HPLC application note

Determination of DDT by C18 RP-HPLC

Abstract

A method is described for the determination of DDT by C18 reversed phase HPLC with UV detection. Sensitivity of the method is at the sub-ppm level with the retention time for DDT being approximately 5 minutes.

Keywords:
DDT, Chlorophenothane, Insecticide, Chlorinated Hydrocarbon, Agrochemical, Environmental

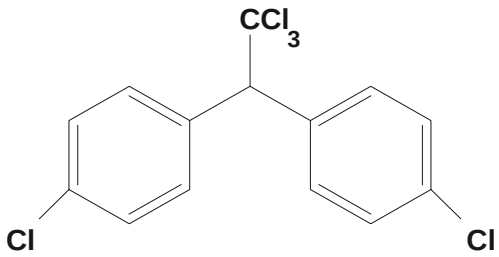
'...low vapor pressure of DDT is the cause of its remarkable persistence, killing insects for months and years on treated surfaces...'

Since the discovery of its insecticidal effects in 1939 by Paul Muller, p,p'-DDT (Chlorophenothane U.S.P.) has been phenomenally successful and for many years, has been considered the ideal insecticide. It is cheap, 'nontoxic',[1] persistent, and has a wide spectrum of insecticidal activity. The mechanism of action of DDT is by no means clear. It probably interferes with nerve conduction. It has been postulated that DDT orients itself in a special fit in a nerve-membrane-pore channel that distorts the ion passage through the nerve membrane.[2]

More recently however DDT has fallen into disfavor because of its potentially harmful effects on wildlife, the steady increase in insect resistance, and its accumulation in plants and animals. Its use is currently banned in many countries.[3] The low vapor pressure of DDT is the cause of its remarkable persistence, killing insects for months and years on treated surfaces.

It is unusually nonpolar, making it extremely oil-soluble and water-insoluble, which contributes to its accumulation in the food chain. It is chemically stable and insensitive to sunlight, which is beneficial for its insecticidal effects but undesirable from an ecological point of view.

Commercially available DDT contains approximately 80% of the p,p'-isomer and about 20% of the o,p'-isomer.[4] Some of the toxic effects of DDT have been attributed to the presence of the o,p'-isomer. HPLC offers a simple but effective method for the analysis of DDT at sub-ppm levels under isocratic conditions with UV detection.



Conditions

Column:	Spherisorb S5 ODS2, 250 x 4.6 mm ID
Mobile Phase:	Water/Acetonitrile (10:90)
Flow Rate:	1.0 ml/min
Temperature:	30°C
Detection:	UV at 254 nm
Injection Vol:	20 µl
Standard Conc.:	500 µg/ml

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GBC HPLC Instrumentation

LC1110 Dual Piston HPLC Pump
LC1200 Variable Wavelength UV/Vis Detector
LC1445 System Organiser
LC1650 Advanced Autosampler
WinChrom Chromatography Data Management System

References

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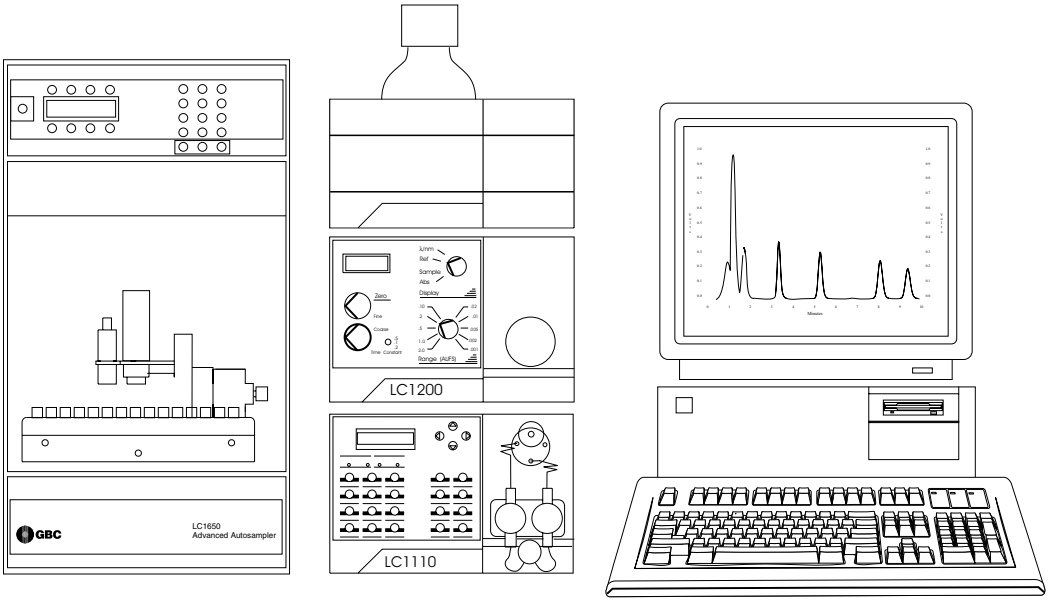
2. L.J.Mullins, Science, 122, (1955), 118.

3 'Report of the Secretary's Commission on Pesticides and Their Relationship to Environmental Health', U.S. Department of Health, Education and Welfare, Washington D.C., U.S. Government Printing Office, 1969.

4. Haller, J. Am. Chem. Soc., 67, (1945), 1591.

'...HPLC offers a simple but effective method for the analysis of DDT at sub-ppm levels under isocratic conditions with UV detection...'



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GBC publication number 01-0331-00
September, 1995

HPLC application note

MCPA Herbicide

Abstract

For quality control of commercial formulations containing MCPA, a simple but effective method for analysis of the active principle is described. HPLC is ideal for this task, since MCPA preparations may be assayed rapidly under isocratic conditions with UV detection, as shown in Figure 1.

Keywords:
4-Chloro-2-methylphenoxyacetic acid, herbicide, MCPA, substituted phenoxy acids

'...HPLC is ideal for this task, since MCPA preparations may be assayed rapidly under isocratic conditions with UV detection...'

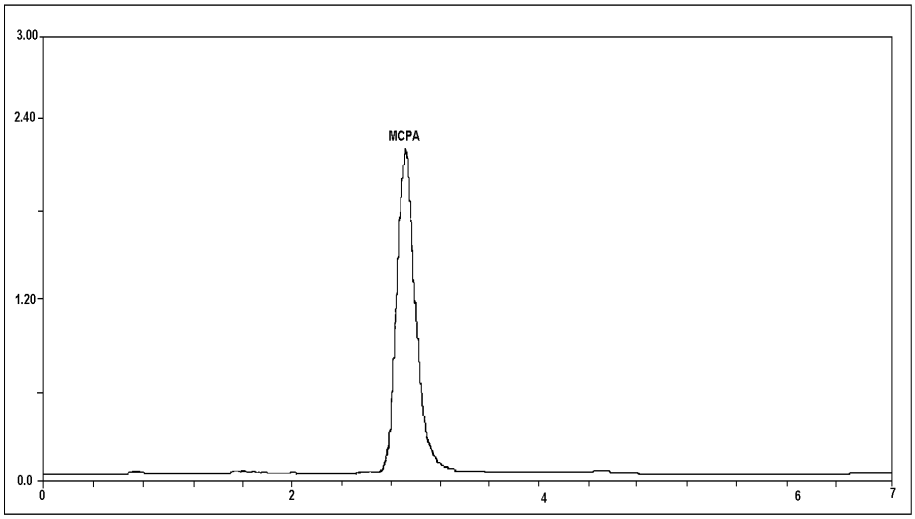

Figure 1 *Three minute separation of a standard solution of MCPA*

The herbicide 4-chloro-2-methylphenoxyacetic acid, commonly know as MCPA, is a fairly typical member of the substituted phenoxy acid group of synthetic plant growth hormones.

When applied to plant foliage, MCPA is absorbed and translocated throughout the whole plant. It acts by stopping cell division at the meristems and producing an abnormal structure in leaves, stems and roots by interfering with the normal processes of cell elongation. The vascular system of the plant is disrupted and it slowly dies.

Synthetic growth hormones like MCPA are rapidly degraded in both the soil and in the plant and are not considered to be highly toxic to mammals. The main problem in their use lies in the possibility that during spraying operations there may be some drifting onto other crops which may be susceptible to the action of such herbicides (Reference 1).

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GBC HPLC Instrumentation

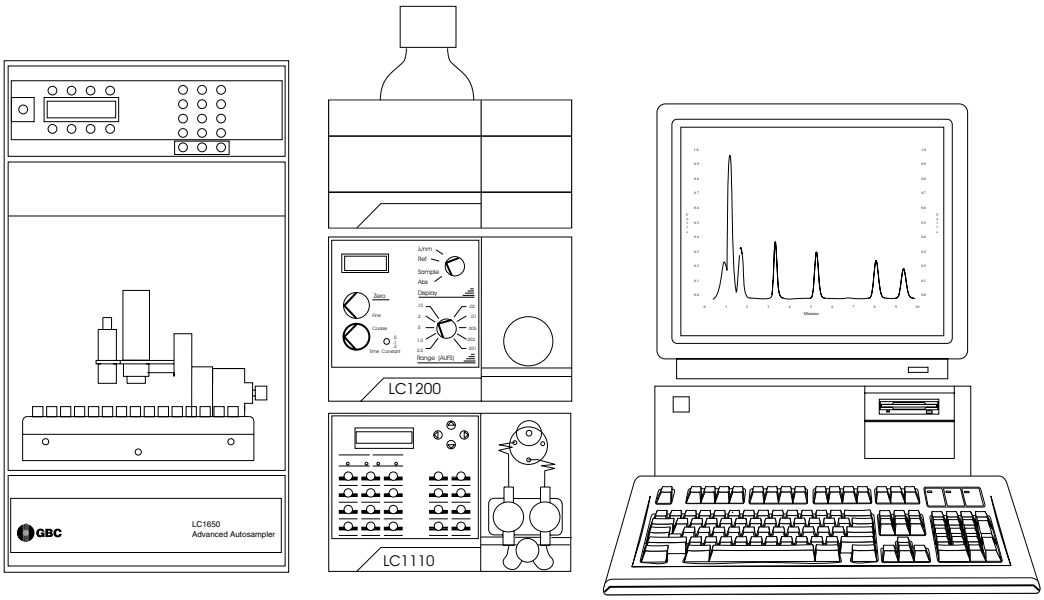
LC1110 Dual Piston HPLC Pump
LC1200 Variable Wavelength UV/Vis Detector
LC1445 System Organiser
LC1650 Advanced Autosampler
WinChrom Chromatography Data Management System

Conditions

Column:	Spherisorb S5 ODS2, 50 mm x 4.6 mm ID
Mobile Phase:	0.1% Aqueous phosphoric acid: methanol (40:60)
Flow Rate:	1 ml/min
Detection:	UV at 280 nm

References

1. 'The Pharmaceutical Codex' (11th Edition, 1979), pp. 668-9.



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September, 1995

HPLC application note

Morphine and Codeine in Wild Poppies

'...HPLC analysis of these poppies revealed that both species contained significant levels of opiate alkaloids...'

Keywords:
Codeine, morphine, Papaver setigerum, Papaver hybridum, wild poppies

For over one hundred years, following the introduction of European cereal crops to Australia, wild poppies have been a problem weed, especially in oatfields after good seasonal rainfall. Two species of poppy, tentatively identified by botanical experts as Papaver setigerum and Papaver hybridum, with mauve and red flowers respectively, are often seen during spring harvest. Contrary to popular belief that only the 'Opium Poppy', Papaver somniferum, produces morphine and related alkaloids, these wild poppies may also contain narcotic alkaloids.

HPLC analysis of these poppies revealed that both species contained significant levels of opiate alkaloids, mainly morphine and codeine, with the Papaver setigerum being particularly potent.

In Figure 1, a methanolic extract of dried P.setigerum capsules shows morphine and codeine at 0.02% and 0.04% by weight, respectively. More efficient extraction of these alkaloids from the same plant material may be achieved by using aqueous alkali, as shown in Figure 2, where the observed levels of morphine and codeine are 0.06% and 0.1% respectively.

