HPLC application note

Determination of Ammonia in Water by Pre-Column Derivatisation

'...FMOC-Cl has been employed for the derivatisation of amino acids...'

Abstract

A semi-automated method is described for the analysis of ammonia in water by pre-column derivatisation with FMOC-Cl (Fluorenylmehyl chloroformate), followed by reversed phase chromatography with UV detection. This method is highly selective and sensitivity is at the 0.1 ppm level. The derivatisation procedure is straightforward and no costly reagent is required.

Keywords:
Ammonia, Water, Pre-column Derivatisation, FMOC-Cl

Methods for the analysis of ammonia in water are numerous, ranging from relatively simple colorimetric methods[1] to sophisticated microcomputer-controlled flow-analysis system[2]. Among various procedures, HPLC offers the versatility of modifying a standard LC procedure to suit the requirements of particular analyses. These requirements include criteria such as sensitivity, selectivity and degree of instrument automation.

FMOC-Cl has been employed for the derivatisation of amino acids[3]. This derivatisation procedure does not require any solvent extraction and gives stable derivatives. An automated analysis system based on this chemistry has also been reported[4]. By employing similar chemistry, a method has been developed for the selective analysis of ammonia in water by pre-column derivatisation, followed by reverse-phase HPLC with binary gradient and UV detection. In addition, the procedure offers the possibility of further automation, including sample preparation by customisation of the GBC WinChrom Data Station and LC1610 Advanced Autosampler.

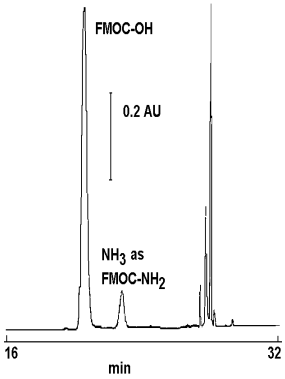

Figure 1 *Ammonia Standard (5 ppm)*

Conditions

Column:	Spherisorb S5 ODS2, 150 x 4.6 mm ID
Mobile Phase:	Solvent A - Water, Solvent B - methanol (Helium Sparging)

Gradient Program:

Time (min)	A (%)	B (%)
0.0	60.0	40.0
1.0	60.0	40.0
25.0	35.0	65.0
26.0	0.0	100.0
30.0	0.0	100.0
31.0	60.0	40.0

Injection Vol:	20 µl
Flow Rate:	1.50 ml/min
Temperature:	Ambient
Detection:	UV at 263 nm

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Derivatisation

Derivatisation Buffer:
0.25 M Sodium Borate, pH 9.6
Derivatisation Reagent:
FMOC-Cl (25 mg/ml) in Acetonitrile (prepare daily)
Derivatisation Procedure:
A sample of water is collected under clean conditions and filtered through a 0.2 µ filter. A 100 µl aliquot is added to a 1.5 ml Eppendorf tube. To this is added 450 µl of borate buffer and 450 µl of acetonitrile, followed by 50 µl of the derivatisation reagent.

The vial is capped and allowed to react at room temperature for 5 minutes. At the end of the reaction period, 10 µl of glacial acetic acid is added to the mixture to quench the reaction. The mixture is recapped and mixed thoroughly. A 20 µl aliquot of the mixture is subjected to HPLC analysis.

GBC HPLC Instrumentation

LC1110 Dual Piston HPLC Pump (x 2)
LC1200 Variable Wavelength UV/Vis Detector
LC1431 System Organiser
LC1650 Advanced Autosampler
WinChrom Chromatography Data Management System

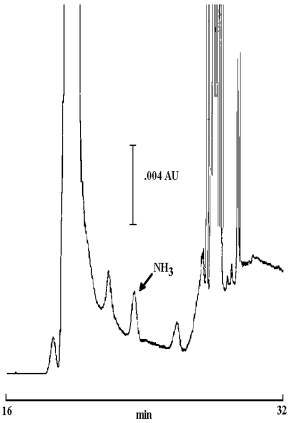

Figure 2 *Analysis of Ammonia in Hail Water*

References

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4. P.A. Haynes, D. Sheumack, L.G. Greig, J. Kibby and J.W. Redmond, in press.

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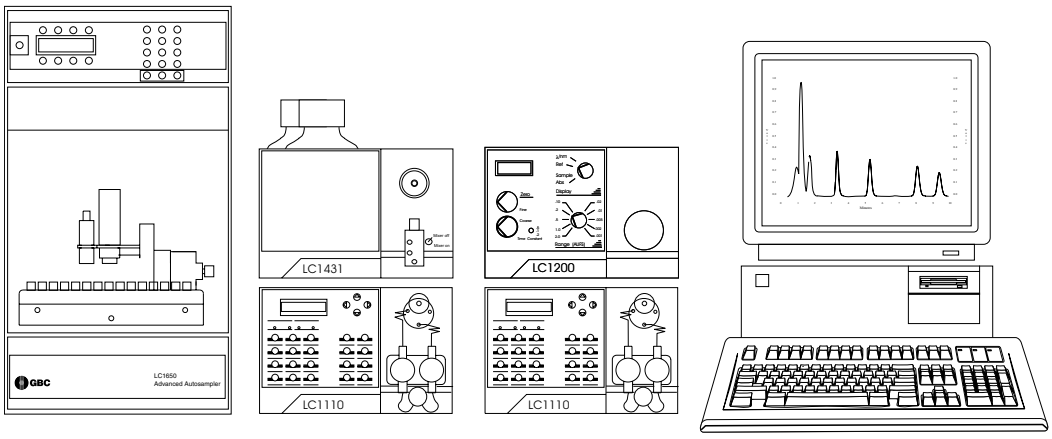

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