VERITY® Compact LCMS System: Mass-based Peptide Purification

TECHNICAL NOTE TN220

GILSON APPLICATIONS LABORATORIES

INTRODUCTION

Liquid chromatography coupled with mass spectrometry (LCMS) is widely used in both analytical and preparative chemistry applications. In this process, compounds in a complex mixture are separated based on their size or chemical properties using LC, and the mass signal from the MS detector is used to confirm compound identity and trigger fraction collection. This mass-directed fraction collection approach allows the end user to collect fewer fractions, as compared to collecting based on UV signal alone, saving time and reducing the number of fractions that need to be dried down and processed in downstream steps of the workflow.

The VERITY Compact LCMS system[1] (Figure 1) provides efficient and reproducible sample purification. This system features a PLC Purification System and a VERITY® 1910 MS Detector controlled by Gilson Glider Prep (GGP) Software. The VERITY 1910 MS Detector is a single quadrupole mass spectrometer that has a mass range of 50 to 1400 *m/z*, making it ideal for detection of peptides and small molecules. Ionization with the nano-flow electrospray ionization (ESI) ion source can be set to positive or negative mode. The chip-based technology of the VERITY 1910 MS Detector includes the spraychip® ionization source and vac-chip™, which are customer replaceable, making the system easy to clean and maintain. The MiDas™ module, which functions as the makeup pump and splitter, rests on top of the mass detector to save bench space. Low nitrogen consumption leads to a low flow rate, which allows the system to run with a gas cylinder and does not require an external pump or expensive plumbing of a dedicated N_2 line.

The VERITY 1910 MS Detector has scan modes including full scan, selected ion monitoring (SIM), or full scan and SIM simultaneously. When operated with the PLC Purification System and GGP Software, the VERITY 1910 MS Detector can employ full scan (TIC: Total Ion Current Chromatogram) and up to three target masses (XIC: eXtracted Ion Chromatogram) in a run. Fraction collection settings include conditional settings for AND, OR, and AND/OR logic. This technical note describes LCMS purification of selected compounds from a mixture of five peptides, using conditional fraction collection logic to collect only the peaks that met the criteria set for both UV signal and MS signal.

Figure 1
VERITY® Compact LCMS System

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MATERIALS & METHODS

Materials

All chemicals were ACS-grade quality. HPLC peptide standard mixture containing five peptides (Sigma-Aldrich #H2016)[2] was dissolved in water + 0.1% formic acid to a final concentration of 2.5 mg total peptide per mL and filtered through a 0.45 µm filter. Table 1 lists the peptides in the mixture, their molecular weight, and the associated molecular ions produced in positive mode using methanol + 0.1% formic acid as makeup solvent.

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

- Gilson Glider Prep (GGP) Software
- VERITY Compact LCMS System
  - PLC 2250 Purification System with DAD 200-400 nm
  - Sample Loop 500 µL
  - VERITY 1910 MS Detector with MiDas
- Dr. Maisch ReproSil 100 C18 column (250x10 mm, 5µm, part number r15.96.s2510)

