CESI-MS performance check and MS calibration using PepCalMix standard for silica surface OptiMS cartridges

Featuring PepCalMix standard, CESI 8000 Plus ESI-MS System coupled to TripleTOF® 6600+ LC-MS/MS System

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Introduction

CE-MS methodology provides flexibility in addressing new and challenging applications that sometimes cannot be accomplished by LCMS. It also provides an orthogonal solution to LC-MS applications. The OptiMS cartridge for the CESI 8000 Plus system is directly connected to the mass spectrometer without additional fluidic connections. Instead of having multiple methods to check performance for each feature, it is desirable to have one single analysis with a single set of standards. A group of standards can monitor the successful CESI-MS integration, performance of the CESI-OptiMS cartridge and mass spectrometer.

The system suitability test is an essential check of the CESI-MS specifications; monitoring of the cartridges' separation performance, optimizing CESI tip position, and calibrating the mass spectrometer on MS and MS/MS level.

Here, we demonstrated using PepCalMix standards (a mixture of peptides) to monitor the silica surface OptiMS cartridge's performance. We showed excellent repeatability of the PepCalMix measure on the same cartridge, across different batches of cartridges, and day-to-day measurements, totaling >100 sample injections across three random BFS cartridges.

CESI 8000 Plus coupled with Triple TOF® 6600+ system and PepCalMix standard

Furthermore, to ensure good mass spectrometer performance, the PepCalMix was used for automatic calibration between sample measurements.

Key features of CESI-MS performance check

- A single system integration test with the PepCalMix standard to evaluate the CESI and MS performances
- The PepCalMix standard is robust and stable over time
- The cartridge performance can be monitored with two pairs of closely migrating peptides
- The PepCalMix standard can be used for both manual and automatic calibration of the MS

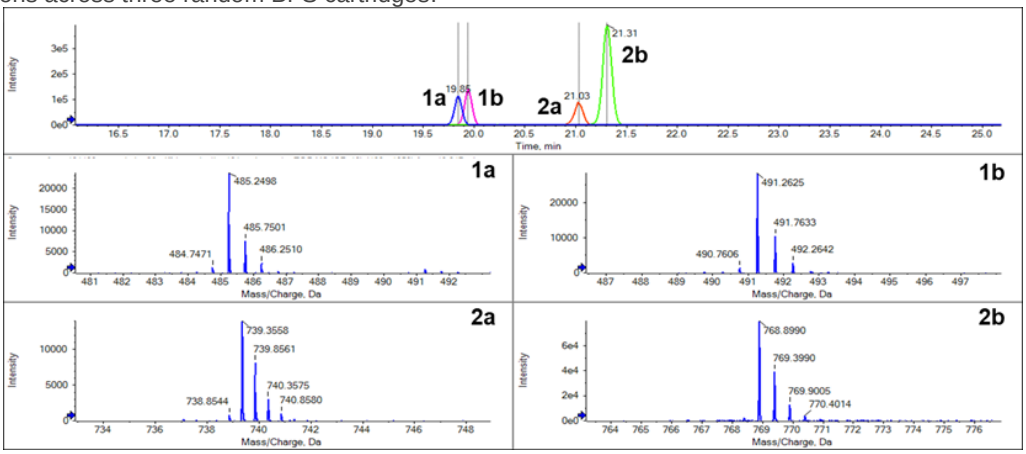

Figure 1. Electropherogram showing separation and m/z ions monitored in two key peptide pairs (blue/pink and orange/green). a) Separation of two pairs (1a/1b and 2a/2b; blue/pink and orange/green) of key peptides shown in XIC; m/z observed for each peptide are shown and marked as 1a, 1b, 2a, and 2b.

Materials and Methods

Sample preparation: The PepCalMix (SCIEX, P/N 5045759) stock solution contains one pmol/µL of each of the 20 different stable isotope-labeled peptides. The stock solution was aliquoted into 5 µL and stored at -20°C.[1] For the manual MS and MS/MS calibration via CESI direct infusion, the PepCalMix (SCIEX, P/N 5045759) was diluted 1:1 (v:v) with 10% acetic acid (background electrolyte solution, BGE). For the separation analysis and auto-calibration, the PepCalMix was 10-fold diluted with leading electrolyte (LE; 100 mM ammonium acetate, pH 4). Before analysis, every sample was vortexed and centrifuged for 5 min at 12 000 g to remove any possible precipitant.