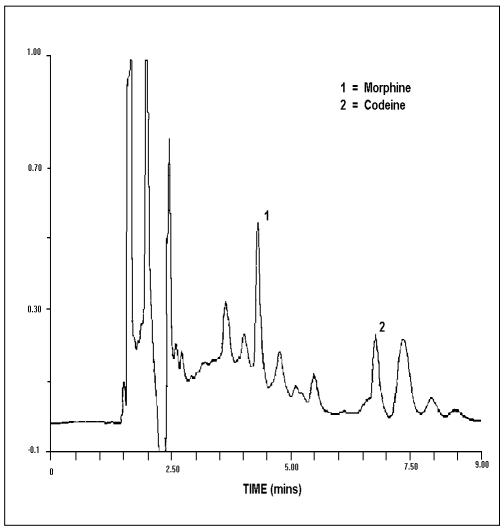

Figure 1 *Methanolic extract of P.setigerum capsules*

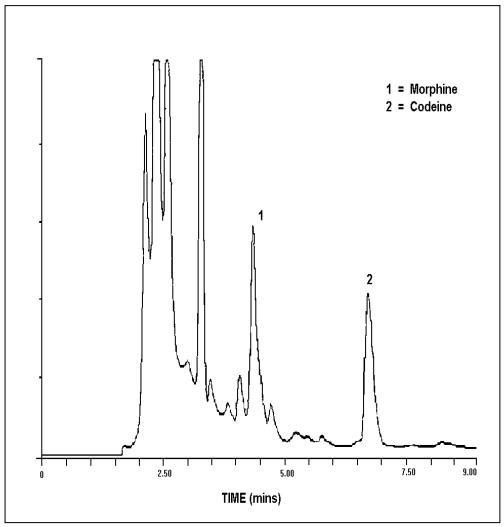

Figure 2 *Alkaline extract of P.setigerum capsules*

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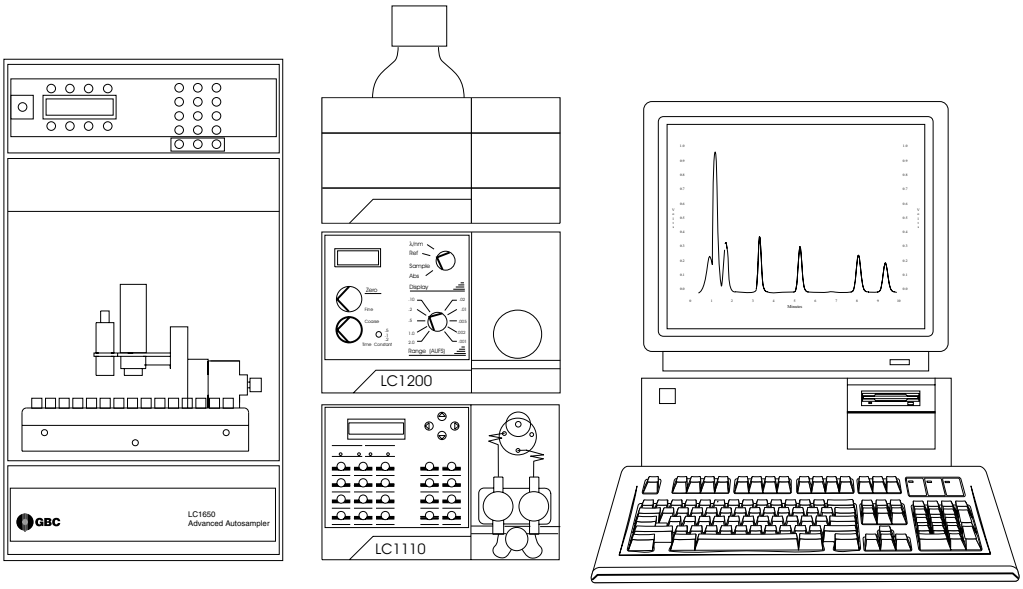
GBC HPLC Instrumentation

LC1110 Dual Piston HPLC Pump
LC1200 Variable Wavelength UV/Vis Detector
LC1445 System Organiser
LC1650 Advanced Autosampler
WinChrom Chromatography Data Management System

Conditions

Column:	Spherisorb S5 octyl, 250 mm x 4.6 mm ID
Guard Column:	Spherisorb S5 Octyl, 50 mm x 4.6 mm ID
Mobile Phase:	20% acetonitrile in water containing 0.01 M octane sulphonic acid and 0.2% triethylammonium phosphate, pH 3
Flow Rate:	1 ml/min
Wavelength:	285 nm



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GBC publication number 01-0333-00
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HPLC application note

Automated Amino Acid Analysis of Peptide Hydrolysates

Abstract

The amino acid analyses of two peptides, Angiotensin II and Neurotensin, by AMINOMATE are described. Each derivatisation has been performed on 10 pmol of hydrolysate and analysis on 5 pmol of derivatised sample. the results are in excellent agreement with the expected value including Histidine and Tyrosine.

'...sensitivity of the system is at the femtomole levels...'

Keywords:
Amino Acid, AMINOMATE, Angiotensin II, Neurotensin, Peptide, Hydrolysate

AMINOMATE, GBC Automated Amino Acid Analyser,1 has been developed with the accurate amino acid determination of peptide and protein2 hydrolysates in mind. Sensitivity of the system is at the femtomole levels, allowing analyses to be performed with as little as 5 pmol of derivatised samples and 10 pmol of the peptides or proteins.

Human Angiotensin II is an oligopeptide with eight amino acid residues (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) and a molecular weight of 1047. It has been chosen to demonstrate AMINOMATE's accuracy in the analysis of tyrosine, histidine and aspartic (Figure 1) which have been found to be problematic with existing methods3. The results (Table 1) are in excellent agreement with the expected values.

Table 1: Amino Acid Composition of Human Angiotensin II

Peak No.	Amino Acid	Molar Ratio	Expected Value determined
1	Asx[a]	1.2	1.0
2	His	0.8	0.9
3	Pro	1.1	1.0
4	Tyr	1.0	0.9
5	Arg	1.0	1.0
6	Val	0.9	0.9
7	Ile	0.9	0.9
8	Phe	1.0	1.1

a: Asp + Asn

Neurotensin is a basic tridecapeptide (Glu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro -Tyr-Ile-Leu) with a molecular weight of 1673. It is found in mammalian brain and gut, having a wide variety of hormone-like activities. The analysis results (Table 2) is again in excellent agreement with the expected values.

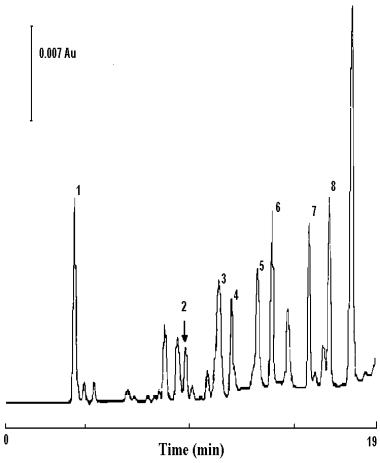

Figure 1 *Separation of Angiotensin II Hydrolysate (5 pmol)*

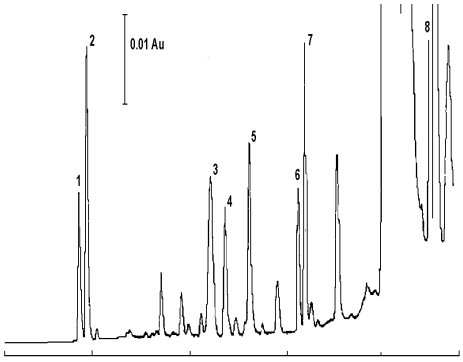

Figure 2 *HPLC Separation of Neurotensin Hydrolysate (5 pmol)*

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Table 2: Amino Acid composition of Neurotensin

Peak No.	Amino Acid	Molar Ratio	Expected Value determined
1	Asxa	1.1	1.0
2	Glxb	2.0	2.0
3	Pro	2.0	2.0
4	Tyr	2.0	2.0
5	Arg	2.0	2.0
6	Ile	1.0	1.0
7	Leu	2.0	2.0
8	Lys	1.0	1.0

a: Asp + Asn

b: Glu + Gln

GBC HPLC Instrumentation

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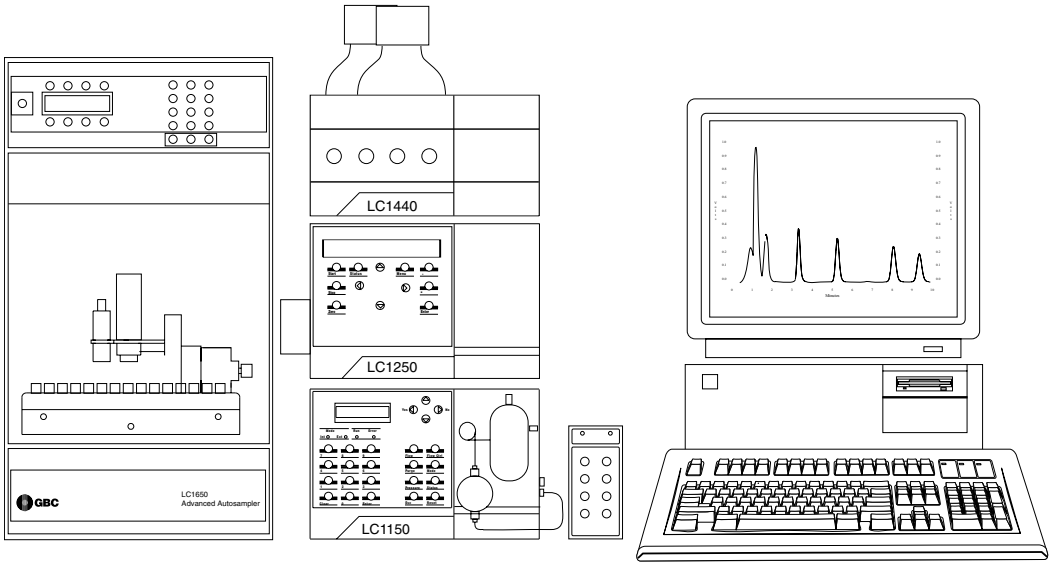
LC1250 Fluorescence Detector
LC1445 System Organiser
LC1650 Advanced Autosampler
LC1120/LC1150 HPLC Column Oven Option



Instrumentation

Hydrolysis Procedure: see Reference 4.

References

1. 'AMINOMATE: Automated Amino Acid Analysis by Precolumn Derivatisation', GBC HPLC Application B11.

2. 'Automated Amino Acid Analysis of Protein Hydrolysates', GBC HPLC Application B3.

3. P. Furst, L. Pollack, T.A. Graser, H. Godel and P. Stehle, J. Chromatogr., 499, (1990), 557.

4. P. Haynes, D. Sheumack, J. Kibby and J.W. Redmond, J. Chromatogr., 540, (11991), 177.

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September, 1995

HPLC application note

Automated Amino Acid Analysis of Protein Hydrolysates

Abstract

The amino acid analysis of three protein hydrolysates, Lysozyme, Chymotrypsinogen A and Pepsin, are described. Derivatisations have been performed on 10 pmol of hydrolysates, and separations conducted on 5 pmol of derivatised samples. The results are in very good agreement with expected values, including those of histidine and tyrosine.

'...the rapid, sensitive and fully automated analysis of amino acids in protein hydrolysates...'

Keywords:
Amino Acid, AMINOMATE, Lysozyme, Chymotrypsinogen A, Pepsin, Protein, Hydrolysate

AMINOMATE, GBC Automated Amino Acid Analyser,1 has been developed for the rapid, sensitive and fully automated analysis of amino acids, especially those in peptide2 and protein hydrolysates. Due to the high sensitivity of the system, with detection limit at 50 fmol, valuable protein hydrolysates as little as 10 pmol is sufficient for analysis. Accurate quantitation of histidine and tyrosine residues, which have proved problematic with existing methods, is also achieved.3

Lysozyme is an enzyme in egg white and human tears which catalyses the hydrolytic cleavage of the bacterial cell wall polysaccharides. It has 130 amino acid residues, with a molecular weight of 14300, but contains only one histidine and three tyrosine residues. The analysis results (Figure 1, Table 1) are in good agreement with the expected values, with accurate determination of both histidine and tyrosine even though they are present at low levels.

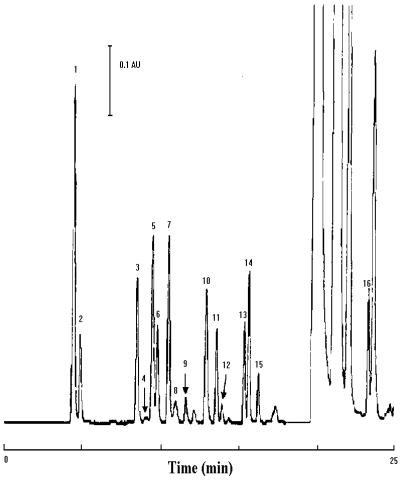

Figure 1 *HPLC Separation of Lysozyme Hydrolysate (5 pmol)*

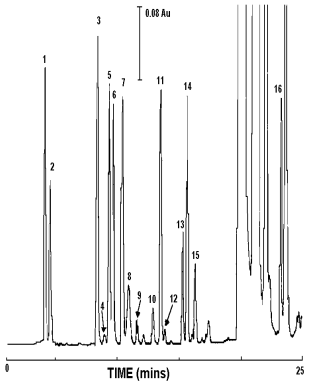

Figure 2 *HPLC Separation of Chymotrysinogen A Hydrolysate*

Chymotrysinogen A is the inactive precursor of chymotrypsin which is a proteolytic enzyme secreted into the small intestine. Chymotrypsinogen A has 244 residues and a molecular weight of 26400. However, it only contains two histidine and four tyrosine residues. Pepsin is an enzyme found in the stomach which is responsible for the hydrolysis of the peptide bonds of aromatic amino acids or ingested proteins. It has 327 residues, with a molecular weight of 34700, but contains only one histidine. The analysis results by AMINOMATE on the hydrolysates of both proteins (Figure 2 & 3, Table 1) are again in good agreement with the expected values.

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Table 1: Amino Acid Composition of Lysozyme, Chymotrysinogen A and Pepsin

No.	Amino acid	Lysozyme		Molar Ratioa Chymotrysinogen A		Pepsin	
1	Asxb	21.4	(21)	23.1	(23)	44.1	(42)
2	Glxc	5.2	(5)	14.4	(15)	26	(26)
3	Ser	9.3	(10)	23.2	(28)	40.7	(44)
4	Zhis	0.8	(1)	2.0	(2)	1.0	(1)
5	Gly	11.8	(12)	21.7	(23)	34.9	(35)
6	Thr	7.0	(7)	21.9	(22)	26.4	(26)
7	Ala	12.6	(13)	22.3	(22)	17.2	(16)
8	Pro	2.1	(2)	8.9	(9)	14.8	(15)
9	Tyr	2.9	(3)	4.0	(4)	13.7	(16)
10	Arg	11.0	(11)	4.3	(4)	2.3	(2)
11	Val	5.6	(6)	2.04	(23)	19.9	(22)
12	Met	1.7	(2)	1.9	(2)	3.6	(4)
13	Ile	5.5	(6)	9.0	(10)	21.9	(26)
14	Leu	8.0	(8)	19.8	(19)	25.8	(26)
15	Phe	3.1	(3)	6.4	(6)	13.8	(14)
16	Lys	5.9	(6)	13.8	(14)	1.3	(1)
17	Trp	nad	(6)	na	(8)	na	(5)
18	Cys	nad	(8)	na	(10)	na	(6)

a: Expected values given parentheses (protein compositions from Swiss protein data bank)
b: Asp + Asn
c: Glu + Gln
d: Not analysed

'...the analysis results on the hydrolysates of both proteins are again in good agreement with the expected values...'

