Application Note

INTEGRA

Easy 2-step sample preparation for LC-MS analysis on the ASSIST PLUS pipetting robot

Introduction

Liquid chromatography mass spectrometry (LC-MS) is commonly used in analytical chemistry workflows to separate, identify, and quantify complex samples. It plays a vital role in areas such as environmental monitoring, cosmetics industry, and pharma. Varying pipetting and dilution steps during sample preparation, however, are time consuming and prone to error when performed manually.

The VOYAGER adjustable tip spacing pipette on the ASSIST PLUS pipetting robot can access hazardous compounds from any tube format without exposing the user to danger. In addition, the ASSIST PLUS can take advantage of the D-ONE single channel pipetting module's broad volume range and in combination with VIALAB's worklist step, individual high volume sample dilutions are easier than ever.

Reward yourself with reproducible results, additional hands-free time and profit from the versatility of the ASSIST PLUS.

Key benefits:

- More walk-away time and reproducible results with automated sample preparation for LC-MS analysis on the ASSIST PLUS pipetting robot.
- Efficient VIALAB parameter settings for pipetting speeds guarantee high precision when working with non-aqueous solutions. Adjustable mixing cycles help with the varying solubility of compounds.
- Unbeatable very high volume sample dilutions with the D-ONE single channel pipetting module for the ASSIST PLUS. Two GRIPTIP® sizes in one module enable high diluent and small sample volume pipetting without changing tools.
- Foolproof liquid handling with automatic GRIPTIP® selection and liquid level detection (LLD), ensuring precision and accuracy when using the D-ONE on the ASSIST PLUS.
- Effortless sample dilutions with VIALAB's worklist step allows the direct creation and import (e.g. LIMS) of .csv files. Instant worklist exchange without the need to change the program helps when adapting to different sample volumes.
- Risk-free automated compound handling on the ASSIST PLUS with the VOYAGER adjustable tip spacing pipette, because samples are reached from any tube or plate.

Overview: How to make sample preparation for LC-MS analysis easy

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Experience reproducible results while having your hands free by using our easy sample preparation protocols for peptides, including high volume dilutions for LC-MS analysis (**Figure 1**).

Dissolve sample in DMSO

Dilute sample in methanol

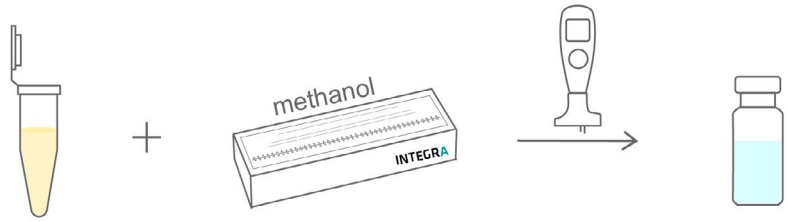

Figure 1: Experimental set-up of sample preparation for LC-MS analysis.

Step by step procedure:

1. Dissolve samples in DMSO

STEP: Dissolve freeze-dried peptides in 100 % DMSO.

HOW TO: Place a 25 ml multichannel reagent reservoir filled with 10 ml of 100 % DMSO on deck position A (**Figure 2**, blue), and an INTEGRA rack with 1.5 ml microcentrifuge tubes containing freeze-dried peptides on deck Position B (**Figure 2**, pink).

Select and run the VIALAB program 'Dissolve_samples_in_DMSO'. With 1250 µl low retention, sterile, filter GRIPTIPS®, the 8 channel 1250 µl VOYAGER adjustable tip spacing pipette aspirates 1 ml of DMSO from the reservoir (**Figure 3a**) and dispenses it into the 1.5 ml centrifuge tubes on deck Position B (**Figure 3b**). Mixing 10 times at a gentle speed (6) ensures a homogeneous solution of peptides.

TIPS:

- Easy adjustment of mixing parameters in VIALAB helps mix reagents of different solubilities.
- Accurate pipetting of DMSO with low retention GRIPTIPS® and a reduced pipetting speed.

