

Metabolic Fingerprinting to Determine Tryptophan Contamination

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Key Words

3-(phenylamino)alanine (3PAA), Eosinophilia Myalgia Syndrome, HPLC-ECD, Tryptophan

Goal

To develop an HPLC-electrochemical detection (ECD) method to measure and categorize contaminated tryptophan samples

Introduction

Ingestion of toxin contaminated food (or food supplements) is responsible for a variety of aggressive, debilitating and often lethal diseases. Many of the toxins are highly modified amino acids or amines and include domoic acid (causing amnesic shellfish poisoning), β -N-oxalylamino-L-alanine (lathyrism), β -N-methylamino-L-alanine (Guam amyotrophic lateral sclerosis with secondary Parkinsonism and Alzheimer's dementia), and anilides (toxic oil- or Spanish oil-syndrome).

Tryptophan is often used as a dietary supplement to aid insomnia and depression. Abnormalities in tryptophan metabolism are associated with a number of clinical syndromes including carcinoid syndrome, scleroderma, and eosinophilic fasciitis. In the late 1980s, an epidemic of a new disease occurred in the USA, termed eosinophilia myalgia syndrome (EMS). This was caused by consumption of contaminated tryptophan produced by a Japanese manufacturer Showa Denko KK. Evidence to date suggests that EMS is probably triggered by an impurity formed when the fermentation conditions used in the manufacture of tryptophan were modified.¹ World-wide, more than 1600 people have been affected by the illness, including 38 deaths.



A number of techniques have been used to investigate tryptophan contamination. Radiochemical, microbiological and elemental analysis failed to detect any significant contamination.² A number of contaminants were detected using HPLC-UV but due to poor sensitivity this technique was limited. Several trace contaminants (typically <0.1%) have now been identified and their structures elucidated using LC-MS-MS (Figure 1).³⁻⁵ Of these ethylidenebis(tryptophan) (EBT; tryptophan animal; [E]), a dimeric form of tryptophan, and 3-(phenylamino)alanine (3PAA), which is a non-tryptophan derivative, received the most attention. While the administration of EBT to humans caused eosinophil activation⁶, in animal studies 3PAA was without effect⁷, suggesting that of these two EBT is the major toxin. Interestingly, similar contaminants are also found in over-the-counter melatonin^{8,9} and 5-hydroxytryptophan¹⁰ suggesting that tighter control and regulation of nutritional supplements is warranted.

