

Quantitative Detection of RAS and KKS Peptides in COVID patient serum by Stable Isotope Dimethyl Labeling LC-MS

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Materials and Methods

Peptides and Reagents:

Angiotensin I human (human acetate salt hydrate), Angiotensin II human (93% HPLC), Angiotensin 1-7, Bradykinin (acetate salt), Bradykinin 1-7, normal formaldehyde solution (37% stock concentration), ammonium hydroxide solution, sodium cyanoborohydride, HPLC grade water, HPLC grade methanol, HPLC grade acetonitrile, and HPLC grade formic acid were purchased from MilliporeSigma, (Burlington, MA, USA). Bradykinin fragment 1-8 (acetate salt) was bought from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA), and Angiotensin 1-5 was supplied by Echelon Biosciences (Salt Lake, UT, USA). Deuterated formaldehyde (formaldehyde-D₂, 20% stock concentration) was supplied by Cambridge Isotope Laboratories, Inc. (Tewksbury, MA, USA); Oasis HLB solid phase extraction microelution plate, 1.5 mL centrifuge tubes, HPLC sample vials and inserts were purchased from Waters Corporation (Millford, MA, USA), batches of pooled human serum were supplied by Innovative Research, Inc. (Novi, MI, USA), Sigma-Aldrich, Inc. (St. Louis, MO, USA), and Fisher Scientific.

Scheme of Work_Dimethyl Labeling

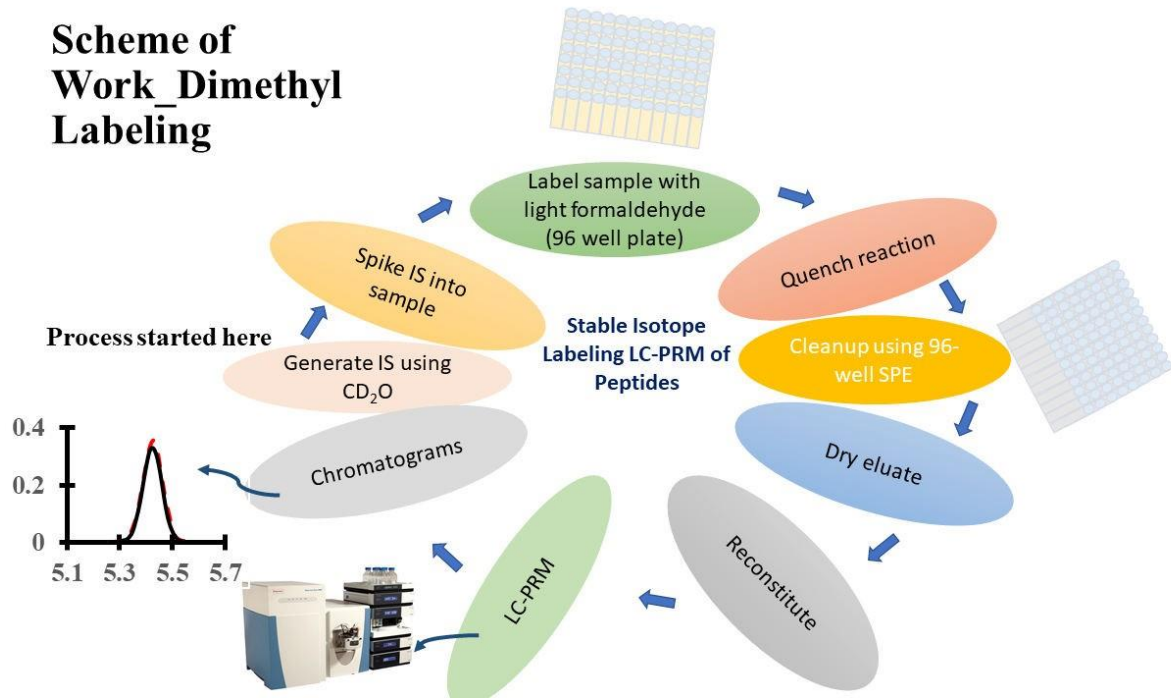


Figure S1. A Scheme showing the steps involved in dimethyl labeling of peptides and LC-MS analysis.

Table S1: Parameters for the parallel reaction monitoring (PRM) method. Precursor list, retention time windows, collision energy and orbitrap resolution for the respective analytes. L and H indicate formaldehyde and deuterium-formaldehyde labeled versions, respectively.

Compound	m/z	z	t start (min)	T stop (min)	HCD Collision Energy (%)	Orbitrap resolution
Ang 1 L	442.2435	3	5.1	7.2	27	30000
Ang 1 H	443.5852	3	5.1	7.2	27	30000
Ang 2 L	358.8625	3	4	5.5	25	15000
Ang 2 H	360.2043	3	4	5.5	25	15000
Ang 1-7 L	464.256	2	0.8	2.4	27	15000
Ang 1-7 H	466.2685	2	0.8	2.4	27	15000
Ang 1-5 L	347.2001	2	1.8	3	27	15000
Ang 1-5 H	349.2127	2	1.8	3	27	15000
Brad L	363.5382	3	2.7	4.7	20	30000
Brad H	364.8799	3	2.7	4.7	20	30000
Des-R9- Brad L	466.7531	2	4.6	6.1	20	15000
Des-R9- Brad H	468.7656	2	4.6	6.1	20	15000
Brad 1-7 L	393.2189	2	0.8	2.5	20	30000
Brad 1-7 H	395.2314	2	0.8	2.5	20	30000

