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A fully automated offline solution for the analysis of amino acids by GC-MS

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Introduction

Amino acids are both key metabolites in primary metabolism and the structural building blocks of proteins. With a diverse range of structures and properties, routine amino acid analysis in a range of sectors including:

- Diagnosis & management of metabolic diseases
- Biomedical research & epidemiological studies in animal/ human health & nutrition (e.g., branched chain amino acids are predictive biomarkers of metabolic syndrome)
- Routine food testing for compliance with regulations or food labelling requirements (e.g. histamine, collagen, protein, aspartame/phenylalanine, protein content)
  Large scale field/breeding trials for plant quality or nutritional traits
- Food & agricultural science: studying flavour formation during cooking; managing/predicting acrylamide formation potential of raw materials (grain or potatoes) for baked or fried products.
- Metabolic flux analysis (fluxomics) in biochemical studies
- Fermentation reaction monitoring (biopharma, industrial biotechnology, synthetic biology)
- Peptide/protein characterisation & QC

Amino acid analysis is usually done using Ion Exchange Chromatography (IEC) and High Performance Liquid Chromatography (HPLC) techniques, with post-column or pre-column derivatization respectively. For certain sample types, ion chromatography with electrochemical detection or GC-MS may also be used.

Clearly there are lots of options for amino acid analysis however, each approach has its limitations. The nature of the matrix can significantly affect pre-column derivatization reactions or separations of underivatized amino acids, requiring extensive sample preparation. In addition, post-column derivatization methods, require long analysis times, limiting sample throughput.

Many of the methods also have performance limitations in one or more areas (sample stability, resolution, dynamic range, precision and detection limits) and all of these approaches involve the use of labour intensive, complex, manual sample preparation protocols that are intolerant of even small changes to matrix composition.

The work in this application note builds on some proof-of-concept work done by Dr Katja Dettmer[1,2] to develop a fully automated offline solution for amino acid analysis by GC-MS.

The alkyl choroformate derivatization used benefits from being fast, robust and matrix tolerant. The method can be applied to a range of aqueous samples including soil, plant & food extracts, beverages, plasma, urine, cell culture/broths and protein hydrolysates; without the need for either sample clean-up or urea/protein removal.

The derivatives are analytically versatile and (depending on the application, target metabolites and required detection limits), analysis can be done by GC-FID, GC-MS or potentially LC-MS/MS. The EI spectra of these derivatives are particularly valuable in 15N metabolic flux studies.

The automation of on-line SPE employs SmartSPE™ (Figure 1) single-use miniaturised cartridges from ITSP Solutions, packed with a proprietary sorbent. The small scale of the SmartSPE™ format makes it compatible with fully automated online sample preparation and trace level amino acid determination in limited volume samples (e.g., dried blood spots or rodent plasma).

Figure 1: Instrument Top Sample Prep (ITSP) miniaturised cartridges for smart SPE and 96 well plate format ITSP tray.

The most unique aspect of SmartSPE™ is the ability to precisely control flows and achieve high levels of chromatographic performance. In contrast to manual, larger volume SPE, SmartSPE™ flow profiles follow the expected Van Deemter curves with clearly defined optima. As an result, absolute recoveries of >99% can be achieved with high precision and effective background matrix removal.

Instrumentation

The fully automated workflow for the extraction and alkyl chloroformate derivatisation of amino acids was developed on a GERSTEL Dual Head Multipurpose Sampler (MPS2-xt) (Figure 2) equipped with the following objects:

- 4 x VT54-2 mL Vial Trays
- 1 x VT98-2mL Vial Tray
- 1 x VT32-10mL Vial Tray
- Standard Large Wash Station (2 x 100 mL wash and waste)
- SmartSPE™ kit from ITSP Solutions
- GERSTEL Multiposition Vortexer (mVORX)
- Anatune CF-200 Robotic Centrifuge
- SmartSPE™ Custom Cartridges Cation Exchange

GC-MS analysis was performed using the Agilent 7890B Gas Chromatograph coupled to an Agilent 5975C MSD.

GC-MS conditions

GC:

- Column: Agilent DB1701 15 m x 0.25mm x 0.25µm
- Injection mode: Splitless
- GC ramp: 75 °C for 2 min, 16.3 °C/min to 280 °C, held for 5.5 min

MS:

- Inert EI source
- Scan/SIM mode

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Figure 2: GERSTEL Dual Head MPS for the extraction and derivatisation of amino acids for GC-MS analysis

Methods

Optimised automated sample preparation and spiking:

Approximately 10 mg of sample (flour, gound cashew nut, amino acids supplement powder) was transferred manually into 2 mL glass vials with magnetic screw cap.

The sample was loaded onto the GERSTEL Multipurpose Sampler and from this point the sample extraction, Amino Acid purification and derivatization was fully automated within a GERSTEL Maestro PrepSequence.

