

## Introduction



Figure 1 – Anatune Amino Acid Solution comprising GERSTEL Dual Head Multi Purpose Sampler with GERSTEL Multi-position Vortexer (mVORX), Anatune Robotic Centrifuge (CF-200) and SmartSPE™ (ITSP Solutions Inc) options

Amino Acids are both key metabolites in primary metabolism and the structural building blocks of proteins.

Robust and reproducible, routine amino acid analyses are important across a range of sectors including crop breeding, agri-food, diagnostic medicine, biomedical and epidemiological (nutrigenomic) studies, routine food testing (labelling and regulatory compliance), industrial biotechnology, synthetic biology and protein/peptide characterisation/quality control.

Current approaches to amino acid analysis (HPLC or ion chromatography with pre-/post- column derivatization) have a range of limitations including variable recoveries and poor precision (matrix effects); long analysis times, limited throughput and slow turnaround times. Furthermore, many of the methods have further performance limitations in one of more areas (sample stability, resolution, dynamic range, and detection limits).

Building on initial proof-of-concept work, we have developed a fully automated end-to-end solution for amino acid analysis by GC-FID/GC-MS, using SmartSPE™, that has the potential for further development to include an LC-MS/MS platform.

The solution provides accurate and precise results and high recoveries of up to 50 amino acids and related compounds in a wide range of sample matrices (soils, plant & food extracts, beverages, bio-fluids and fermentation broths). Sample throughput is up to 72 samples per day, delivering increased capacity and faster turnaround times than other amino acid analysis platforms.

## Experimental

The extraction of amino acids by solid phase extraction with subsequent derivatization to form the alkyl chloroformates is a widely used metabolomics protocol for the manual sample preparation of amino acids and biogenic amines.

Automation of the derivatization has previously been done, as a proof-of-concept study, by Dr Katja Dettmer<sup>1,2</sup>.

The extraction, isolation and derivatization of amino acids was fully automated using a GERSTEL Multi Purpose Sampler.

Amino acids (and biogenic amines) were extracted from 10 mg samples (whole-flour, gluten free flour, sports supplements and ground cashew nuts) with 20% propanol in water. The samples were centrifuged and basic metabolites isolated from the supernatant by SmartSPE™ (ITSP Solutions) with a custom (SCX) packing. Acidic and neutral components were removed by washing with 20% n-propanol, and basic metabolites were eluted with an eluting medium.

A two-step derivatization was then done and the derivatives formed were extracted, by automated liquid-liquid extraction, using the GERSTEL MultiPosition Vortexer (mVORX) and Anatune Robotic Centrifuge (CF-200). Amino acids were analysed by GC-MS using an Agilent 7890A/5975C GC-MSD.



Figure 2 – SmartSPE™ Instrument Top Sample Preparation micro-scale (μSPE) solid phase extraction cartridge and 96-well format tray

## Results

Full automation of amino acid extraction, purification/isolation and derivatization, was done using the GERSTEL Multi Purpose Sampler with mVORX, CF-200 and SmartSPE™ modules.

Good linearity (Figure 3) was obtained for all analytes in the calibration mixture and precision (% RSD) was comparable to the reported values for manual sample preparation.

Initial recovery data (not shown) demonstrated that recoveries from the SmartSPE™ step were between 90-110% however, as the reaction volumes were not matched this work needs to be repeated.

<sup>3</sup>Precision data obtained from the samples tested were comparable with those obtained from amino acid standard mixtures demonstrating that there were no significant matrix effects on precision.

We will shortly be collaborating Curtis Analytics Ltd to do a larger scale comparison of manual and automated amino acid analysis in plant samples.

