

Asparagine Analysis in Food Products

Tivadar Farkas and Jafar Toulouee, Phenomenex Inc., Torrance, California, USA.

The current concern over acrylamide levels in food has unexpectedly brought amino acid analysis to the forefront. Acrylamide (2-propenamide) is a known mutagen in rats and also a neurotoxin and probable carcinogen in humans. Swedish researchers have discovered surprisingly high levels of this toxic compound in common food products such as chips and french fries.¹ The source of acrylamide in processed foods is believed to be linked to the Maillard reaction involving amino acids and sugars.² The current understanding is that all food-borne acrylamide originates with free asparagine (ASN), perhaps with minor contributions by other free amino acids.³ For this reason, monitoring of precursors such as asparagine in raw food ingredients, and also the selection of low amino acid containing food varieties, is of great interest.

Most methods used for the analysis of amino acids in biological matrices are laborious, time consuming and expensive. We present in this application note data on the analysis of ASN in potato samples using the EZ:faast® procedure. The total time for the sample preparation and analysis of ASN in food with this kit is 15 min. This procedure involves a simple solid phase extraction (SPE) step, followed by a rapid derivatization reaction and GC (FID/NPD) analysis. Alternative kits are available for GC/MS or LC/MS analysis. The method demonstrates excellent sensitivity (limit of

quantification 88 ng/mL ASN), reproducibility, linearity, recovery and derivative stability. Good within-the-day (3.1%) and day-to-day (RSD 1.94%) reproducibility ensures high-quality analysis of ASN and other amino acids in food products.

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Experimental

Lyophilized potato samples were extracted in 25% acetonitrile in water (v/v) for 1 h at 40 °C at an extraction ratio of 0.05 g solid sample to 1 mL liquid extractant. Additionally, dilute extracts were prepared at the ratios 0.05 g:10 mL and 0.05 g:100 mL, respectively. All extracts were filtered with 0.45 µm pore size syringe filters, and refrigerated until use. An aliquot of 50 µL extract was prepared in each case for amino acid analysis by SPE using sorbent cartridges provided in the kit. Amino acids are eluted in aqueous phase and derivatized in one step at room temperature. The derivatization reagent is added in organic phase

Figure 1: A typical chromatogram showing the separation of 33 amino acids in a standard mixture prepared by EZ:faast®, and analysed by GC-FID. Experimental conditions: inlet 250 °C; injecton 2 µL with split 15:1; column 10 m × 0.25 mm i.d. ZB AAA; flow 1.5 mL/min He; oven 110–320 °C, 32 °C/min; detector 320 °C.

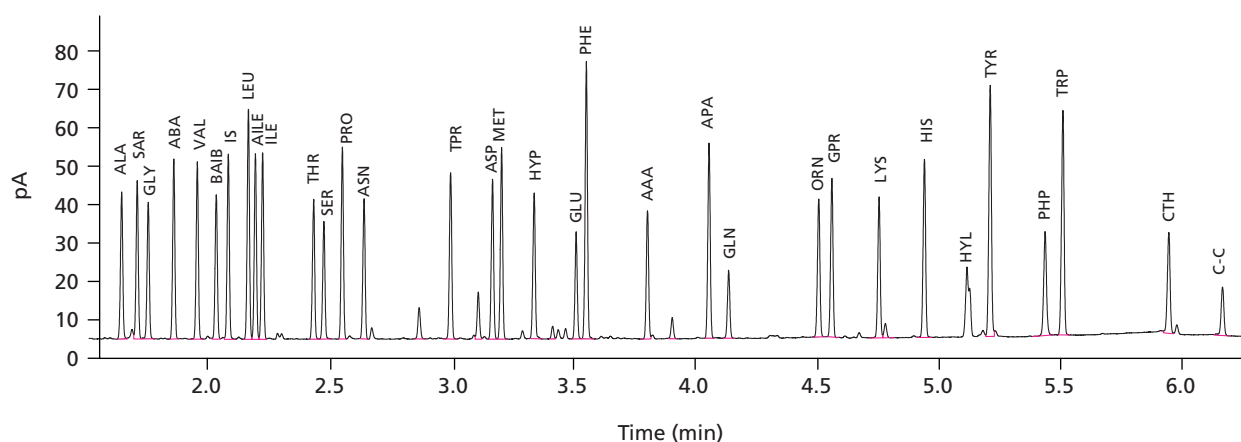
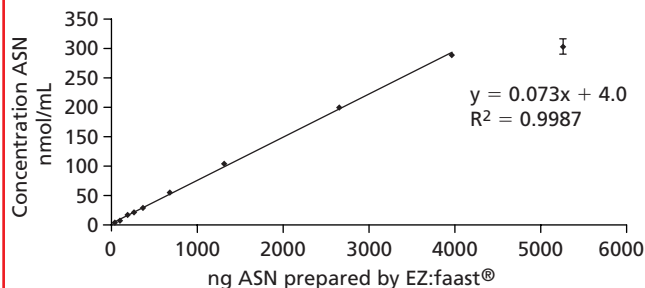


