VERITY® 271 LCMS System: Mass-based Peptide Purification

TECHNICAL NOTE (TN230)

TECHNICAL FEATURES
- The ability to collect targeted fractions using MS signals

TECHNICAL BENEFITS
- Improve information on collected fractions
- Never miss targeted compounds
- Reduce number of fractions thus re-analysis time

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INTRODUCTION

Preparative LC coupled with mass spectrometry (MS) is a well-established method for purifying target compounds. The VERITY® LCMS system (Figure 1) leverages the separating power of HPLC with the detection capabilities of MS to isolate fractions with high purity and achieve excellent recovery for target molecules. The system incorporates signal data from both ultraviolet (UV) and mass-based detectors.

The VERITY® 1920 MS Detector is a single quadrupole MS with a mass range (*m/z*) from 10 to 2000. It provides both electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) capabilities with positive or negative polarity. When integrated into Gilson VERITY Purification systems, the VERITY 1920 MS is an essential tool to selectively purify active ingredients from small to large molecules, and natural products from lab to pilot scale. VERITY Purification systems are scalable depending on throughput.

This technical note demonstrates the use of a VERITY 271 LCMS system to perform mass-based purification of target peptides from a mixture. The system is controlled using TRILUTION® LC Software,[1] which provides real-time and post-run MS and UV data.

The method takes advantage of the software's powerful conditional fraction collection feature, which can utilize UV signal and up to five MS channels (full scan or selected ion monitoring) simultaneously.

MATERIALS & METHODS

Materials

All chemicals were ACS grade quality. HPLC peptide standard mixture containing five peptides (Sigma-Aldrich #H2016)[2] was dissolved in water to a final concentration of 2.5 mg total peptide per milliliter. Table 1 lists the peptides in the mixture and the associated molecular weight.

Figure 1
VERITY® LCMS System

Table 1
HPLC Peptide Standard Mixture

Peptide	Molecular Weight (g/mol)	Expected Molecular Ions (*m/z*)
Angiotensin II	1046.2*	1047.2 524.1*
Gly-Tyr	238.2	239.2
Leucine enkephalin	555.6	556.6
Methionine enkephalin	573.7	574.7
Val-Tyr-Val	379.5	380.5

*Angiotensin II fragments during ionization into doubly charged 523.1 and singly charged 1046.2

Software and Hardware

- TRILUTION LC v4
- VERITY 271 LCMS System
  - GX-271 Liquid Handler
  - GX Prep Solvent System
  - GX Direct Injection Module (1/16”, 5 mL loop)
  - 333 HPLC Pump
  - 334 HPLC Pump
  - VERITY 1741 UV-VIS Detector (0.5 mm flow cell)
  - 506C Interface Module
  - VERITY 1920 MS Detector
- Phenomenex® Luna® Preparative HPLC column (50 x 21.2 mm, 5µm, product code 00B-4252-P0-AX) with guard column

Run Parameters

The run parameters are shown in Table 2. One data channel of the VERITY 1741 Detector was utilized (set to 220 nm). The VERITY 1920 MS was operated in positive mode and data were collected on five channels, one scan channel (200 – 1100 *m/z*) to display a trace of the total ion count (TIC) and four selected ion monitoring (SIM) channels.

Objective

The objective of the purification run is to isolate peptides using targeted mass-based fraction collection. Run parameters were set to use Conditional Fraction Collection to collect four of the five peptides in the HPLC Peptide Standard Mixture, demonstrating the ability to selectively collect only the peptides of interest from a mixture.

Table 2
Run Parameters

Parameters	Conditions
Mobile Phase	Solvent A: Water Solvent B: Methanol Make Up Solvent: Methanol with 0.1% Formic Acid
Gradient Conditions	0 min: 5% B 2 min: 5% B 19.5 min: 40% B 20.5 min: 40% B 22.7 min: 50% B 26.15 min: 50% B 28.1 min: 5% B 30 min: 5% B
Flow Rate	15 mL/min
Injection Volume	500 µL
UV Detection	Channel 1: 220 nm Data rate: 20 pt/sec
Mass Detection	Channel 1: Scan Channel 2: SIM1, Angiotensin II Channel 3: SIM2, Leu enkephalin Channel 4: SIM3, Met enkephalin Channel 5: SIM4, Gly-Tyr Data rate: 5 pt/sec
Fraction Collection Conditions	UV signal at 220 nm, slope >= 25 (front and back slope), peak width 0.2 AND one of the following: MS Detector SIM1 Level >= 1500000 TIC MS Detector SIM2 Level >= 1500000 TIC MS Detector SIM3 Level >= 1500000 TIC MS Detector SIM4 Level >= 1500000 TIC

This purification run also illustrates the benefit of including a MS detector in the system to identify co-eluting compounds and to confirm compound identity using mass spectral data conditions. Run parameters were set to use gradient conditions such that two of the five peptides co-elute. When peaks co-elute, the MS data is used to confirm the presence of more than one compound in a single UV peak.

