIX.DGTFAVDGPGVIAK.+2Y7.HEAVY	677.86	649.41	33.6
PEPCALMIX.GAYVEVTAK.+2Y5.HEAVY	473.26	555.32	23.9
PEPCALMIX.GFTAYYIPR.+2Y5.HEAVY	549.29	721.39	26.9
PEPCALMIX.IGNEQGVSR.+2Y8.HEAVY	485.25	856.41	26.7
PEPCALMIX.LDSTSIPVAK.+2Y4.HEAVY	519.8	422.29	34
PEPCALMIX.LGLDFDSFR.+2Y6.HEAVY	540.27	796.35	26.7
PEPCALMIX.LVGTPEAER.+2Y7.HEAVY	491.27	769.37	23.4
PEPCALMIX.SAEGLDASASLR.+2Y7.HEAVY	593.8	729.38	30
PEPCALMIX.SGGLLWQLVR.+2Y5.HEAVY	569.83	711.42	29.6
PEPCALMIX.SPYVITGPGVVEYK.+2Y9.HEAVY	758.91	957.51	37.8
PEPCALMIX.TVESLFPPEAETPGSAVR.+2Y6.HEAVY	964.98	596.34	42.4
PEPCALMIX.VFTPLEVDVAK.+2Y8.HEAVY	613.35	878.51	26.3
PEPCALMIX.VGNEIQYVALR.+2Y6.HEAVY	636.35	759.44	33.5
PEPCALMIX.YDSINNTEVSGIR.+2Y9.HEAVY	739.36	999.51	40.3
PEPCALMIX.YIELAPGVDNSK.+2Y7.HEAVY	657.34	724.37	29

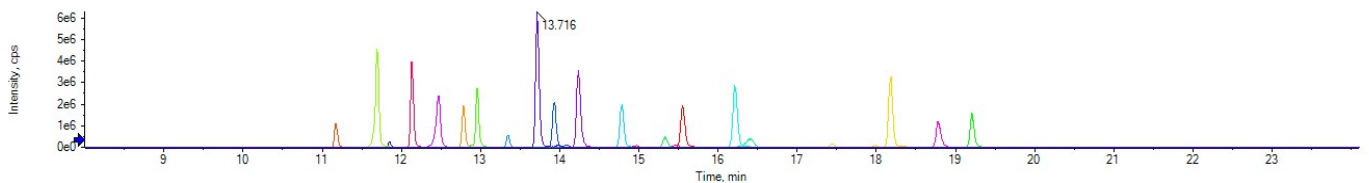
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**Figure 1.** Extracted ion chromatograms from a mixture of 20 isotopically labeled peptides run on 3 different bioZen™ nano 2.6 μm XB-C18 150 x 0.075 mm columns.

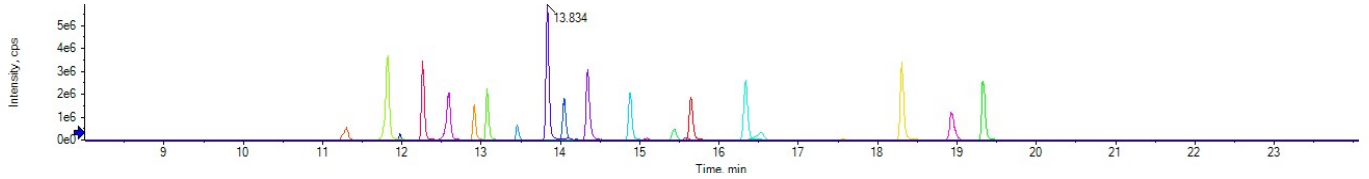
Column 1



Column 2



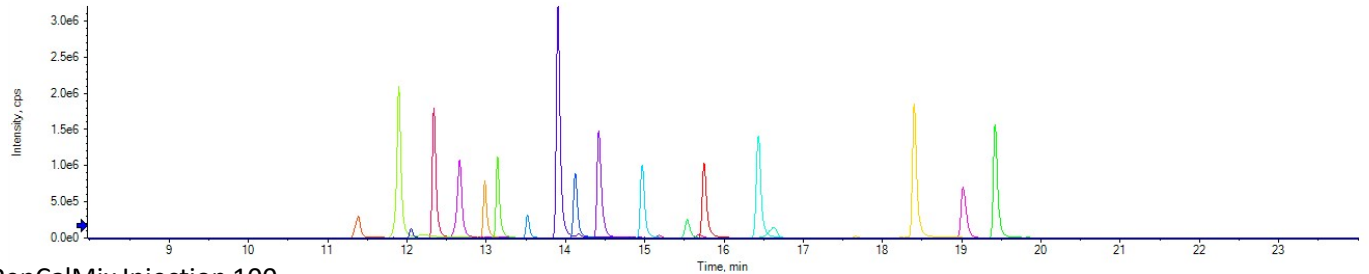
Column 3



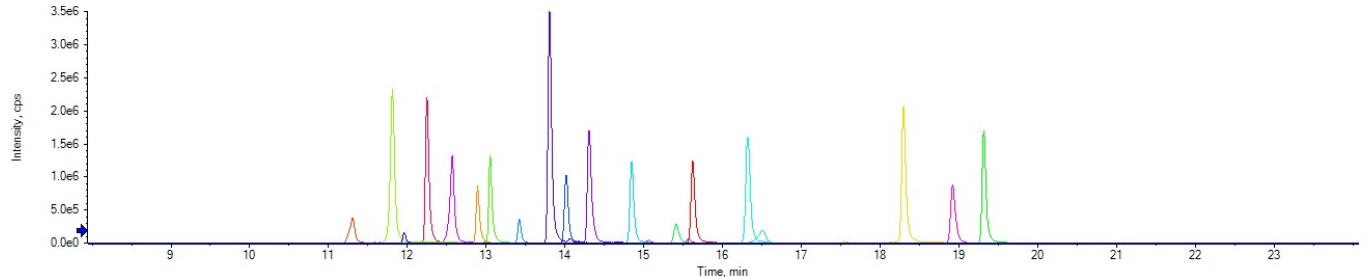
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**Figure 2.** Extracted ion chromatograms of injection 1 and 100 from a mixture of 20 isotopically labeled peptides run on a bioZen nano 2.6 μm XB-C18 150 x 0.075 mm column.

PepCalMix Injection 1



PepCalMix Injection 100



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