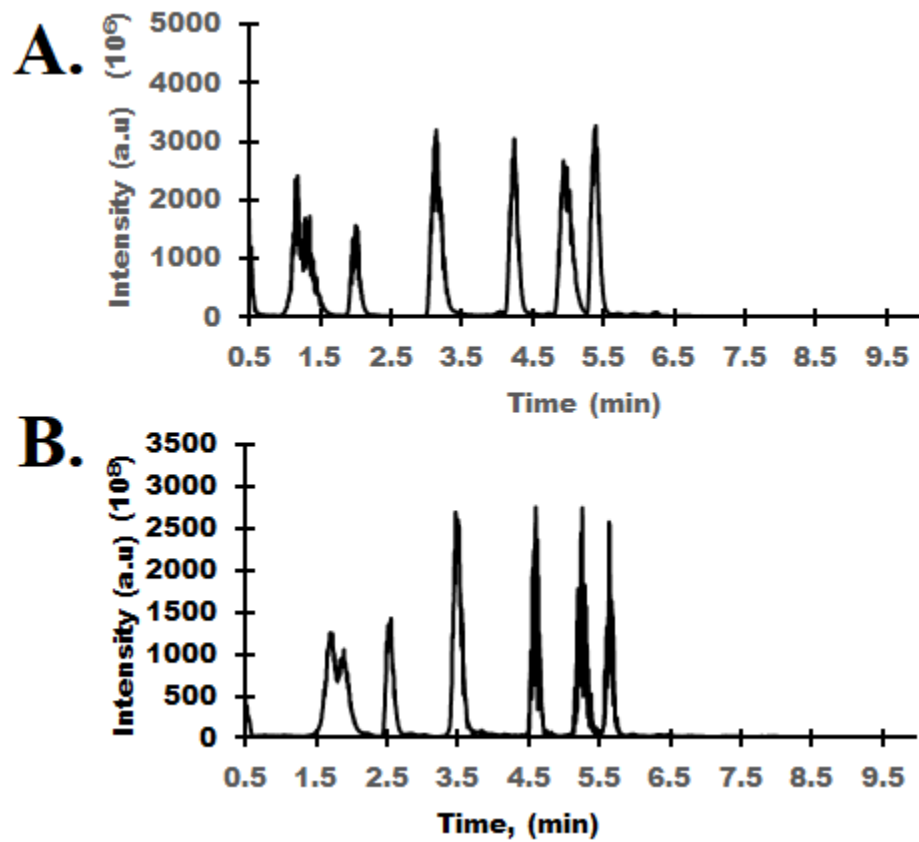


Figure S2. A representative Chromatogram illustrating a successful labeling of RAS-KKS peptides with minimal side-products formation. A. A full MS1 chromatogram of unlabeled peptides at 5 ng/mL. B. A full MS1 chromatogram of formaldehyde-labeled RAS-KKS peptides at 5 ng/mL, showing a complete labeling of analytes with minimal reaction side products.

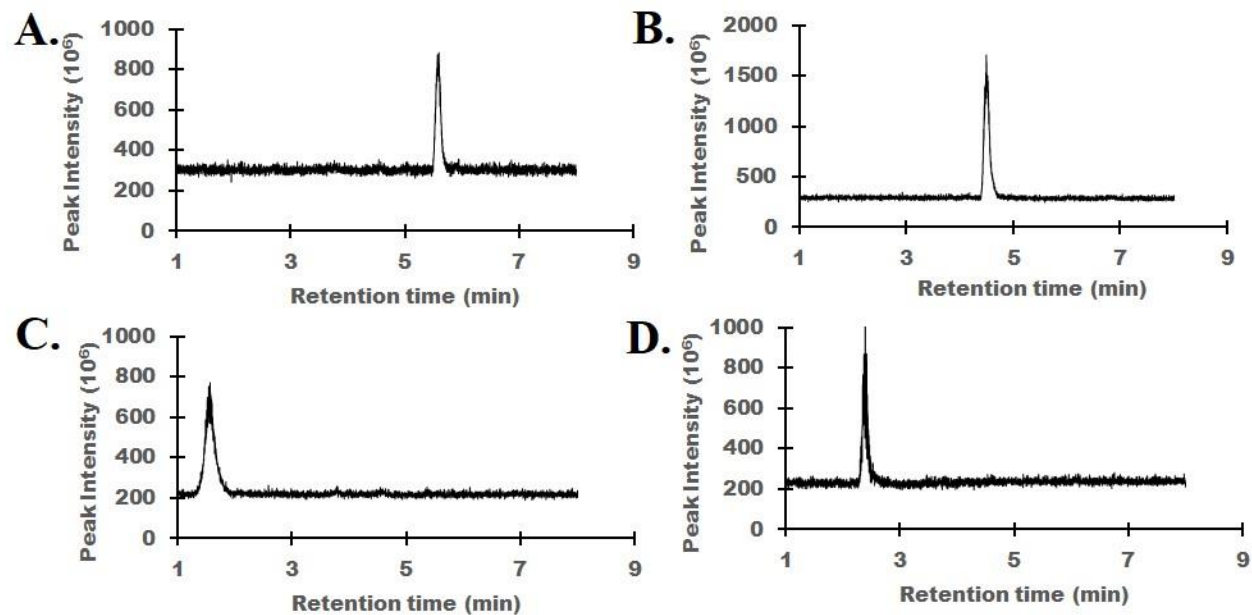


Figure S3. Representative full scan chromatograms of RAS peptides showing a successful dimethyl labeling with no side products. A. A full scan chromatogram of formaldehyde-labeled Ang 1. B. Full MS scan chromatogram of formaldehyde-derived Ang 2. C. Full scan chromatogram of formaldehyde-derivatized Ang 1-7. D. A chromatogram of formaldehyde-coded Ang 1-5.