RESULTS AND DISCUSSION

This technical note demonstrates the use of the VERITY 271 LCMS system, featuring a VERITY 1920 MS detector, for mass-based peptide purification using a mixture of five peptides. The chromatogram (Figure 2) shows the mobile phase gradient (dark red) as well as the UV signal (dark blue), and mass scan from 200 – 1100 m/z (dark green). In addition, the signals from four SIM channels are plotted (orange, light green, aqua, and red). The run parameters were selected to illustrate key features of the instrumentation and software.

First, LC gradient conditions were selected that separated the five peptides into four peaks distinguishable by UV. Two of the peptides, angiotensin II and leucine enkephalin, co-elute at approximately 24 minutes as shown in the chromatogram in Figures 2 and 3. Whereas the UV trace shows one peak (blue trace in Figures 2 and 3), the SIM traces from the MS detector show that two components are present (aqua and orange traces in Figure 2).

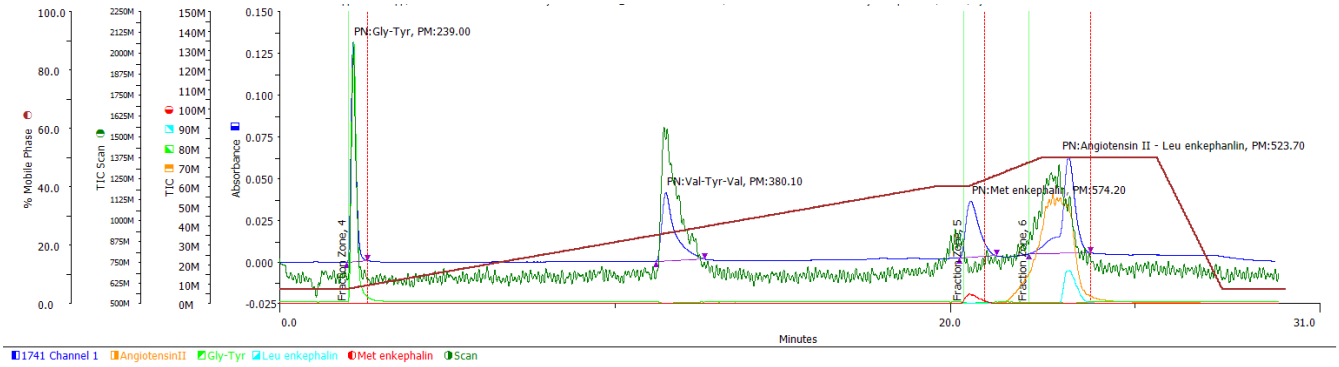

Figure 2
Separation and conditional fraction collection of peptide mixture (UV and MS traces)

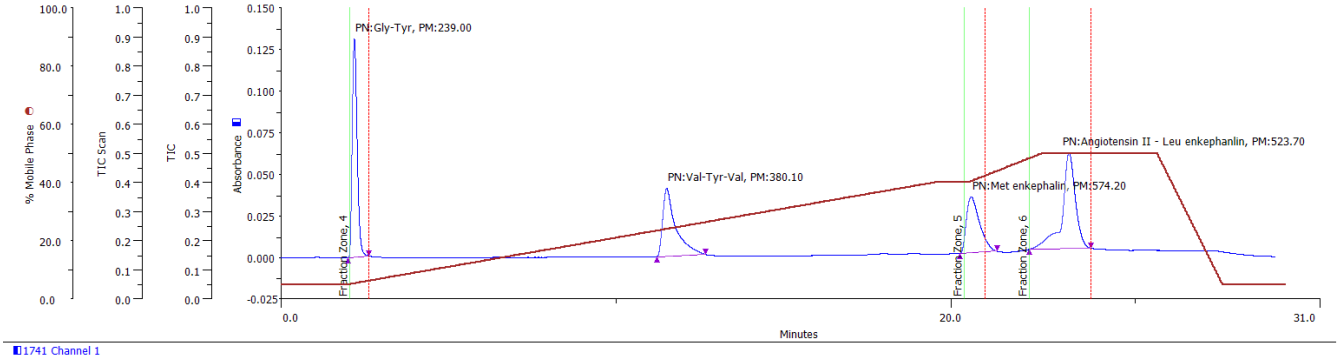

Figure 3
Separation and conditional fraction collection of peptide mixture (UV trace only)

The mass spectral data of the peak from the MS scan channel confirms the presence of the two components (Figure 4). In addition, the MS results in Figure 4 show that angiotensin II is fragmented during ionization and the abundance of the doubly charged ion (523.1) is greater than the abundance of the singly charged ion (1046.2). These data illustrate the benefit of having an MS detector in the system to identify peaks containing co-eluting compounds and to confirm compound identity using mass spectral data.