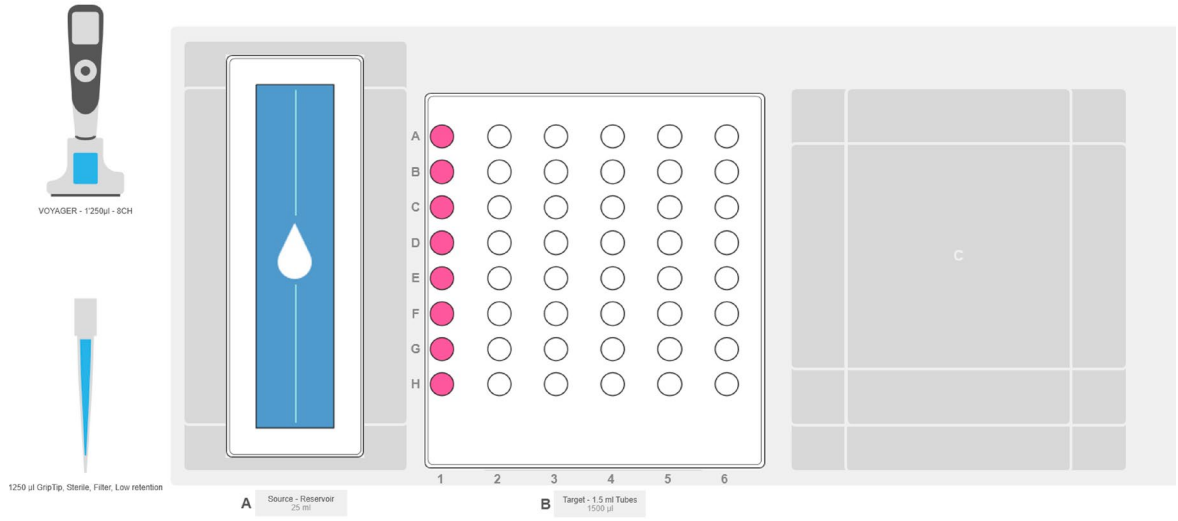

Figure 2: Deck set-up for dissolving samples in DMSO. **Position A**: Source – 25 ml multichannel reagent reservoir containing 100 % DMSO (blue). **Position B**: Target – INTEGRA tube rack for 1.5 ml centrifuge tubes with freeze-dried peptides (pink). **Position C**: Empty.

a)

b)

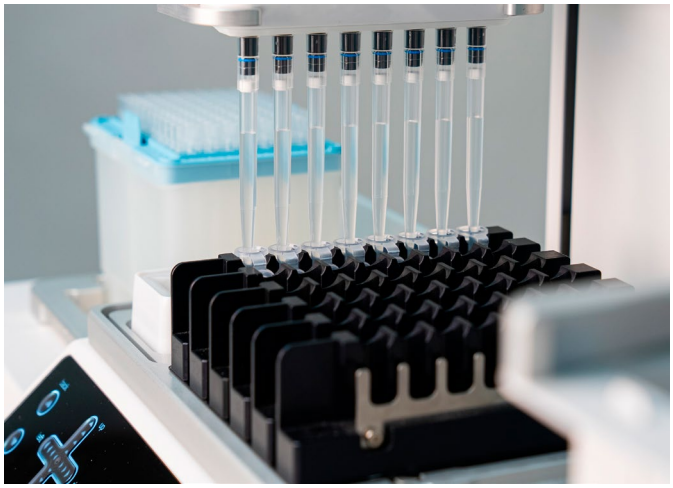

Figure 3: The VOYAGER transferring DMSO from a) a reservoir to b) microcentrifuge tubes.

2. Dilute samples in methanol

STEP: Worklist set-up for individual peptide dilutions.

HOW TO: .csv files are created using the VIALAB worklist template (**Figure 4**) or imported directly into the VIALAB worklist step. The first worklist step includes the worklist for diluent distribution to analyze eight peptides in triplicates, and the second worklist step, the worklist for the different peptides.

TIPS:

- Worry-free exchange of worklists for different sample dilutions without changing the VIALAB program.
- Quick adaption to varying sample counts when deselecting samples in the worklist step.

