

# IMPROVING GC-MS QUANTITATIVE ANALYSIS OF FREE AMINOACIDS BY PTV INJECTION AND DYNAMIC BACKGROUND COMPENSATION ON THE ACQUIRED CHROMATOGRAMS

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The analysis of free amino acids (FAA) by GC-MS has gained interest since the introduction of the EZFAAST™ procedure which implements a cation exchange micro spe-extraction and purification followed by chloroformate derivatization of both carboxylic and amidic groups. The major limitation of this procedure is the narrow linear quantitation range (5–20 nmoles of each AA) which very often force the analysts to repeat the analyses with two different aliquots of sample extracts, (a small one for the most concentrated and a larger one for the least concentrated FAA) given the large difference in the concentration of some FAA in true sample. This was verified, in samples of different origin, from human plasma to green plant extracts, where the amino acids involved in the pristine steps of nitrogen cycle generally exceed all the others by 10–20 fold especially following water stress conditions, or in ripening of lard or other fat meat products.

*Is it possible to extend the linear calibration over a wider range and speed up some steps in the EZFAAST™ procedure?*

## Experimental

### Injection conditions:

Regular EZFAAST™ procedure: Split/splitless injector, split ratio 1:10 injection volume 2 µl with almost complete solvent change after derivatization (last preparation step in EZFAAST™ procedure)

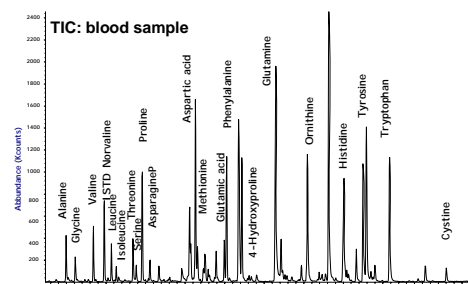
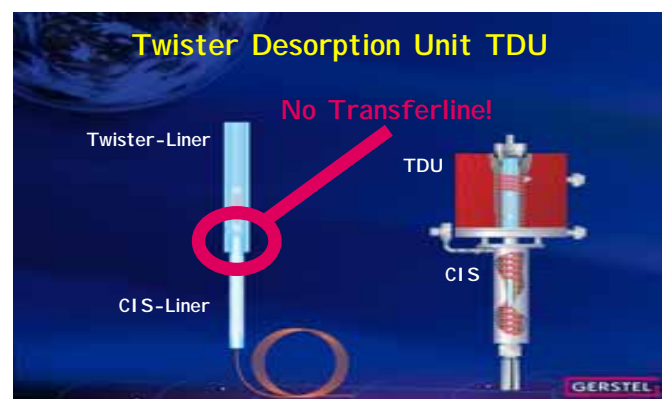
Modified procedure: Gerstel CIS4 liquid CO<sub>2</sub>-cooled injector, 50°C standby temperature, solvent vent mode, 25 ml min<sup>-1</sup> flow, then 12°C min to 300°C, hold time 3 min.

Gerstel TDU: 300 °C for 5 min, splitless mode, direct injection of the derivatization solution by means of

"liquid adapter" TDU tube holders

Dynamic background compensation algorithm (ALMSO Clear view™) on acquired chromatograms before data analysis

All others conditions were the same as for EZFAAST™ procedure.

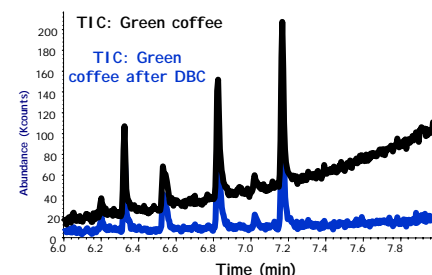
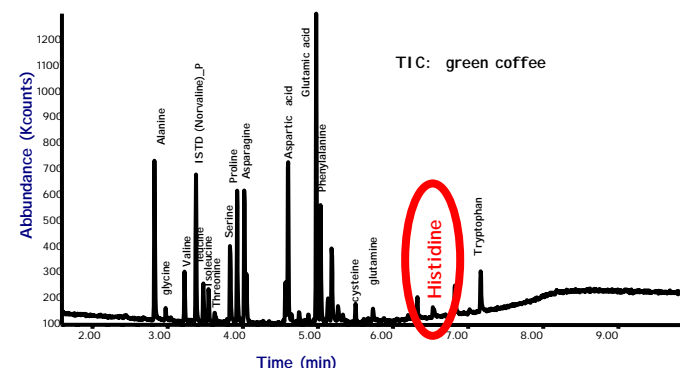


