

Determination of Amino Acids in Feeds by Acquity AAA UPLC®

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- Non-essential amino acids (non-EAA) are those which can be synthesized by the body of the animal.
- Essential amino acids (EAA) are those which cannot be created from other compounds by the body because the carbon skeletons are not synthesized from non-EAA of animals and therefore must be added to feed and taken in by the animal.

<http://www.jasbsci.com/content/5/1/34>

- 11 EAA (cysteine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine, and valine) are classified as nutritionally essential and must be added to feeds in order to maintain physiological functions of cells, tissues, and the whole body.

Baker DH: Advances in protein-amino acid nutrition of poultry. *Amino Acids* 2009, 37:29-41

Overview



- Sulfur containing AA, methionine and cysteine must be pre-oxidized prior to hydrolysis.
- Cysteine is oxidized to cysteic acid and methionine to methionine sulfone to prevent partial destruction during acid hydrolysis.
- Pre-oxidation is done by the addition of 6 ml performic acid to ground sample and chilled at 1°C for 16 hours.
- Sodium metabisulfate is added post oxidation to decompose the remaining performic acid.

Methodology – Pre-oxidation of Sulfur AA



- All AA must be acid-hydrolyzed in order to cleave the peptide bonds which hold them together to form polypeptide chains
- This is performed by the addition of 30 ml 6N HCl containing 0.1% phenol. Samples are hydrolyzed on reflux condensers for 24 hours at 110-120°C
- Hydrolysates are then filtered and HCL is evaporated prior to derivatization and analysis on the Acquity AAA UPLC®

Methodology – Acid Hydrolysis



- This is a “canned” system that has been optimized by Waters.
Instrument parameters, columns, Waters mobile phases cannot be changed by the user.
- AA standard mix supplied by Waters is not working well as the retention times of amino acids in mix are quite different from the retention times seen in samples.
- Concentrations are in picomoles

Waters UPLC® Amino Acid Analysis



- LC System: Waters ACQUITY UPLC System
- Column: AccQ•Tag Ultra, 2.1 x 100 mm, 1.7 μ m
- Column Temp: 55°C
- Sample Temp: 20°C
- Flow Rate: 700 μ L/min.
- Mobile Phase A: 1:20 Dilution of AccQ•Tag Ultra Eluent A with water
- Mobile Phase B: AccQ•Tag Ultra Eluent AB
- Gradient: AccQ•Tag Ultra Hydrolysate Method (pre-programmed in Empower)
- Injection volume: 1 μ L
- Detector: UV (TUV), 260nm

