ZipChip Protocol for Peptide Mixture Analysis

Objective
Mixed peptide analysis separated by charge using ZipChip.

Material Information
- ZipChip Peptide Kit (908 Devices Inc., p/n 850-00034)
- ZipChip HR (908 Devices Inc., p/n 810-00194)
- HPLC Peptide Standard Mixture (Sigma Aldrich, p/n H2016)
- Milli Q or LC/MS Grade or equivalent water

References
1. For system set up, refer to the ***Let's get back to it – Your ZipChip Essentials Checklist Guide***.
2. For chip treatment information, refer to the ***Tech Note 7.0 – Chip Care Guide***.
3. Adjust the ZipChip and Mass Spec to the Base Analysis Conditions and optimize Mass Spec. ***See Tech Note 8.0 – Mass Spec Settings***.
4. For system shut down, refer to the ***System Shutdown and Storage*** support article.

Base Analysis Conditions

ZipChip Parameters	Settings
Field Strength (V/cm)	500
Injection (nL)	5.0
Chip Type	HR
Pressure Assist Enabled (min)	0.5
Analysis Time (min)	6 [a]

a The standard peptides in the example migrate before 6 minutes. Peptides with less charge may require longer times. For example, a peptide map of the NIST mAb can take 12 minutes for full migration.

Sample Considerations
- Samples formulated in slow-moving cations such as TRIS, guanidine, or TCEP **must be buffer exchanged into associated kit diluent** or a formulation with acceptable alternatives before preparation. These additives will disrupt focusing.

Note: Alternative peptide digests can be performed in neutrals and anions such as Urea or SDS.

- Samples formulated with **non-volatile salts** can be digested and diluted in the **associated kit diluent** if the dilution step results in **less than 10 mM** concentration of the salt. Otherwise, samples should be buffer exchanged into the **associated kit diluent** prior to digestion.
- Refer to ***Protocol for SPE Clean-up of Protein Digests*** for suggestions to clean up samples after digestion in non-volatile salts or slow-moving cations.

For more sample guidance and consideration, review the ***ZipChip Sample Guide***.

Standard Preparation
Use a standard solution to ensure operation of the device and data output from the Mass Spec.

1. Reconstitute the standard peptide mixture in 1.0 mL of LC/MS water to obtain a working concentration of 0.25 mg/mL. Working concentration can be aliquoted and frozen for future analysis.
2. Prepare the injection standard by diluting the working concentration 100x in the **kit diluent**.

For example: Add 5 µL of the working solution to 495 µL of the **associated kit diluent**. Note: the diluent must be used as it includes the leading electrolyte at the correct working concentration. **Do not dilute with BGE or water**.

Sample Preparation
1. Ensure the formulation for the samples is appropriate within the list of sample considerations (above).
2. Perform the digestion without use of any of the prohibited additions listed in the sample considerations (above).
3. Target **a peptide concentration >0.1 mg/mL after dilution** in the **associated kit diluent**.

Note: Samples must be diluted at least 5x in the

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associated kit diluent, so minimal digest concentration should be 0.5 mg/mL or performed in the associated kit diluent. For questions or recommendations when performing digests, contact help@908devices.com.

To optimize sample concentration targets

1. Ensure the base peak intensity is within the dynamic range for the instrument. Example electropherograms of the standard peptide mixture are provided in Figure 1 and display typical intensity values.
2. If peak shapes appear overloaded or display fronting, reduce the injection volumes until optimal peak shapes are achieved.
3. If later migrating peaks appear broad and unfocused, there may not be a high enough concentration of the leading electrolyte contained in the **associated kit diluent** in the injection. Samples should be reprepared with at least 5x dilution in the **associated kit diluent**.

Example Data

The following images are the extracted peptides from the peptide standard mixture. Peptides extracted are 523.77/349.52, 239.10, 380.21, 556.37 and 574.23.