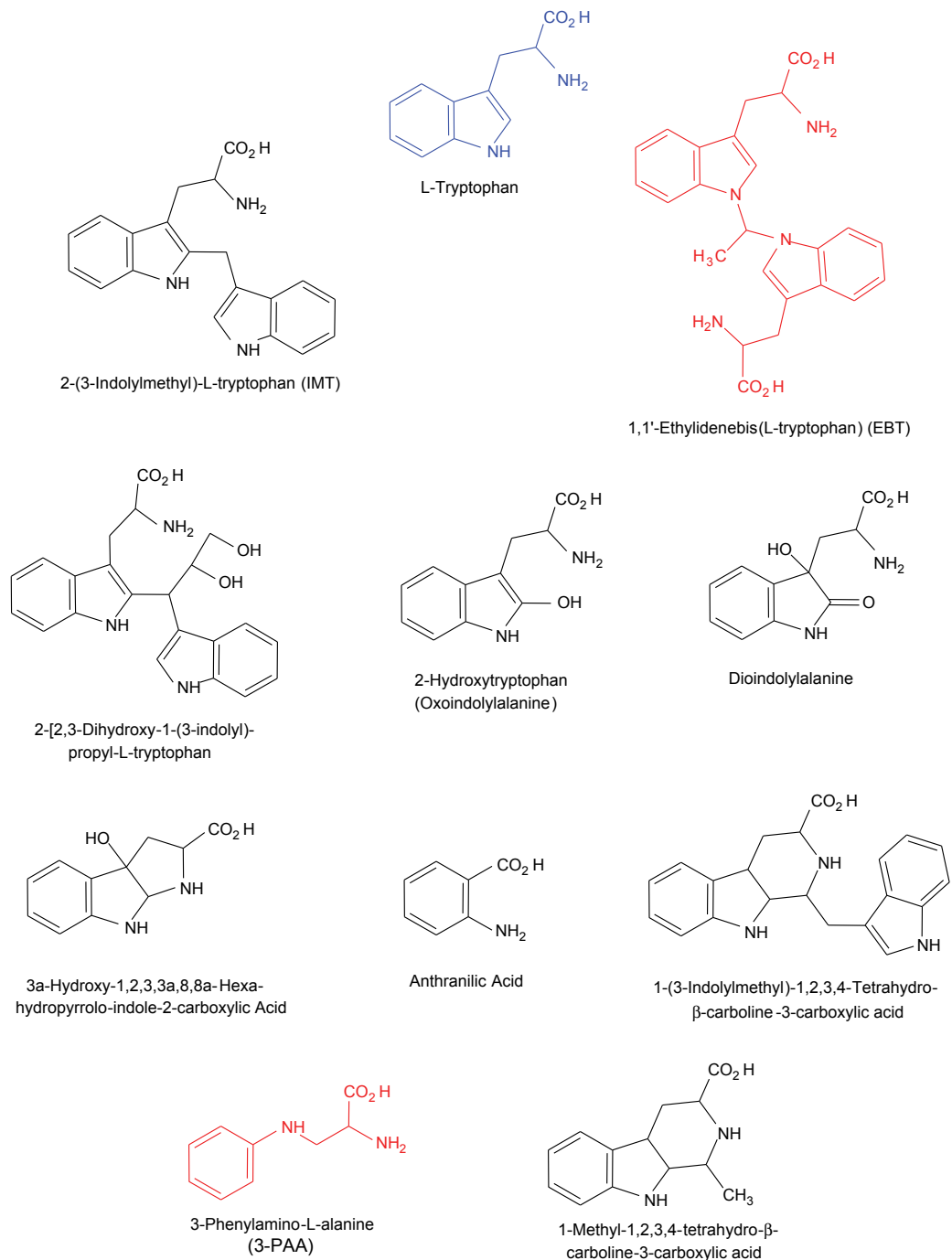


Figure 1. The structure of tryptophan and some potentially toxic compounds found in contaminated tryptophan samples.

This application uses gradient HPLC with coulometric array detection to measure >300 analytes, including EBT and 3PAA, in contaminated tryptophan samples. Using pattern recognition software this metabolic finger-printing is then used to unambiguously categorize contaminated from control tryptophan samples. This is of great use whenever identification of product contamination is paramount.

Materials and Methods

The gradient system consisted of two pumps, an autosampler, a thermal chamber and a sixteen channel Thermo Scientific™ Dionex™ CoulArray™ Coulometric Array Detector.

LC Conditions

Column:	C18, 4.6 × 150 mm, 5 μm
Mobile Phase A:	0.1 M Monobasic Sodium Phosphate, 10 mg/L Sodium Dodecyl Sulfate, 50 nM Nitrilotriacetic Acid; pH 3.35 with Phosphoric Acid
Mobile Phase B:	0.1 M Monobasic Sodium Phosphate, 50 mg/L Sodium Dodecyl Sulfate, 50 nM Nitrilotriacetic Acid: 35% Methanol: 30% Acetonitrile. pH 3.35 with Phosphoric Acid
Gradient Conditions:	0–6 minutes hold at 0% B; 6–10 minutes linear to 20% B; 10–20 minutes linear to 50% B; 20–25 minutes linear to 65% B; 25–40 minutes linear to 95% B; 40–45 minutes linear to 100% B; 45–48 minutes linear to 0% B
Flow Rate:	1.0 mL/min
Temperature:	35 °C
Injection Volume:	10 μL

Detector and Conditions

Detector:	Model 5600A, CoulArray
Applied Potentials:	0 to 900 mV (vs. Pd) in 60 mV increments

Results and Discussion

The chromatographic profile of tryptophan, EBT and 3PAA is presented in Figure 2. The electrochemical behavior of the analytes is presented in Figure 3. The assay had a limit of detection in the low pg range and was linear from 0–1000 ng/mL.

