Easy Quantification of Tryptophan

Technical Overview

Introduction

The Agilent Evaporative Light Scattering Detector (ELSD) can outperform traditional detectors when analyzing non-chromophoric samples such as tryptophan and its metabolites by HPLC, as the detection mechanism does not rely on the optical properties of the analyte.

Tryptophan metabolism is of significant biochemical importance, since altered levels of metabolites have been noted in patients suffering from illnesses ranging from depression and schizophenia to Down's Syndrome and alcoholism[1].

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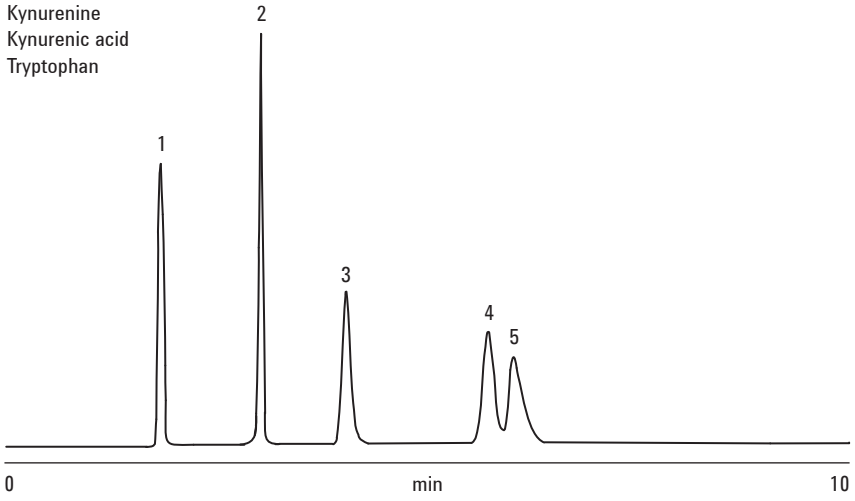
The major and minor routes of tryptophan catabolism lead to a wide range of related compounds which may be acidic, basic or zwitterionic. High performance liquid chromatography with fluoresence or electrochemical detectors has been traditionally used for these compounds. However, when using these detectors with silica-based columns, analysis of some of the compounds can prove difficult. The Agilent evaporative light scattering detector with PLRP-S HPLC columns offer a number of advantages here. Provided volatile salts and buffers are chosen (such as ammonium acetate), all of the metabolites are detected without the need for derivatization and with similar response factors, leading to easier quantification.

Conditions

Column: PLRP-S 100Å 5 µm, 4.6 x 250 mm (p/n PL1512-5500)
Eluent: 40 mM Ammonium acetate, pH 6, 7% (v/v) Acetonitrile
Flow Rate: 1 mL/min
Detector: Agilent ELSD

Agilent ELSDs recognise all compounds less volatile than the mobile phase, and with the advantage of low temperature operation, the benefits of ELSD compared to UV or RI now apply to an even wider range of HPLC applications.

Peak Identification
1. Quinolinic acid
2. 3-Hydroxykynurenine
3. Kynurenine
4. Kynurenic acid
5. Tryptophan



References

[1] A. M. Krstulovic *et al.* (1984) Analytical methodology for assays of serum tryptophan metabolites in control subjects and newly abstinent alcoholics: preliminary investigation by liquid chromatography with amperometric detection. *J. Chrom.* **297**, 271-281.

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