Run Parameters

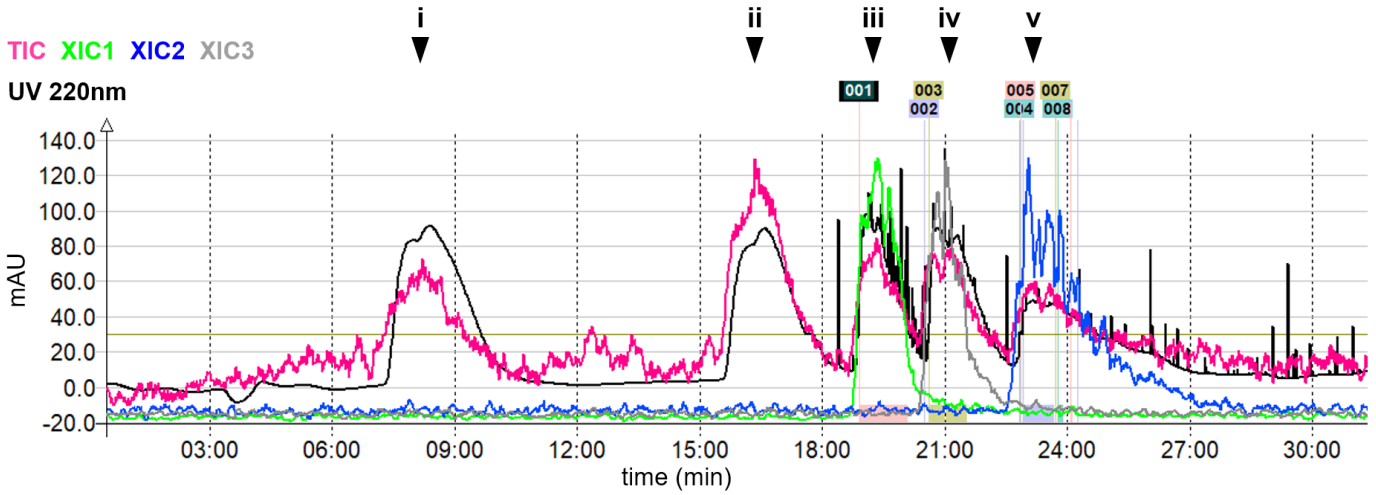
The run parameters are shown in Table 2. One UV channel was set to 220 nm and a second channel was used to scan wavelengths between 200 and 400 nm. The VERITY 1910 MS Detector was operated in positive mode and data was collected on four channels. The first channel was the total ion current (TIC) signal and the three other channels were the extracted ion chromatograms (XIC) for selected compounds according to their ionization mass range. Conditional fraction collection logic was set to collect fractions (up to 10 mL per tube) when the UV signal at 220 nm was ≥ 30 mAU AND any one of the XIC channels had a signal ≥ 100 Kc/s.

Table 2
Run Parameters

Parameters	Conditions
Mobile Phase	A = Water + 0.1% formic acid B = Acetonitrile + 0.1% formic acid
Equilibration	5%B for 25 min
Injection Volume	500 µL
Gradient Conditions	0 min: 5%B 15 min: 25%B 30 min: 25%B 50 min: 95%B 60 min: 95%B 65 min: 5%B
Elution Flow Rate	5 mL/min
UV Detection	• 220 nm • Scan 200 – 400 nm
Mass Detection	• TIC (200-1100 m/z) • XIC1 522-526 (Angiotensin II) • XIC2 554-558 (Leucine enkephalin) • XIC3 573-577 (Methionine enkephalin)
Fraction Collection Conditions	• UV signal (220 nm) >= 30 mAU AND any one of the following: • XIC1 522-526, signal >= 100 Kc/s • XIC2 554-558, signal >= 100 Kc/s • XIC3 573-577, signal >= 100 Kc/s
Ionization Mode	Positive
MiDas parameters	• Make-up pump solvent: methanol + 0.1% formic acid • Make-up pump flow rate: 0.9 mL/min • Split: 10:1

RESULTS AND DISCUSSION

This technical note demonstrates the use of the VERITY Compact LCMS System, featuring a VERITY 1910 MS Detector, for mass-based peptide purification. Figure 2 shows the chromatogram from the separation of five peptides. The conditional fraction collection logic was set to collect fractions only when the UV signal was greater than 30 mAU AND any one of the three XIC channel conditions was met (see Table 2). Whereas peaks i and ii met the UV condition, the detected mass did not match one of the XIC channels and therefore no fractions were collected for peaks i and ii. Peaks iii, iv, and v each met both the UV condition and one of the mass conditions as shown in Figure 2.



Peak #	Peptide	UV > 30mAU	Mass (*m/z*) Detected	Mass conditions met?	Peak collected?
i	Gly-Tyr	Yes	239	No	No
ii	Val-Tyr-Val	Yes	380	No	No
iii	Angiotensin II	Yes	524	Yes: XIC1 > 100	Yes (tube 1)
iv	Methionine enkephalin	Yes	574	Yes: XIC3 > 100	Yes (tube 2-3)
v	Leucine enkephalin	Yes	556	Yes: XIC2 > 100	Yes (tube 4-10)

Figure 2
Chromatogram showing separation of five peptide peaks (i through v). Peaks iii through v met the conditions as indicated and were collected as fractions.