Figure 2: Linear dynamic range for the quantification of asparagine.

and the reaction products (alkylated amino acid derivatives) are extracted into the same phase. The entire sample preparation takes 7–8 min.

Potato extracts and amino acid standard mixtures were prepared and analysed in the same way. Standard mixtures were used for both instrument calibration and for linearity measurements. Potato extracts were analysed as such, and also spiked with 20 nmols ASN. Recovery was calculated at three different concentrations of ASN in potato extracts.

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A typical chromatogram showing the separation of 33 amino acids in a standard mixture is shown in Figure 1. The amino acid derivatives are analysed by GC in 7 min. Either FID or NPD can be used for detection. Alternative kits are available for either GC/MS or LC/MS analysis. The total cycle time for sample preparation followed by GC analysis is 15 min. An Agilent 6890 gas chromatograph equipped with an FID was used for sample analysis. The chromatographic column with the dimensions 10 m × 0.25 mm i.d. of a proprietary phase is provided with the kit. The temperature programme starts at 110 °C, includes a linear ramp of 32 °C/min up to 320 °C, and a hold time of 1 min. The injector temperature is 250 °C, while the detector is kept at 320 °C.

The sample preparation for LC/MS analysis is similar to the sample preparation for the GC/FID and GC/MS analysis kits. The LC/MS analysis takes 12 min, and it's followed by a 3 min column re-equilibration time. All materials required for sample preparation and for chromatographic analysis are provided with the kit, except for common laboratory consumables and utilities. One kit provides

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for the analysis of 384 samples and standard mixtures used for instrument calibration.

Results

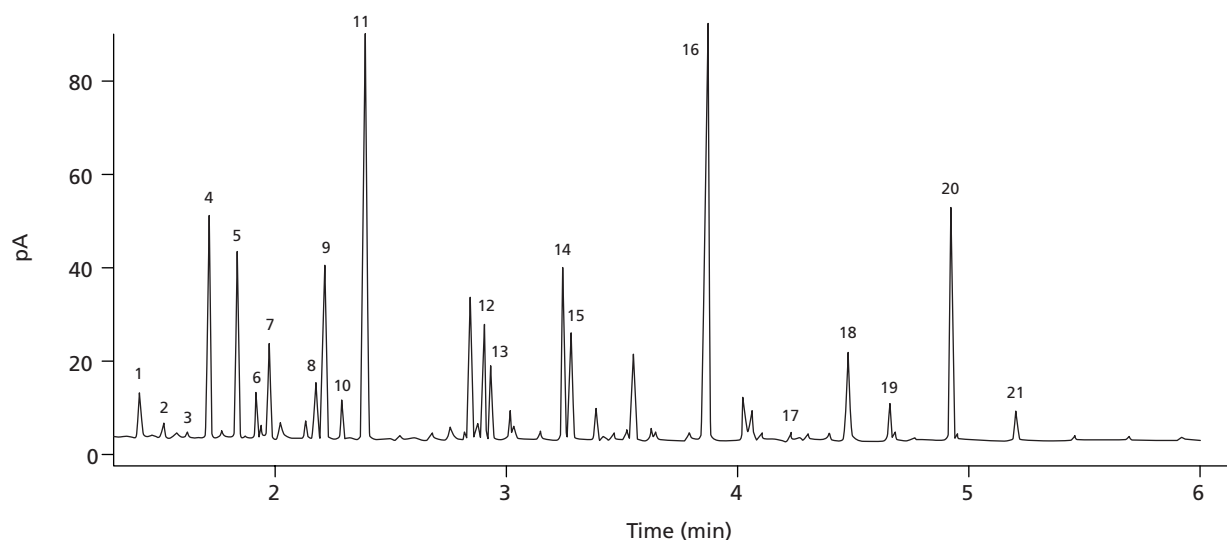
The ASN analysis method based on the EZ:faast® procedure proved linear in the 88–4000 ng/mL (0.6–30 µM) concentration range, with a correlation coefficient of 0.998 (Figure 2). The linearity range can be further extended by either adjusting the solid-liquid extraction ratio prior to SPE, or by processing a smaller/larger volume of extract depending on ASN level. A simple dilution of a highly concentrated extract will practically bring any sample high in ASN within the linearity range.

