Application note
Neuroscience

Large Neutral Amino Acids

Introduction

The large neutral amino acids (LNAAs) include tryptophan, tyrosine, phenylalanine, and the branched-chain amino acids leucine, isoleucine and valine. LNAAs are the precursors for various brain neurotransmitters [1]. For instance, tryptophan is a precursor of the 'feel good' neurotransmitter serotonin. Studies have shown that LNAAs as dietary supplement support modulation of mood in mammalians. For example, pigs receiving high dietary tryptophan levels were found to be less affected by social stress caused by aggressive behavior between pigs in intensive pig farming [2].

This note highlights the analysis of LNAAs based on automated in-needle OPA-sulfite pre-column derivatization using the system and method principles that are described in detail in reference [3]. The chromatograms were provided by the Univ. of Utrecht, where a study was conducted with chickens in the context of agricultural animal welfare improvement.

Method

Chicken blood plasma LNAA content was investigated for a study at the University of Utrecht. The LNAAs were extracted with methanol from the sample together with

Fig. 1. *ALEXYS Analyzer*

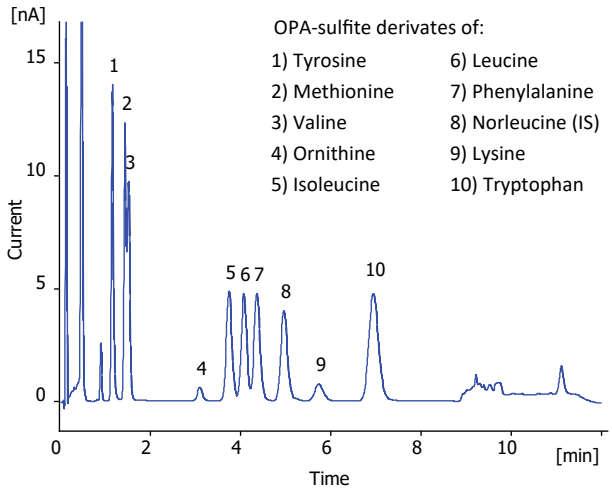

Fig. 2. Analysis of a 10 µM standard mix of 10 LNAAs and related compounds. Conditions according to Table 1. Chromatogram courtesy of Mrs. Gerdien Korte-Bouws, Department of Pharmaceutical Sciences, division of Pharmacology, University of Utrecht, The Netherlands.

Table 1. Recommended LC-ECD conditions

UHPLC	ALEXYS Analyzer
Column	Acquity UPLC HSS T3 1.0 x 50 mm, 1.8 µm (Waters)
Pre-column filter	Acquity in-line filter kit (Waters)
Mobile phase A (separation)	50 mmol/L phosphoric acid, 50 mmol/L citric acid and 0.1 mmol/L EDTA.Na_2, set to pH 4.5 with NaOH solution, 8% acetonitrile and 10% methanol
Mobile phase B (post-separation)	Mobile phase A, but with 50% acetonitrile
Flow rate	200 µL/mL: 100% A during separation, 12% A 88% B during 2-min post-separation flush
Temperature	40 °C for separation and detection
$V_{injection}$	1.5 µL, full loop injection as part of automated in-needle derivatization program using 10 µL sample and 1 µL reagent
OPA-sulfite reagent	125 mM OPA, 125 mM sodium sulfite, 90 mM sodium borate buffer pH 10.4
Needle wash solution	20 % methanol
Flow cell*	SenCell™ with 2 mm GC working electrode and saltbridge reference electrode, AST setting 1
Ecell	850 mV
Range	50 nA
ADF	0.5 Hz

*) The original work was done with a (now obsolete) 0.7 mm GC electrode vs ISAAC (Ecell 0.7 V/8 mmol/L chloride).

norleucine as internal standard. After centrifugation, the supernatant was diluted with borate buffer (pH 10.4) to assure efficient pre-column derivatization with OPA. The final dilution factor of the sample was 24. This specific type of sample contains high amounts of various (competing) amino acids: the strength of the derivatization reagent was therefore increased relative to the original protocol [3].

The ALEXYS system is a versatile UHPLC-ECD platform that is dedicated to run the method in a fully automated way. The recommended LC-ECD settings and conditions (adapted from reference [3]) are listed in Table 1. The method showed a linear response (corr. coeff. >0.998) in the range up to at least 20 µM, a detection limit of about 80-100 nM for ornithine and lysine (both these components have a side chain with a primary amine), and a detection limit in the range of 5-15 nM for the other LNAAs and related compounds.

Results

A chromatogram of LNAA analysis in blood plasma extract is given in Fig. 3. The LNAAs are clearly detectable and the concentration of LNAAs in plasma was calculated to be in the range of 0.08 - 0.35 mM (using the standard given in Fig. 2).

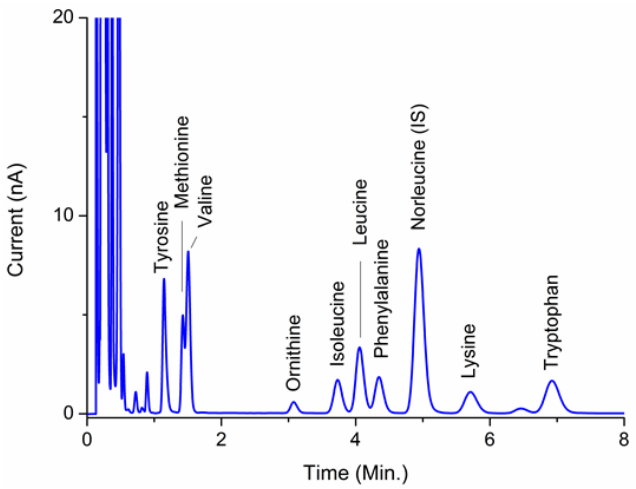

Fig. 3. Analysis of extracted chicken plasma (with 20.8 µM NLeu as internal standard). Conditions according to Table 1. Chromatogram courtesy of Mrs. Gerdien Korte-Bouws, Department of Pharmaceutical Sciences, division of Pharmacology, University of Utrecht, The Netherlands.

Ordering information

180.0091UA	ALEXYS Neurotransmitters SCC base
180.0504UA	Add-on parts for (2-pump) HPG option
116.4120	SenCell with 2 mm GC WE and sb REF
250.1160*	Acquity UPLC C18 HSS T3, 1x50 mm 1.8 µm (186003535)
250.1165*	Acquity UHPLC in-line filter kit + 6 frits (205000343)

*) Columns are products of Waters Corporation (Milford, USA). The Waters part numbers are given between parenthesis for reordering purposes.

Conclusion

This short note highlights the analysis of LNAAs in blood plasma with the versatile ALEXYS Analyzer. The method, based on automated OPA/sulfite pre-column derivatization, offers a user -friendly and fast solution for the analysis of various amino acids and related compounds.

References

1. Fernstrom, J.D. Large neutral amino acids: dietary effects on brain neurochemistry and function. *Amino Acids* **45,** 419–430 (2013).
2. Koopmans, S. J., Ruis, M., Dekker, R., van Diepen, H., Korte, M., & Mroz, Z. (2005). Surplus dietary tryptophan reduces plasma cortisol and noradrenaline concentrations and enhances recovery after social stress in pigs. *Physiology & behavior*, *85*(4), 469-478.
3. Antec Scientific, ALEXYS Neurotransmitter Analyzer for GABA and glutamate and other amino acids, Application note, 213.020

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