SampleID	SourceDeckPosition	SourceWell	TargetDeckPosition	TargetWell	TransferVolume [µl]
1.1	A	A1	C	A8	990
1.2	A	A1	C	B8	990
1.3	A	A1	C	C8	990
2.1	A	A1	C	A7	995
2.2	A	A1	C	B7	995
2.3	A	A1	C	C7	995
3.1	A	A1	C	A6	980
3.2	A	A1	C	B6	980
3.3	A	A1	C	C6	980
4.1	A	A1	C	A5	970
4.2	A	A1	C	B5	970
4.3	A	A1	C	C5	970
5.1	A	A1	C	A4	975
5.2	A	A1	C	B4	975
5.3	A	A1	C	C4	975
6.1	A	A1	C	A3	990
6.2	A	A1	C	B3	990
6.3	A	A1	C	C3	990
7.1	A	A1	C	A2	985
7.2	A	A1	C	B2	985
7.3	A	A1	C	C2	985
8.1	A	A1	C	A1	995
8.2	A	A1	C	B1	995
8.3	A	A1	C	C1	995

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Instructions	
Step 1:	Enter your data
Step 2:	Press the button below to generat a Worklist
Step 3:	Save the Worklist file on your PC
Step 4:	Import the file into VIALAB

Generate a Worklist for Import into VIALAB

Figure 4: VIALAB worklist template for creating individual .csv files with example (grey).

2. Dilute samples in methanol

STEP: Sample dilution with methanol in triplicates.

HOW TO: A 25 ml multichannel reagent reservoir filled with 25 ml of 100 % methanol is placed on deck position A (**Figure 5**, blue), and dissolved peptides in 1.5 ml tubes with an INTEGRA rack on deck position B (**Figure 5**, pink). An INTEGRA rack with 24 empty HPLC vials is placed on deck position C (**Figure 5**, green).

Select and run the VIALAB program ‘Dilute_samples_with_methanol’. The D-ONE single channel pipetting module on the ASSIST PLUS pipetting robot with 1250 µl sterile, filter GRIPTIPS distributes diluent from position A to position C (**Figure 6a**) as indicated in ‘worklist_diluent’. An initial tip pre-wetting prevents dripping when pipetting volatile liquids by saturating GRIPTIPS® with vapor. Decreasing the speed to 5 with a one second delay after pipetting followed by a tip touch against the side of the HPLC vial, increases accuracy when dispensing methanol. The D-ONE then individually dilutes peptides from position B to position C (**Figure 6b**) in triplicates using 125 µl low retention, sterile, filter GRIPTIPS®, as indicated in ‘worklist_samples’. Every peptide is mixed five times with the full 125 µl GRIPTIP® volume.

TIPS:

- Worry-free use of different sample aliquots with the D-ONE's LLD function.
- Avoid dripping caused by vibrations with reduced movement speeds during pipette travel.
- Risk-free methanol pipetting because the ASSIST PLUS fits easily in a fume hood.

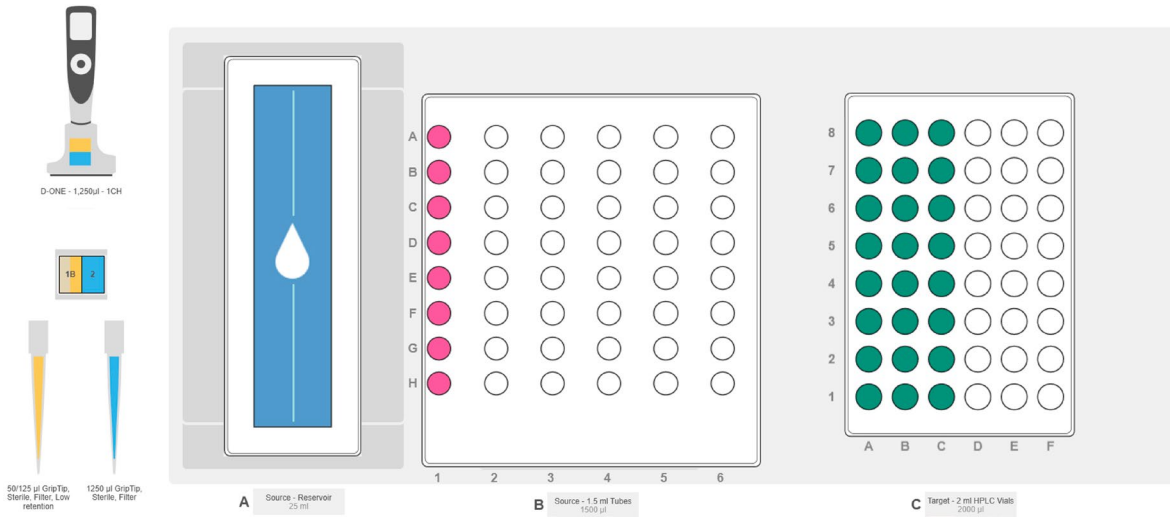

Figure 5: Deck set-up for diluting samples in methanol. **Position A**: Diluent – 25 ml multichannel reagent reservoir containing 100 % methanol (blue). **Position B**: Source – INTEGRA rack for 1.5 ml centrifuge tubes with dissolved peptides (pink). **Position C**: Target - INTEGRA rack for 2 ml HPLC vials (green).