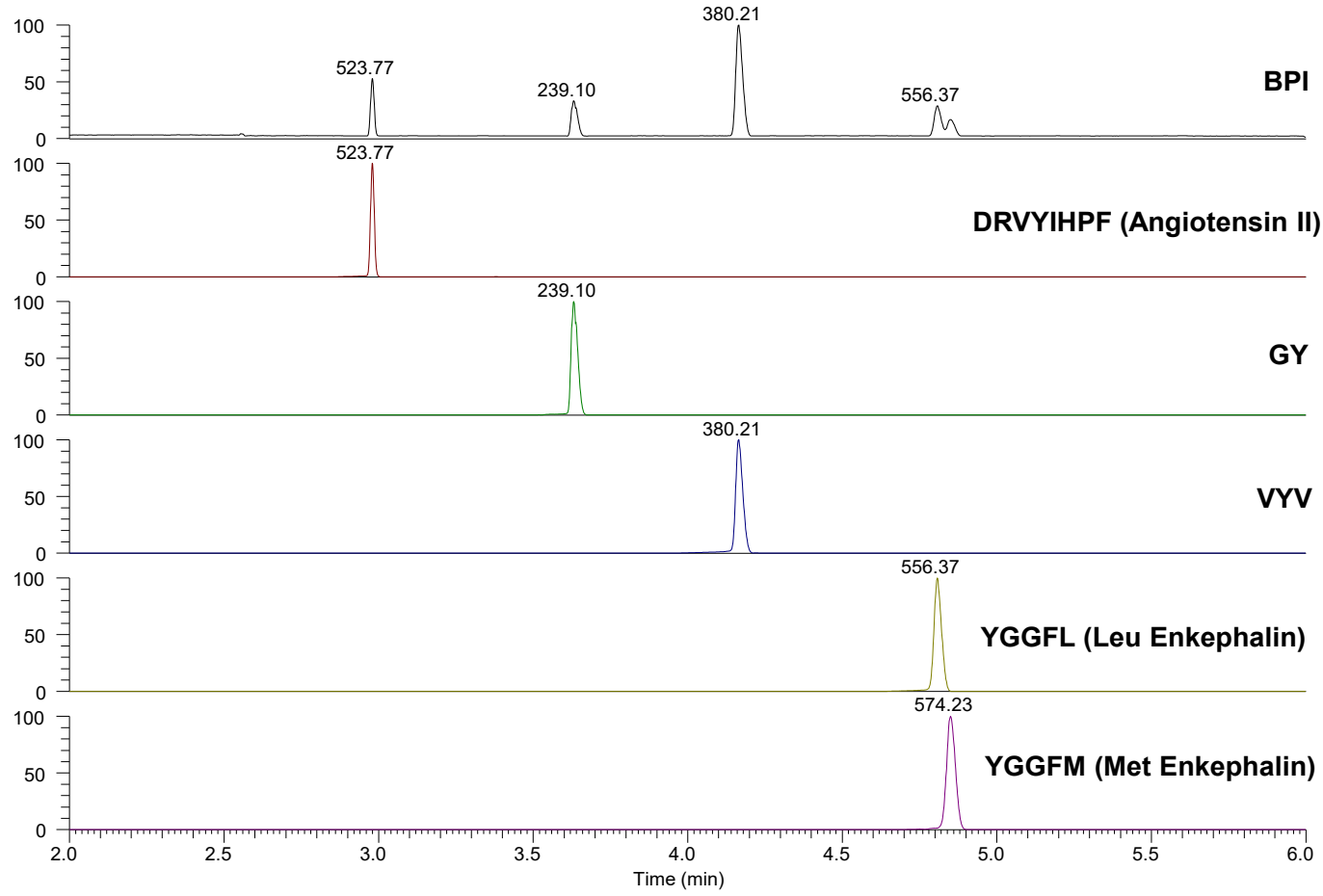

Figure 1: Basic species will migrate before acidic species. Peak shapes should be gaussian (Data was collected on an Exactive Plus EMR operated in Normal mode).

Common Peptides Analysis Troubleshooting Items

Issue Seen	Steps to Take
Peaks are broad/not well resolved in standards	• Ensure that the sample was diluted 5 times or more using the **associated kit diluent** for any sample preparation. Do **not** dilute in Water or BGE. • Reduce injection volume and confirm sample has not been overloaded. • Check the ZipChip Method settings are correct to requirements for the chip used and BGE composition.
The Standards look fine, but the samples are not coming out	• Buffer exchange samples to confirm salt content/formulation is not an issue. • If the samples look over or under loaded and injection volume adjustments do not improve, target a different concentration for digestion.
I see a lot of noise/signal sensitivity is low	• Inject a known standard, such as the peptides standard to ensure instrument settings are appropriate. • Ensure that samples formulated in TRIS, guanidine, or TCEP are **buffer exchanged** into **associated kit diluent** and that the final concentration of non-volatile salts is 10 mM or less.
The spray is poor, sputtering, or creating droplets	• Confirm the chip edge is clean and not damaged prior to testing the spray. • Refer to **Tech Note 7.0: Chip Care Guide**. • Confirm that TRIS or other slow-moving cations are NOT present in formulation. • Collect and send the service report to **help@908devices.com**.
Peak fronting/tailing	• Ensure that the samples do not contain TRIS, TCEP or guanidine. If present, refer to ***Protocol for SPE Clean-up of Protein Digests*** to clean up your samples.
I cannot get good sequence coverage for my proteins	• Process the data at MS level (no MSMS) only to check if all peptides of interest are detected. • Refer to **Tech Note 3.0: Using field strength gradients to enhance ZipChip analysis** to increase peak widths for maximizing sequence coverage.

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