Table 1. CESI conditions were used to separate the PepCalMix.

	Event	Time (min)	Pressure (psi)	Voltage (kV)	Direction	Solution/Note
1	Rinse	2.5	100		Forward	0.1 M NaOH
2	Rinse	2.5	100		Forward	0.1 M HCl
3	Rinse	4	100		Forward	water
4	Rinse	3	75		Reverse	10% acetic acid
5	Rinse	4	100		Forward	10% acetic acid
6	Injection	60 s	5		Forward	sample
7	Injection	25 s	0.5		Forward	10% acetic acid
8	Separation	30	0	20	Forward	10% acetic acid
9	Separation	5	0	1	Forward	ramp down

Instrument configuration was as follows, the CESI 8000 Plus system with the silica surface OptiMS cartridge (SCIEX, P/N B07367) was coupled with the TripleTOF 6600+ System through a NanoSpray® III source and a CESI adapter (SCIEX, P/N B07363). 32 Karat™ and Analyst® TF Software 1.8.1 Software were used to operate the CESI-MS.

Capillary electrophoresis and mass spectrometry: *CESI separation method parameters*: The separation conditions are listed in Table 1. Sample was injected hydrodynamically at 5 psi for 60 s, resulting in approximately 44 nL injected volume, which is about 2.2 fmol of each peptide on the cartridge. The BGE (10% acetic acid) was prepared freshly on the analysis day.

Direct infusion of the PepCalMix: The standard was infused through the cartridge using 5 psi to optimize the CESI sprayer position, ion spray voltage, and to preform TOF MS and MS/MS manual calibration.[2] For the fine-tuning of the CESI tip sprayer position, 20 kV voltage and 0.5 psi pressure on both cartridge ends were applied. The PepCalMix references ions for mass calibration are listed in the SWATH acquisition performance protocol[1] (Table 2-3 and Table 2-4, page 16-17).

MS method parameters: The PepCalMix was measured using a TOF MS acquisition method with a looped MS/MS scan. The acquisition TOF MS mass range was from 400 to 1250 m/z, with a cycle time of 0.8 s. Method details are listed in Table 2.

Data processing: SCIEX OS Software was used to visualize and analyze measured data.

