Comparison of Different Methods for Determination of Amino Acids Contents in Food and Feed

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Analytical Methods Used

- HPLC – ion exchange with ninhydrin post-column derivatization (3 labs)

- HPLC – ion exchange with o-phthaldialdehyde post-column derivatization (1 lab)

- HPLC – pre-column derivatization (ACCQ-Fluor Reagent Kit) with FLD (1 lab)

- UPLC – pre-column derivatization (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) (2 labs)

- LC/MS/MS – pre-column derivatization (iTRAQ™ reagent, an amine reactive compound) (2 labs).

Amino Acid Round Robin Design

- ## 15 amino acids:

  Alanine, arginine, aspartic acid, glycine, glutamic acid, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine and valine

- ## Seven samples:

  1 amino acid hydrolysate standard (NIST)

  1 peanut butter (NIST)
  5 DDGS hydrolysates (triplicate and 2 NIST-STD spiked hydrolysates)

- ## Performance Criteria:

  Precision (DDGS hydrolysate triplicate)

  Accuracy (amino acid hydrolysate standard from NIST, food hydrolysate from NIST, DDGS hydrolysate spiked with NIST standard)

Amino Acid Round Robin Design
(AOCS)

15 amino acids:

Alanine, arginine, aspartic acid, glycine, glutamic acid, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine and valine

Five samples:

soybean, soybean meal, swine diet, poultry diet and BSA

Test Methods:

GC/MS/MS (1 lab)

UPLC – pre-column derivatization (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) (2 labs)

Test Scheme:

Test each sample three times a week in 5 consecutive weeks
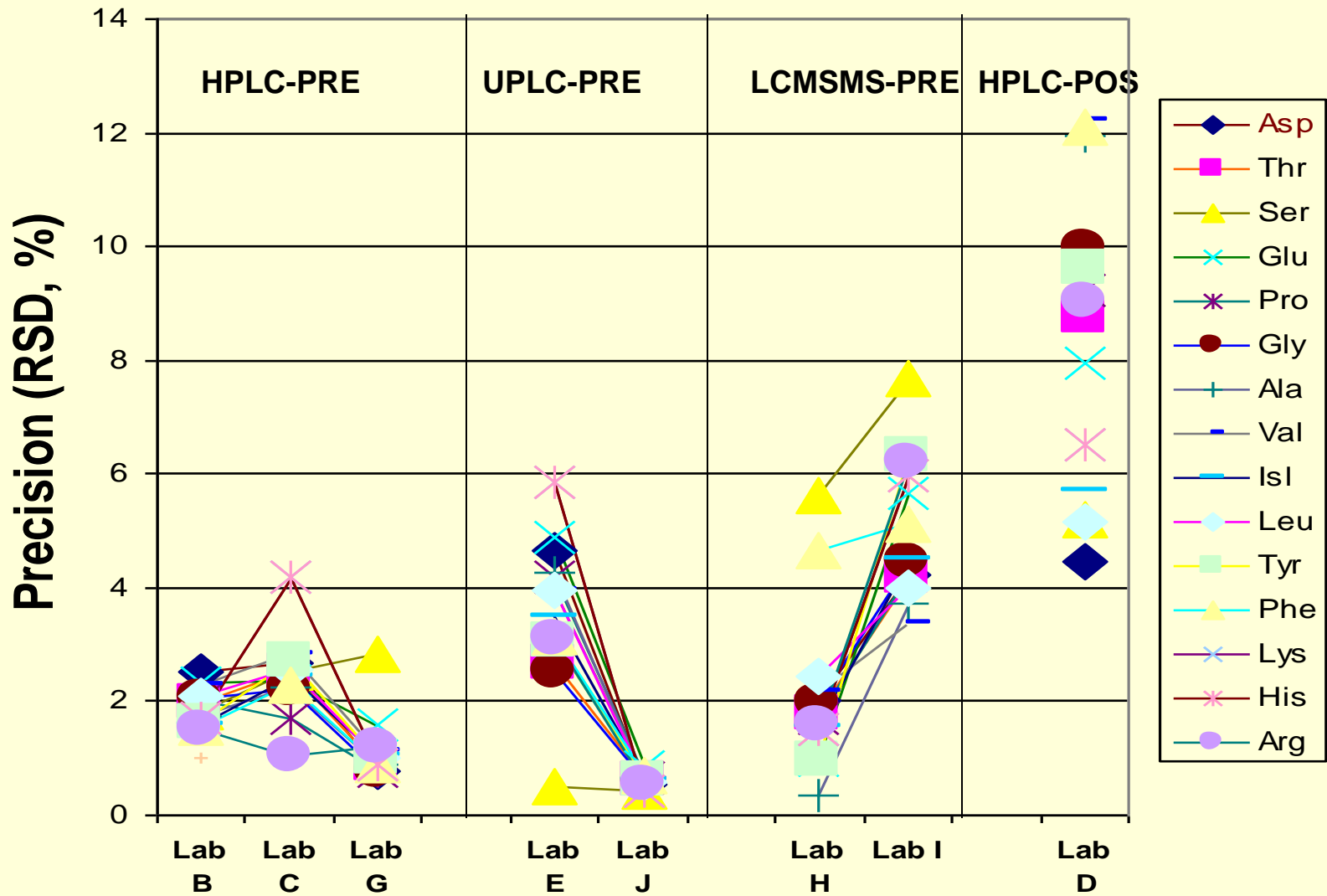
Data Evaluation