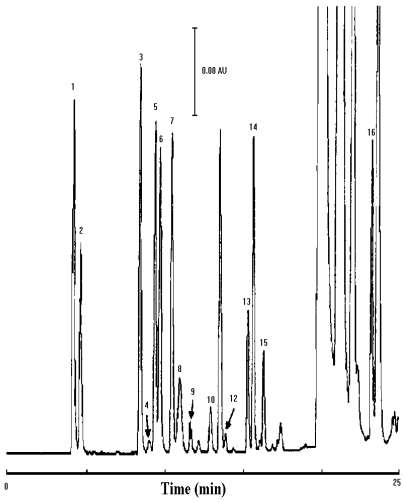


Figure 3 *HPLC Separation of Pepsin Hydrolysate (5 pmol)*

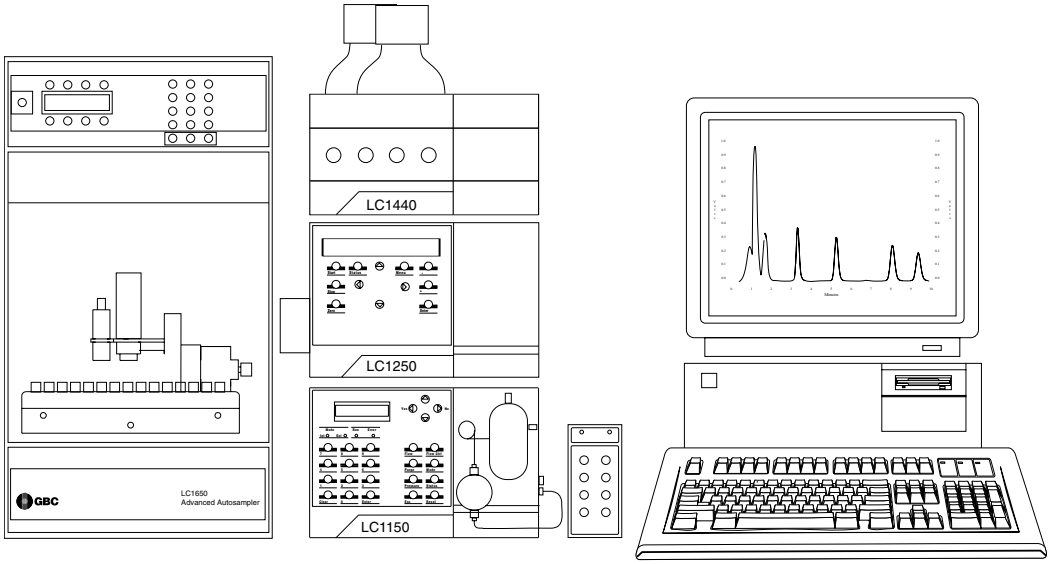
GBC HPLC Instrumentation

LC1150 Quaternary Gradient HPLC Pump
LC1250 Fluorescence Detector
LC1445 System Organiser
LC1650 Advanced Autosampler
LC1120/LC1150 HPLC Column Oven Option

Hydrolysis Procedure: see Reference 4.

References

1. 'AMINOMATE: Automated Amino Acid Analysis by Precolumn Derivatisation', GBC HPLC Application Note B11
2. 'Automated Amino Acid Analysis of Peptide Hydrolysates', GBC HPLC Application Note B2
3. P. Furst, L. Pollack, T.A. Graser, H. Godel and P. Stehle, J. Chromatogr., 499, (1990), 557.
4. P. Haynes, D. Sheumack, J. Kibby and J.W. Redmond, J. Chromatogr., 540, (1991), 177.



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GBC publication number 01-0336-00
September, 1995

HPLC application note

Automated Glycosylated Hemoglobin (HbA1c) Analysis for Diabetes Monitoring

Abstract

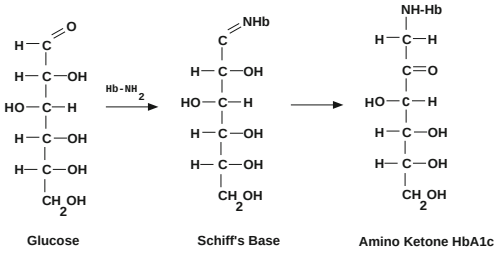
A rapid, sensitive and automated method for the determination of glycosylated hemoglobin HbA1c is described. In the assay, HBA1c is separated from HBA0 with excellent resolution, allowing for accurate quantitation. HbA1c is also resolved from other 'minor' hemoglobins: HbF and HbA3, eliminating any of their possible interference. The labile Schiff base precursor of HbA1c, which can elevate test results and produce inaccurate quantitation, is also removed in the sample preparation. Chromatography is based on the proven cation-exchange technique, using a binary gradient, with a column lifespan exceeding 1000 runs. Each analysis is completed within 8 minutes, with the generation of a fully validated report at the completion of each analysis. Up to 160 samples can be batched for unattended analysis. Reproducibility of the assay is excellent with an RSD of 1.2% for retention time and 1.6% RSD for peak area. The system also offers automatic setup/shutdown sequence and intelligent diagnostics to ensure optimal performance and protection of precious samples.

Keywords:
HbA1c, Glycosylated Hemoglobin, DIABETES, Cation-exchange

'..HBA1c is separated from HBA_0 with excellent resolution, allowing for accurate quantitation...'

Hemoglobin A1c is a minor component of the adult hemoglobin. HbA1c is formed by the reaction of glucose with the terminal amino group of a valine residue of HbA0 as follows.

A labile Schiff's base (labile HbA1c) is



initially formed in the biochemical process, followed by its subsequent chemical rearrangement to the corresponding stable HbA1c, an amino ketone. Since HbA1c is irreversibly bound to the red blood cells, its blood concentration offers a measure of the average blood glucose level for the past 4 to 6 weeks. In fact, the determination of HbA1c in a single blood sample has been considered as a more reliable indication of an individual's glucose tolerance as compared with alternate conventional assays.1

In the case of diabetic patients, HbA1c can be 2-3 times higher than normal. An accurate quantitation of the HbA1c level thus provides a reliable measure of the long-term metabolic control in the subjects, allowing clinicians to make the appropriate adjustments to treatment.

In addition, diabetes affects some 0.2% of pregnant women and gestational diabetes affects about 2% of pregnant women. Both forms of diabetes can be fatal to the baby unless the conditions are detected early and precautions are taken. Diabetes is also associated with pregnancy-induced hypertension in the mother and congenital defects in the newborn. These problems can most often be avoided if a diabetic woman receives the appropriate treatment and advice before conception. In this regard, the measurement of HbA1c has provided a very effective means for the desired diagnosis and monitoring of diabetes.

A proven cation exchange methodology for HbA1c analysis has been chosen for our system.2 A strong cation exchange column is adopted with a binary LiCl gradient using sodium malonate as a buffer. The column resin does not shrink or swell, and has been designed for the specific HPLC of biomolecules. The gradient generates little noise in its formation, excluding the need to employ complex multi-wavelength detection as is in some existing systems. The pH of the buffer has been optimised to provide maximum difference in the electrophoretic mobility and net charge among various different hemoglobins. This

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results in exceptional separation with well-defined peaks, making accurate, reproducible integration of peak areas possible. The labile HbA1c Schiff base precursor is also removed in the sample preparation, eliminating its possible interference in the assay. Typical separation of HBA1c is illustrated in Figure 1.

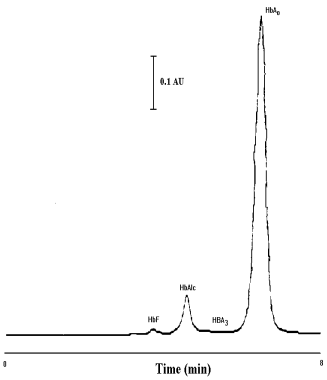

Figure 1 *HPLC Separation of HbAlc*

The reproducibility of the analysis is 1.2% RSD for retention time and 1.6% RSD for peak area based on ten consecutive analyses. Each separation is completed within 8 minutes, with automatic re-equilibration of the column at the end of the run for the next analysis. With good laboratory practice, such as the filtration of all buffers before use, each column can be used for more than 1000 runs.

Automation of the hemoglobin analyser is accomplished by control of the LC1150 Quaternary Gradient HPLC Pump and the robotic functions of LC1650 Advanced Autosampler via the WinChrom Chromatography Data Management System. The system utilises a comprehensive set of validation parameters, enabling single and group validation during analyses. Validation of results includes calculation of criteria such as variance on peak area/height, retention time, plate count, resolution and other commonly used parameters. Command sequence has been pre-programmed, but can also be easily modified, to allow the execution of different analytical routines depending on the validation results obtained. This ensures optimal performance of the analyser and avoids wastage of analysis time and precious samples during unattended operations. Multi-tasking of the management system also permits the execution of other software programs, e.g., for report preparation, while analyses are being processed.

The LC1650 Advanced Autosampler has a sample capacity of 160. A programmed sequence can also be conveniently interrupted for priority samples. The LC1150 Quaternary Gradient HPLC Pump has exceptionally low delay (dwell) volume and provides rapid on column gradient formation. Increased reliability is ensured with the dual in-series pistons arrangement utilising only two check valves. In addition, the configuration of the analyser offers maximum flexibility in allowing easy modification of the existing system for the analyses of other biochemicals such as catecholamines and amino acids.

'...each separation is completed within 8 minutes, with automatic re-equilibration of the column at the end of the run for the next analysis...'

GBC HPLC Instrumentation

LC1150 Quaternary Gradient HPLC Pump
LC1650 Advanced Autosampler
WinChrom Chromatography Data Management System

Acknowledgments

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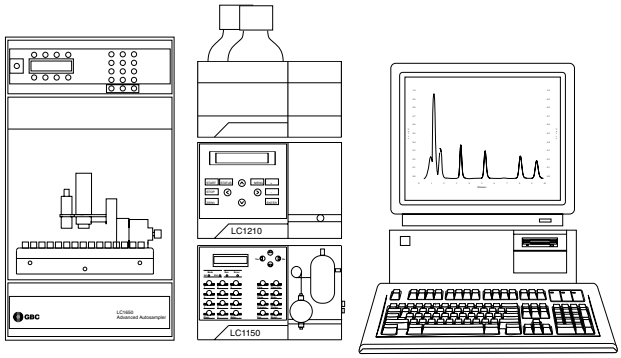
References

1. D.E Goldstein, *N. Engl. J. Med.*, 310, (1984), 284.
2. J-O. Jeppsson, P. Jerntorp, G. Sundkvist, H. Englund and V. Nylund, *Clin. Chem.*, 32(10), (1986), 1867.



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01-0337-00

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GBC publication number 01-0337-00
September, 1995

HPLC

application note

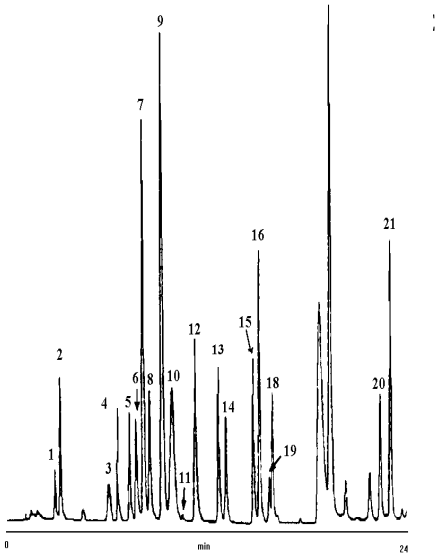
Amino Acid Determination in Intravenous Solution with Automated Pre-column FMOC Derivatisation and UV Detection

'...the sample is diluted with derivatisation buffer and filtered; the system takes care of the rest...'

Sample

1. Aspartic Acid
2. Glutamic Acid
3. OH-Proline
4. Asparagine
5. Serine
6. Histidine
7. Glycine
8. Threonine
9. Alanine
10. Proline
11. Tyrosine
12. Arginine
13. Valine
14. Methionine
15. Isoleucine
16. Leucine
17. Tryptophan
18. Phenylalanine
19. Cystine
20. Ornithine
21. Lysine

Conditions



Intravenous Solution.

Preparation

Diluted with derivatisation buffer (1:100), filtered.

Amino Acid Analysis

These notes illustrate the flexibility of the GBC chromatography equipment in performing complicated pre-column chemistries automatically via the LC1650 and data management station. There is a need in the clinical, food, and Biotechnological industries for the analysis of different sample matrices containing amino acids. The sample matrix can provide the largest problems for most chromatography equipment. The combination of excellent chromatography, precise flow control and sensitive, selective detection, give GBC the edge over most post-column systems as well as many of the pre-column derivative systems.

	AMINOMATE Column (150 x 4.6 mm ID)
Mobile Phase:	Solvent A: 30 mM Ammonium Phosphate (pH 6.5) (85%) and Methanol (15%). Solvent B: Water (85%) and Methanol (15%). Solvent C: Acetonitrile (90%) and Water (10%).
Gradient:	Equilibrate for 5 min. 0.00 to 2.00 min 42% B and 16% C, to 39% B and 22% C in 1.0 min, to 38.5% B and 23% C in 3.0 min, to 31% B and 38% C in 10 min, to 27.5% B and 45% C in 1.0 min, at 27% B and 45% C for 3 min, to 22.5% B and 55% C in 2 min, to 100% C in 1 min, at 100% C for 4 min, to 42% B and 16% C in 1 min.
Flow Rate:	1.0 ml/min
Temperature:	38°C.
Sample:	Intravenous Solution diluted 100 fold with derivatisation buffer.
Injection Vol:	5 µl.
Setting:	263 nm.