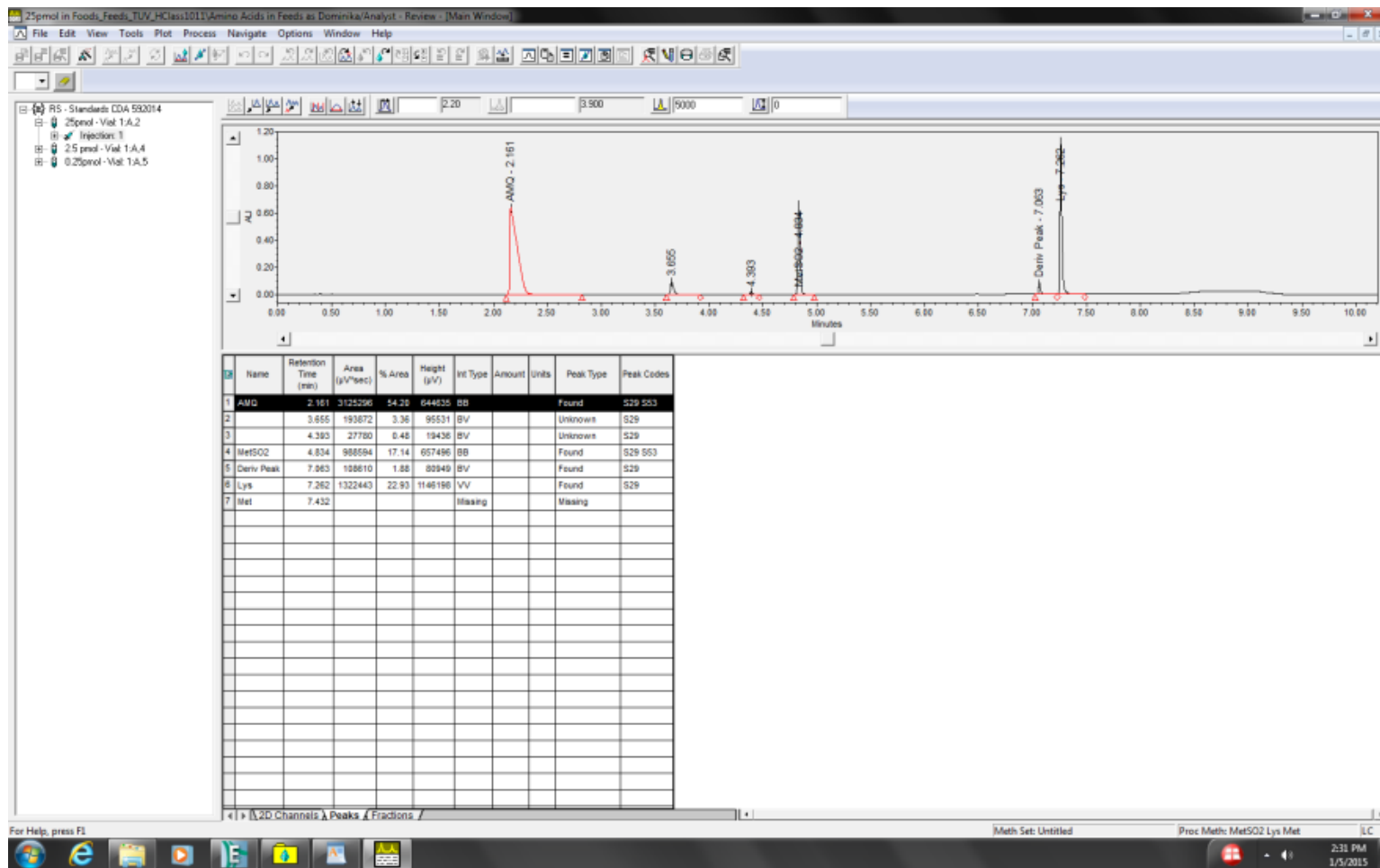
Chromatographic Conditions

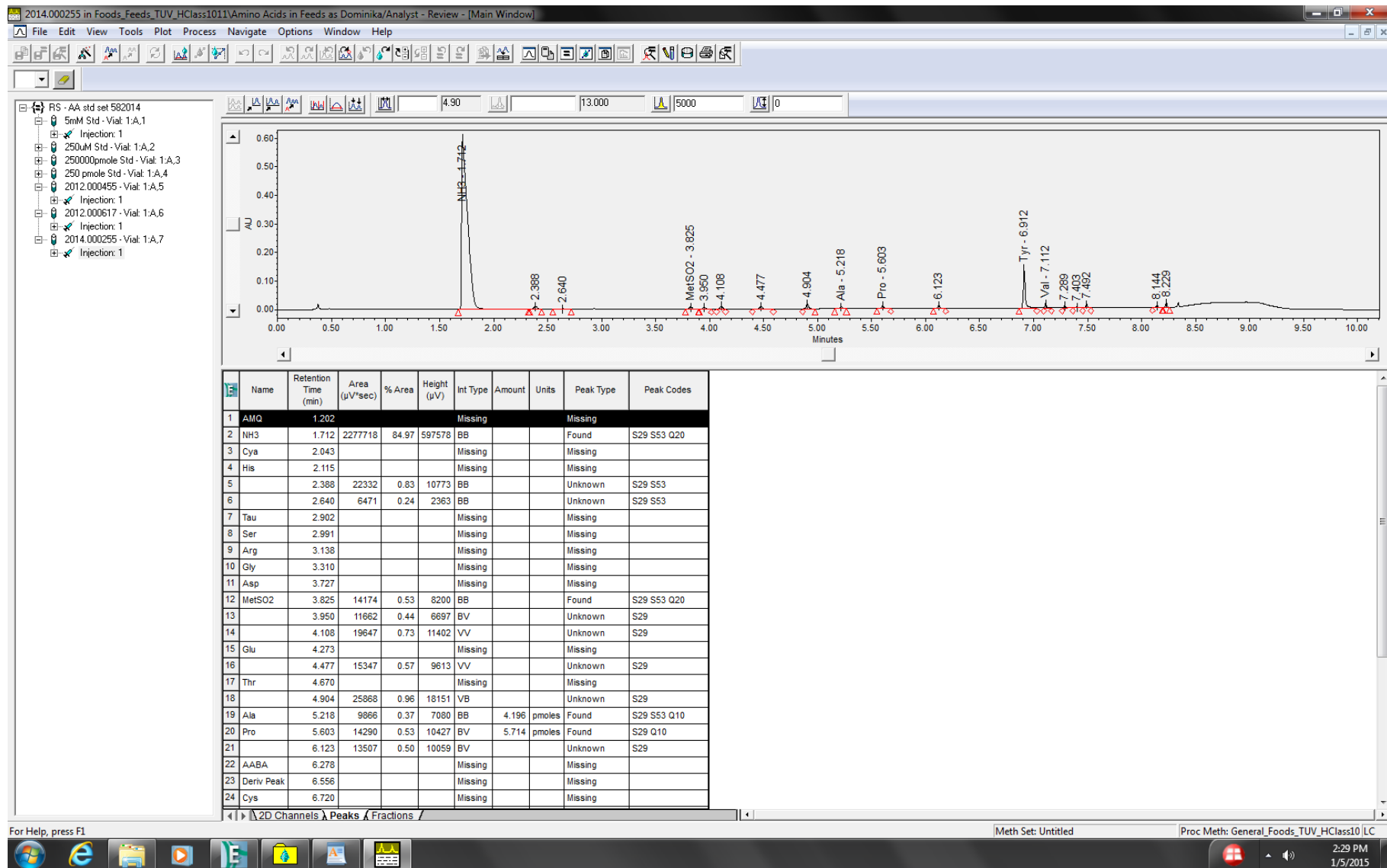


- Successfully hydrolyzed several samples and we are seeing methionine sulfone peak.
- Addition of norvaline as an internal standard to samples (post-hydrolysis) should help and is needed to measure peak response
- Try NIST 2386 reference standard mix and dilute to 5, 100 and 250 pmol/ul.
- Future work needed in correct peak identification and calculating response factor for individual AA.

Work completed
thus far and next
steps







- 2011 Apr;40(4):1159-68. doi: 10.1007/s00726-010-0740-y. Epub 2010 Sep 15. Composition of amino acids in feed ingredients for animal diets. [Li X¹](#), [Rezaei R](#), [Li P](#), [Wu G](#).
- *Journal of Animal Science and Biotechnology* 2014, 5:34 doi:10.1186/2049-1891-5-34. Dietary requirements of synthesizable amino acids by animals: a paradigm shift in protein nutrition. Guoyao Wu
- Waters Application Note. Application of the UPLC Amino Acid Analysis Solution to the Analysis of Animal Feed Hydrolysates. Hillary B. Hewitson, Thomas E. Wheat, Diane M. Diehl. Waters, September 2008.

References