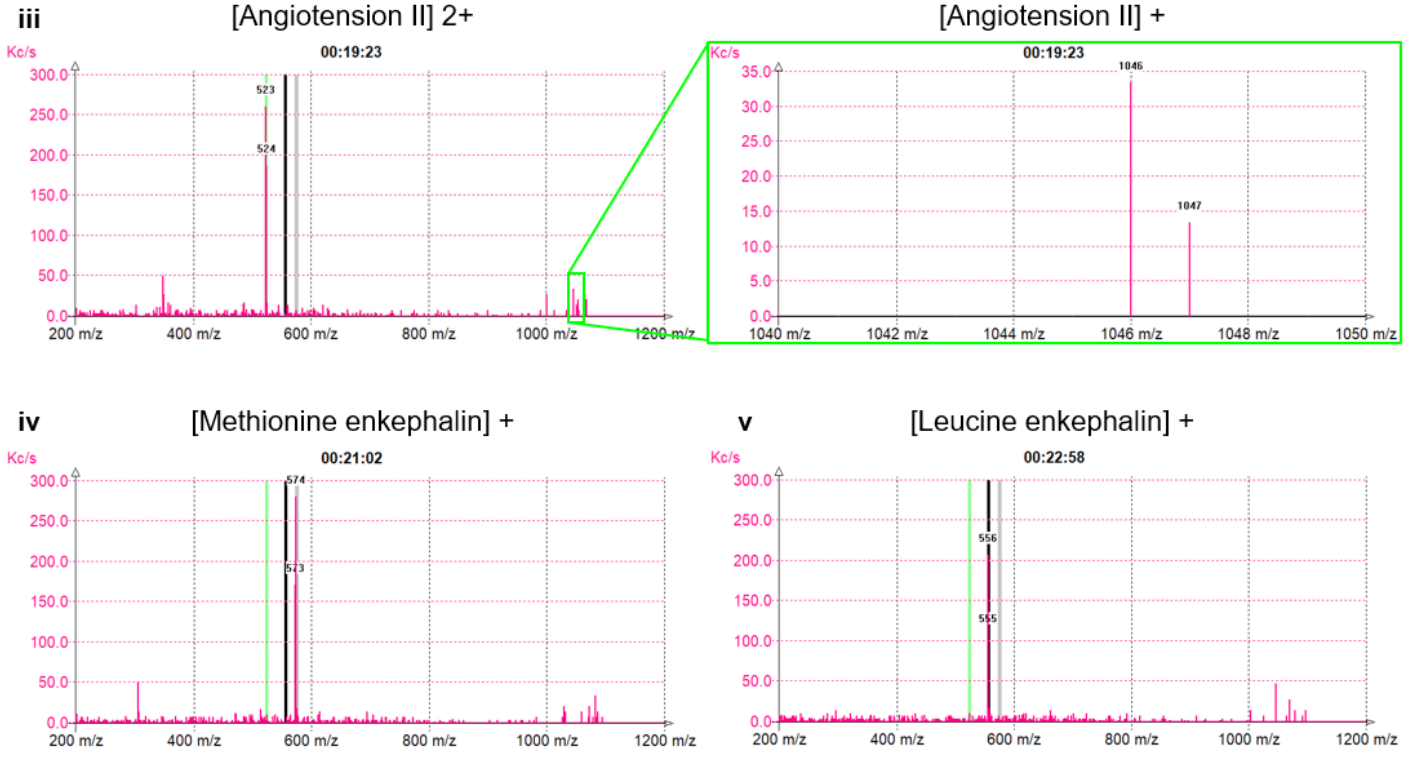


Figure 3
MS spectra of collected peaks iii, iv, and v

Therefore, fractions were collected for peaks iii, iv, and v. Due to the targeted ionization mass collection, it is possible during the purification step to identify the collected peaks as angiotensin II (peak iii, XIC1), methionine enkephalin (peak iv, XIC3), and leucine enkephalin (peak v, XIC2). The determination of the three collected compounds is confirmed by the MS spectra obtained at each peak retention time (Figure 3).

GGP Software provides many useful features including real time and post-run display of total ion count (TIC) and UV data, which allows users to recover information on collected and non-collected compounds.