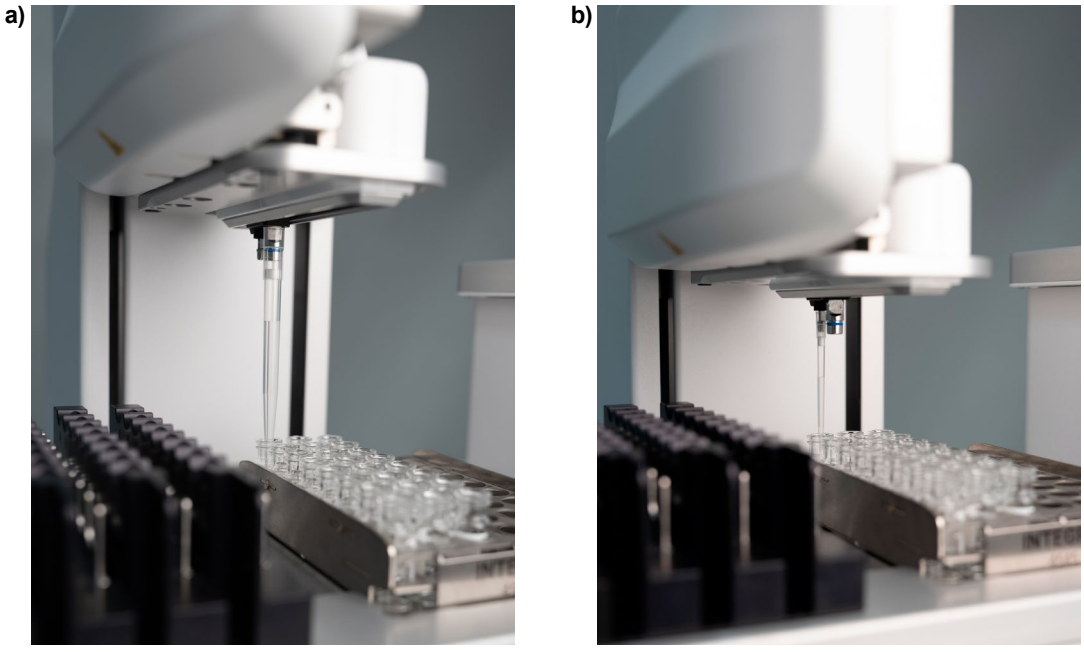

Figure 6: The D-ONE pipetting a) methanol and b) peptides into HPLC vials.

Remarks

- **Low volume HPLC vials:** Special low volume HPLC vials with inlets are compatible with the INTEGRA rack for HPLC vials and accessible by the D-ONE (**Figure 7**).
- **VIALAB software:** The VIALAB programs can be easily adapted to your specific labware and protocols, for instance when partial plates are needed.
- **Partial plates:** Programs can be adapted at any time to a different number of samples, giving laboratories total flexibility to meet current and future demands.

