ZipChip

Evaluation of Sample Carryover from a ZipChip-Autosampler System

PURPOSE

The aim of this work is to demonstrate low levels of injection-to-injection carryover with the use of a ZipChip® system with autosampler.

INTRODUCTION

Sample carry-over in liquid chromatographic systems is a common and unwanted side effect leading to lost time, lost productivity and increased cost. It can lead to false positives, erroneous analytical results, and even costly misinterpretation of data. While sample carryover has many sources, inadequate cleaning of the sample injector and nonspecific adsorption to the stationary phase and system components are the most likely causes. Unlike liquid chromatography analysis, ZipChip separations occur freely in solution via capillary zone electrophoresis. The lack of a retentive stationary phase minimizes the possibility of carryover on the chip. Therefore, the overall carryover on a ZipChip system simply depends upon how effectively the sample transfer line, sample well and needle assembly are rinsed. ZipChip systems, and their associated autosamplers, have been designed with very low carryover in mind, as we will demonstrate in this white paper.

METHODS

All experiments were conducted using a ZipChip system coupled with a Thermo Scientific Q ExactiveTM HF Biopharma mass spectrometer. To determine carryover, two separate experiments were designed. The first experiment was conducted on a commercially available peptide standard to assess carryover in the low mass range (<1500 Da). These measurements were made on 20 different ZipChip-Autosampler systems to ensure that these results represent the performance of the overall ZipChip platform. The second set of experiments were carried out using a commercially available antibody reference standard to assess carryover for large proteins (~150,000 Da)

For the first set of experiments, an HPLC Peptide Standard Mixture (Sigma-Aldrich, p/n H2016) was analyzed using an HS chip and the background electrolyte (BGE) from the ZipChip Peptides Assay Kit. The sample was diluted using sample diluent from the Peptides Assay Kit to a final concentration of 0.25 mg/mL for each peptide in the sample. An injection of working standard was performed followed by a blank consisting of only sample diluent. Carryover was calculated for each peptide by generating peak areas for extracted ion electropherograms with a tolerance of ±30 ppm for every peptide in the working standard and blank. Carryover for the system was determined by averaging carryover of all 5 peptides. The same experiment was repeated on 20 ZipChip systems.

A second set of experiments were performed analyzing the NIST mAb Reference Standard (National Institute of Standards and Technology, p/n 8671) with a ZipChip HR chip and BGE from the ZipChip Metabolites Assay Kit as described in the "Intact mAb analysis using denaturing conditions" protocol by 908 Devices. The NIST mAb standard was diluted in water to 2.0 mg/mL using LCMS grade water. This is more than twice the recommended sample concentration for this assay and was used to increase the possibility of carryover. 1.5 nL of 2 mg/mL standard was analyzed followed by a blank consisting of LCMS grade water. Three sets of sample and blank injections were performed. Extracted ion electropherograms were generated for working standard and blank for m/z 3154 which was found to be the most abundant charge state in the mass spectrum of NIST mAb data. Mass tolerance of ±5 Da was used. Carryover was determined for each set of standard and blank injections by comparing the integrated peak areas in the extracted ion electropherograms for the m/z 3154 charge state. In total, three sets of standard and blank injections were performed.

RESULTS

As discussed above, a ZipChip device does not have a packed stationary phase in the separation channel and analytes have little to no interaction with the walls of the separation channel as they migrate under the influence of the electric field. Therefore, sample carryover, if observed, on a ZipChip system likely occurs only in the autosampler needle assembly, sample flow path or in the chip sample well.

Representative data for carryover for peptides is shown in Figure 1. Extracted ion electropherograms were generated for each of the five peptides for both standard and blank. The average carryover for the five peptides shown was 0.013%. The average carryover data for the five peptides from 20 different ZipChip interfaces for the same experiment is shown in Table 1

Figure 2 shows the carryover data for the NIST mAb under denaturing conditions. Extracted ion electropherograms were generated for the most abundant peak of m/z