Second, to illustrate the conditional fraction collection capabilities of the VERITY 271 LCMS system, SIM channels were assigned to four out of five of the peptides (Table 2), and then the logic was set to collect fractions only when the UV signal condition was met and any of the SIM channel signals (tracking individual masses) was met. As shown in Figure 2, three fractions were collected corresponding to four of the peptides: Gly-Tyr (Fraction Zone, 4), methionine enkephalin (Fraction Zone, 5), and co-eluted angiotensin II/ leucine enkephalin (Fraction Zone, 6). See Figure 5 for mass spectral data from the collected fractions, demonstrating how the system can be used for run time confirmation of fractions. As expected, although the Val-Tyr-Val peak can be clearly seen in the UV trace and the MS full scan trace, it was not collected because it did not have an associated SIM channel and thus did not meet the conditions set in the Conditional Fraction Collection task.

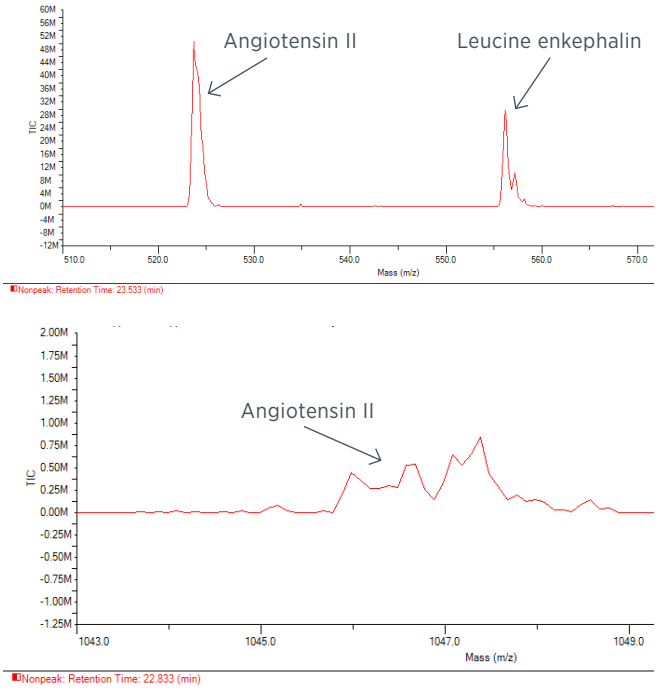

Figure 4
MS spectral data of peak at 23.234 min. Top panel: leucine enkephalin (MW: 555.6) and angiotensin II (doubly charged: 523.1). Bottom panel: angiotensin II (MW: 1046.2).