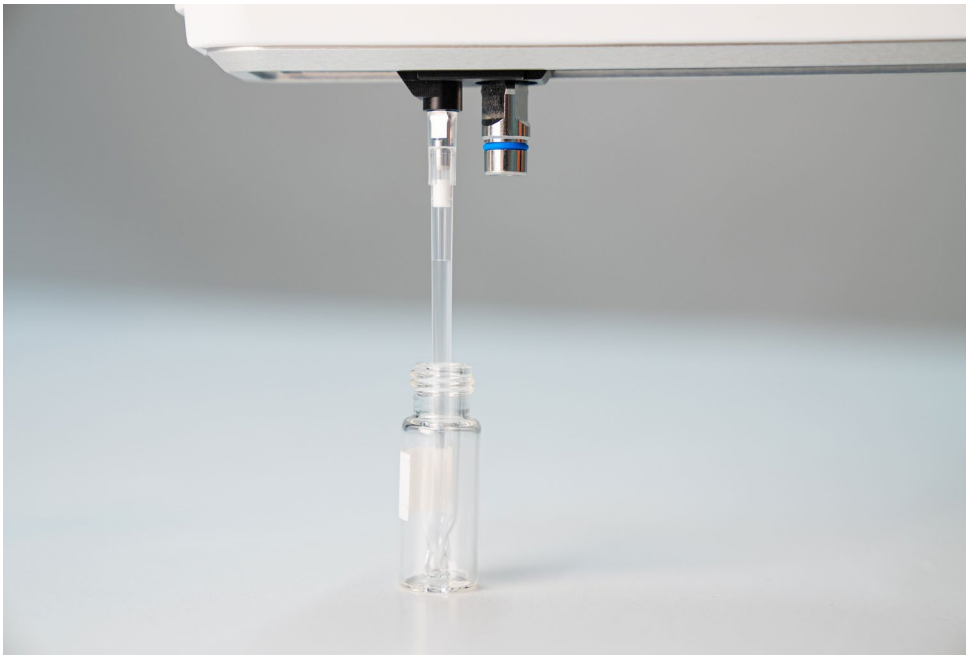

Figure 7: HPLC vial with inlet for low volume LC-MS analysis accessed by the D-ONE.

Conclusion

- Remarkable dilution factors achieved with the D-ONE single channel pipetting module and the ASSIST PLUS pipetting robot, thanks to automatic selection of two different GRIPTIP® sizes.
- Save precious samples by using special low volume HPLC vials that are compatible with INTEGRA's tube rack and accessible by the D-ONE.
- Reliable results with triplicate sample preparation and simplified individual dilutions using VIALAB's worklist step. The creation, import or exchange of .csv files is easier than ever providing fast adaptation to changing sample count.
- Experience more flexibility when transferring liquids between different types of tubes and plates with the VOYAGER adjustable tip spacing pipette on the ASSIST PLUS.

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Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	4505	ASSIST PLUS base unit	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus
INTEGRA Biosciences	4724	8 channel 1250 µl VOYAGER adjustable tip spacing pipette	https://www.integra-biosciences.com/global/en/electronic-pipettes/voyager
INTEGRA Biosciences	4532	D-ONE single channel pipetting module, 5-1250 µl	https://www.integra-biosciences.com/global/en/pipetting-robots/d-one-for-assist-plus
INTEGRA Biosciences	4535	Tip deck for D-ONE on ASSIST PLUS	https://www.integra-biosciences.com/global/en/pipetting-robots/d-one-for-assist-plus
INTEGRA Biosciences	4540	Rack for 1.5/2 ml microcentrifuge tubes	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus
INTEGRA Biosciences	4545	Rack for 2 ml HPLC vials	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus
INTEGRA Biosciences	4310	25 ml reservoir with base unit	https://www.integra-biosciences.com/global/en/reagent-reservoirs/multichannel-reagent-reservoirs
INTEGRA Biosciences	6565	125 µl low retention, sterile, filter GRIPTIPS	https://www.integra-biosciences.com/global/en/pipette-tips/griptip-selector-guide
INTEGRA Biosciences	6545	1250 µl low retention, sterile, filter GRIPTIPS	https://www.integra-biosciences.com/global/en/pipette-tips/griptip-selector-guide
INTEGRA Biosciences	6445	1250 µl sterile, filter GRIPTIPS	https://www.integra-biosciences.com/global/en/pipette-tips/griptip-selector-guide
Greiner Bio-One International	616201	Reaction tube, 1.5 ml, PP, natural, attached cap	https://shop.gbo.com/en/switzerland/products/bioscience/reaction-tubes-analyser-cups/bs-reaction-tubes/616201
Carl Roth	E159.1	Sample vials ROTILABO® 2 ml with thread, clear glass	https://www.carlroth.com/com/en/autosampler-vials-sample-bottles/sample-vials-rotilabo-2-ml-with-thread/p/e159.1
Carl Roth	TY82.1	Sample vials ROTILABO® 0.2 ml with insert clear glass	https://www.carlroth.com/com/en/autosampler-vials-sample-bottles/sample-vials-rotilabo-with-snap-on-ring-nd11/p/ty82.1

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