GBC

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Major Features

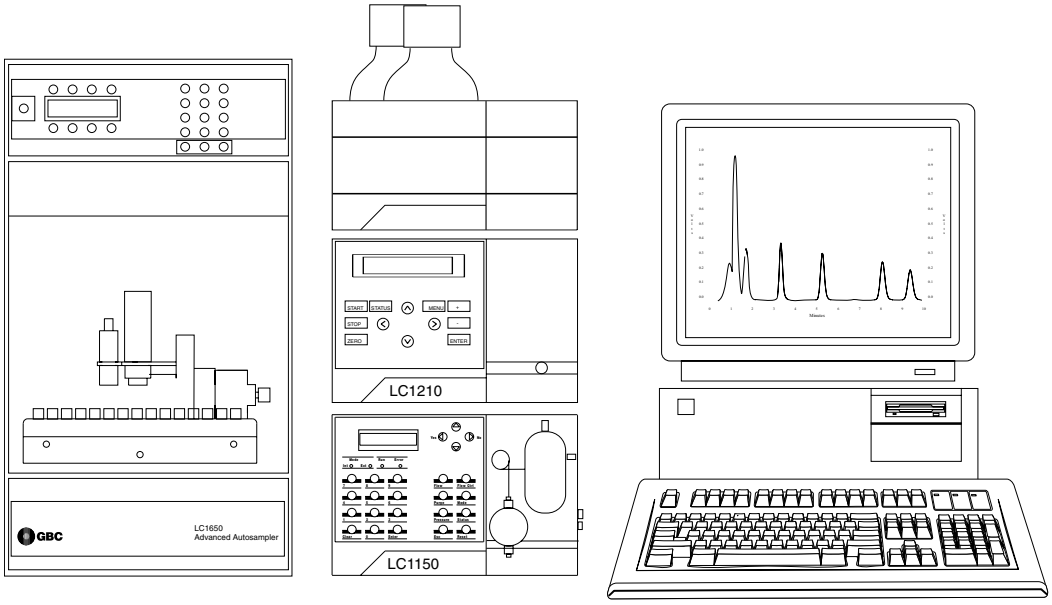
Resolution
Sensitivity
Selectivity
Flexibility
Precision
Accuracy

Relevant Industries

Biotechechnological
Pharmaceutical
Academic (Biochemistry)
Food (Quality Control)

GBC HPLC Instrumentation

LC1150 Quaternary Gradient HPLC Pump
LC1210 Programmable Dual Wavelength Scanning UV/Vis Detector
LC1120/LC1150 HPLC Column Oven Option
LC1650 Advanced Autosampler
WinChrom Chromatography Data Management System



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GBC publication number 01-0338-00
September, 1995

HPLC

application note

'...the sample is diluted with a derivatisation buffer and filtered; the system takes care of the rest...'

Amino Acid Determination in Intravenous Solution with Automated Pre-column FMOC Derivatisation and Fluorescence Detection

Sample

1. Aspartic Acid
2. Glutamic Acid
3. OH-Proline
4. Asparagine
5. Serine
6. Histidine
7. Glycine
8. Threonine
9. Alanine
10. Proline
11. Tyrosine
12. Arginine
13. Valine
14. Methionine
15. Isoleucine
16. Leucine
17. Phenylalanine
18. Ornithine
19. Lysine

Intravenous Solution.

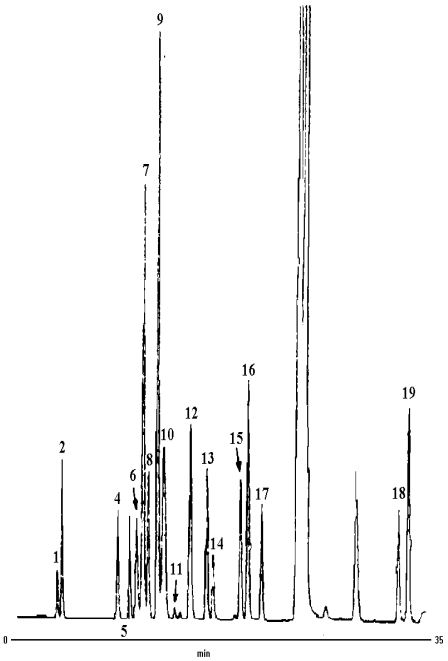
Preparation

Diluted with derivatisation buffer (1:100), and filtered.

Amino Acid Analysis

These notes illustrate the flexibility of the GBC chromatography equipment in performing complicated pre-column chemistries automatically via the LC1650 and data management station. There is a need in the clinical, food, and Biotechechnological industries for the analysis of different sample matrices containing amino acids. The sample matrix can provide the largest problems for most chromatography equipment. The combination of excellent chromatography, precise flow control and sensitive, selective detection, give GBC the edge over most post-column systems as well as many of the pre-column derivative systems.

Conditions



Column:	(150 x 4.6 mm ID)
Mobile Phase:	Solvent A: 30 mM Ammonium Phosphate (pH 6.5) (85%) and Methanol (15%). Solvent B: Water (85%) and Methanol (15%). Solvent C: Acetonitrile (90%) and Water (10%).
Gradient:	Equilibrate for 5 min. from 68% B and 15% C to 43.2% B and 46% C in 32 min, to 100% C in 0.01 min, at 100% C for 3 min., to 68% B and 15% C in 0.01 min.
Flow Rate:	1.0 ml/min
Temperature:	38°C.
Sample:	Intravenous Solution diluted 100 fold with derivatisation buffer.
Injection Vol:	5 µl.
Setting:	263 nm and emission 315 nm.

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GBC HPLC Instrumentation

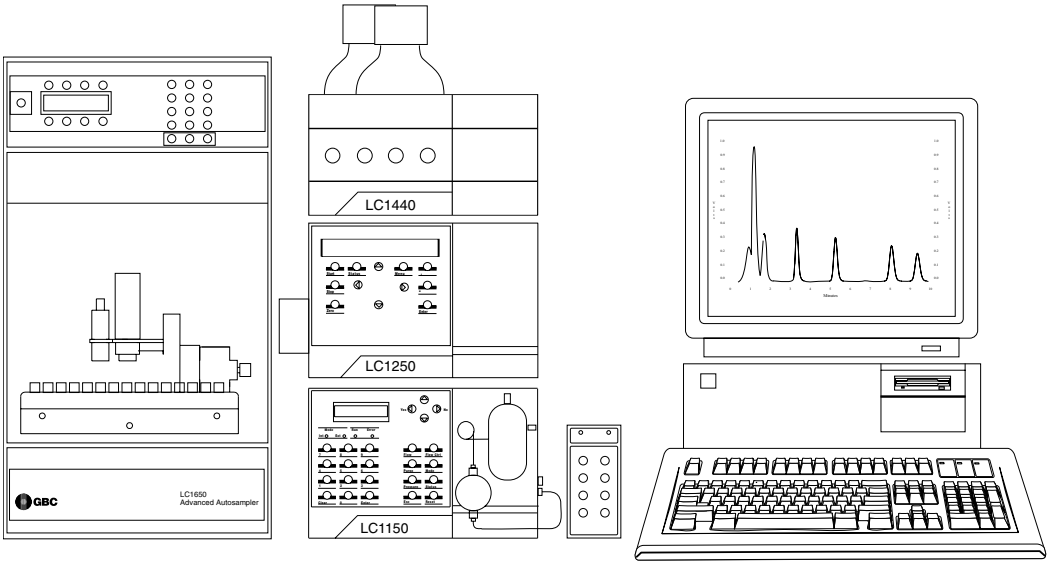
LC1150 Quaternary Gradient HPLC Pump
LC1250 Fluorescence Detector
LC1120/LC1150 HPLC Column Oven Option
LC1650 Advanced Autosampler
WinChrom Chromatography Data Management System

Major Features

Resolution
Sensitivity
Selectivity
Flexibility
Precision
Accuracy

Relevant Industries

Biotechnological
Pharmaceutical
Academic (Biochemistry)
Food (Quality Control)



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GBC publication number 01-0339-00
September, 1995

HPLC

application note

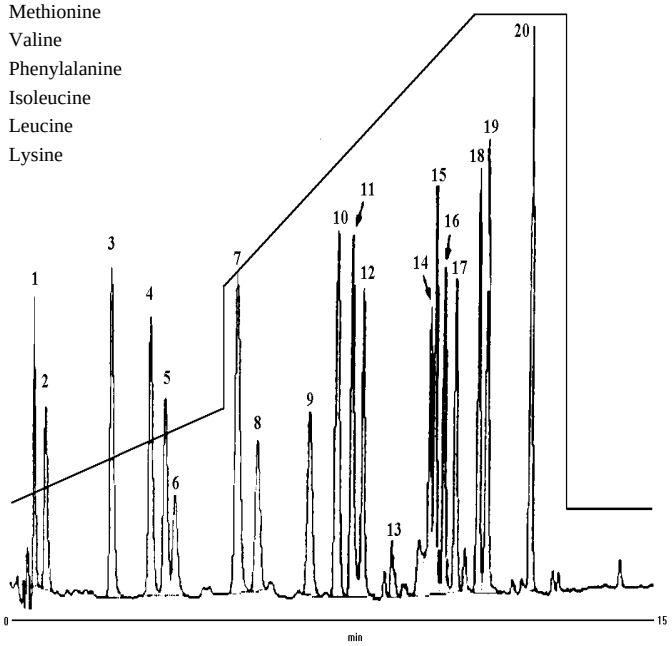
Amino Acid Analysis with Automated Pre-column OPA Derivatisation and UV Detection

'...flexibility of the GBC chromatography equipment in performing complicated pre-column chemistries...'

Sample

1. Aspartic Acid
2. Glutamic Acid
3. Asparagine
4. Serine
5. Glutamine
6. Histidine
7. Glycine
8. Threonine
9. Arginine
10. Taurine
11. Alanine
12. Tyrosine
13. Cystine
14. Tryptophan
15. Methionine
16. Valine
17. Phenylalanine
18. Isoleucine
19. Leucine
20. Lysine

Conditions



Hydrolysate Standard + Asparagine, Glutamine and Taurine.

Amino Acid Analysis

These notes illustrate the flexibility of the GBC chromatography equipment in performing complicated pre-column chemistries automatically via the LC1650 and data management station. There is a need in the clinical, food, and Biotechechnological industries for the analysis of different sample matrices containing amino acids. The sample matrix can provide the largest problems for most chromatography equipment. The combination of excellent chromatography, precise flow control and sensitive, selective detection, give GBC the edge over most post-column systems as well as many of the pre-column derivative systems.

Column:	Spherisorb ODS2 (C18) 5 µ Column (150 x 4.6 mm ID) Mobile Phase: Solvent A: 20 mM Potassium Phosphate (pH 7.0) (89%), Methanol (10%) and THF (1%). Solvent B: 20 mM Potassium Phosphate (pH 7.0) (19%), Methanol (80%) and THF (1%).
Gradient:	0.00 to 1.0 min 20% B, to 35% B in 3.30 min, to 55% B in 0.01 min, to 99% B in 5.29 min, at 99% B for 2 min, to 20% B in 0.01 min, at 20% B for 7 min.
Flow Rate:	1.0 ml/min
Temperature:	40°C
Sample:	50 mM Standard Mixture
Injection Vol:	10 µl
Setting:	340 nm.

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GBC HPLC Instrumentation

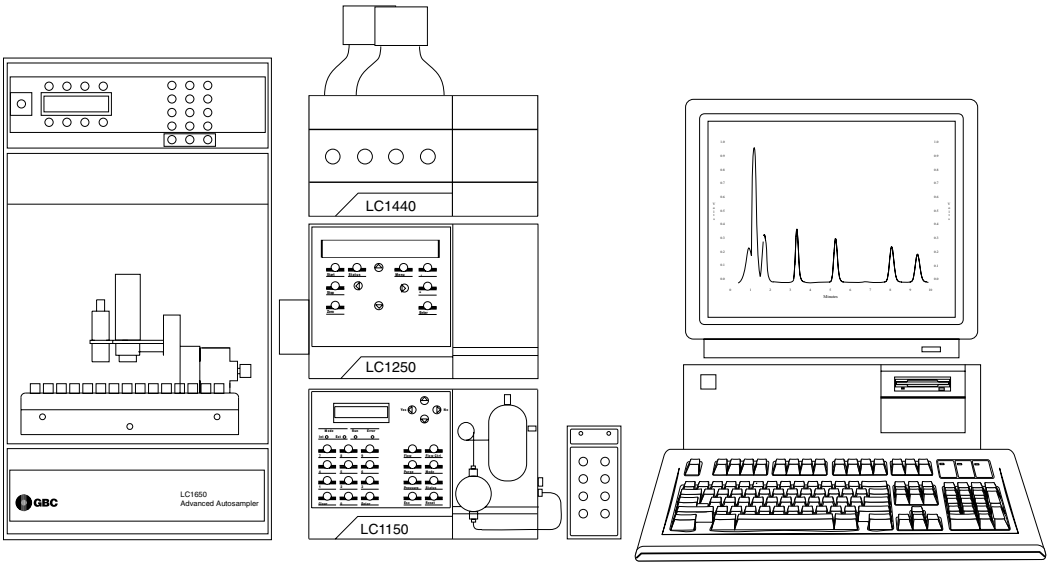
LC1150 Quaternary Gradient HPLC Pump
LC1205 Programmable UV/Vis Detector
LC1120/LC1150 HPLC Column Oven Option
LC1445 System Organiser
LC1650 Advanced Autosampler
WinChrom Chromatography Data Management System

Major Features

Resolution
Sensitivity
Selectivity
Flexibility
Precision
Accuracy

Relevant Industries

Biotechnological
Pharmaceutical
Academic (Biochemistry)
Food (Quality Control)



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GBC publication number 01-0331-00
September, 1995

HPLC

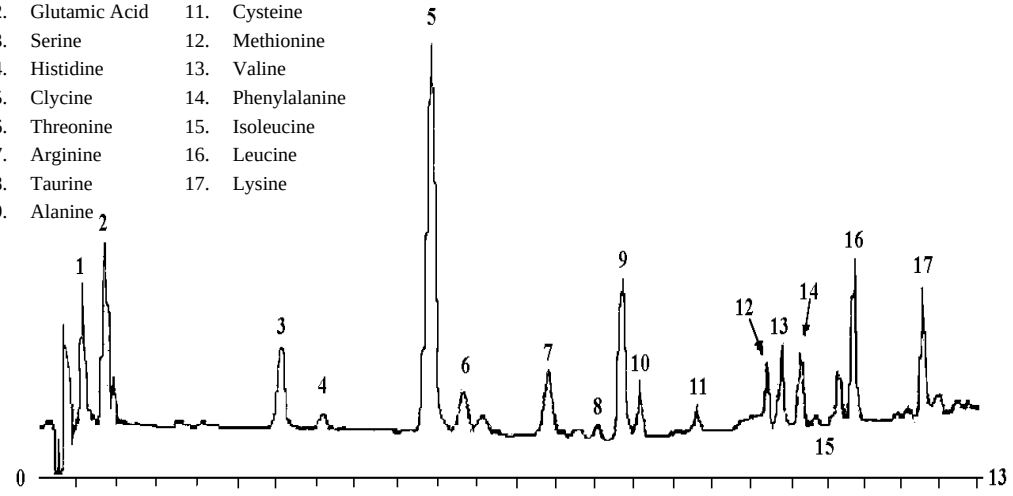
application note

Amino Acid Determination in Protein Rich Food Sample with Automated Pre-column OPA Derivatisation and UV Detection

'...there is a need in the clinical, food, and biotechnological industries for the analysis of different sample matrices containing amino acids...'