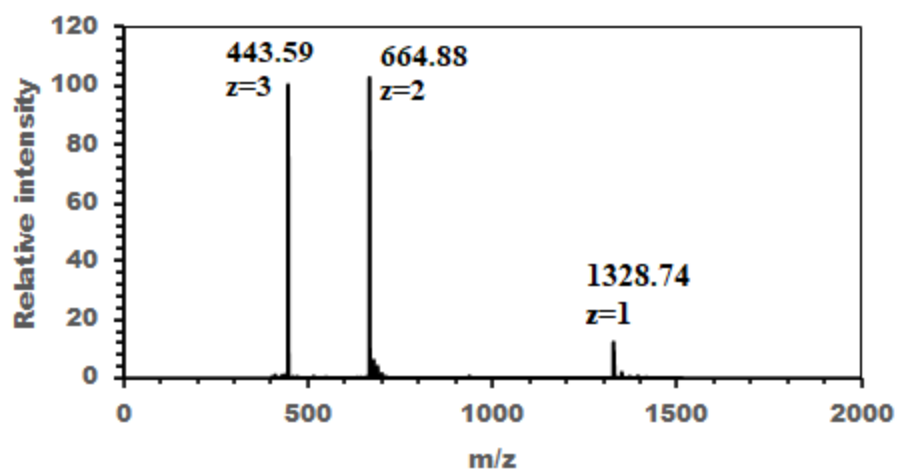
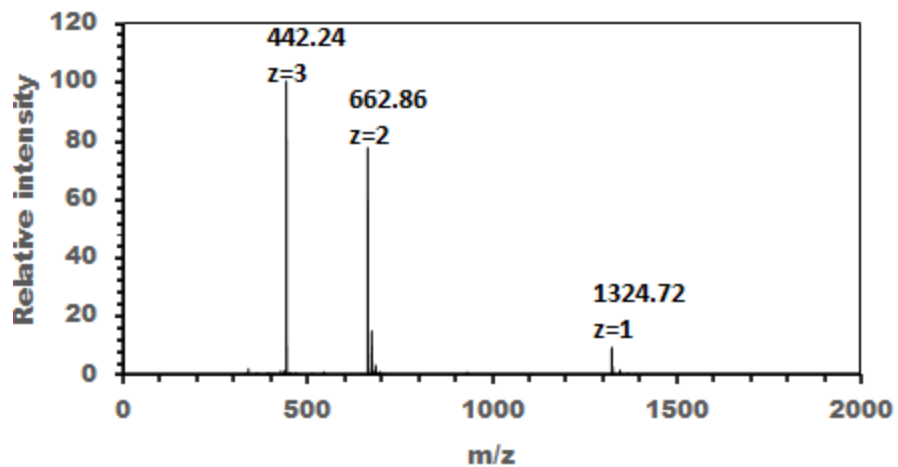


Figure S4. Full MS1 scans showing a successful generation of A) formaldehyde- and B) deuterio-derivatized Ang 1 with no side-products.

