Quantitative Peptide Mapping

Application Note

(U)HPLC: Peptides

Author

Andrew Jones
Activated Research Company
7561 Corporate Way
Eden Prairie, MN 55344
andrew.jones@activatedresearch.com

Abstract

Protein and peptide quantification require expensive and often unavailable standards to calibrate detector (e.g., mass spectrometer) response. The use of a detector with a uniform or universal response to carbon could allow for quantification with surrogate standards of virtually any type and concentration. Here we demonstrate the detection and quantification of 10 peptides and albumin using a combination of thermal evaporation to remove mobile phase and catalytic-laser decomposition to gasify and detect compounds with a flame ionization detector (FID). The resulting detector response is similar for different compounds on a per carbon basis.

Introduction

Protein[1] and peptide-based[2] therapeutics represent a growing number of effective treatments for metabolic and oncological disorders. Peptide analysis is a key step in the characterization and development of protein (including monoclonal antibodies) and peptide-based drugs. Peptide mapping of partially digested proteins is used to confirm a protein's primary structure (amino acid sequence) for identification, primary structural characterization, and quality control (QA/QC). Analysis of peptide-based drugs, on the other hand, yields information about the purity of the active ingredient, the presence and amount of genotoxic impurities, and quality.

High-performance liquid chromatography (HPLC) is an essential tool for the separation and analysis of peptides and proteins. Concentration dependent detectors, such as electrospray mass spectrometry and UV are typically used, but they have several drawbacks. Only 3 of the 22 amino acids that make up peptides and proteins have chromophores that are visible via UV at 220 nm, so many peptides are invisible to the detector. Mass spectrometry is indispensable for qualitative peptide information, however, response factors can vary by several orders of magnitude depending on the ionization efficiency of the peptide/protein, and the cost and complexity of operation and maintenance preclude its use in most quality laboratories.

A detector with uniform sensitivity to various peptides, a linear response and simple operation would solve many of the problems. Here, we analyze a standard with 10 peptides using flame ionization detection (FID) coupled with thermal evaporation and catalytic-laser decomposition within the Solvere™ HPLC detector.

Experimental

A sample of 10 peptides (5190-0583, Agilent) was dissolved in 0.1181 g of demineralized water and vortexed for 5 min. Sample information is given in Table 1. A mass of 0.0546 g of bovine serum albumin (A6003-1G, Sigma Aldrich) was dissolved in 5.0808 g of demineralized water and shaken. An aliquot of 0.9954 g of this solution was added to 10.0737 g of demineralized water and shaken to yield a concentration of albumin of 956 ppm. The column outlet from an Agilent 1290 Infinity II LC System was connected to the Solvere (v1, ARC) with a 300 mm x 0.12 mm ID 316SS tube and the following configuration:

HPLC conditions

Column	Agilent AdvanceBio Peptide Map (2.1 x 100mm, 2.7µm)
Column temp.	55 °C
Test Standard	Peptide Mix (5190-0583)
Injection volume	5 µL
Pump flow rate	0.3 mL/min
Solvent A	Water, 0.1% formic acid
Solvent B	Acetonitrile, 0.1% formic acid

Gradient profile:

Time (min)	Percent B
0	15
25	65
25.1	95
26	95
26.1	15
35	15

Solvere™ conditions

Cell Temperature	150 °C
FID Temperature	400 °C
H_2	50 sccm
Air	350 sccm
Makeup	1000 sccm (air)
Acquisition rate	3.125 Hz
Power	70%
Catalyst	S1-M

Results and Discussion

The responses of 10 peptides (Table 1) from 30 to 110 ppm (µg/mL) are shown in the chromatogram in Figure 1. The response of each peptide is similar, yielding similar minimum detection limits (defined as the response at a signal-to-noise ratio of 3; MDL) between 11-18 ppm depending on the peak width. This equates to detection limits of 53-92 ng on-column per peptide.

The integrated FID response of each peptide is plotted as a function of its carbon concentration in Figure 2. Peptide response increases linearly with carbon concentration or amount of carbon on column, consistent with an equimolar carbon response independent of peptide identity. The equimolar response, or 'carbon counting,' is the result of a catalytic combustion of peptides to CO_2 and subsequent reduction and detection by FID. The scatter in the data is presumed to be due to small differences in the purity of the peptides and integration errors due to analysis near the MDL.