Sample

1. Aspartic Acid
2. Glutamic Acid
3. Serine
4. Histidine
5. Clycine
6. Threonine
7. Arginine
8. Taurine
9. Alanine
10. Tyrosine
11. Cysteine
12. Methionine
13. Valine
14. Phenylalanine
15. Isoleucine
16. Leucine
17. Lysine



Protein Rich Food Sample Hydrolysed with 4 M Methansulfonic Acid.

Amino Acid Analysis

These notes illustrate the flexibility of the GBC chromatography equipment in performing complicated pre-column chemistries automatically via the LC1650 and data management station. There is a need in the clinical, food, and biotechnological industries for the analysis of different sample matrices containing amino acids. The sample matrix can provide the largest problems for most chromatography equipment. The combination of excellent chromatography, precise flow control and sensitive, selective detection, give GBC the edge over most post column systems and many of the precolumn derivative systems as well.

Conditions

	Spherisorb OD S2 (C18) 5 µ Column (150 x 4.6 mm ID)
Mobile Phase:	Solvent A: 20 mM Potassium Phosphate (pH 7.0) (89%), Methanol (10%) and THF (1%). Solvent B: 20 mM Potassium Phosphate (pH 7.0) (19%), Methanol (80%) and THF (1%).
Gradient:	0.00 to 1.0 min 20% B, to 35% B in 3.30 min, to 55% B in 0.01 min, to 99% B in 5.29 min, at 99% B for 2 min, to 20% B in 0.01 min, at 20% B for 7 min.
Flow Rate:	1.0 ml/min
Temperature:	40°C
Sample:	Food sample hydrolysed with 4 M methanesulfonic acid.
Injection Vol:	10 µl.
Setting:	340 nm.

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01-0341-00

GBC HPLC Instrumentation

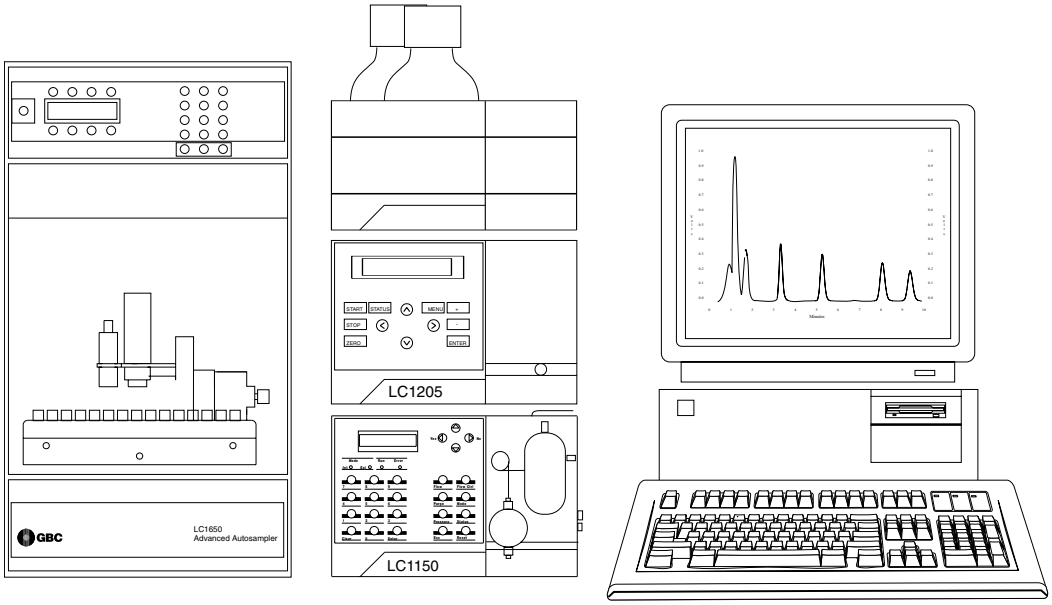
LC1150 Quaternary Gradient HPLC Pump
LC1205 Programmable UV/Vis Detector
LC1120/LC1150 HPLC Column Oven Option
LC1650 Advanced Autosampler
LC1445 System Organiser
WinChrom Chromatography Data Management System

Major Features of these Notes

Resolution
Sensitivity
Selectivity
Flexibility
Precision
Accuracy

Relevant Industries

Biotech
Pharmaceutical
Academic (Biochemistry)
Food (Quality Control)



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GBC publication number 01-0341-00
September, 1995

HPLC application note

Diagnosis of Neuroblastoma, a Childhood Cancer, by LCEC

Abstract

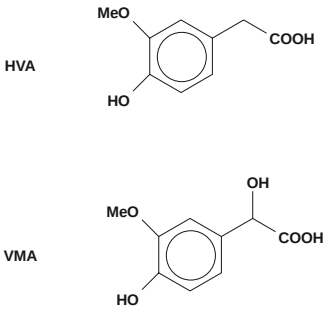
A rapid, sensitive and automated method for the diagnosis of Neuroblastoma is described. In the assay, Homovanillic acid (HMA) and Vanillylmandelic acid (VMA) in infant urine are determined by LCEC. The method is highly selective and sensitivity is in the ppm range. Each analysis is completed within 10 minutes. Results can be individually and/or group validated to assure the precision and accuracy of assays. Sample preparation is minimal, and up to 160 samples can be batched for unattended operation. The system also offers intelligent diagnostics to ensure optimal chromatographic performance and protection of precious samples.

'...the electrochemical reaction involves the selective oxidation of the vanillyl nucleus to the corresponding benzoquinone...'

Neuroblastoma (NB) is the most common solid tumour in young children and the second most common cause of infant mortality under the age of five. NB is a tumour of the sympathetic nerves or nerve ganglia. It usually occurs in the thoracic or abdominal regions close to the spine and can spread rapidly into other areas including adrenal, kidney, liver and bone.1 NB is an embryonic neoplasm estimated to be present in 0.4% of new borns. The tumour remains dormant initially but will eventually develop in approximately one in 7000 children before the age of five2. However, NB, is curable when detected early in babies less than 12 months old. In contrast, the chances of recovery dimish markedly in children over the one despite costly therapy. As NB is easily curable when detected early, mass screening programs have been initiated in Japan, UK, Canada, France and USA.

Neuroblastomas are unique tumours biochemically because they possess metabolic pathways for catecholamine synthesis and catabolism. Homovanillic acid (HVA) and vanillylmandelic acid (VMA), metabolites of dopamine, are re-excreted in excessive amounts in patients' urine and present as the most useful markers for the tumour.

Keywords:
Neuroblastoma, Homovanillic Acid (HMA), Vanillylmandelic Acid (VMA), Electrochemical Detection, Reversed Phase HPLC



Both HVA and VMA contain the electroactive vanillylmoiety, and Liquid Chromatography with Electrochemical Detection (LCEC) is the method of choice for their determination. The electrochemical reaction involves the selective oxidation of the vanillyl nucleus to the corresponding benzoquinone. The anodic current generated can then be used for accurate quantitation of the analytes. This method of LC detection is highly selective with little interference from other metabolites. The GBC LC1260 Electrochemical Detector, with its unique 'Wall Jet' design, allows shorter equilibration time and increased reliability. The use of a MicroComposite Glassy Carbon Electrode results in increased sensitivity when compared with conventional 3 mm glassy carbon electrodes. Sensitivity has also been enhanced through the use of low noise electronic circuitry with active and digital filtering. In addition, the automatic self-cleaning mode of the detector extends the electrode's operating life by avoiding contamination of the electrode surface.

GBC

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01-0342-00

Automation of the HPLC system is accomplished by control of the LC1120 Advanced Spindle Driven HPLC Pump, the LC1260 Electrochemical Detector and robotic functions of the LC1650 Advanced Autosampler via the WinChrom Chromatography Data Management System. The system utilises a comprehensive set of validation parameters, enabling single and group validation during analyses. Command sequence can be easily pre-programmed to direct the execution of different analytical routines depending on the validation results. This ensures optimal performance of the analyser and avoids wastage of analysis time and precious samples during unattended operations. Multi-tasking of the management system also permits the running of other software programs, e.g., for report preparation, while analyses are being processed.

The LC1650 Advanced Autosampler has a maximum sample capacity of 160. The low cost LC1120/LC1150 HPLC Column Oven Option Kit delivers excellent flow rate accuracy and precision. In addition, the configuration of the system offers maximum flexibility, offering easy modification of the instrumentation for other HPLC applications.

'...the use of a MicroComposite Glassy Carbon Electrode results in increased sensitivity...'

GBC HPLC Instrumentation

LC1260 Electrochemical Detector.
LC1440 System Organiser
LC1650 Advanced Autosampler
LC1120/LC1150 HPLC Column Oven Option
WinChrom Chromatography Data Management System

Sample Preparation

Infant urine is collected on a filter paper strip. It is diluted and filtered (2 µm) before injection.

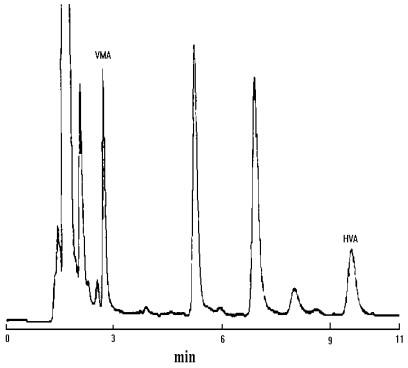


Figure 1 *Infant Urine Sample*

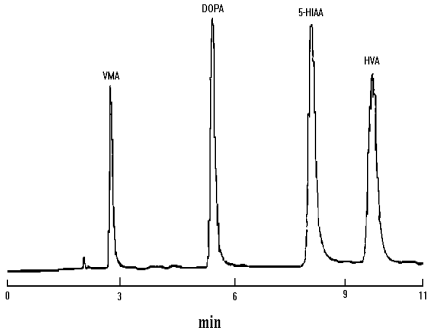
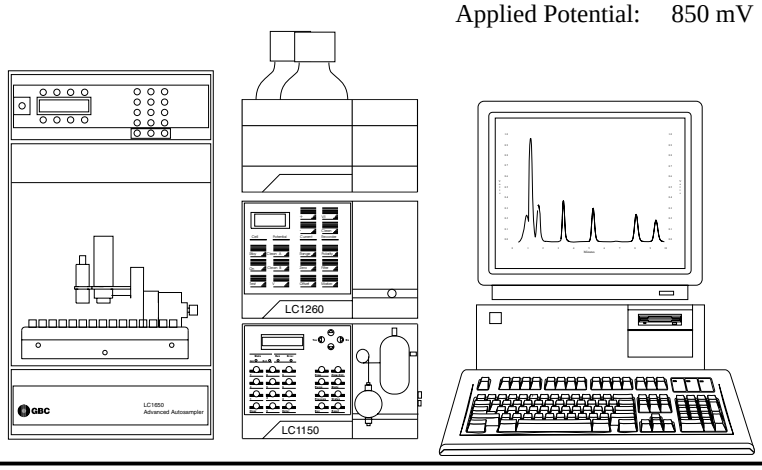


Figure 2 *15 µM Standard (5-HIAA = 5-OH-indoleacetic acid)*

Conditions

Column:	Spherisorb S5 OD S2, 150 x 4.6 mm ID
Mobile Phase:	0.05 mM Sodium Phosphate Buffer, pH 2.5, with 0.1 mM EDTA Disodium Salt/Acetone (90:1) (Helium sparging)
Flow Rate:	1.0 ml/min
Temperature:	35°C
Detection:	
Working Electrode:	3 mm Micro Composite Glassy Carbon.
Reference Electrode:	Ag/AgCl (3M KC1)
Auxiliary Electrode:	Cell Body
Applied Potential:	850 mV



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GBC publication number 01-0342-00
September, 1995

HPLC application note

Catecholamine Analysis for the Diagnosis of Neural Crest Tumours

Abstract

A method is described for the analysis of catecholamines—dopa, dopamine, nor-adrenaline and adrenaline, by LCEC. Assay of these biogenic amines in biological fluids has been utilised for the diagnosis of neural crest tumours and other human metabolic disorders. The method is highly selective and sensitivity is at the ppm range. Each assay is completed within 6 minutes.

'...biogenic amines are secreted only by tumours occurring in embryological neural crest derived tissues...'

The analysis of catecholamines, an important class of neurotransmitters, has been utilised in clinical laboratories for the diagnosis of tumours of the neural crest and for the investigation of neurological and neuropsychiatric disorders. The specific and successful diagnosis of these tumours has been possible as these biogenic amines are secreted only by tumours occurring in embryological neural crest derived tissues. Plasma and urine samples from patients are analysed for catecholamines and their metabolites.