Analyte	R <sup>2</sup>	% RSD
Sarcosine	0.997	7
Alanine	0.996	3
Glycine	0.990	4
α-amino-butyric acid	0.997	3
Valine	0.998	4
β-alanine	0.996	3
β-amino-iso-butyric acid	0.994	3
Leucine	0.996	5
Isoleucine	0.999	7
Proline	0.998	3
Threonine	0.981	6
Serine	0.968	4
Asparagine	0.990	7
Methionine	0.994	18
Aspartic acid	0.998	19
Hydroxyproline	0.983	6
Phenylalanine	0.985	2
Glutamic acid	0.987	10
2-amino-adipic acid	0.976	15
Tyrosine	0.984	10
Tryptophan	0.958	13

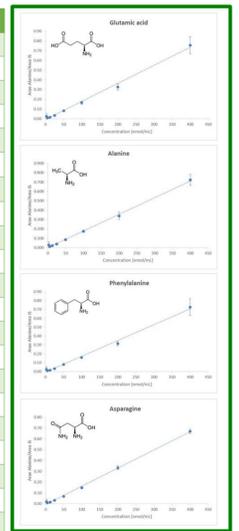
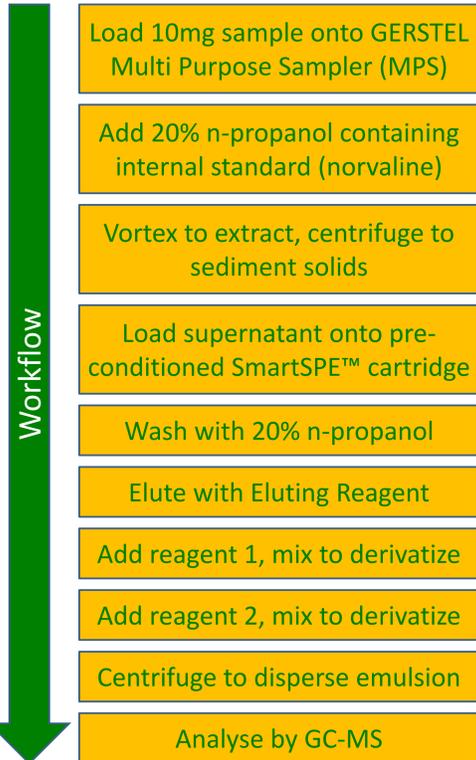


Figure 3 – Summary statistics for calibration standards (R<sup>2</sup> and % RSD). Tabulated values obtained from mid-level calibration standards (n = 3) Calibration curves for amino acids with a range of side chain functional groups (acidic, basic, aliphatic & aromatic)



## Conclusions

A fully automated amino acid solution for the extraction, isolation, derivatization and analysis of amino acids has been developed that:

- Is suitable for the analysis of amino acids in both physiological samples and protein hydrolysates by GC-FID or GC-MS.
- Delivers equivalent/improved performance (Linearity, precision, recoveries and sample throughput (72 samples per day) compared to the manual protocol.
- is highly matrix tolerant and unaffected by the presence of folates, lipid, protein, starch and sample pH.
- is scalable from trace (e.g., capillary micro-sampling or small mammal studies) to bulk (e.g., nutritional composition) sample analysis.

The solution is currently available both as an offline PrepStation and an online solution with fully automated, just-in-time, sample preparation.

With online solutions, sample prep time matches the (rate limiting) GC cycle time for maximum productivity.

The derivatives may also be analysed by LC-MS/MS, extending the range of metabolites that can be determined.

**The amino acid solution is available from Anatune in the UK and Ireland, and worldwide through GERSTEL, its subsidiaries and distributors**

### References:

1. Dettmer, K Fully Automated GC-MS Determination of Amino Acids, GERSTEL Solutions Worldwide 10 (2010) 18-19
2. Kaspar et al. Automated GC-MS Analysis of Free amino acids in Biological Fluids, Journal of Chromatography B, 870 (2008), 222-232
3. Liscio, C & Hawkins N., A fully automated offline solution for the analysis of amino acids by GC-MS. Anatune Chromatography Technical Note, AS175 (2017)

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Anatune Ltd, Unit 4, Wellbrook Court, Girton Road, Cambridge, CB3 0NA, UK

Tel: +44 (0) 1223 279210 Fax: +44 (0) 1223 279253 Email: [info@anatune.co.uk](mailto:info@anatune.co.uk) Internet: [www.anatune.co.uk](http://www.anatune.co.uk)

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