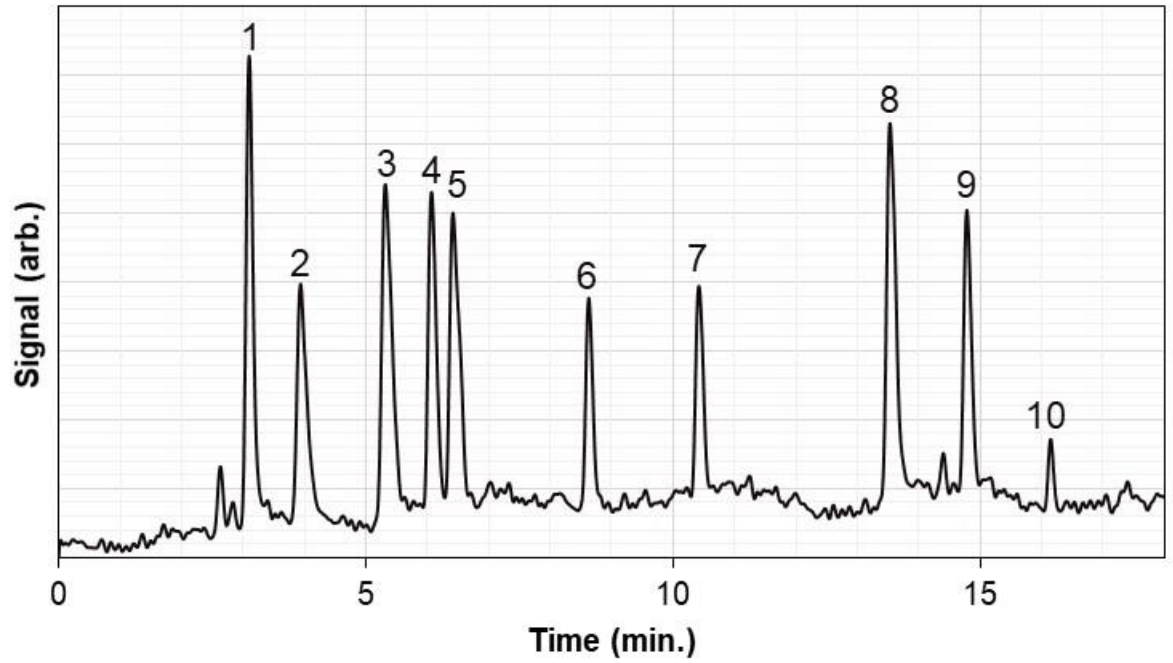

Figure 1. Solvere™ response of peptides from Table 1.

Table 1. Peptide information

Number	Peptide Name	Peptide Sequence	MW (g/mol)	#C	Conc. (µg/g)	Carbon (µgC/g)
1	Bradykinin frag 1-7	RPPGFSP	757	35	49.1	27.3
2	Bradykinin	RPPGFSPFR	1060	50	44.8	25.3
3	Angiotensin II (human)	DRVYIHPF	1046	50	56.4	32.4
4	Neurotensin	Glp-LYENKPRRPYIL	1673	78	56.4	31.6
5	Angiotensin I (human)	DRVYIHPFHL	1296	62	52.0	29.8
6	Renin substrate porcine	DRVYIHPFHLLVYS	1759	85	46.2	26.8
7	[Ace-F-3,-2 H-1] Angiotensinogen (1-14)	Ace-FFHDRVYIHPFHLLVYS	2232	101	57.8	31.4
8	Ser/Thr Protein Phosphatase (15-31)	EIFLSQPILLELEAPLK	1952	93	109.8	62.8
9	[F14] Ser/Thr Protein Phosphatase (15-31)	FEIFLSQPILLELEAPLK	2099	102	98.3	57.3
10	Melittin	GIGAVLKVLTTGLPALISWIKRKRQQ	2846	131	30.3	16.7

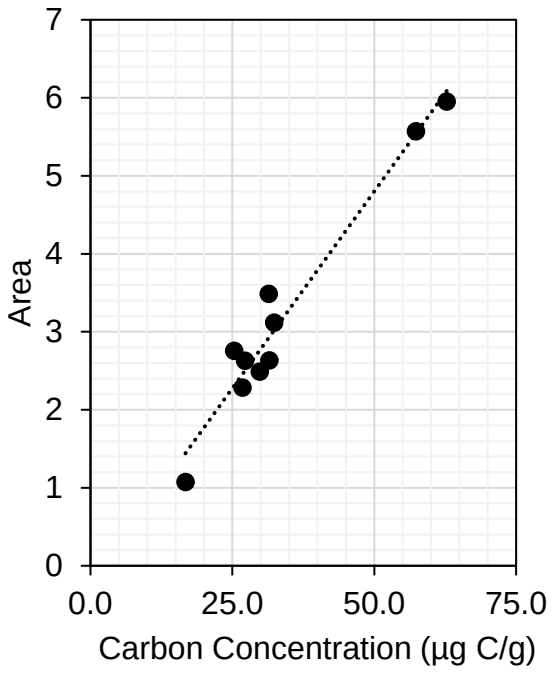

Figure 2. Integrated response of peptides per carbon

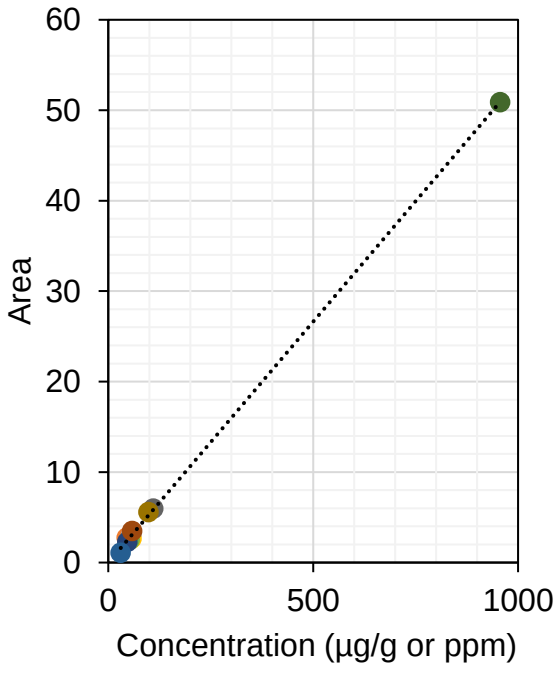

Figure 3. Integrated response of peptides and protein (albumin at 960 ppm; green circle) versus mass concentration.