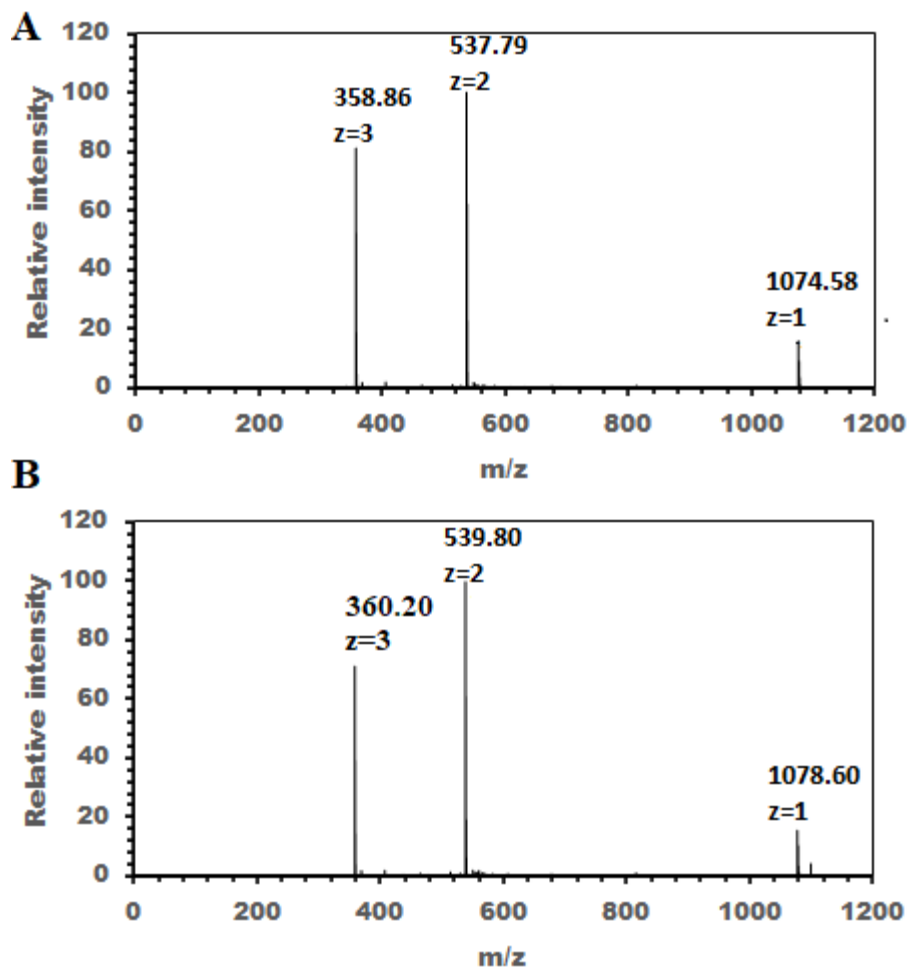


Figure S5. Full MS1 scans showing a successful generation of A) formaldehyde- and B) deuterio-derivatized Ang 2 with no side-products.

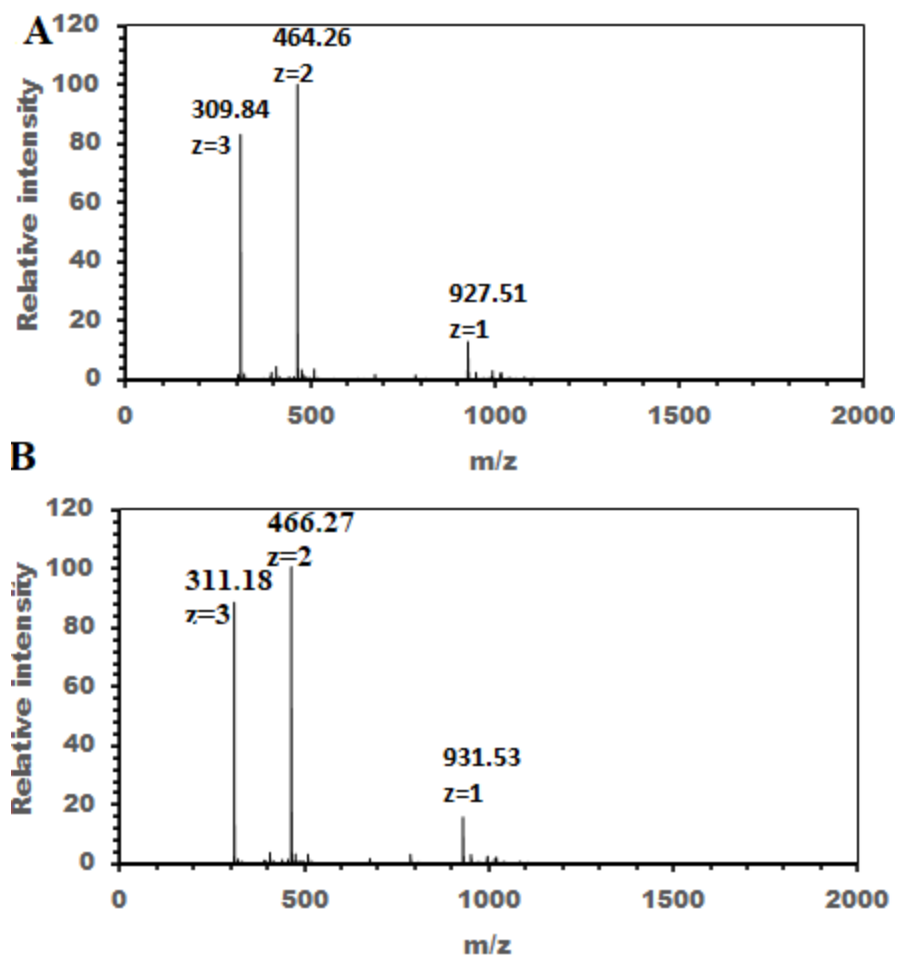


Figure S6. Full MS1 scans showing a successful generation of A) formaldehyde- and B) deuterio-derivatized Ang 1-7 with no side-products.