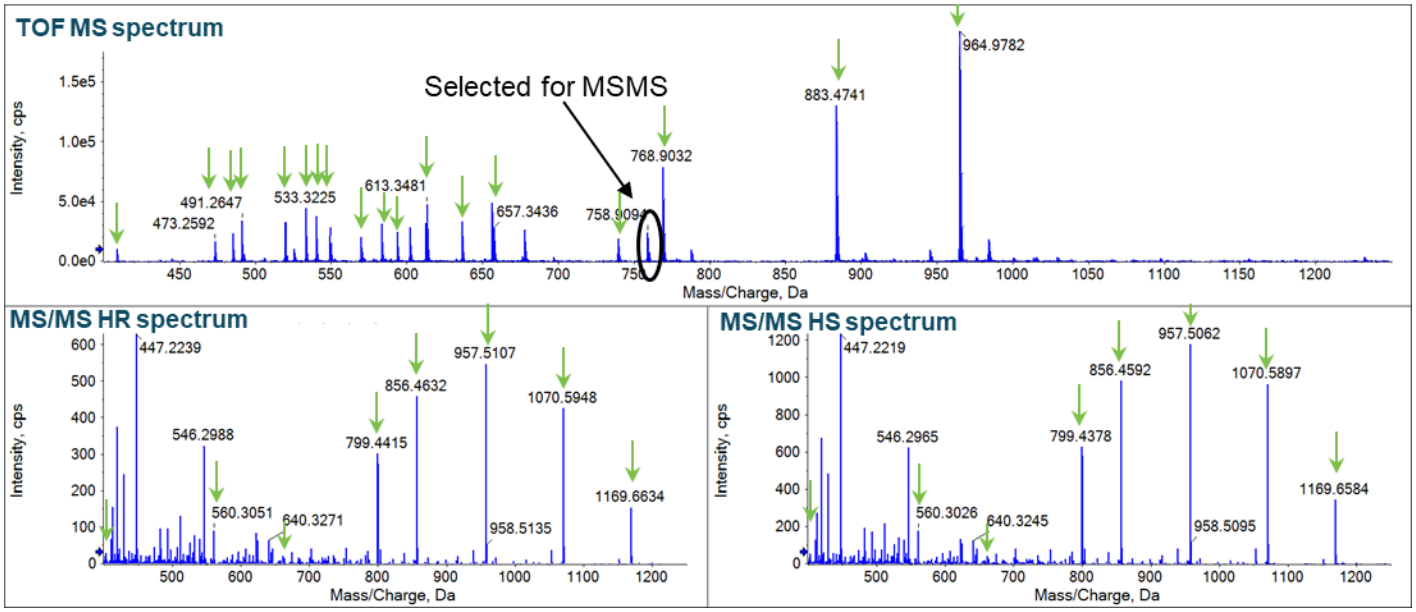

Figure 2. Example spectrum from silica surface OptiMS cartridge infusion. a) Example Data for TOF-MS Spectrum; b) Example Data for TOF-MS/MS Spectrum for Peptide m/z 758.91 in high-resolution (HR) and high sensitivity (HS) mode. Arrows highlight the m/z values corresponding to the peptides in the mixture and values used for TOF-MS and MS/MS calibration.

Table 2. Summary of mass spectrometry parameters.

Parameter	Value
MS mass range	400-1 250 m/z
MS accumulation time	250 ms
MS/MS mass range	150-2 250 m/z
MS/MS accumulation time	500 ms
Curtain gas:	5 psi
Polarity:	positive
Source temperature:	50 °C
Collision energy (CE)	42 V
CE spread	± 5 V
Ion spray voltage	1 850 V*

***Value is tuned during direct infusion of the PepCalMix**.

Results and Discussions

When the peptide solution in BGE is infused through the separation capillary, a spray variation should be less than 40% within 2-5 minutes time frame. The example data for the infusion TOF MS spectrum from the silica surface OptiMS cartridge, and for the MS/MS high-resolution and MS/MS high-sensitivity spectrum for peptide m/z 758.91 are shown in Figure 2. The peptides used for the calibration of MS and MS/MS are highlighted with arrows (Figure 2). Peptide standard infusion is an easy and quick way to perform manual MS and MS/MS tuning and calibration.

In addition to direct infusion, the peptide standards can be diluted into 100 mM leading electrolyte (LE) solution and use 10% acetic acid in water as BGE to perform the high-resolution separation. The separation method utilizes the tITP (transient Isotachophoresis) technique to concentrate and separate peptides in the mixture. These peptides are scanned with TOF MS, and peptide 758.91 m/z is selected for further MS/MS fragmentation. Overlay of peptides extracted ion chromatogram (XIC) from three consecutive injections is shown in Figure 3.

The migration time of peptides can be different when multiple cartridges are used. They may also shift when the sample and BGE used for other injections are different from those used for the peptide standard separation. Nevertheless, the relative separation of the key peptides should not be affected (Figure 4 and Figure 5).

An extensive evaluation of the PepCalMix and silica surface cartridges was executed. Overall, >100 measurements were conducted to cover the inter-day, intra-day, and cartridge lot-lot performance. The RTs of 20 standard peptides remained consistent among the entire study.