Amino acid	retention time	target ion	response factor	amount (nmoles)
Alanine	3.498	130	2161533	29.08
Glycine	3.614	116	951450	16.78
Valine	3.843	158	1246907	18.24
Leucine	4.071	172	1252004	8.49
Isoleucine	4.132	172	358986	4.05
Threonine	4.343	101	993529	5.95
Serine	4.385	146	267702	6.92
Proline	4.459	156	3701555	16.47
Asparagine	4.558	155	448794	4.19
Aspartic acid	5.131	216	235850	0.76
Methionine	5.161	203	292554	2.15
Glutamine	6.147	84	8409437	42.85
4-Hydroxyproline	5.498	172	159176	2.61
Glutamic acid	5.499	230	737587	4.74
Phenylalanine	5.527	148	1289044	4.49
Ornithine	6.55	156	4068515	6.65
Lysine	5.498	170	307926	1.16
Histidine	7.01	282	1018202	7.19

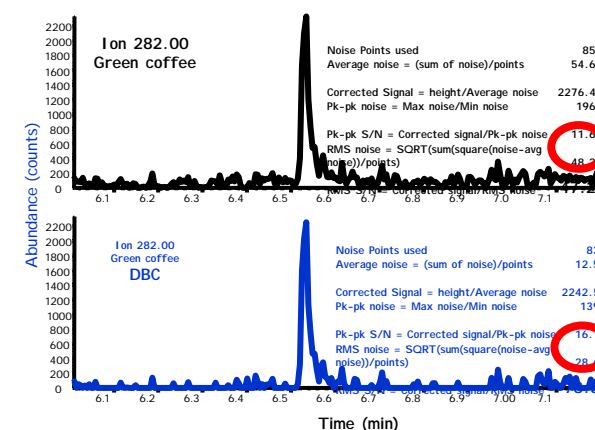
A large variability was recorded in the concentration of different amino acids even in blood samples.

## Results

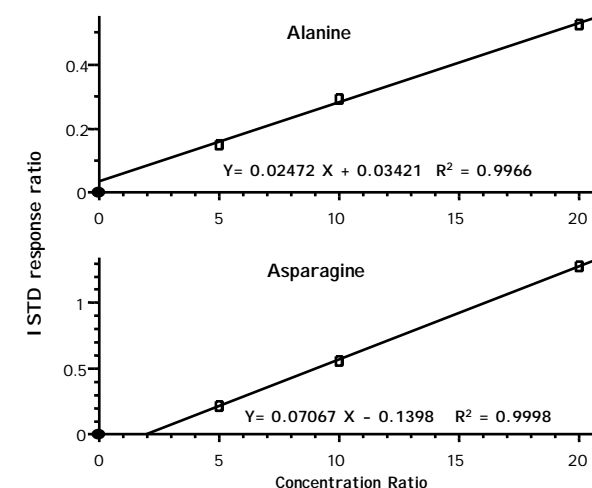
Least concentrated amino acids can be conveniently quantified in complex matrices along with the most concentrated ones after Clear view™ DBC which improves the S/N ratio



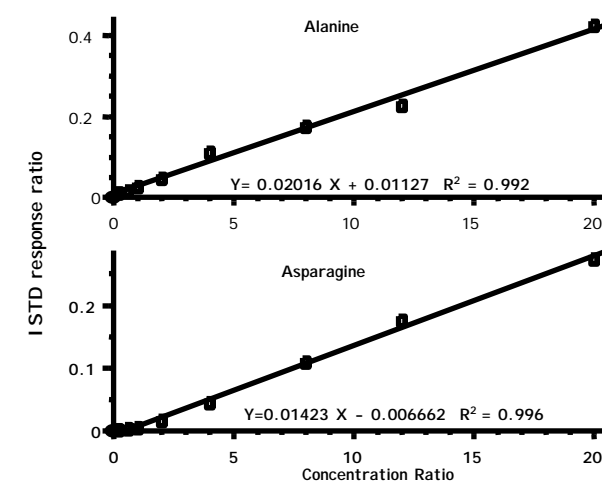
After DBC the baseline is improved....



With a better Signal/Noise ratio also for the specific ion



Calibration curves after the regular EZFAAST™ procedure are linear in the 5–20 nmoles range.



Calibration curves with the modified injection procedure are linear from 0.2 to 20 nmoles

## Conclusions

- ✓ An improved linear calibration range and speed up of the EZFAAST™ procedure was obtained using a CIS 4 injector.
- ✓ The ALMSO Clear view™ algorithm resulted in an improved Signal/Noise ratio which allows an unbiased AA quantitation at low concentration in the EZFAAST™ procedure.
- ✓ A further improvement could be obtained by the use of large volume injection with CIS 4 applying an appropriate initial solvent venting.