Three catecholamines are important for the biochemical diagnosis: Dopamine, Noradrenaline (Norepinephrine) and Adrenaline (Epinephrine). Noradrenaline is derived from dopamine by hydroxylation of its side chain, while N-methylation of noradrenaline gives adrenaline (Figure 1). Dopamine is synthesized in abnormal quantities by neuroblastomas and ganglioneuromas, resulting in elevated levels of the amines and its metabolites in the blood and urinary excretion. On the other hand, phaechromocytomas are characterised by the secretion of an excess amount of noradrenaline and adrenaline. Since none of these tumours produce any significant clinical symptoms until they reach advanced stages and intrude upon other surrounding tissues, an accurate measurement of these amines and their metabolites offers a rapid, reliable and non-invasive diagnosis.

Keywords:
Catecholamines, Dopa, Dopamine, Noradrenaline, Adrenaline, Norepinephrine, Epinephrine, Electrochemical Detection

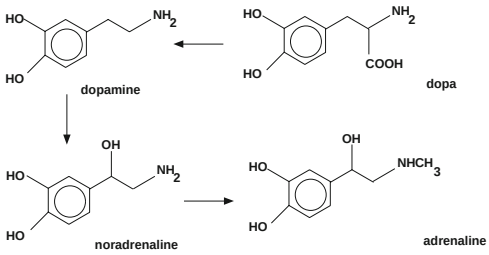

Figure 1 *Biotransformation of Catecholamines*

Traditionally, the detection of catecholamines in biological fluids has been difficult due to their low concentrations in complex matrices. The high capital and running costs for dedicated systems, such as GCMS, precludes their use in all but a few laboratories. In recent years, the advent of LCEC (Liquid Chromatography with Electrochemical Detection) has delivered the specificity and sensitivity required for the HPLC analysis of catecholamines and their 3-methylated metabolites.1 Derivatisation is not required and instrumentation is relatively inexpensive. Common to all of these biogenic amines is a 3,4-dihydroxyphenyl moiety (hence the name 'catechol') which can be selectively oxidised to the corresponding benzoquinone on the surface of the electrode (Figure 2). The anodic current generated, which is directly proportional to the number of analyte molecules in contact with the electrode surface, can be used for the accurate quantitation of the analytes.

Figure 2 *Oxidation of Catecholamines*

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'...the exceptionally low background noise level of the detector permits on-column detection of catecholamines down to low picogram levels...'

As the sensitivity and the reliability of the detector employed for the analysis are of great importance, the GBC LC1260 Electrochemical Detector has been designed with these criteria in mind. The LC1260 detector, with its unique 'Wall Jet' design, allows shorter equilibration time and increased reliability. Unlike traditional flowcells, this design also reduces the requirement for the detector flowcell to be dismantled for cleaning. Sensitivity has been enhanced through the application of low noise electronic circuitry featuring active and digital filtering. The exceptionally low background noise level of the detector permits on-column detection of catecholamines down to low picogram levels. In addition, the detector's self-cleaning mode extends the electrode operating life by avoiding contamination of the electrode surface.

Sample Preparation

Various extraction procedures are available in the literature based on cation-exchange followed by clean-up on alumina.2 Solid phase extraction procedures are also available from major SPE column manufacturers.

GBC HPLC Instrumentation

LC1110 Dual Piston HPLC Pump
LC1260 Electrochemical Detector
LC1440 System Organiser
LC1650 Advanced Autosampler
LC1120/LC1150 HPLC Column Oven
WinChrom Chromatography Data Management System

Conditions

Column: Spherisorb S5 ODS2, 150 x 4.6 mm ID

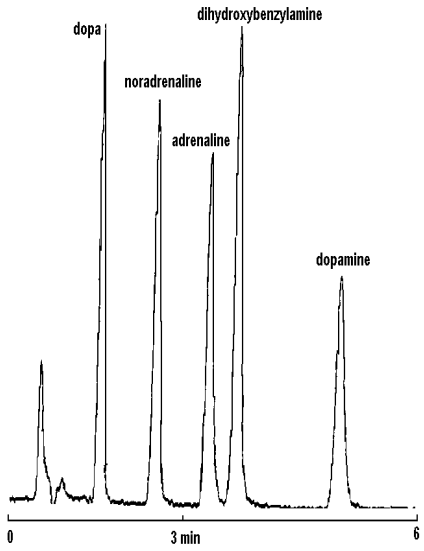


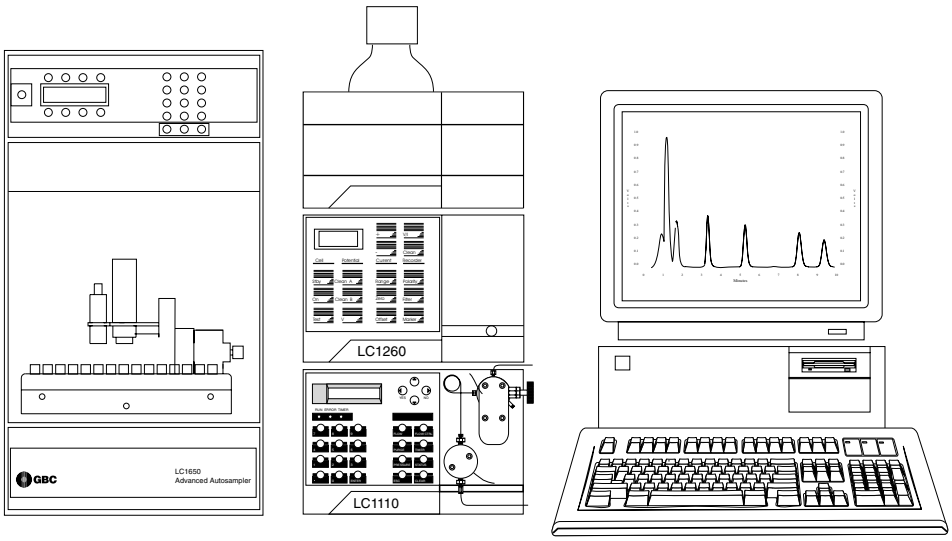
Figure 3 *Catecholamine Standards (5 picomoles on column)*

Mobile Phase: 75 mM Phosphate buffer with 1 mM sodium octyl sulfate and 0.05 mM EDTA, pH 3/ Acetonitrile (90:10) (Helium sparging)
Flow Rate: 1.0 ml/min
Temperature: 35°C
Detection:
Working Electrode: 3 mm Glassy Carbon.
Reference Electrode: Ag/AgCl (3 M KC1)
Auxiliary Electrode: Cell Body
Applied Potential: 650 mV

References

1. 'Diagnosis of Neuroblastoma, a Childhood Cancer, by LCEC', GBC Application Note B9.

2. E. Gerlo and R. Malfait, J. Chromatogr., 343, (1985), 9; G.C. Davis, P.T. Kissinger and R.E. Shoup, Anal. Chem., 53 (1981), 156; R.M. Riggin and P.T. Kissinger, Anal. Chem., 49, (1977), 2109.



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September, 1995

HPLC application note

Amino Acid Analysis by Precolumn Derivatisation using a New FMOC Procedure

'...the resultant derivatives are very stable and highly fluorescent, offering assay sensitivity in the fmol range...'

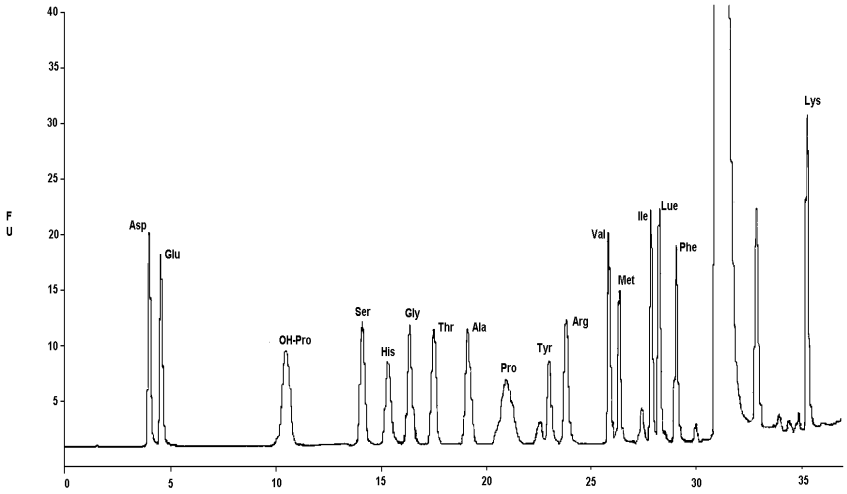

Figure 1 *Separation of Hydrolysate Amino Acid Standard with Fluorescence Detection*

Abstract

A rapid, sensitive method for the determination of primary and secondary common protein amino acids is described. This is based on a newly developed, simple and effective procedure for precolumn derivatisation using FMOC-Cl (9-fluorenylmethyl chloroformate). The method is applicable to both manual and automated protocols. It does not require any solvent extraction or incubation and gives stable single adducts including histidine and tyrosine. The HPLC conditions have been developed to deliver high assay accuracy and precision. Application of the technique has been demonstrated on hydrolysate samples and procedures for the execution of the application is described in detail.

Keywords:
Amino Acid, AMINOMATE, Automated Precolumn Derivatisation, FMOC

Traditionally, the determination of amino acids has been conducted by ion-exchange chromatography, followed by postcolumn derivatisation with ninhydrin or o-phthalaldehyde (OPA). In recent years, with developments in LC instrumentation and methodology, precolumn derivatisation and reversed-phase HPLC have been used as an alternate method for amino acid analysis.

The FMOC precolumn derivatisation method is attractive as it is applicable to both primary and secondary amino acids. The derivatisation is rapid and is conducted at ambient temperature. It is also not susceptible to any major matrix interferences. The resultant derivatives are very stable and highly fluorescent, offering assay sensitivity in the fmol range.

Our newly developed FMOC method has incorporated a modified procedure[1,2] which enhances the effectiveness of the FMOC chemistry by removal of the inherent setbacks of the traditional technique.

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The new derivatisation protocol and HPLC conditions allow:

1. Elimination of any requirement for solvent extraction, thus simplifying derivatisation and providing accurate quantitation of the hydrophobic amino acids.
2. Generation of single, stable adducts, allowing reliable quantitation of all amino acids including histidine and tyrosine.
3. Elimination of interference from reagent by-products.

'...a comparison of experimental results and literature values for angiotensin II, neurotensin, chymotrysinogen A and pepsin is presented...'

Several different peptide and protein hydrolysates have been chosen as examples to confirm the viability of this new FMOC method, especially its accuracy in the quantitation of the histidine and tyrosine residues. A comparison of experimental results and literature values for the analysis of two synthetic peptides, angiotensin II and neurotensin, and three proteins, lysozyme, chymotrysinogen A and pepsin is presented in the following table (Table 1).

Each derivatisation was performed on 10 pmol of hydrolysate and 5 pmol of the derivatised samples were analysed. The results agree very well with the expected values, including the determination of histidine and tyrosine at low levels of complex amino acid mixtures.

Reproducibility and Linearity of Automated Derivatisation

In an experiment using the recommended hardware configuration, the reproducibility of the automated procedure was established by analysing a series of 10 and 20 consecutive amino acid standards at a concentration of 5 pmol and 100 pmol on column (i.e., 20 and 40 µM respectively) with hydroxyproline as an internal standard. The reproducibility of the retention time was less than or equal to 0.5. The RSD for peak area (corrected to OH-Pro) is in the range of 0.5 – 2.2 at the 100 pmol level and 1.5 – 4.1 at 5 pmol level.

The linearity of the automated procedure was established over a 200-fold concentration range between 1 µM and 200 µM. All of the amino acids were found to give linear derivatisation over this range, with correlation coefficients.

Limit of Detection

The FMOC derivatives of amino acids are highly fluorescent and can be detected at very low levels. The detection limit for hydroxyproline, chosen for this study as it was not present in reagent blank derivatisation, was 50 fmol at a signal to noise ration of 3:1.

Table 1: Amino Acid Composition of Angiotensin II, Neurotensin, Lysozyme, Chymotrysinogen A and Pepsin

			*Molar Ratio**		
Acid	***Angiotensin***	***Neurotensin***	***Lysozyme***	***Chymotrysinogen***	***Pepsin***
Asn**	1.0 (1.2)	1.0 (1.1)	21.4 (21)	23.1 (23)	44.1 (42)
Clx***		2.0 (2.0)	5.2 (5)	14.4 (15)	26.9 (26)
Ser			9.3 (10)	23.2 (28)	40.7 (44)
His	0.9 (0.8)		0.8 (1)	2.0 (2)	1.0 (1)
Gly			11.8 (12)	21.7 (23)	34.9 (35)
Thr			7.0 (7)	21.9 (22)	26.4 (26)
Ala			12.6 (13)	22.3 (22)	17.2 (16)
Pro	1.0 (1.1)	2.0 (2.0)	2.1 (2)	8.9 (9)	14.8 (15)
Tyr	0.9 (1.0)	2.0 (2.0)	2.9 (3)	4.0 (4)	13.7 (16)
Arg	1.0 (1.0)	2.0 (2.0)	11.0 (11)	4.3 (4)	2.3 (2)
Val	0.9 (0.9)		5.6 (6)	20.4 (23)	19.9 (22)
Ile	0.9 (0.9)	1.0 (1.0)	5.5 (6)	9.0 (10)	21.9 (26)
Leu		2.0 (2.0)	8.0 (8)	19.8 (19)	25.8 (26)
Phe	1.1 (1.0)		3.1 (3)	6.4 (6)	13.8 (14)

* Expected Values given in parentheses (peptide sequences from suppliers data and Protein compositions from Swiss protein data bank)

** Asp + Asn

*** Glu + Gln

In routine analysis of protein hydrosylate samples, lower µM concentrations are achievable with fluorescence detection. UV detection is typically 25 times less sensitive than fluorescence, but is useful for analysis in the higher µM concentration range and the determination of tryptophan and cystine which form non-fluorescent FMOC derivatives.

Summary

The new FMOC method enables common protein amino acids to be accurately determined in lower µM concentrations. This methodology is easy to execute and is applicable to both manual and automated derivatisation. The method delivers stable adducts, is linear and reproducible within the stated concentration range. Its viability has been confirmed by the analysis of various hydrolysate samples.