- Variation by individual methods
- Variation by individual amino acids
- Variation by individual labs
- Variation by sample matrix

Amino Acid Round Robin Studies

Precision

Precision (RSD, %)
## (Based on DDGS hydrolysate triplicate)

Precision (RSD, %)
(Based on DDGS hydrolysate triplicate)

Methods	Lab 1	Lab 2	Lab 3	Mean (of all labs)
HPLC Post–Column Deriv.	1.9* (0.3)	2.4 (0.7)	1.1 (0.5)	1.8 (0.7)
UPLC Pre-Column Deriv.	3.5 (1.3)	0.6 (0.1)		2.1 (2.1)
LC/MS/MS Pre-Column Deriv.	2.1 (1.4)	4.9 (1.2)		3.5 (2.0)
HPLC Pre-Column Deriv.	8			8

* Mean of 15 amino acids

Precision (RSD, %)
(Soy bean sample, tested in 5-week period, mean of 15 data points)
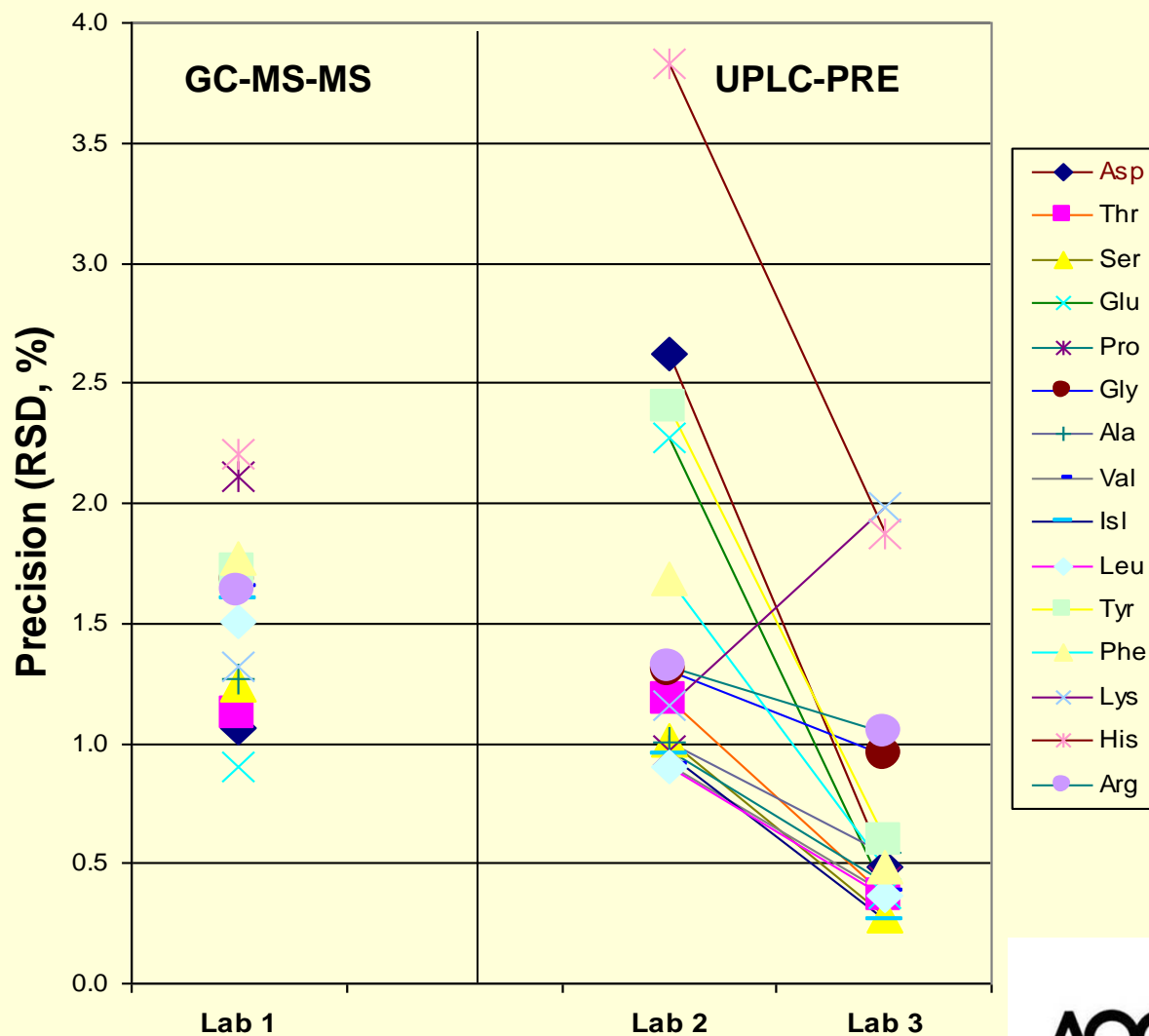
Precision (RSD, %)

(Soy bean sample, tested in 5-week period, mean of 15 data points)

Methods	Lab 1	Lab 2		Mean (of all labs)
GC-MS-MS	1.6* (0.5)			1.6 (0.5)
UPLC Pre-Column Deriv.	1.6 (0.9)	0.8 (0.7)		1.2 (0.5)

* Mean of 15 amino acids

Summary on Precision

- Precision is method dependent: HPLC post–column derivatization has the best performance (RSD < 2%) and HPLC pre-column derivatization has the highest RSD.

- Most methods investigated show satisfying precision for within batch and between batch runs.

- More data points needed to produce statistically sound conclusion.

Amino Acid Round Robin Study

Accuracy

Accuracy (Recovery, %)
(Based on NIST amino acid hydrolysate standard)

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(Based on NIST amino acid hydrolysate standard)

Methods	Lab 1	Lab 2	Lab 3	Mean (of all labs)
HPLC Post–Column Deriv.	98* (8)	104 (8)	105 (8)	102 (3)
UPLC Pre-Column Deriv.	96 (9)	105 (7)		100 (6)
LC/MS/MS Pre-Column Deriv.	93 (5)	107 (8)		100 (10)
HPLC Pre-Column Deriv.	105			105