Clicking on a fraction tube in the schematic will display the data related to that time in the run. This is illustrated in Figure 4, which shows a screenshot from the GGP Software highlighting the data from 21:05 min the run, when a compound of 574 *m/z* was collected in fraction tube 3. The Synoptic view of the run conditions is shown in panel (A), chromatograms showing UV peaks, mass peaks and fractions collected in panel (B), the UV spectral data at 21:05 min in panel (C), the mass spectral data at 21:05 min in panel (D), and the schematic of the collection tube racks, with tube 3 highlighted in panel (E).

Clicking on non-collected peaks on the chromatogram allows determination of the two other compounds injected according to their mass spectra. Mass spectral data at 8:26 min shows peak i is the expected molecular ion of glycine-tyrosine peptide, whereas mass spectral data at 16:33 min shows peak ii is the expected molecular ion of valine-tyrosine-valine peptide (Figure 5).

In conclusion, GGP Software has an easy-to-use touchscreen interface that allows run-time adjustment of parameters including flow rate and gradient conditions. The real time and post-run MS and UV signal display are very useful and intuitive for identification of separated molecules. Taken together, the VERITY Compact LCMS system provides a powerful system for separation, purification, and identification of compounds over a mass range of 50 to 1400 *m/z*.

In addition to use with preparative HPLC as described in this technical note, the VERITY 1910 MS Detector can be used with flash chromatography and centrifugal partition chromatography (CPC) while operated with GGP Software. The VERITY 1910 MS Detector can also be controlled using TRILUTION® LC software as part of a Gilson modular VERITY® HPLC system (see technical note TN221).