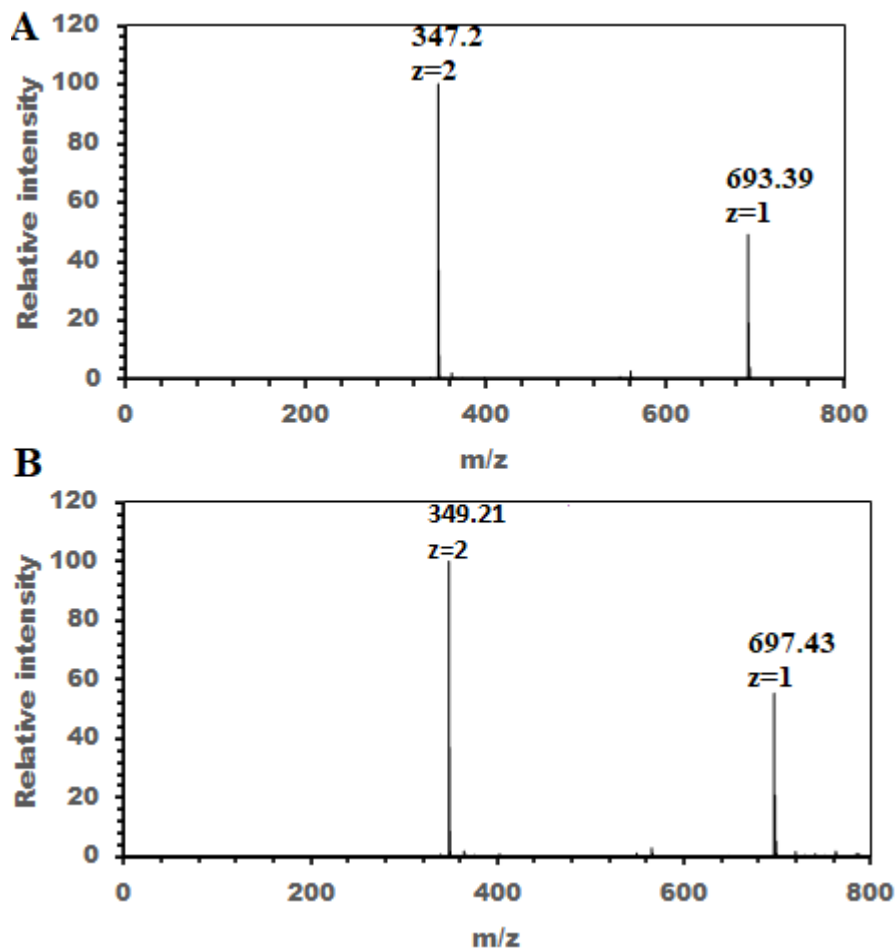


Figure S7. Full MS1 scans showing a successful generation of A) formaldehyde- and B) deuterio-derivatized Ang 1-5 with no side-products.

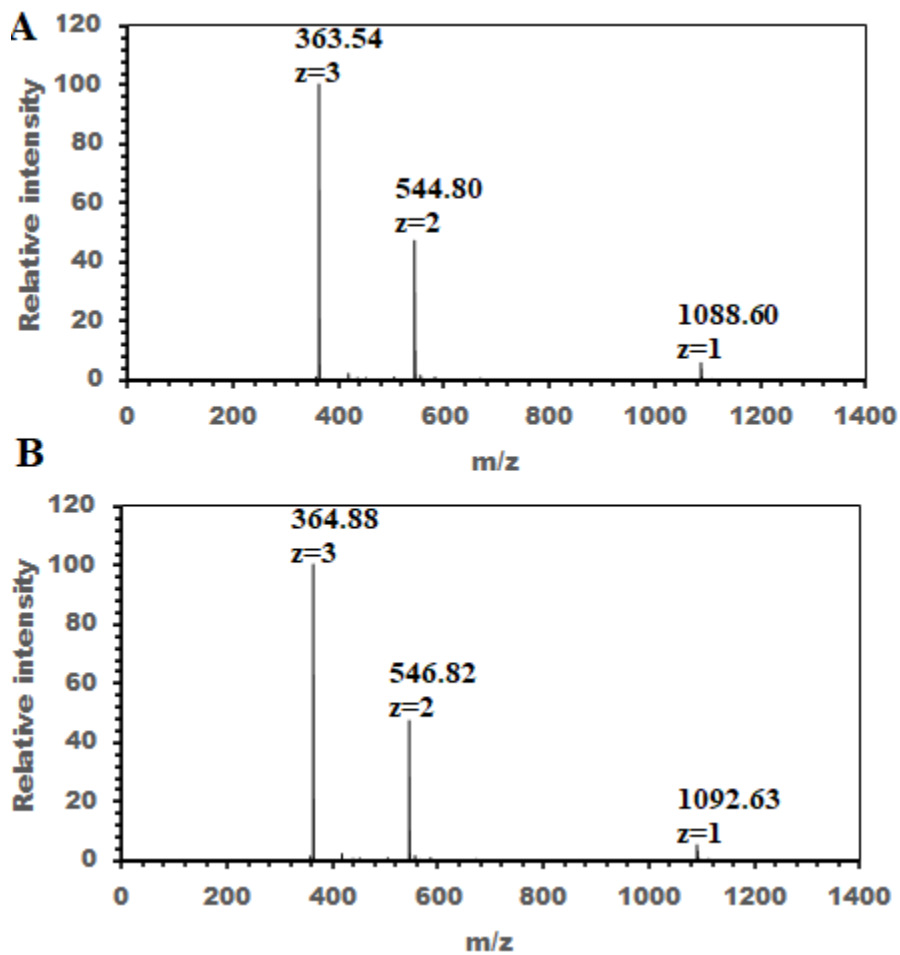


Figure S8. Full MS1 scans showing a successful generation of A) formaldehyde- and B) deuterio-derivatized Brad with no side-products.