Table 1: Amino acid levels in a lyophilized potato sample analysed by the EZ:faast® procedure.

Amino Acid	nmol/mL	ppm	Percent %
ALA	92.77	330.58	0.03
GLY	49.23	147.84	0.01
VAL	494.41	2316.79	0.23
LEU	68.84	361.23	0.04
ILE	227.05	1191.38	0.12
THR	150.32	716.23	0.07
SER	1028.93	4325.20	0.43
PRO	56.18	258.70	0.03
ASN	2816.25	14892.35	1.49
ASP	194.63	1036.23	0.10
MET	120.16	717.16	0.07
GLU	898.36	5287.03	0.53
PHE	174.83	1155.18	0.12
AAA	143.81	927.02	0.09
GLN	1622.04	9482.47	0.95
LYS	259.60	1518.04	0.15
HIS	78.13	484.91	0.05
TYR	285.08	2066.17	0.21
TRP	47.72	389.83	0.04

Table 2: Recovery data for the analysis of asparagine in potato extracts with the EZ:faast® AAA Kit.

ASN concentration level	Concentration in potato extract, nmol/mL	Concentration in spiked extract, nmol/mL	Recovery (%)	RSD (%)
1	59.2	259.2	100	0.53
2	554.9	759.8	102	1.86
3	5796	5996	99.2	1.38

Figure 3: A typical chromatogram for the analysis of amino acids in potato.

Peaks: 1 = ALA; 2 = GLY; 3 = ABA; 4 = VAL; 5 = ISO; 6 = LEU; 7 = ILE; 8 = THR; 9 = SER; 10 = PRO; 11 = ASN; 12 = ASP; 13 = MET; 14 = GLU; 15 = PHE; 16 = GLN; 17 = ORN; 18 = LYS; 19 = HIS; 20 = TYR; 21 = TRP (experimental conditions as in Figure 1).

The limit of quantitation of 88 ng/mL (90 pg on column) was determined as the lowest point of the linearity curve, three times analysed, for which the relative standard deviation is less than 20%, and the accuracy is between 80 and 120%.

The repeatability of the EZ:faast® procedure was evaluated based upon multiple injections of the same preparation of a standard mixture, and also upon multiple preparations of the same potato extract. The RSD for injections of the same preparation was 0.72% (for nine consecutive runs). The RSD for four preparations of the same extract was 3.1%. A typical chromatogram for the analysis of amino acids in potato is shown in Figure 3.

Quantitative results on the amino acid composition of a lyophilized potato sample are shown in Table 1. The accuracy of asparagine analysis using the EZ:faast® procedure was evaluated based on per cent recovery calculated from spiked potato extracts, at three different levels of concentration. The recoveries at all three levels were 99–102% (Table 2).

Other food products besides cooked potatoes have been reported to contain high levels of acrylamide, although significantly less than found in cooked potatoes. Corn and tomato have been shown to also generate acrylamide when cooked at high temperatures. Corn and tomato samples analysed with the EZ:faast® procedure showed high levels of ASN, GLU, SER and ASP in corn, and GLU, GLN, ASP, ASN and SER in tomato.

Conclusions

The EZ:faast® amino acid analysis procedure is a sensitive, accurate and reliable method for the analysis of asparagine and other amino acids in food products. It requires generic instrumentation available in most laboratories. The kit developed based on this procedure is the most rapid and cost effective product on the market.

Phenomenex has a complete SPE method for the extraction of acrylamide from french fries using strata-X-C, the new polymeric mixed-mode sorbent. Please contact Phenomenex for application note SPE TN-007.

References:

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Phenomenex Inc.,
411 Madrid Avenue,
Torrance, California 90501-1430, USA
tel. +1 310 212 0555, fax +1 310 328 7768.

Phenomenex Germany
Zeppelinstr. 5, D-63741,
Aschaffenburg, Germany
tel. +49 06021 58830-0, fax +49 06021 58830-11

Phenomenex UK
Queens Ave., Hurdsfield Ind. Est.,
Macclesfield, Cheshire SK10 2BN, UK.
tel. +44 01625 501367, fax +44 01625 501796

For other inquiries, contact Phenomenex USA or
e-mail: international@phenomenex.com
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