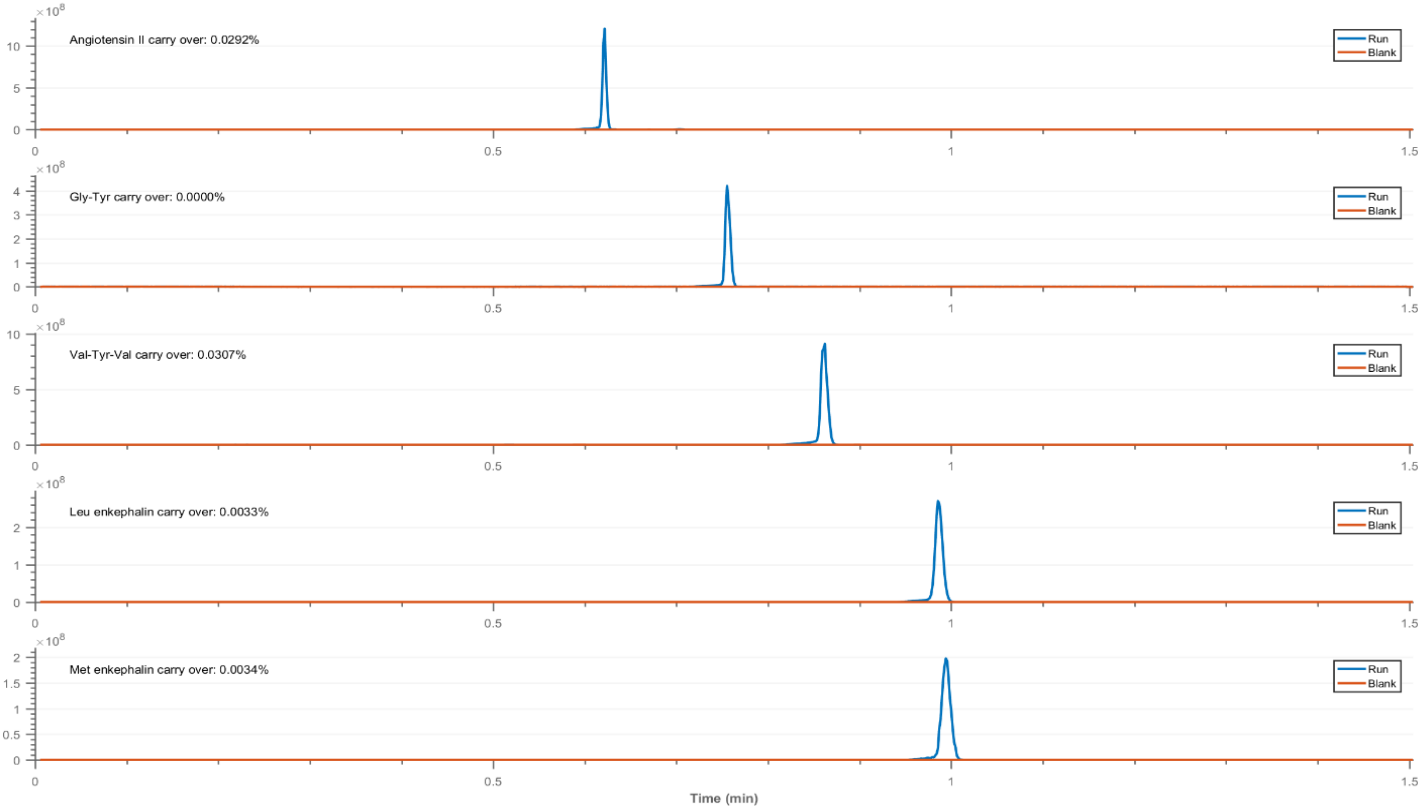

Figure 1: Representative data for carryover of peptides (Extracted Ion Electropherograms with ±30 ppm mass tolerance)

#	Carryover (%)	#	Carryover (%)
1	0.02	11	0.04
2	0.06	12	0.06
3	0.04	13	0.05
4	0.07	14	0.00
5	0.04	15	0.03
6	0.03	16	0.00
7	0.03	17	0.07
8	0.01	18	0.04
9	0.01	19	0.09
10	0.01	20	0.01
Average Carryover: 0.04%			

Table 1: Average carryover for twenty different ZipChip systems

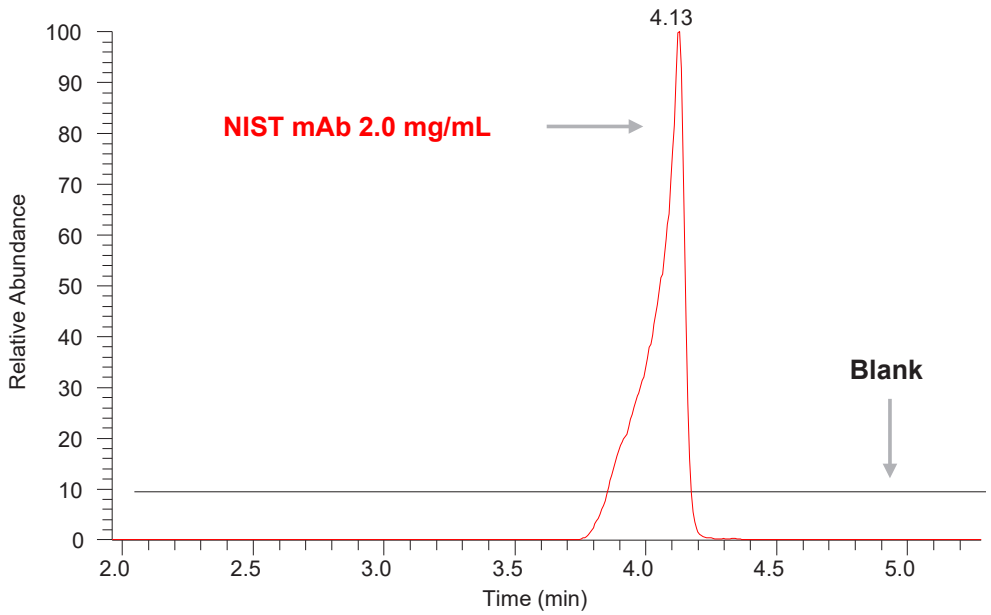

Figure 2: Representative data showing extracted ion electropherogram for a NIST mAb ion with m/z 3154.26 ±5 Da

#	Carryover (%)
1	0.000
2	0.010
3	0.002
Average Carryover: 0.004%	

Table 2: Carryover for three sets of standard and blank injections are shown

3154.26 in the NIST mAb mass spectrum. The NIST mAb sample was deliberately overloaded for this experiment to maximize the chance of seeing carrryover. That is why the electropherogram for intact NIST mAb showed fronting. Carryover for three sets of standard and blank injections are shown in Table 2.

CONCLUSIONS

In conclusion, the two separate experiments were conducted to assess carryover of peptides and intact proteins. The average carryover for all the peptides on twenty different ZipChip systems was 0.04% and in the case of the NIST mAb, the average carryover was only 0.004%. Both of these values are well below the carryover specification of 0.2% for the ZipChip-Autosampler system. It should be noted that the conclusions regarding carryover from this study are also applicable to manual ZipChip systems provided that the sample well is rinsed thoroughly by the user in between runs.

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