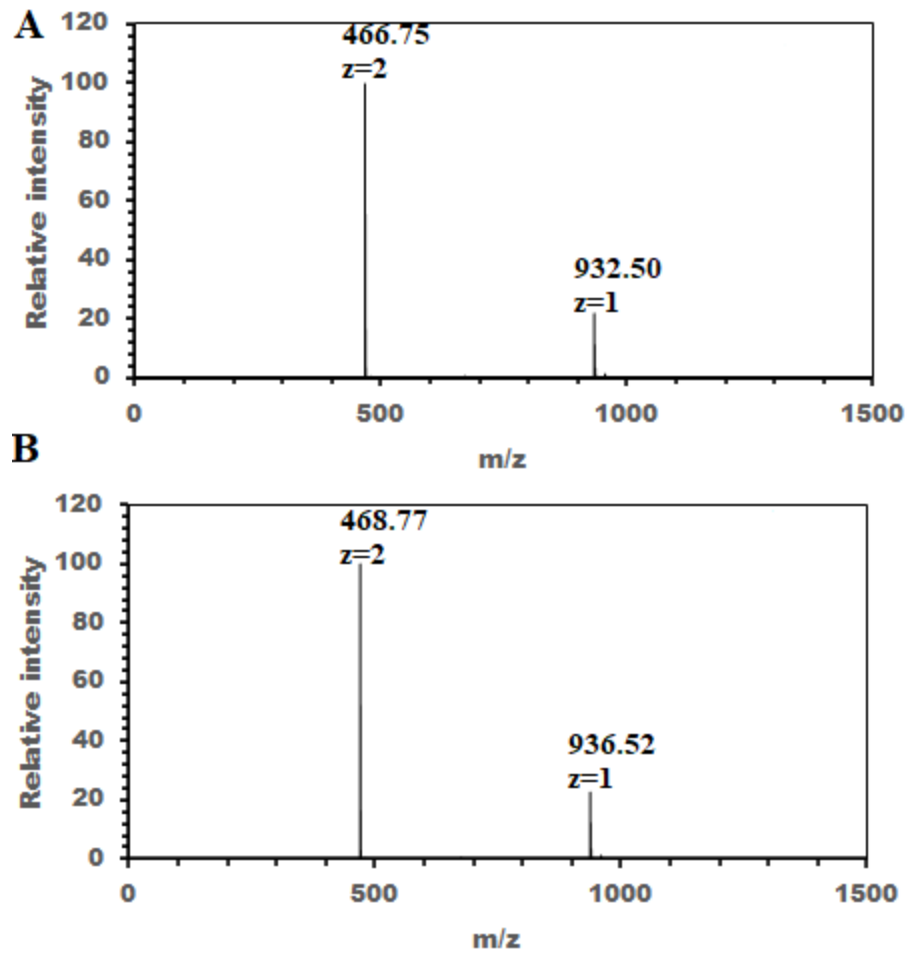


Figure S9. Full MS1 scans showing a successful generation of A) formaldehyde- and B) deuterio-derivatized des-R9-Brad with no side-products.