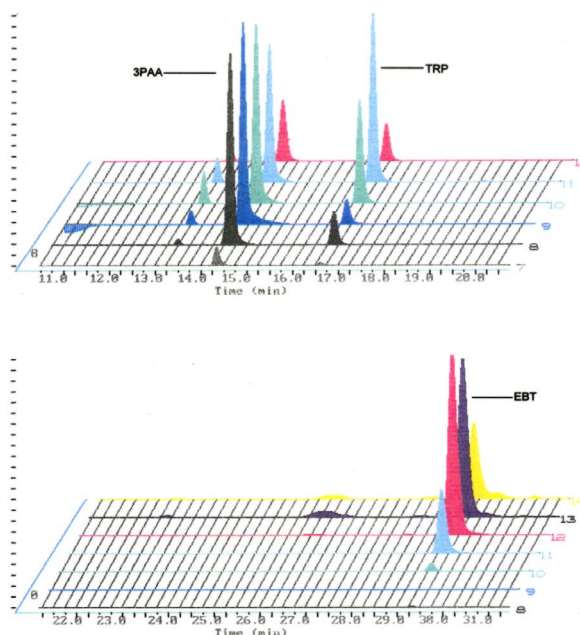


Figure 2. Analysis of tryptophan (TRP), 3PAA and EBT standards. 3PAA and TRP are at 1 μA range, EBT is at 2 μA.

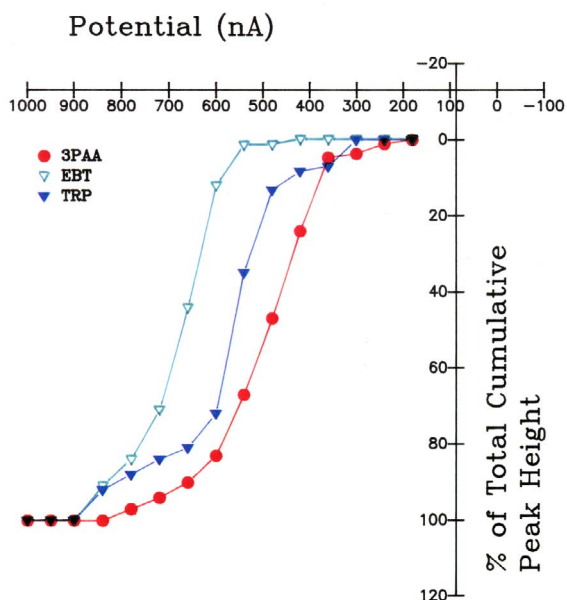


Figure 3. The current-voltage behavior of TRP, 3PAA and EBT.

Eight control and six contaminated samples were analyzed. The presence of EBT and 3PAA in control and contaminated (case) samples is presented in Figure 4. All tryptophan samples contained between 350–400 unknown analytes: Levels of 92% of the analytes were <0.01%; the remainder were <0.1%. Using conventional UV detection <100 peaks were detected. In contaminated samples the levels of EBT were 60 ± 20 ng/mg tryptophan compared to 3 ± 1 in controls. 3PAA levels were 102 ± 59 ng/mg tryptophan in case samples and 15 ± 6 in controls.

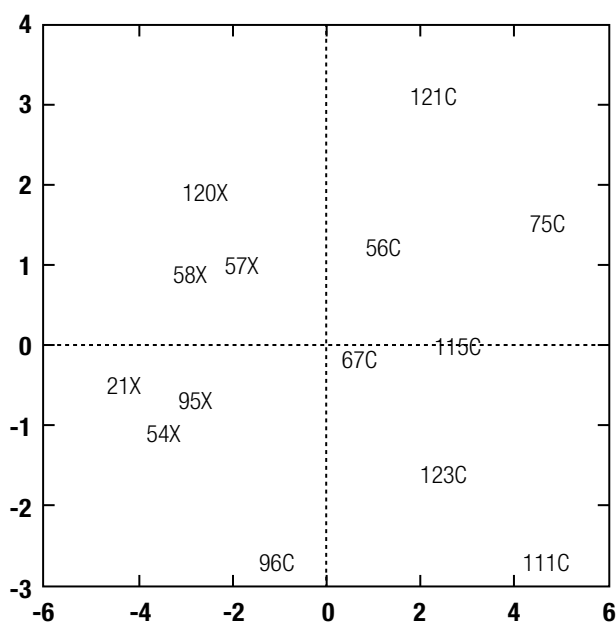


Figure 4. Principle component plot of metabolite patterns from control (C) and contaminated (X) samples.