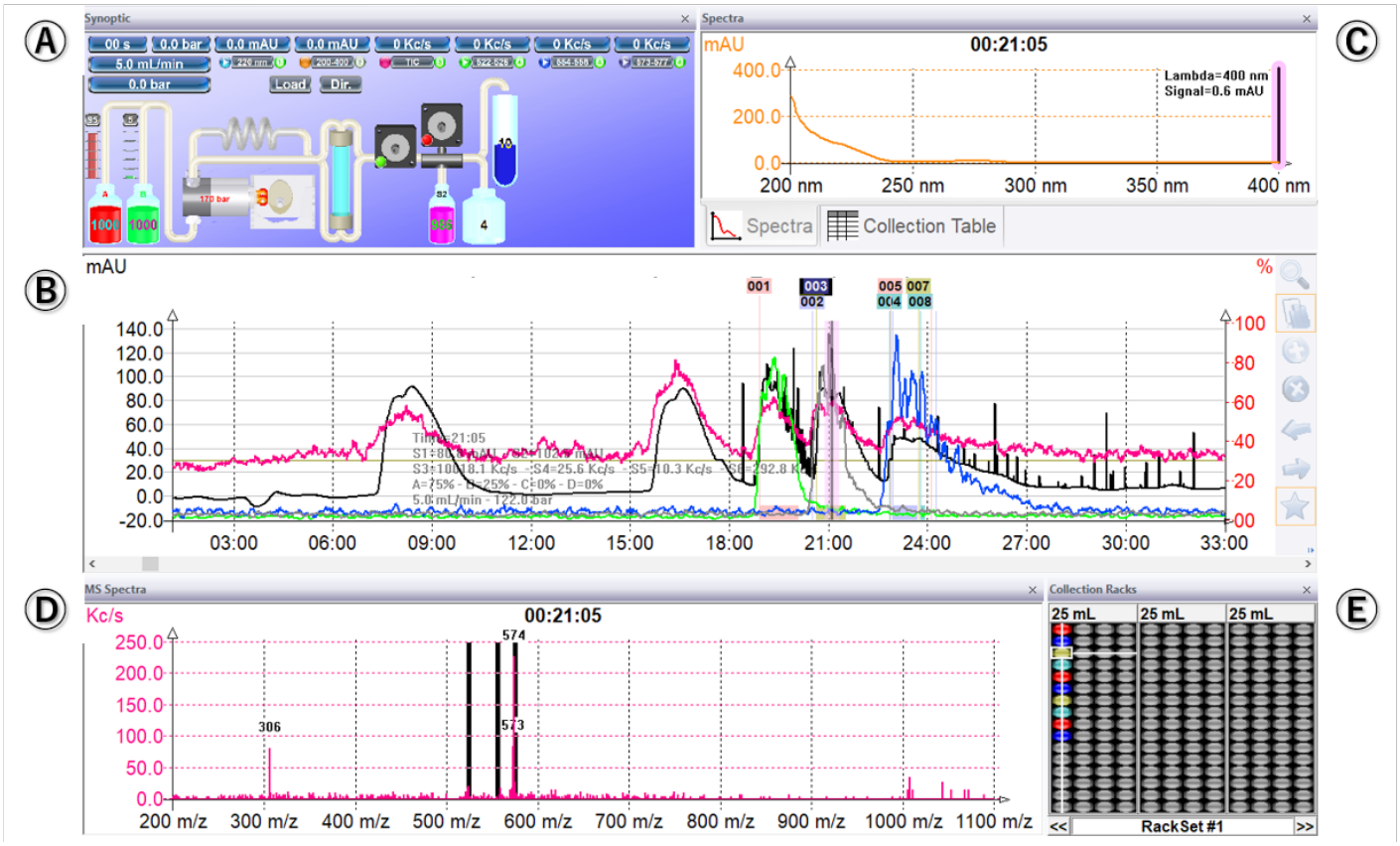


Figure 4
Screenshot from Gilson Glider Prep (GGP) Software

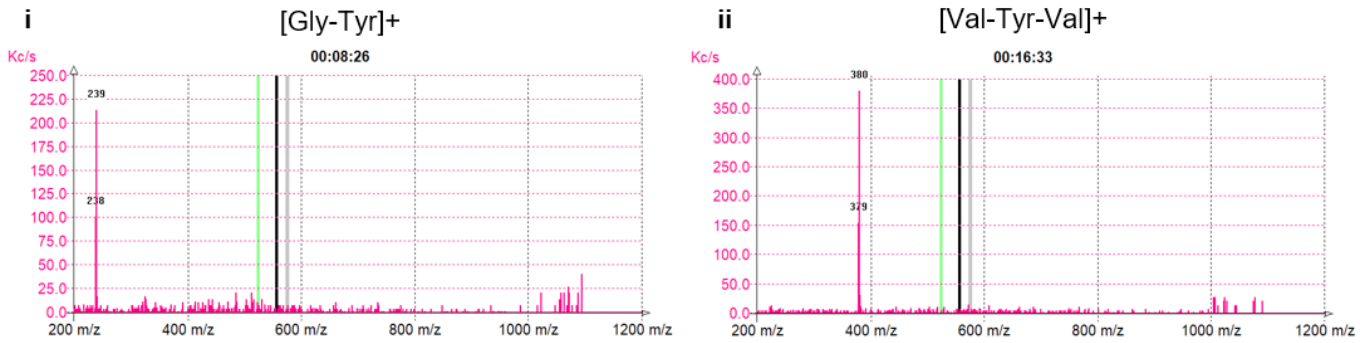


Figure 5
MS spectra of non-collected peaks i and ii

CONCLUSIONS AND BENEFITS

- The VERITY Compact LCMS is a compact and powerful system that can be used for purification of compounds within a mass range of 50 to 1400 *m/z*.
- Combining MS detection with the PLC Purification System allows direct identification of compounds and thus reduces the needs for post run analysis of fractions generally performed after the purification step.
- The intuitive software offers flexibility and an easy-to-use touchscreen interface for run-time adjustment of parameters.
- The conditional fraction collection logic available in GGP Software means fewer fractions to dry down, reconstitute, and analyze, which translates into savings in time and money.

REFERENCES

1. VERITY® MS Purification Systems https://www.gilson.com/pub/static/frontend/Gilson/customtheme/en_US/images/docs/VERITY1910_SellSheet_FLYR_LT380169-1.pdf
2. Product information for Sigma HPLC peptide standard mixture H2016

Description	PN
VERITY 1910 MS, MIDAS FOR PLC	14410032
PLC 2250 UV-1	21140002
AUTO INJ VLV W/TBNG KIT (PLC 2250/2500)	21040004
DAD-4WL, 200-400 NM, PLCA	21040010