The sample was extracted by Vortexing (GERSTEL mVORX) with 20% n-propanol (containing norvaline as an internal standard) in deionized water. The MPS then transferred the sample to the Anatune robotic centrifuge CF-200 and samples were centrifuged at 4500 rpm to obtain a clear supernatant.

Full procedural blanks (with ISTD) and calibration standards (2.5-400 nmol/mL) were automatically prepared using the GERSTEL Multipurpose sampler and extracted in an identical manner to the samples.

Optimised automated solid phase sample extraction and derivatisation:

A portion of the supernatant was removed from the vial and transferred to a Ion Exchange SmartSPE™ cartridge (preconditioned with 20% n-propanol in water).

The cartridge was eluted to waste and washed with 20% n-propanol in water.

The retained basic fraction (containing amino acids and biogenic amines) was then eluted with Eluting mixture into a clean high recovery vial and the the eluate was transferred to a capped vial for derivatization.

A two-step derivatization was done with derivatization reagent mixtures to form the chlofororomate derivatives.

The sample was vortexed to emulsify and centrifuged to obtain a clear separation between the phases.
The organic layer was transferred to a separate vial, diluted 1:10 with iso-octane/chloroform and analysed by GC-MS.

Results and Discussion

In this study, three replicate sample batches (*n*=3) including blanks with internal standard and eight calibration points were prepared to test robustness and reproducibility of the system.

Three replicate sample batches (*n*=3) including blank and eight calibrator samples were also prepared without being extracted via smart SPE (i.e. derivatisation only) to evaluate ITSP recovery of the target analytes.

The fully automated solution was demonstrated to give very good linearity and reproducibility. Table 1 reports the average (*n*=3) slope, intercept and R^2 for each of the investigated analytes. No linearity could be evaluated for ornithine, histidine and glutamine since limit of detection were in the range of the top calibration points. Further investigation focused on higher concentration ranges will be needed to fully characterize the performances of the method for these three analytes.

	Analyte	R^2	%RSD
1	Sarcosine	0.9966	7
2	Alanine	0.9955	3
3	Glycine	0.9904	4
4	α-Aminobutyric Acid	0.9970	3
5	Valine	0.9984	4
6	b-alanine	0.9960	3
7	b-amino-iso-butyric acid	0.9942	3
8	Leucine	0.9955	5
9	Isoleucine	0.9991	7
10	Proline	0.9980	3
11	Threonine	0.9808	6
12	Serine	0.9682	4
13	Asparagine	0.9898	7
14	Methionine	0.9940	18
15	Aspartic Acid	0.9975	19
16	Ornithine		
17	Hydroxyproline	0.9831	6
18	Phenylalanine	0.9845	2
19	Glutamic acid	0.9868	10
20	Lysine		
21	2-amino-adipic acid	0.9761	15
22	Histidine		
23	Glutamine		
24	Tyrosine	0.9838	10
25	Triptophan	0.9575	13

Table 1: Average (*n*=3) R^2 and % RSD for the investigated amino acids

Figure 3 shows as an example of the calibration plots for four selected amino acids (1 acid, 1 alkyl chained, 1 aromatic and 1 basic).

Three replicates of each selected sample matrix (flour, gluten free flour, cashew nuts and amino acids supplement powder for branched alkyl chain amino acids: were analysed using the described method. Summary of the average concentrations and standard deviation obtained for the target amino acids are reported in Table 3