*   Mean of 15 amino acids

Amino Acid Contents in DDGS Hydrolysate
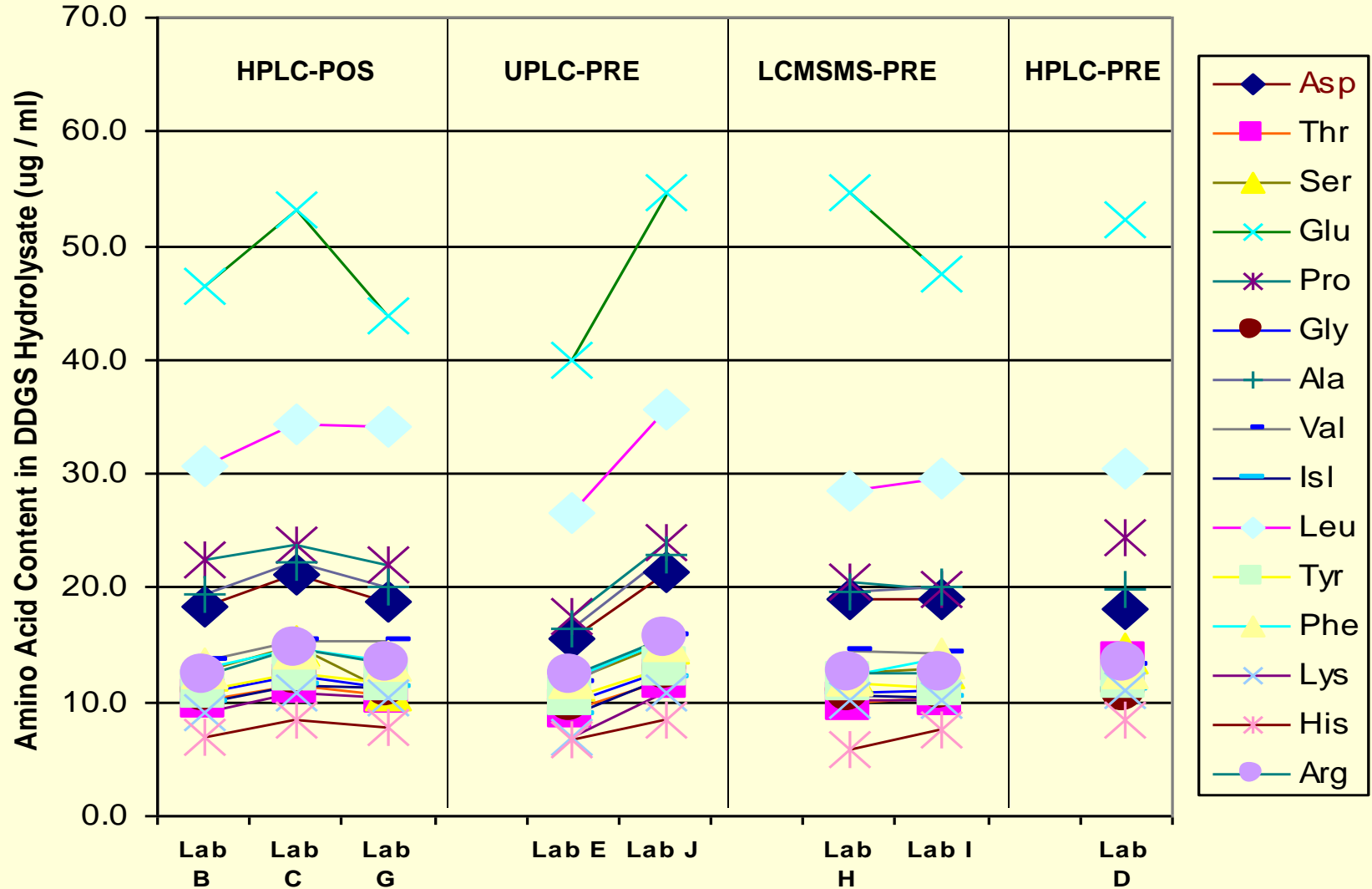