Conclusion

Principal component analysis (Ein*Site software – Infometrics) based on differences in metabolite patterns clearly differentiated the case samples from the controls. Although the identity of majority of compounds in the sample remain unknown, this is irrelevant, since it is the pattern of metabolites that is unique and qualifies the sample as belonging to one group or another. The power of this approach is especially important in quality control where the determination of trace-level contamination is critical and when chemical standards are not available.

References

1. Mayeno, A. N., and Gleich, G. J. (1994). Eosinophilia-myalgia syndrome and tryptophan production: A cautionary tale. *Trends Biotechnol.*, **12**, 346–352.
2. Simat, T., van Wickern, B., Eulitz, K., and Steinhart, H. (1996). Contaminants in biotechnologically manufactured L-tryptophan. *J. Chromatogr. B.*, **685**, 41–51.
3. Naylor, S., Johnson, K.L., Williamson, B. L., Klarskov, K., and Gleich, G. J. (1999). Structural characterization of contaminants in commercial preparations of melatonin by on-line HPLC-electrospray ionization tandem mass spectrometry. *Adv. Exp. Med. Biol.*, **467**, 769–777.
4. Williamson, B. L., Benson, L. M., Tomlinson, A. J., Mayeno, A. N., Gleich, G. J., and Naylor, S. (1997). On-line HPLC-tandem mass spectrometry analysis of contaminants of L-tryptophan associated with onset of eosinophilia-myalgia syndrome. *Toxicol. Lett.*, **92**, 139–148.
5. Williamson, B. L., Benson, L. M., Tomlinson, A. J., Mayeno, A. N., Gleich, G. J., and Naylor, S. (1998). On-line HPLC-tandem mass spectrometry analysis of contaminants of L-tryptophan associated with onset of eosinophilia-myalgia syndrome. *Toxicol. Lett.*, **99**, 139–150.
6. Yamaoka, K. A., Miyasaka, N., Inuo, G., Saito, I., Kolb, J. P., Fujita, K., and Kashiwazaki, S. (1994). 1,1'-Ethlidenebis(tryptophan) (peak E) induces functional activation of human eosinophils and interleukin 5 production from T lymphocytes: Association of eosinophilia-myalgia syndrome with an L-tryptophan contaminant. *J. Clin. Immunol.*, **14**, 50–60.

7. Sato, F., Hagiwara, Y., and Kawase, Y. (1995). Subchronic toxicity of 3-phenylamino alanine, an impurity in L-tryptophan reported to be associated with eosinophilia-myalgia syndrome. *Arch. Toxicol.*, **69**, 444–449.
8. Naylor, S., and Gleich, G. J. (1999). Over-the-counter melatonin products and contamination. *Am. Fam. Physician*, **59**, 287–288.
9. Naylor, S., Williamson, B. L., Johnson, K. L., and Gleich, G. J. (1999). Structural characterization of case-associated contaminants peak C and FF in L-tryptophan implicated in eosinophilia-myalgia syndrome. *Adv. Exp. Med. Biol.*, **467**, 453–460.
10. Johnson, K. L., Klarskov, K., Benson, L. M., Williamson, B. L., Gleich, G. J., and Naylor, S. (1999). Presence of peak X and related compounds: The reported contaminant in case related 5-hydroxy-L-tryptophan associated with eosinophilia-myalgia syndrome. *J. Rheumatol.*, **26**, 2714–2717.

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Ordering Information

Description	Part Number
HPG-3400RS Biocompatible Binary Rapid Separation Pump with two solvent selector valves	5040.0046
WPS-3000TBRS Biocompatible Rapid Separation	5841.0020
CoulArray, Model 5600A – 16 channel	70-4334
CoulArray Organizer with Temp. Control	70-4340T
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