'...the reproducibility of the retention time was less than or equal to 0.5%...'

GBC HPLC Instrumentation

LC1150 Quaternary Gradient Pump
LC1150 Oven Option
LC1250 Fluorescence Detector
LC1650 ACSIS Advanced Autosampler
WinChrom 1-2 Data Management System*

(*plus compatible 486 PC & accessories)

Conditions

Column : Hypersil Column for Amino Acid Analysis, 150 x 4.6 mm ID

Fluorescence Detector:
Ex: 270 nm
Em: 316 nm

Mobile Phase A: 30 mM Ammonium Phosphate (pH 6.5) in 15% Methanol/85% Water

Mobile Phase B: 15% Methanol / 85% Water

Mobile Phase C: 90% Acetonitrile / 10% Water

Equilibration: 5 minutes

Flow Rate: 1.00 ml/min

Temperature: 38ºC

Injection Vol. : 5 ul

References

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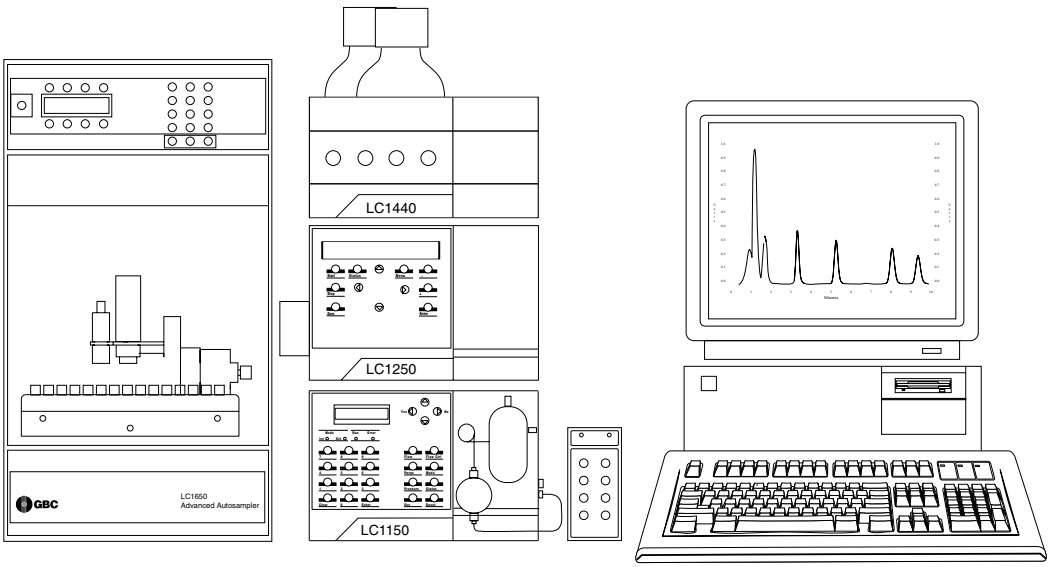

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GBC publication number 01-0344-00
September, 1995

HPLC

application note

Determination of Amino Acids in Beer Samples by Precolumn Derivatisation using the FMOC Procedure

'...this method does not require any solvent extraction or incubation and gives stable single adducts...'

Abstract

The application, 'Amino Acid Analysis by Precolumn Derivatisation using a New FMOC Procedure' was used for the amino acid analysis of beer samples, supplied by a local brewery. This method does not require any solvent extraction or incubation and gives stable single adducts.

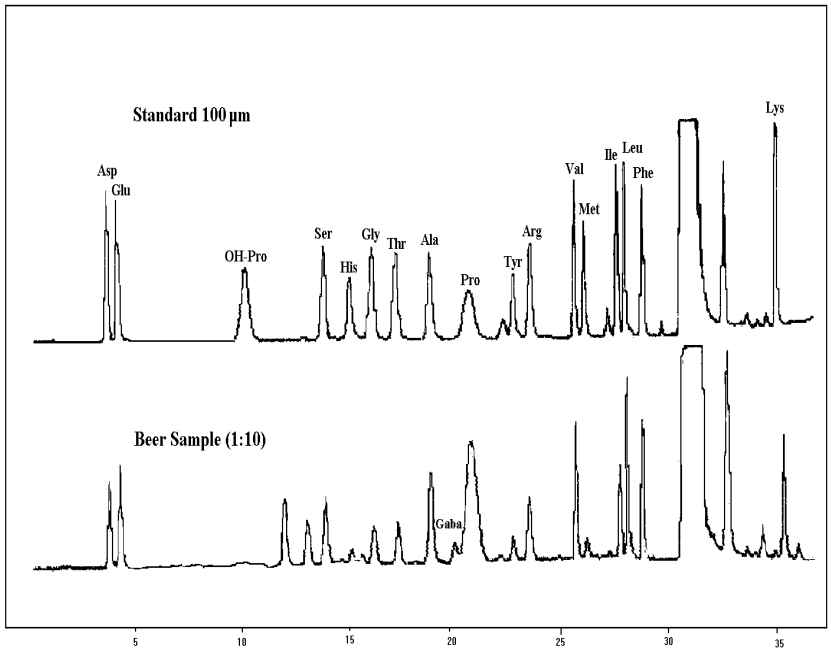
Sample Preparation

Diluted beer sample (1:10) with derivatisation (diluent) buffer, and filtered.

Conditions

Keywords:
Amino Acid, AMINOMATE, Automated Precolumn Derivatisation, FMOC, beer

Column :	Hypersil Column for Amino Acid Analysis, 150 x 4.6 mm ID
Fluorescence Detector	
Ex:	270 nm
Em:	316 nm
Equilibration :	5 min
Flow Rate:	1.00 ml/min
Temperature:	38°C
Injection Vol. :	5 µl



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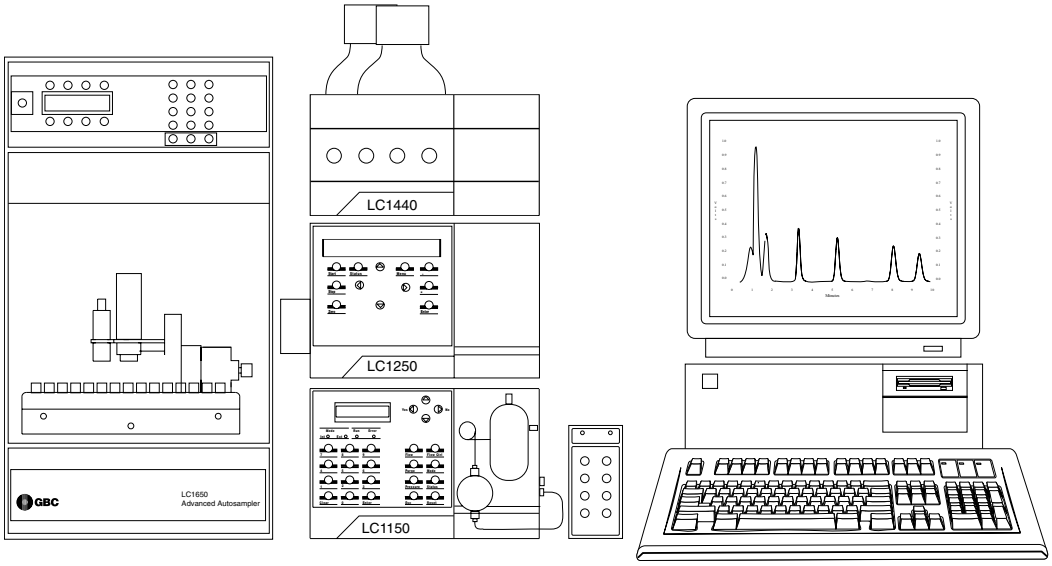
(*plus compatible 486 PC & accessories)



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GBC publication number 01-0345-00
September, 1995

HPLC application note

Determination of Amino Acids in Wine Samples by Precolumn Derivatisation using the FMOC Procedure

Abstract

The application, 'Amino Acid Analysis by Precolumn Derivatisation using a New FMOC Procedure' was used for the amino acid analysis of wine samples, supplied by a local vineyard. This method does not require any solvent extraction or incubation and gives stable single adducts.

Keywords:
Amino Acid, AMINOMATE, Automated Precolumn Derivatisation, FMOC, wine

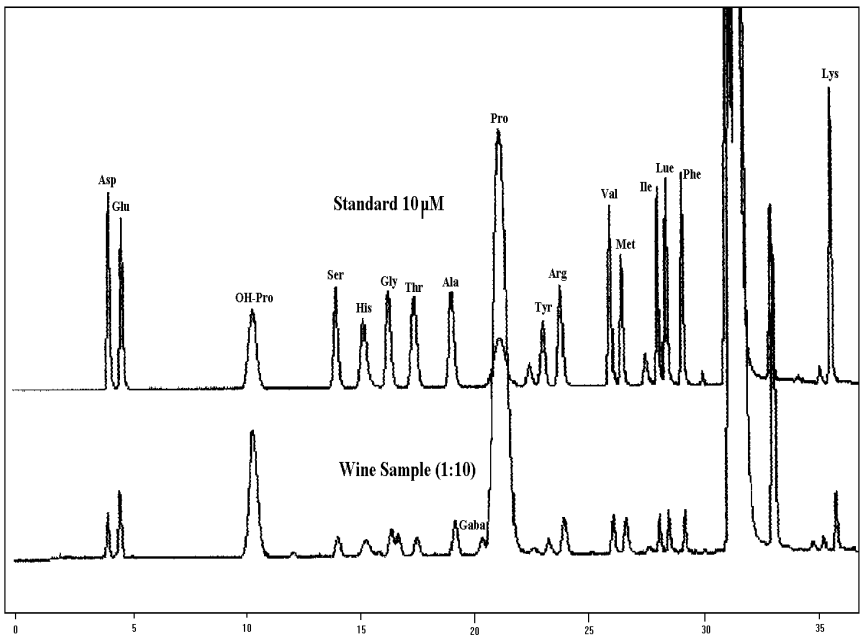
'...this method does not require any solvent extraction or incubation and gives stable single adducts...'

Sample Preparation

Diluted wine sample (1:10) with derivatisation (diluent) buffer, and filtered.

Conditions

Column:	Hypersil Column for Amino Acid Analysis, 150 x 4.6 mm ID
Fluorescence Detector:	
Ex:	270 nm
Em:	316 nm
Equilibration:	5 minutes
Flow Rate:	1.00 ml/min
Temperature:	38ºC
Injection Vol.:	5 µl



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GBC HPLC Instrumentation

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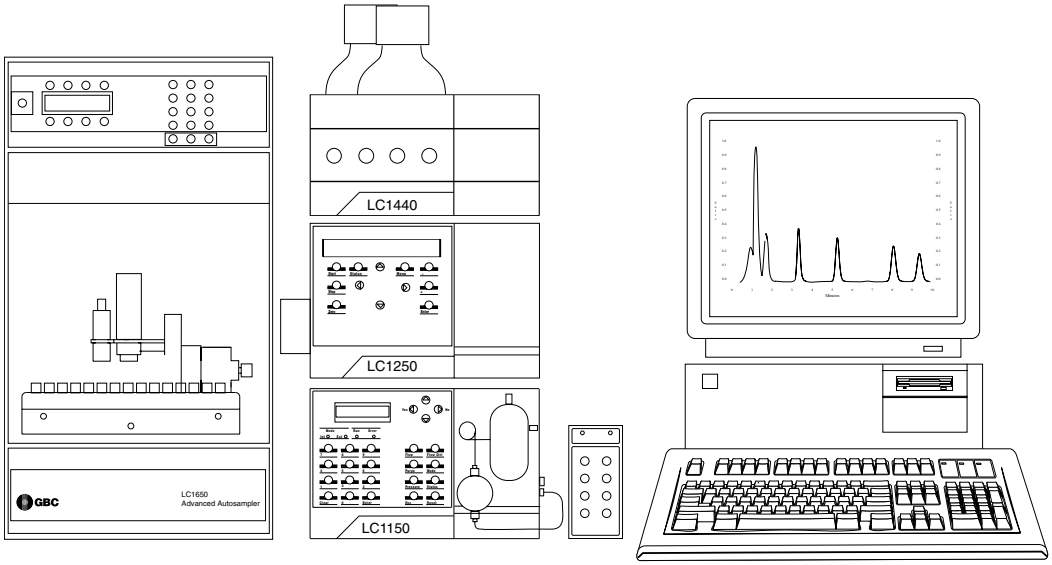
LC1120/1150 Online Degasser
LC1250 Fluorescence Detector
LC1650 ACSIS Advanced Autosampler
WinChrom 1-2 Data Management System*

(*plus compatible 486 PC & accessories)



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GBC publication number 01-0346-00
September, 1995

HPLC application note

Determination of Phenols by Electrochemical Detection

'...there is little fluctuation of the system between injections, achieving good reproducibility...'

Abstract

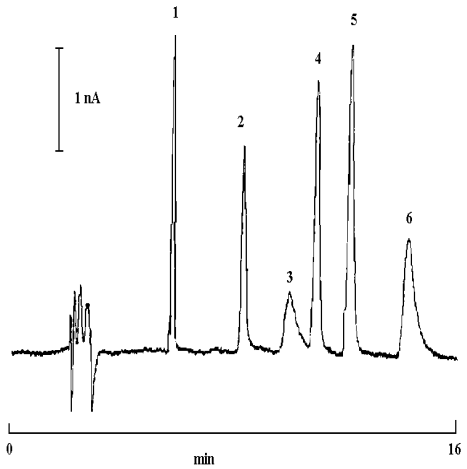
A method is described for the analysis of phenols in waste water by RP-LCEC (Reverse Phase Liquid Chromatography with Electrochemical Detection). The method is highly selective while no derivatisation is required. Sensitivity is at ppb level. There is little fluctuation of the system between injections, achieving good reproducibility and allowing samples to be analyzed every 15 minutes.