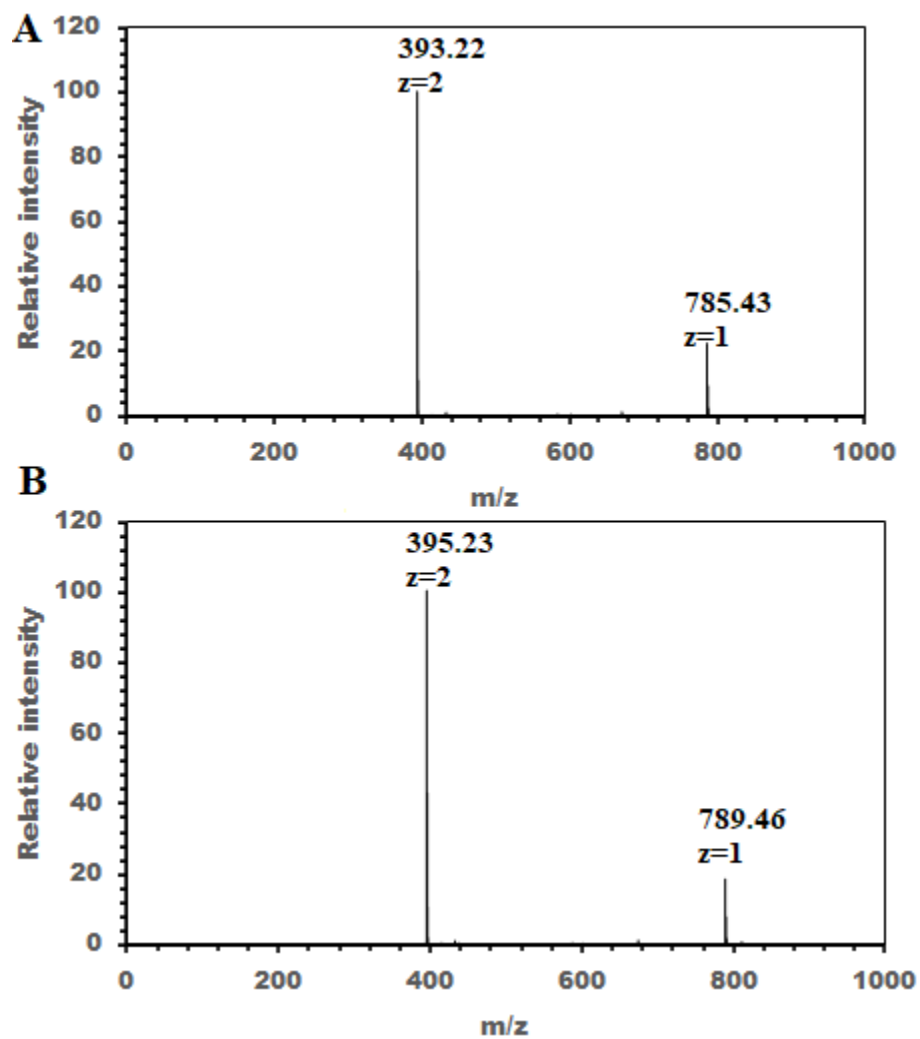


Figure S10. Full MS1 scans showing a successful generation of A) formaldehyde- and B) deuterio-derivatized Brad 1-7 with no side-products.

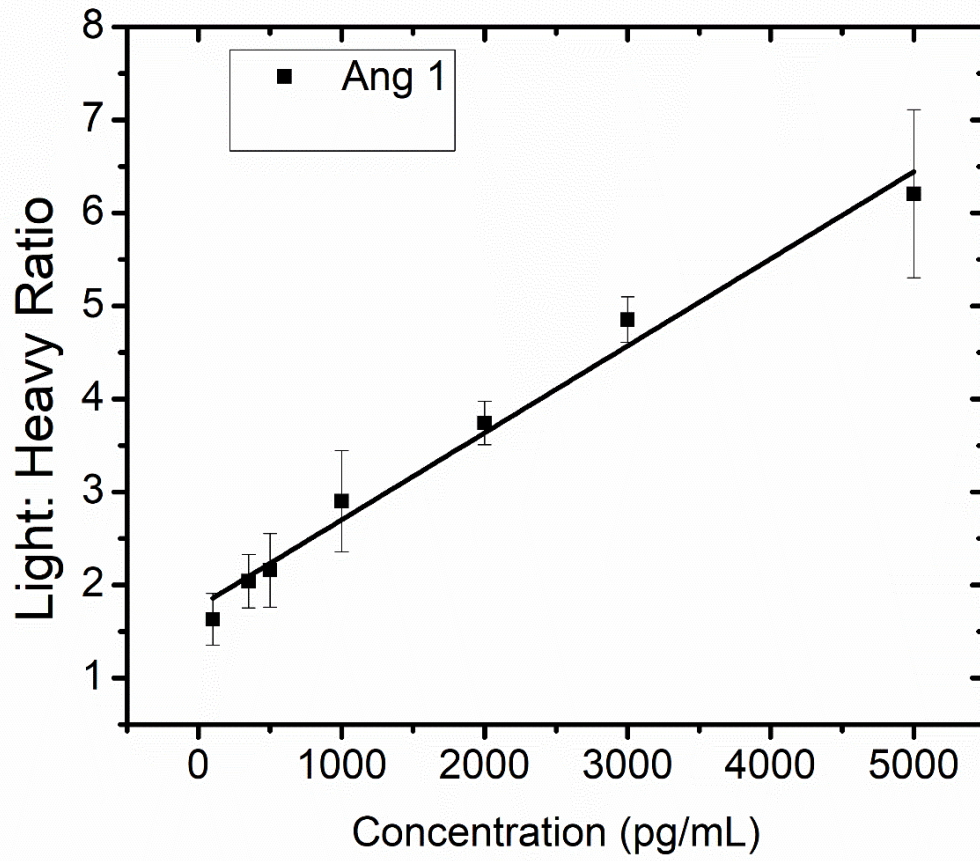


Figure S11. Ang 1 Calibration curve.

Table S2: Accuracy and precision of the method. Accuracy values are indicated for each analyte at the respective quality control (QC) levels.

Peptide	QC Level (pg/mL)	Accuracy		Precision	
		Intra-assay (%)	Inter- assay (%)	Intra- assay (% CV)	Inter- assay (% CV)
Ang 1	50	103.8	92.2	1.2	26
	350	101.8	90.0	7.2	7.4
	800	114.3	105.3	9.1	5.6
Ang 2	50	106.3	120.1	3.4	3.2
	350	103.0	111.8	2.5	1.5
	800	89.6	87.7	3.6	2.0
Ang 1-7	50	100.3	96.0	1.2	3.2
	350	103.9	107.8	11	2.0
	800	98.3	88.5	2.1	12.7
Ang 1-5	10	99.6	77.9	4.6	
	50	89.7	99.3	2.0	7.8
	350	103.5	109.2	9.0	5.7
	800	104.2	97.2	4.7	2.0
Brad	10	85.1	N/A	9.9	2.7
	50	93.1	90.5	3.8	4.2
	350	97.2	96.9	4.9	1.3
	800	85.6	81.5	1.4	5.3
Des-R9-Brad	10	83.4	113.3	13	18.1
	50	101.2	114.3	7.4	2.7
	350	92.3	86.1	6.7	1.7
	800	98.0	95.8	1.2	4.2
Brad 1-7	10	109	97.4	1.76	4.6
	50	95.2	97.8	13	1.5
	350	98.9	85.7	5.6	3.8
	800	101	102.2	7.7	2.9