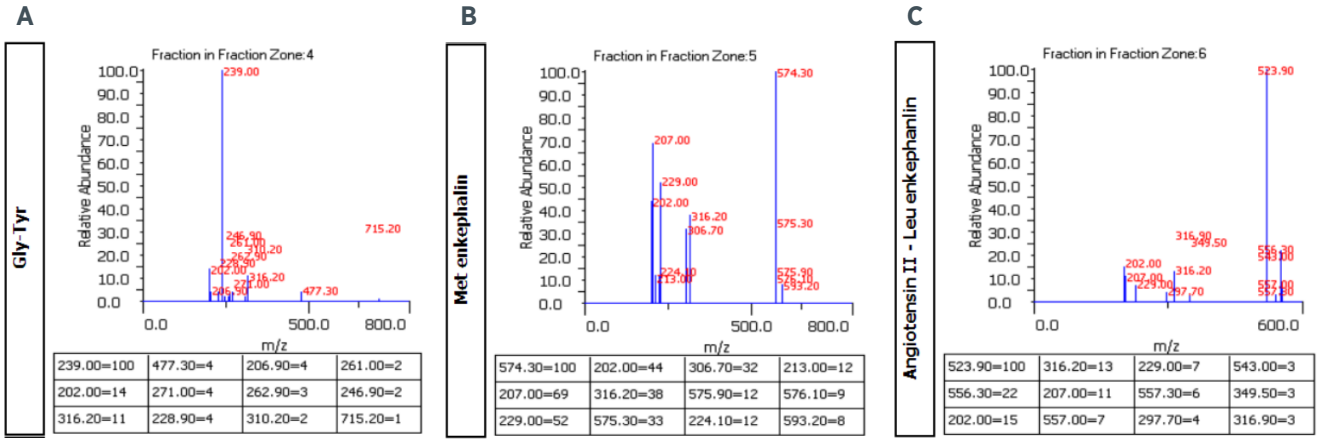

Figure 5
Mass spectra of the three fractions collected

When controlled by TRILUTION LC, the VERITY 1920 can support up to five MS channels, which can be scanned (for a range of masses) or SIM (for a target mass). Conditional fraction collection allows the user to select conditions that must be met before fractions are collected (Figure 6). A UV channel is usually selected as the primary channel, with additional parameters to collect either by slope (slope and peak width of the peak) or by the absorbance level. The MS data channel(s) are then set as secondary conditions. Logical operators including AND, OR, and AND/OR allow flexibility for which conditions are used to trigger fraction collection. In this technical note, the conditions were set to AND/OR, meaning that the UV condition plus any one of the mass conditions must be met in order for fraction collection to begin.

In conclusion, the VERITY 271 LCMS system featuring the VERITY 1920 MS Detector was successfully used to perform a mass-directed purification of peptides. TRILUTION LC v4 controlled all components of the system to perform an injection with fraction collection and collect data from the VERITY 1741 UV-VIS Detector and the VERITY 1920 MS Detector.

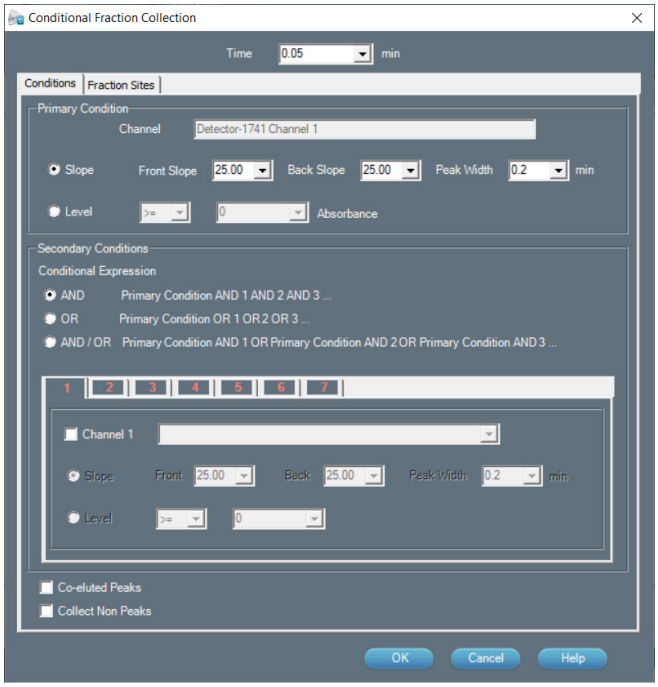

Figure 6
Screenshot from TRILUTION LC v4 showing the Conditional Fraction Collection dialog box.

TRILUTION LC allows real time display of fraction mass spectra during a run, and the post-run analysis report includes peak mass spectra from all analyzed peaks. Two peptides (angiotensin II and leucine enkephalin) eluted at similar retention times, which resulted in a single peak on the UV channel. With the MS data available (scan and SIMs), the presence of both peptides within the single UV peak was confirmed, which demonstrates the advantage of using a VERITY 1920 MS Detector in addition to traditional UV detection.

CONCLUSIONS AND BENEFITS

- The VERITY LCMS system combines Preparative HPLC and MS to achieve mass-directed purification (10 – 2000 m/z) of compounds of interest from a mixture.
- Real-time and post-run UV and MS spectra data allow the user to identify co-eluting compounds.
- Conditional fraction collection allows the user to save time by collecting just the target fractions, which means fewer fractions to reconstitute and analyze (saving time and money).

REFERENCES

1. TRILUTION LC software specifications. https://www.gilson.com/pub/media/docs/TRILUTIONLC_%20SPEC_LT370140-01.pdf
2. Product information for Sigma HPLC peptide standard mixture H2016. https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma-Aldrich/Product_Information_Sheet/1/h2016pis.pdf

ORDERING INFORMATION

Description	PN
GX-271 Prep LH, With Z Drive	2614107
GX Prep Solvent System	261350
GX Direct Injection Module, 1/16, Prep	261354
VERITY 1920 MS Detector (110V) VERITY 1920 ION SOURCE – ESI VERITY 1920 ACTIVE SPLITTER KIT	18105000 18005000 18005005
VERITY 1741 UV-VIS Detector	14161003
333-H3 Pump, Primary Solvent	38103331
334-H3 Pump, Secondary Solvent	38103341
TRILUTION LC Software License	21063134