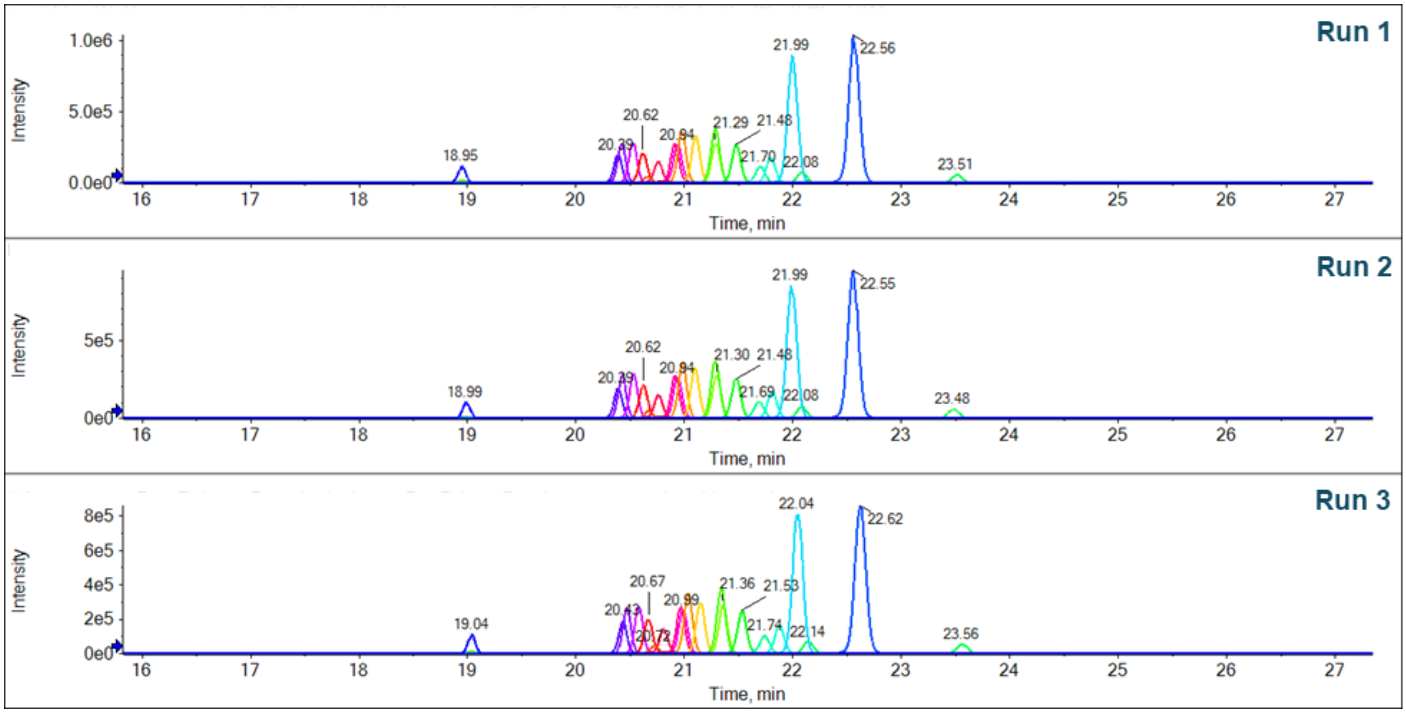

Figure 3. Example of inter-day cartridge separation repeatability. Overlay of three injections of CESI-MS separation results of the PepCalMix on a single silica surface OptiMS cartridges.