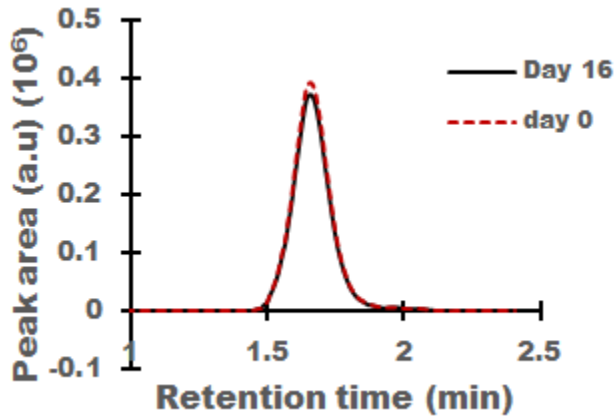
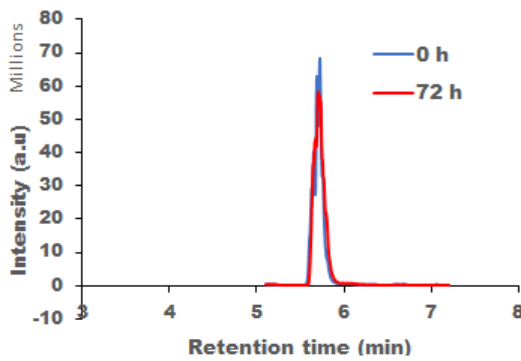


Figure S12. Stability of samples stored at -20 °C for 16 days. A representative signal of heavy-labeled Ang 1-7 in a freshly prepared sample (red peak) and a sample stored at -20 °C for 16 days (black peak).

A Light Ang 1 at 4 °C for 0 and 72 h



B Ang 1-7 at 4 °C for 0 and 72 h

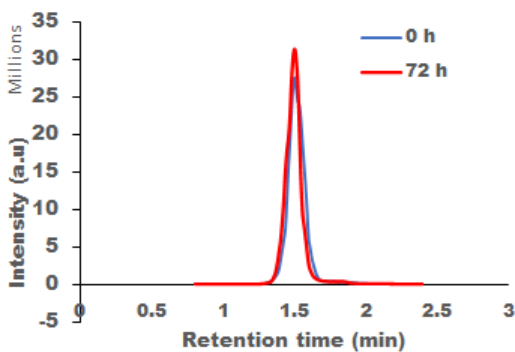


Figure S13. Stability of RAS peptides at the autosampler temperature of 4 °C. A) Light Ang 1 and B) light Ang 1-7 analyzed after 0 h (blue curve) and 72 h (red curve) of storage at 4 °C.

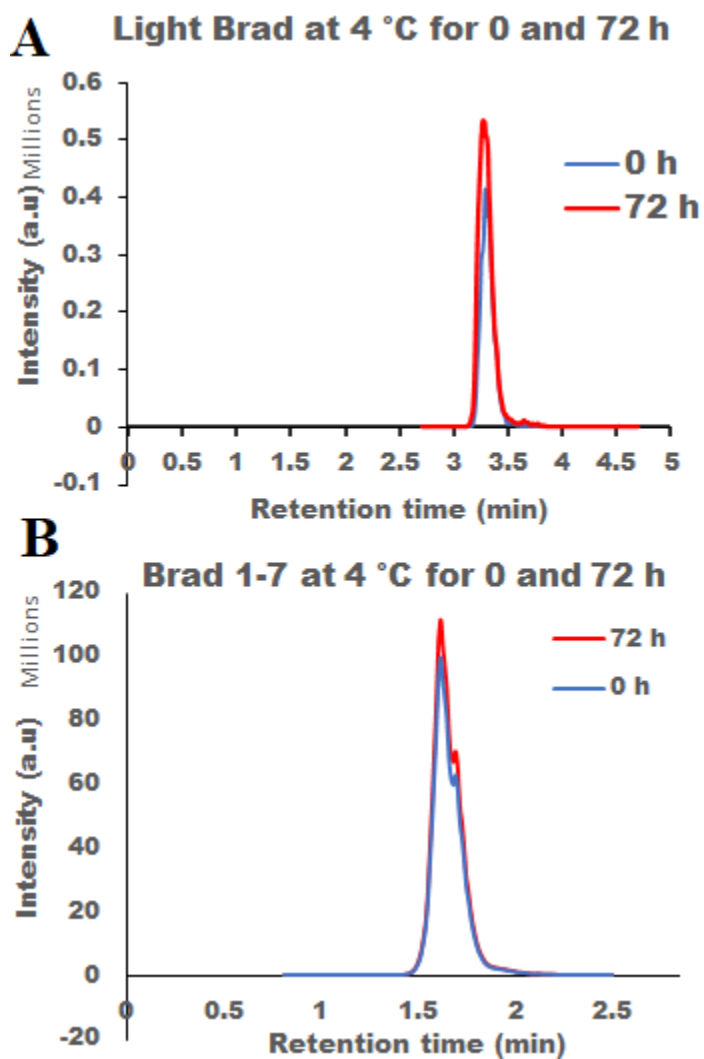


Figure S14. Stability of analytes at the autosampler temperature of 4 °C. A) Light Brad and B) light Brad 1-7 analyzed after 0 h (blue curve) and 72 h (red curve) of storage at 4 °C.