ZipChip

ZipChip performance validation study

INTRODUCTION

This work summarizes migration time and peak area reproducibility across the common variables encountered in routine operation while using ZipChip®.

METHODS

8 production HSX ZipChips were analyzed using a commercially available peptide standard mixture (Sigma Aldrich, PN H2016.) Peptide Background Electrolyte (BGE) Kit was used in accordance with 908 Devices' preparation and operating instructions.

Following initial priming of each chip, 8 replicate analyses of the same sample in the ZipChip sample well were performed, after which the sample reservoir was purged and rinsed in preparation for a new sample. This process was repeated 3 more times for a total of 32 analyses, at which point the chip was dried in the Drydock drying device to emulate typical usage & storage. This 32-analysis cycle was repeated 7 additional times for a total of 256 injections on each chip, at which point the chip was retired. All 8 chips were subjected to the same protocol of 256 runs. The visual run-chart in Figure 1 provides a graphical view of the described protocol. A typical CE-MS run (base peak electropherogram) is shown in Figure 2.

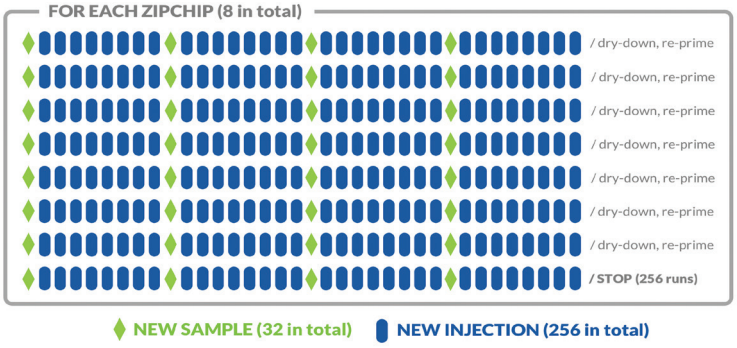

Figure 1. Study protocol diagram for operations on each of 8 chips in the validation study.

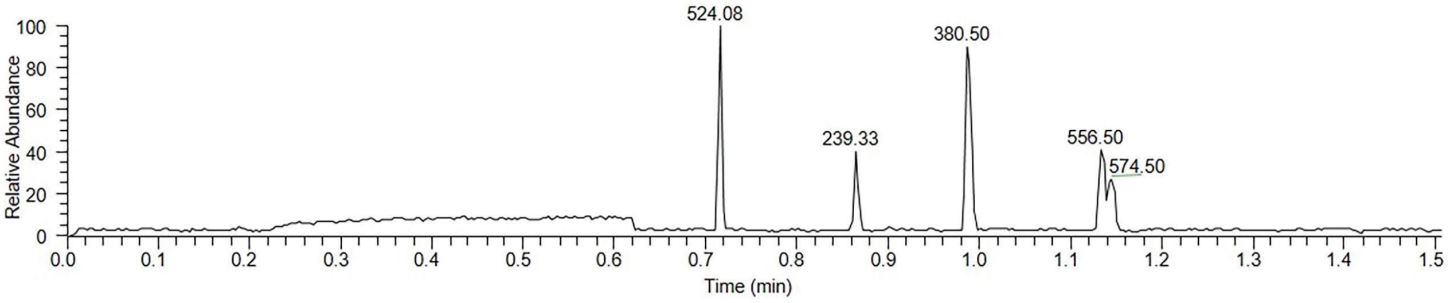

Figure 2. Example peptide separation (base peak electropherogram) acquired on an LTQ-XL mass spectrometer.

Migration times and peak areas were calculated for each analyte. Since a greater degree of variance in peak area and migration time is typically observed in late-migrating components, this analysis reports summary statistics for both the earliest eluting peptide (angiotensin II, 524 m/z) as well as the latest (methionine enkephalin, 574 m/z). From this data set, peak areas and migration times were analyzed to yield:

- Intra-chip RSD between separate drying cycles
- Intra-chip RSD within the same drying cycle

In order to provide a more meaningful and accurate estimate for inter-chip variance in peak areas and migration times, manufacturing QA test data on 400 standard HSX chips was aggregated and evaluated. These 400 production chips were tested with replicate analyses of the same peptide mixture as part of our routine QC testing process.

RESULTS

The aggregated data for a single chip run is shown in Figure 3 below as a visual illustration of performance. The three vertical columns illustrate normalized migration times for each of the five peptide standards. The column on the left shows the raw results, with no normalization based on drying cycle or migration time alignment. The middle column shows the result when migration times are all normalized within a given drying cycle with no migration time alignment. And the column on the right illustrates the result of a simple migration time alignment utilizing leucine enkephalin as a simulated internal standard.