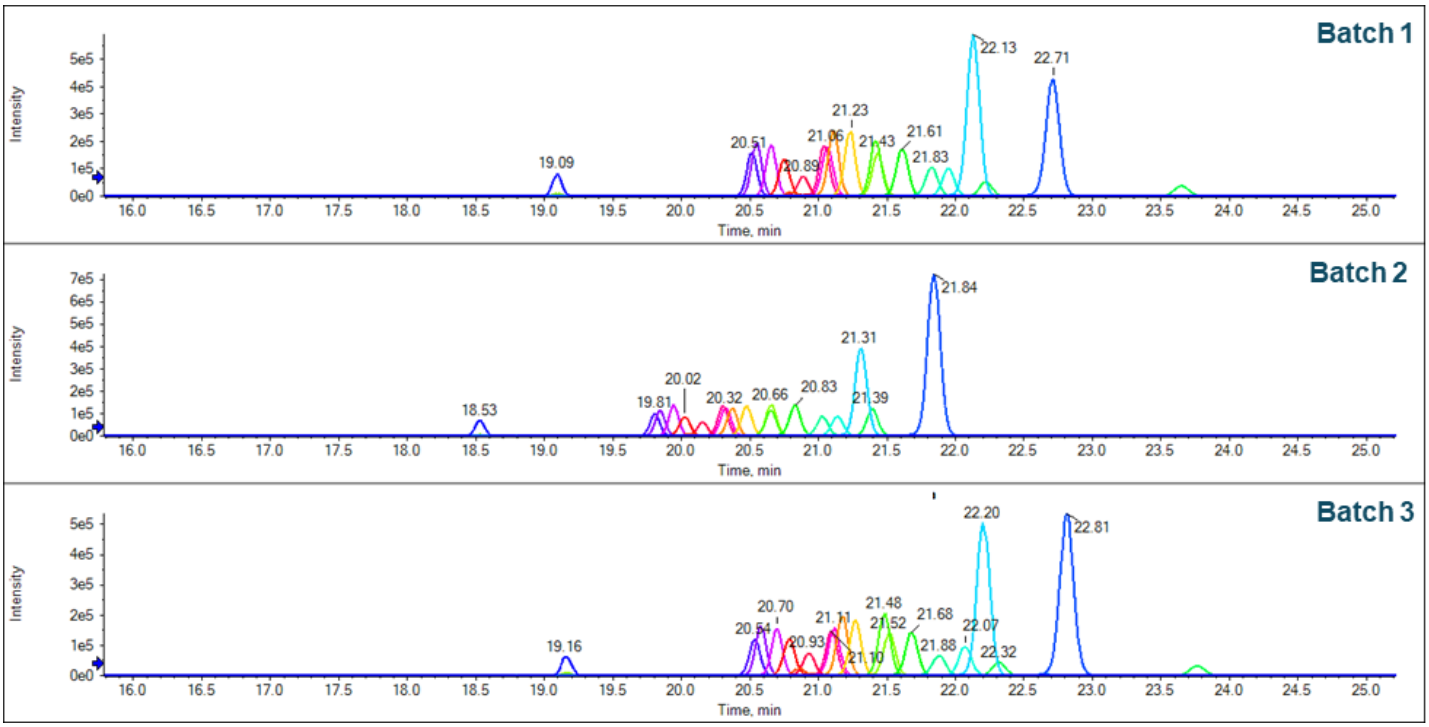

Figure 4. Example of batch-to-batch cartridge separation repeatability. Overlay of three injections of CESI-MS separation results of the PepCalMix on three different batches of silica surface OptiMS cartridge**.**