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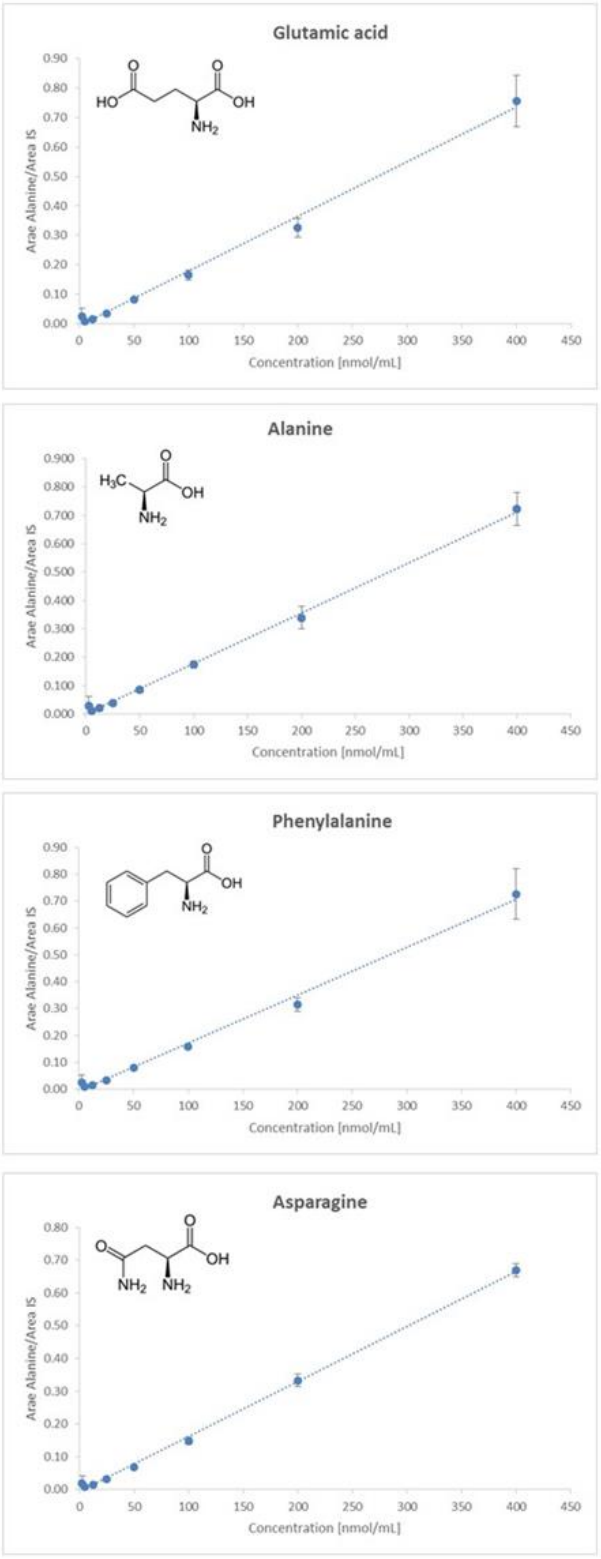

Figure 3: Calibration plots for the four selected amino acids: (acid: glutamic acid, alkyl chained: alanine, aromatic: phenylalanine, basic: asparagine)

	Analyte	Flour	Glute free flour	Cashwe nuts	AAs supplement
1	Sarcosine	n.d.	n.d.	1.5 ± 0.3	29 ± 1
2	Alanine	n.d.	n.d.	n.d.	n.d.
3	Glycine	n.d.	n.d.	1.1 ± 0.1	n.d.
4	α-Aminobutyric Acid	n.d.	n.d.	n.d.	12 ± 1
5	Valine	n.d.	n.d.	1.2 ± 0.2	29 ± 1
6	b-alanine	n.d.	n.d.	n.d.	n.d.
7	b-amino-iso-butyric acid	n.d.	n.d.	n.d.	n.d.
8	Leucine	n.d.	n.d.	1.2 ± 0.1	453 ± 64
9	Isoleucine	n.d.	n.d.	0.7 ± 0.1	504 ± 48
10	Proline	n.d.	n.d.	2.3 ± 0.4	n.d.
11	Threonine	n.d.	n.d.	n.d.	33 ± 4
12	Serine	n.d.	n.d.	n.d.	n.d.
13	Asparagine	0.9 ± 0.1	0.6 ± 0.1	n.d.	n.d.
14	Methionine	n.d.	n.d.	n.d.	3.7 ± 0.4
15	Aspartic Acid	1.3 ± 0.1	0.9 ± 0.1	1.8 ± 0.3	0.8 ± 0.1
16	Ornithine	n.d.	n.d.	n.d.	n.d.
17	Hydroxyproline	n.d.	n.d.	n.d.	7.3 ± 0.7
18	Phenylalanine	n.d.	n.d.	n.d.	0.9 ± 0.1
19	Glutamic acid	1.0 ± 0.1	0.8 ± 0.1	1.6 ± 0.3	0.7 ± 0.1
20	Lysine	n.d.	n.d.	n.d.	n.d.
21	2-amino-adipic acid	n.d.	n.d.	n.d.	n.d.
22	Histidine	n.d.	n.d.	n.d.	n.d.
23	Glutamine	n.d.	n.d.	n.d.	n.d.
24	Tyrosine	n.d.	n.d.	n.d.	1.2 ± 0.2
25	Triptophan	n.d.	n.d.	n.d.	n.d.

Table 3: Average (*n*=3) concentration (nmol/mg) and standard deviation for the investigated amino acids in the target sample matrices

Conclusions

The Amino Acid Analysis solution described in this study delivers fully automated sample preparation for GC-MS analysis.

The solution is available both as an offline Prepstation and an online solution with fully automated just-in-time samples preparation to match GC throughput for maximum productivity.

Throughput is up to 72 samples in a day, exceeding the current manual protocol on which the solution is based.

This method has proved to be matrix tolerant (folates, lipid, protein, starch, pH) and linearity, recoveries and precision met or exceeded those obtained with manual sample preparation.

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

References

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