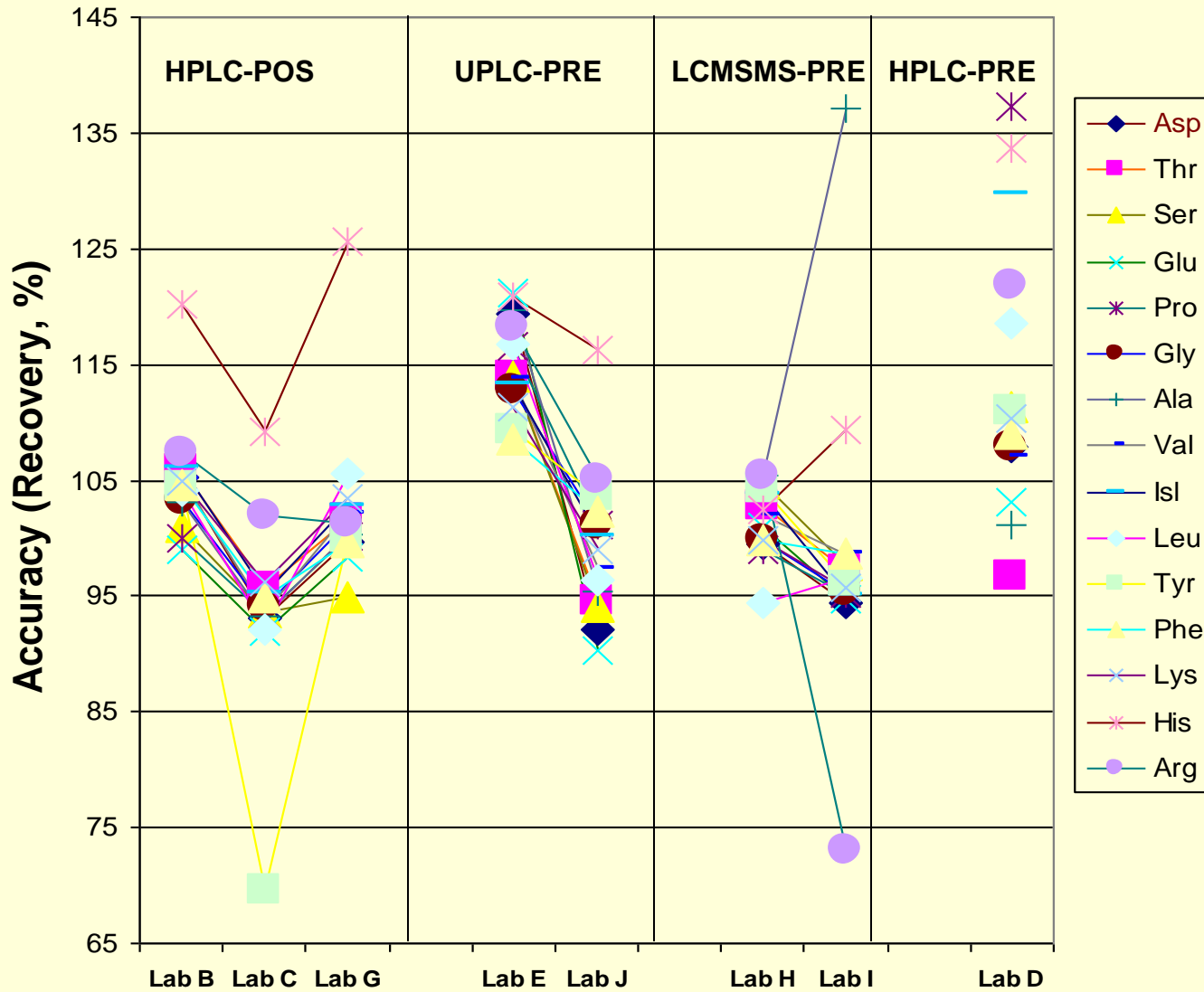
Total Amino Acid Contents in DDGS Hydrolysate
(ug / ml)