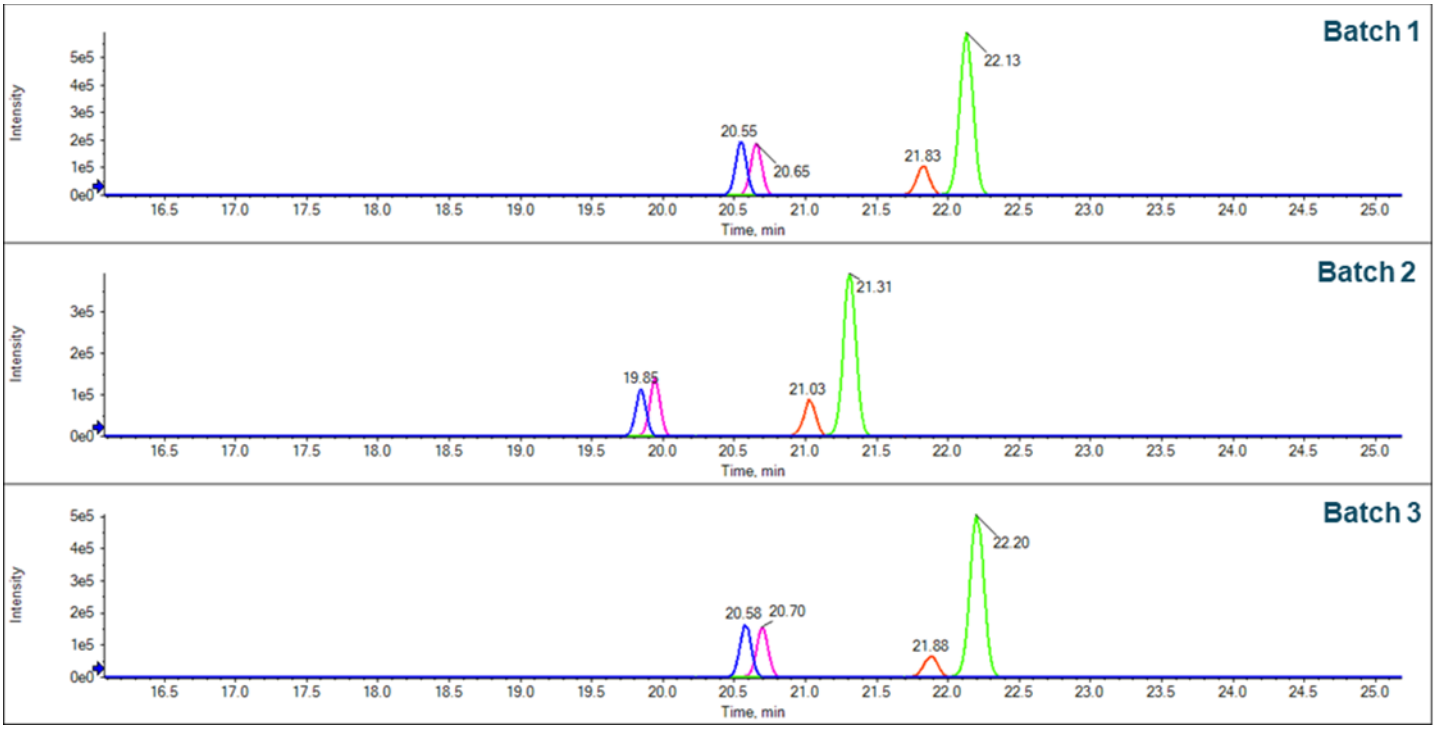

Figure 5. Separation of the two pair selected key peptides of three CESI-MS separation injections on three different batches of silica surface OptiMS cartridges. XIC of four key peptides with difference in the migration time for the corresponding pair (blue/pink and orange/green) is more or equal to 0.08 and 0.25 minutes, respectively.

peptides remained consistent among the entire study.

The RT variation of performance was on average the inter-day 1.8% ±0.12 per cartridge, the intra-day 3.1% ±0.21 and an cartridge lot-lot variation was 3.9% ±0.15 for three cartridges. The intra-day RT variation is higher compare to inter-day due to other complex samples were measured between the PepCalMix which influence the RT shift.

The separation of two pair peptides is selected as an indicator of the silica surface OptiMS cartridge performance (Figure 1). The first pair migrates at the beginning of the entire peptide train, while the second pair migrates towards the end. The efficiency of cartridge performance can be gauged by identifying and clearing separation between the two sets of peptides, 485.25 m/z (blue trace) with 491.25 m/z (pink trace) and 739.35 m/z (orange trace)with 768.90 m/z (green trace), Figure 1 and 5. The difference in the migration time between each peak in the corresponding pair should be more significant than 0.08 minutes and 0.25 minutes, respectively (Figures 1 and 5).

Conclusions

This technote introduces an approach for performing both calibrations of TOF MS and MS/MS and gauges the silica surface OptiMS cartridge's performance. This type of calibration approach can be performed easily over a wide mass range via direct infusion or separation with the silica surface OptiMS cartridge. The performance of the silica surface OptiMS cartridge is gauged by the relative separation between two pairs of peptides of similar migration times.

References

1. SWATH acquisition performance protocol, Protocol, RUO-IDV-05-3363-A May 2016 https://sciex.com/Documents/protocols/SWATH_Acquisition_Performance_Kit_Protocol.pdf

2. CESI 8000 High Performance Separation System, User manual, RUO-IDV-05-3897-C March 2020 https://sciex.com/Documents/manuals/cesi8000_uManual_B11949AD.pdf

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