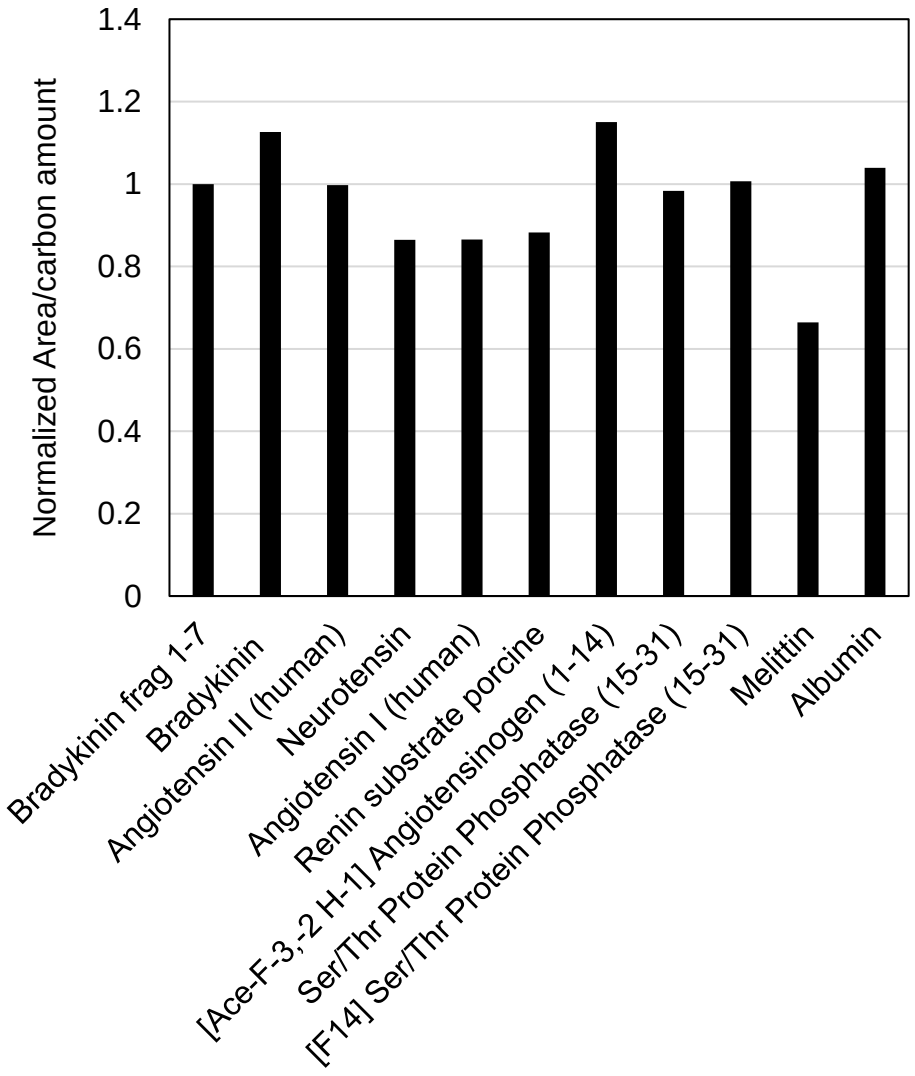

Figure 4. Solvere™ response per mass of carbon on-column for each peptide and albumin normalized to the response per carbon of bradykinin frag 1-7.

The integrated response of each peptide is plotted as a function of its mass concentration in Figure 3 alongside the response of albumin (960 ppm) from a separate run. The linearity of the response per mass (instead of per carbon) is because these peptides, and peptides/proteins in general, have a similar carbon-to-weight ratio. This is useful for the quantification of unknowns or quick purity analysis from Area %, which is roughly equal to mass or carbon %. The equivalent response per carbon or per mass of the albumin protein (66 kDa) and the peptides (0.8-2.8 kDa) demonstrates that the response is independent of molecule size (Figure 4). Furthermore, the equivalent response taken together with the varied elution time and gradient (Figure 1) demonstrates that response is independent of mobile phase composition.

Conclusions

- Solvere™ CSD gives equimolar carbon response for peptides and proteins
- Minimum detection limits are 11-18 ppm
- Mass equivalent response is possible within the class of peptides/proteins due to similar carbon-to-weight ratios of amino acids
- Response is independent of mobile phase composition
- Response is independent of analyte size
- Purity can be estimated from Area % on a weight basis (or carbon-basis is more accurate)

References

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Printed in the USA
October 22, 2020
PA-APP-2035