Methods	Lab 1	Lab 2	Lab 3	Mean (of all labs)
HPLC Post–Column Deriv.	247* (5)	282 (7)	256 (3)	261 (18)
UPLC Pre-Column Deriv.	216 (8)	289 (2)		253 (52)
LC/MS/MS Pre-Column Deriv.	253 (5)	250 (12)		252 (2)
HPLC Pre-Column Deriv.	266			266

*   Mean of triplicate

Accuracy (Recovery, %)
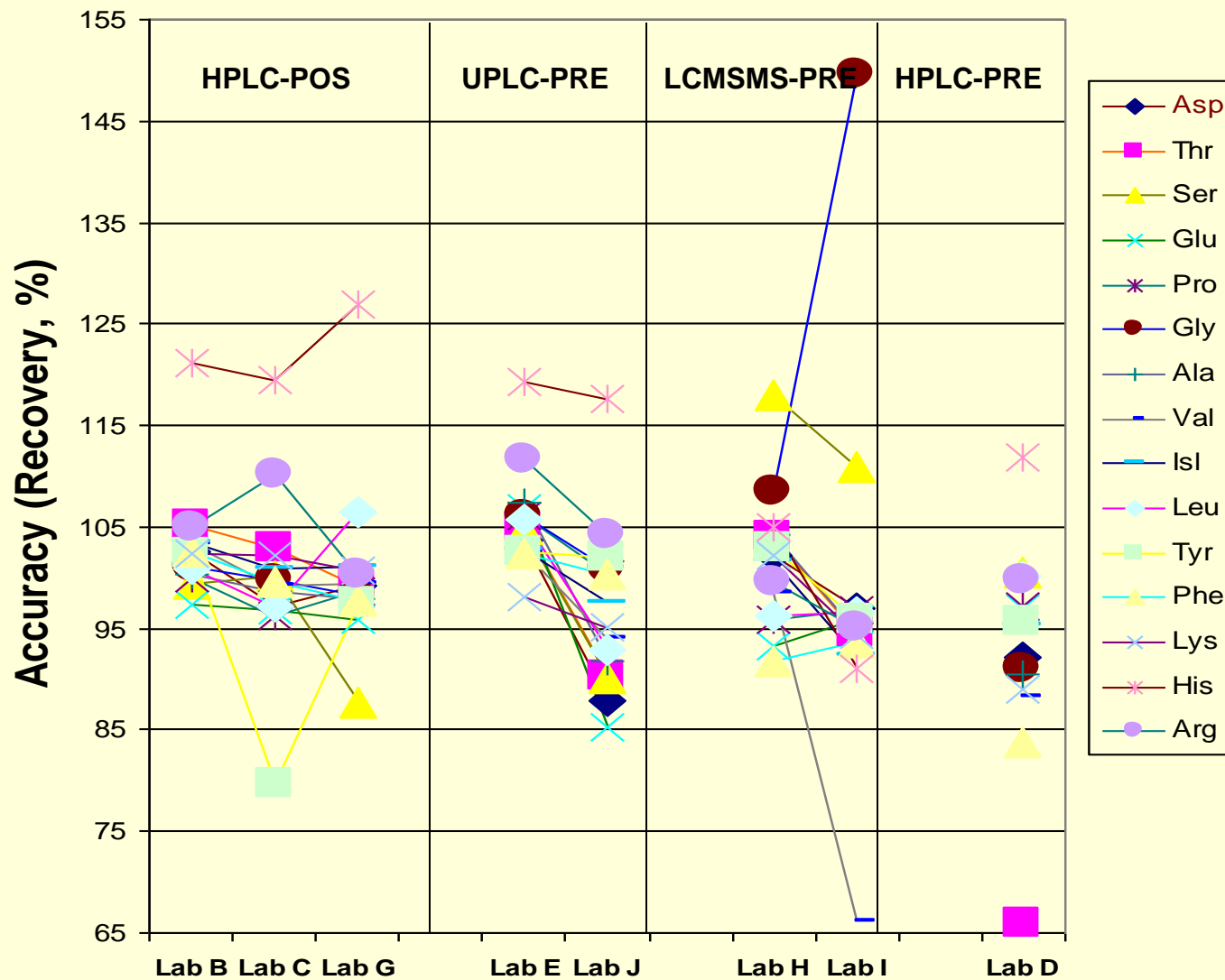## (DDGS hydrolysate spiked I)