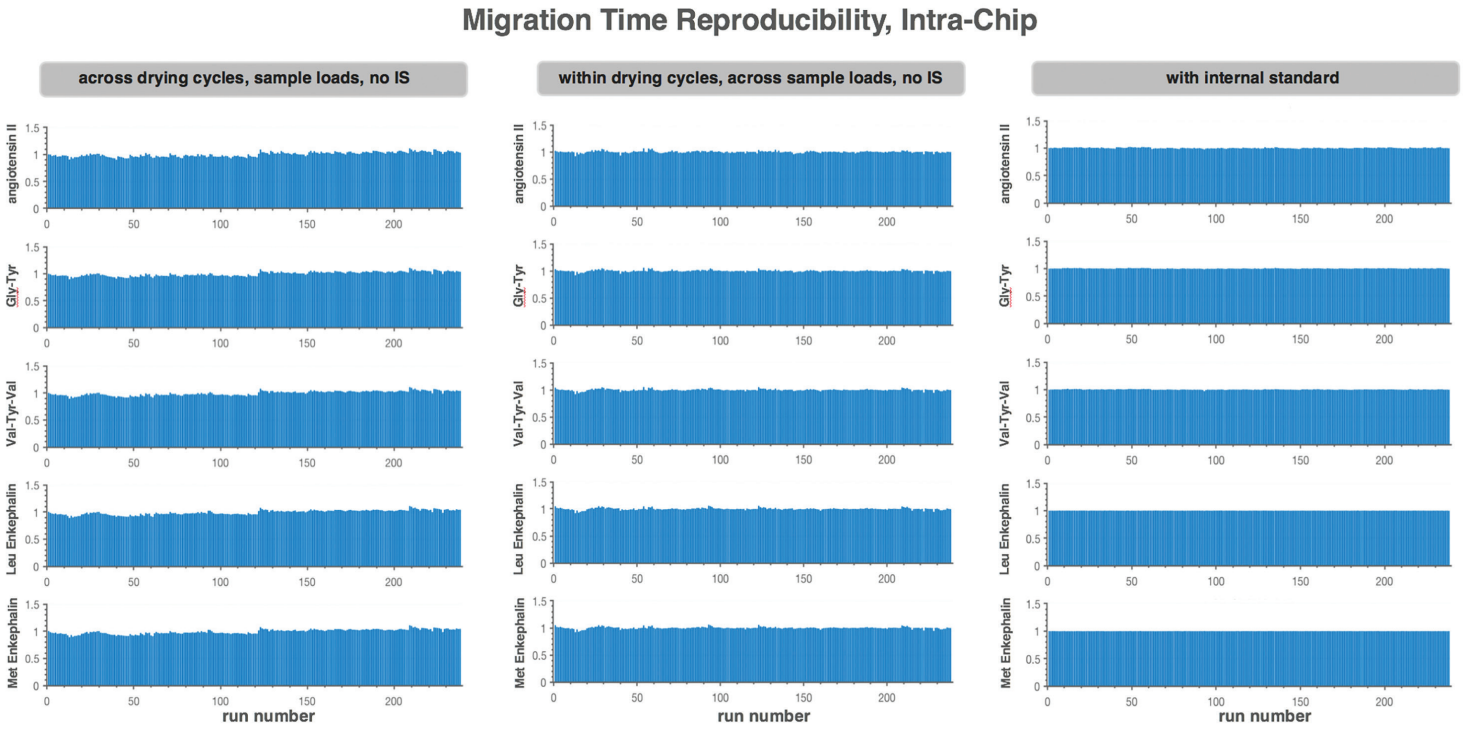

Figure 3. Example data from a single chip run of the peptide standard, from left to right: all sources of variance, variance within a drying cycle, variance with the use of internal standard references. All data has been normalized to mean chip migration time for purposes of scale.

The statistical summary for all of the work presented here is shown in Table 1.

	ANGII		METENK	
Level	**Mig Time RSD**	**Peak Area RSD**	**Mig. Time RSD**	**Peak Area RSD**
Intra-chip across separate dry cycles	3.5%	20.4%	3.8%	11.6%
Intra-chip within the same dry cycle	1.3%	12.0%	1.5%	6.4%
Intra-chip migration time aligned	1.0%	n/a	0.1%	n/a
Inter-chip (400 chips, manufacturing)	3.9%	18.2%	7.5%	12.9%

Table 1. Summary statistics for migration time and peak area % RSD.

The intra-chip results across separate dry cycles indicate that migration time reproducibility is better than 4% RSD and peak area reproducibility is approximately 20% RSD for simulated "real world" use. These runs were performed in groups separated by drying cycles with delays between run groups ranging from hours to days. These data therefore are not only a measure of the variability of the chip's performance, but they are also sensitive to changes in the lab environment and mass spec performance. Normalizing the intra-chip results "within the same dry cycle" corrects for some of the environmental changes which could contribute to variability. We see that this normalization decreases both migration time and peak area variance to about half of their initial values. This level of variance should be expected for ZipChip experiments run continuously with no drying cycles between runs. For all ZipChip quantitation experiments, internal standards could be used to improve both migration time and peak area reproducibility. The crude migration time alignment performed here is sufficient to decrease migration time RSD to 1% or better. Finally, the inter-chip data shows that chip to chip variability is very similar to intra-chip performance. Note that the 400 chips included in this data set were produced and tested over many months of chip production so environmental and system drift are likely to contribute significantly to the observed variances.

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