Accuracy (Recovery, %)
(DDGS hydrolysate spiked I)

Methods	Lab 1	Lab 2	Lab 3	Mean (of all labs)
HPLC Post–Column Deriv.	105* (5)	94 (8)	102 (7)	101 (6)
UPLC Pre-Column Deriv.	115 (4)	99 (6)		107 (11)
LC/MS/MS Pre-Column Deriv.	102 (3)	98 (13)		100 (2)
HPLC Pre-Column Deriv.	114			114

* Mean of 15 amino acids

Accuracy (Recovery, %)
(DDGS hydrolysate spiked II)

Accuracy (Recovery, %)
(DDGS hydrolysate spiked II)

Methods	Lab 1	Lab 2	Lab 3	Mean (of all labs)
HPLC Post–Column Deriv.	103* (5)	100 (8)	100 (8)	101 (2)
UPLC Pre-Column Deriv.	106 (5)	97 (8)		101 (6)
LC/MS/MS Pre-Column Deriv.	102 (6)	98 (17)		100 (3)
HPLC Pre-Column Deriv.	93			93

*   Mean of 15 amino acids

Summary on Accuracy

(in testing hydrolysate)

- The three methods: HPLC post–column, UPLC and LC/MS/MS give similar performance, which have 100% recovery for most amino acids, with the HPLC post–column method being the most consistent.

- Except for LC/MS/MS, all the other methods have higher than 120% recovery for histidine.

- More data points needed to produce statistically sound conclusion.

Amino Acid Round Robin Studies

(Based on food and feed samples)

Accuracy (Recovery, %)
(Peanut Butter Standard, NIST)

Record of Accuracy

(Recovery, %, based on NIST Peanut Butter)

Methods	Lab 1	Lab 2	Lab 3	Mean (of all labs)
HPLC Post–Column Deriv.	105 * (8)	114 (17)	106 (12)	108 (5)
UPLC Pre-Column Deriv.	98 (8)	120 (7)		98 (16)
LC/MS/MS Pre-Column Deriv.	101 (9)	99 (6)		100 (1)
HPLC Pre-Column Deriv.	102			102

*   Mean of 15 amino acids

Amino Acid Contents in Soybean
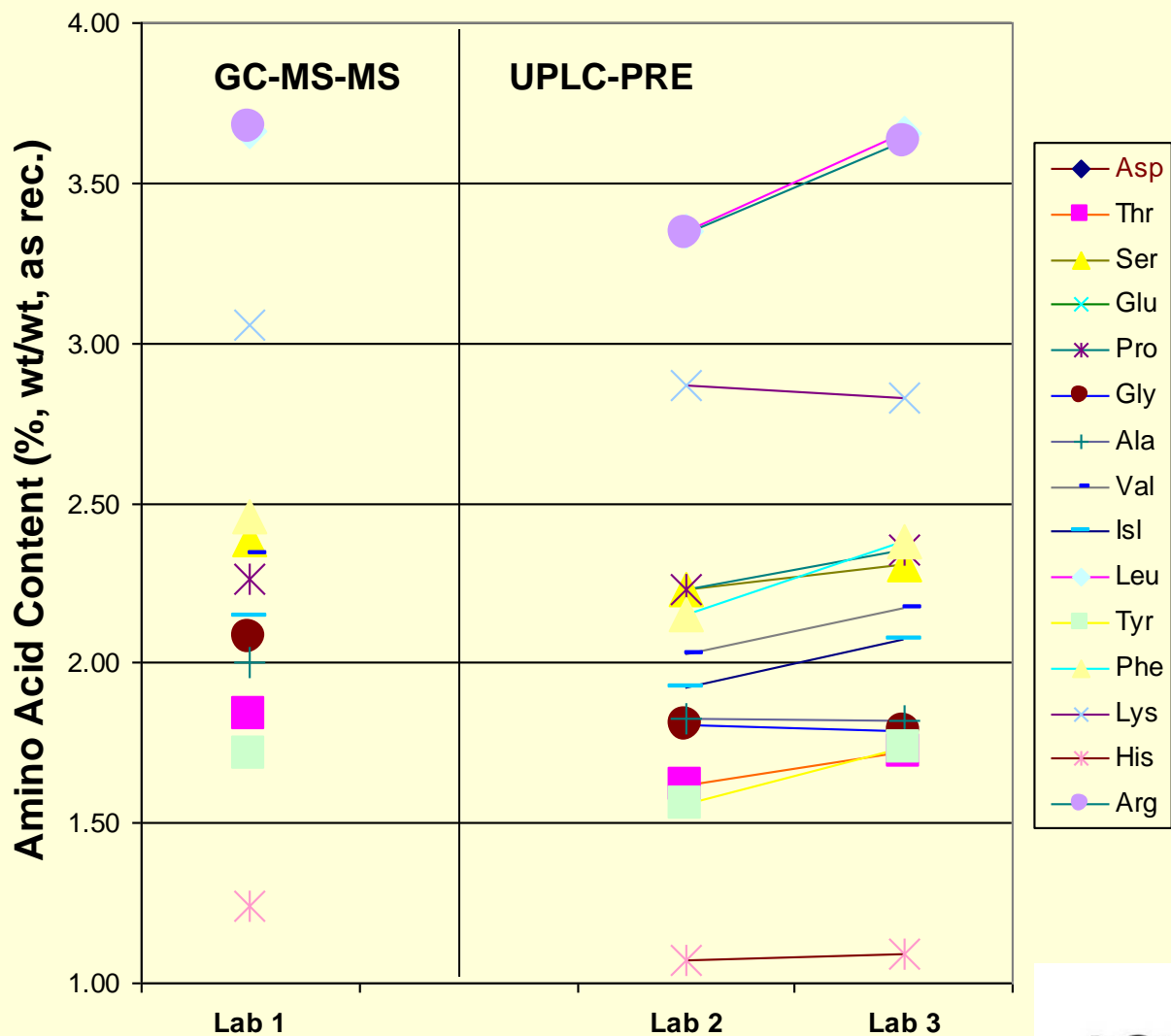
Total Amino Acid Contents in Soy Bean (wt/wt, %, as-rec.)

Methods	Lab 1	Lab 2		Mean (of all labs)
GC-MS-MS	45.6* (0.5)			45.6
UPLC Pre-Column Deriv.	41.7 (0.5)	45.6 (0.2)		43.6 (2.7)

*   Mean of 15 runs

Comparison on other Factors

Methods	Run Time (minutes)	Price ($)
HPLC **Post–Column Deriv.**	60	180
UPLC **Pre-Column Deriv.**	10	100
LC/MS/MS **Pre-Column Deriv.**	24	200
HPLC **Pre-Column Deriv.**	40	250

Summary on Accuracy in testing food and feed

- The three methods: HPLC post–column, UPLC and LC/MS/MS give similar performance, which have 100% recovery for most amino acids, with the HPLC post–column method being the most consistent.

- More data points needed to produce statistically sound conclusion.