Keywords:
Phenols, Electrochemical, RP-LCEC, Wastewater, Environmental

Phenols constitute a large part of the environmental pollutant load of many industrial processes, *e.g.*, oil refining, coal processing, agrochemical manufacturing and wool preserving. Phenols are also found in soil from contaminated sites either as a direct result of past industrial practices or as degradation products.[1]

The once popular colorimetric method of 4-aminoantipyrine derivatisation[2] has been replaced by modern instrumental methods. Gas chromatography employing mass spectrometry, electron capture and flame ionization detection is used but usually requires derivatisation of the phenols to improve volatility and sensitivity.[3] By comparison, LCEC offers very high sensitivity (at pg level) and selectivity, in that detected compounds must be electroactive at the control potential. In addition, only minimal sample clean-up is required in most cases. Phenols, with the electroactive aromatic moiety, have been well studied in their trace analysis for environmental monitoring by LCEC. In fact, reverse phase LCEC has proven to be the most selective and sensitive of all modern techniques.

The optimum oxidation potential range for phenols is 700 to 1100 mV. Passivation of the glassy carbon electrode surface by the phenoxy radical, the oxidation product of phenol, is well documented. As a result of this passivation, the phenol oxidation potential is usually set at the upper limit of the available potential window, typically at 1110 mV. The optimum oxidation potential for substituted phenols tends to be less, depending upon the substituent's ability to stablize the aromatic nucleus.



1. Phenol
2. 2-Chlorophenol
3. 2-Nitrophenol
4. 2,4-Dimethylphenol
5. 4-Chloro-3-methylphenol
6. 2,4-Dichlorophenol

Figure 1 *HPLC Separation of Various Phenols*

The GBC LC1260 Electrochemical Detector, with its unique 'Wall Jet' design, allows shorter equilibration time and increased reliability. During normal operation, it is believed that the high turbulence created in the 'Wall Jet' nozzle decreases chemical fouling of the working electrode surface, thereby reducing the requirement for the detector flow-cell to be dismantled frequently for cleaning. Sensitivity is enhanced through the use of low noise electronic circuitry with active and digital filtering.

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Conditions

Column:	Spherisorb S5 ODS2, 150 x 4.6 mm ID
Mobile Phase:	0.02 M Ammonium Acetate, pH 6.0/Acetonitrile (60:40) (Helium Sparging)
Flow Rate:	1.0 ml/min
Temperature:	Ambient
Detection:	
Working Electrode:	3 mm MicroComposite Glassy Carbon
Reference Electrode:	Ag/AgCl (3M KC1)
Auxiliary Electrode:	Cell Body
Applied Potential:	1100 mV

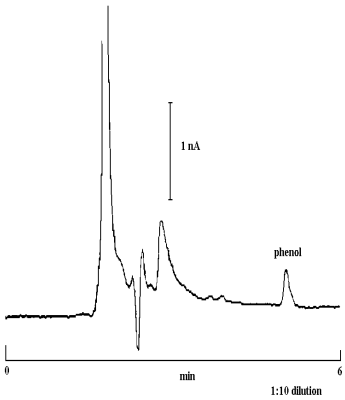

Figure 2

Sample Preparation

1. Dilute sample with mobile phase.
2. Filter diluted sample through a 0.2 µm filter.
3. Transfer small quantity into autosampler vial.

(As with all preparation procedures, the analyst must determine the suitability or otherwise of the above procedure for their sample type. This procedure is for relatively clean wastewater, free of suspended matter.)

GBC HPLC Instrumentation

LC1110 Dual Piston HPLC Pump
LC1260 Electrochmical Detector
LC1440 System Organiser
LC1650 Advanced Autosampler
WinChrom Chromatography Data Management System

'...the high turbulence created in the 'Wall Jet' nozzle decreases chemical fouling of the working electrode surface, reducing cleaning requirements...'

References

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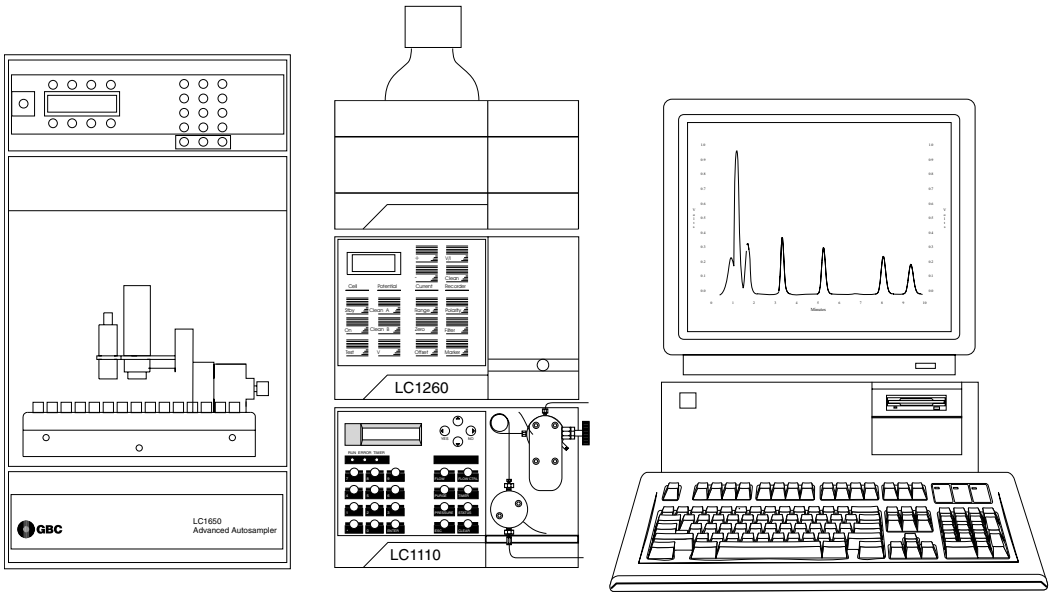

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GBC publication number 01-0347-00
September, 1995

HPLC

application note

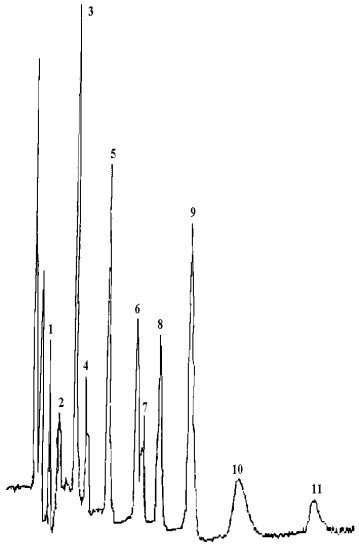
Phenol Separation Using Glassy Carbon Electrode (3 mm) with ppb Sensitivity

'...this detector removes the sample preparation required. This can mean up to a day's labor savings...'

Sample

US EPA Priority Phenol Pollutants (5 ppb) Chromatogram

1. 2,4-Dinitrophenol
2. 2-Chlorophenol
3. Phenol
4. 4-Nitrophenol
5. 2-Methyl-4,6-Dinitrophenol
6. 2-Nitrophenol
7. 2,4-Dinitrophenol
8. 4-Chloro-3-methylphenol
9. 2,4-Dichlorophenol
10. 2,4,6-Trichlorophenol
11. Pentachlorophenol



Conditions

Column:	Spherisorb OD S2 (C18) 5 µM Column (150 x 4.6 mm ID)
Mobile Phase:	20 M Ammonium Acetate, (pH 5.0) (60%): Acetonitrile (40%)
Flow Rate:	1.0 ml/min
Injection Vol:	20 µl
Temperature:	40°C
Electrode:	3 mm Glassy Carbon
Potential:	1.0 V (Ag/AgCl)

This application illustrates the use of the patented impinging wall jet electrochemical detector. Most environmental applications require extractions and derivative formation to ensure sensitivity and selectivity. This detector removes the sample preparation required. This can mean up to a day's labor savings. The sample preparation consists of a filtering of the sample followed by injection or storage on the autosampler carousel.

When sample throughput is required, this application can save the commercial clinical laboratory hundreds of dollars per run. Compared to the cost of a GC-MS at $50,000 to $150,000, payback for this system, which is about one third of the cost of a GC-MS can be recovered in as little as one month.

Key Features

- Sensitivity
- Cost savings
- Selectivity
- New patented technology
- Limited technical expertise

Relevant Industries

- Commercial environmental laboratories
- EPA laboratories
- Industrial monitoring laboratories
- Water quality laboratories
- Water treatment laboratories
- Landfill monitoring

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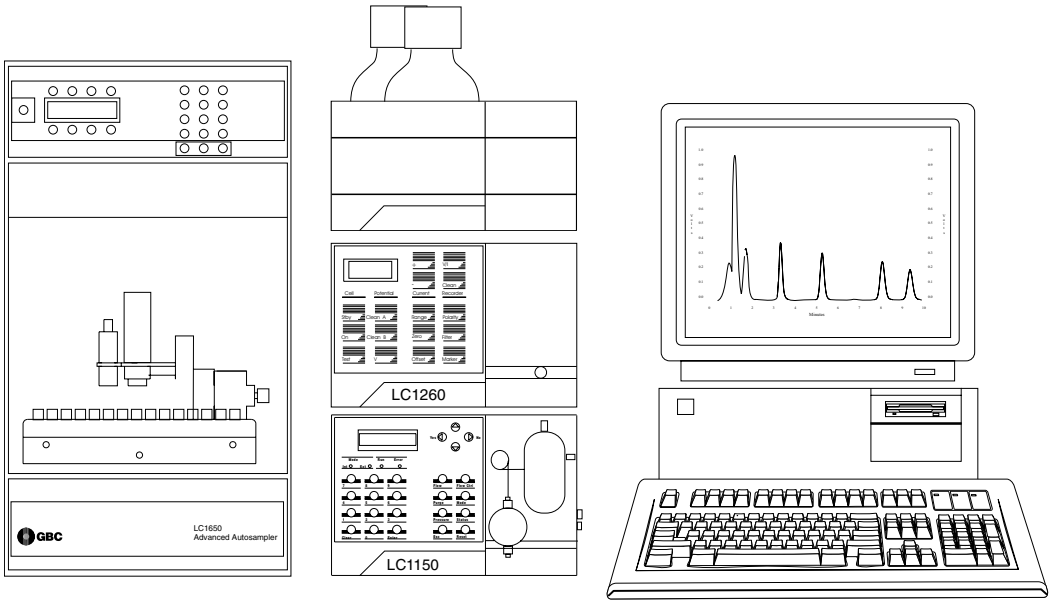
E2
01-0348-00

GBC HPLC Instrumentation

LC1150 Quaternary HPLC Pump
LC1260 Electrochmical Detector
LC1120/LC1150 HPLC Column Oven
LC1650 Advanced Autosampler
WinChrom Chromatography Data
Management System



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GBC publication number 01-0348-00
September, 1995

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.

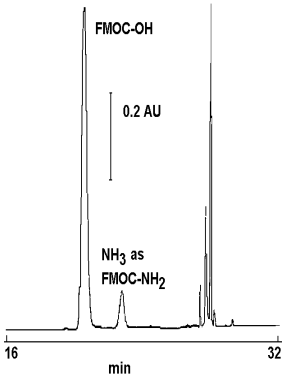

Figure 1 *Ammonia Standard (5 ppm)*

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

GBC

E3
01-0349-00

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

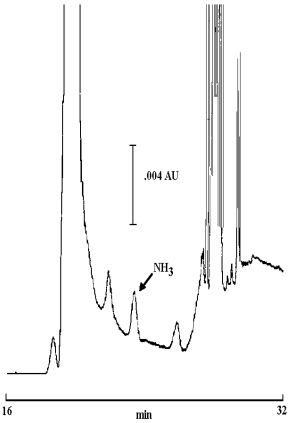

Figure 2 *Analysis of Ammonia in Hail Water*

References

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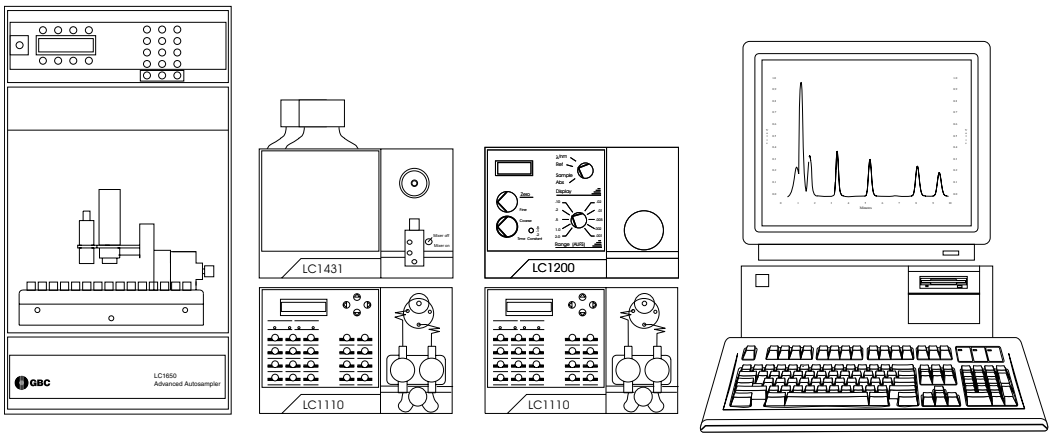
4. P.A. Haynes, D. Sheumack, L.G. Greig, J. Kibby and J.W. Redmond, in press.

'...by employing similar chemistry, a method has been developed for the selective analysis of ammonia...'



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September, 1995

HPLC application note

Azide in Air

Introduction

Concern for the health of workers exposed to toxic vapours in industries where sodium azide is processed, *e.g.*, in the manufacture of explosives, pharmaceuticals and pesticides, has led to the publication of a number of methods for the detection of azide at trace levels in the environment.

One of the most sensitive of these methods was developed by Stephen Swarin and Richard Waldo at General Motors (Reference 1) following a proposal that sodium azide be used as a nitrogen gas generant in 'air bags' for occupant restraint in automobile collisions. They derivatised azide samples with 3,5-dinitrobenzoyl chloride prior to reversed phase analysis by HPLC.

In this communication, automation of their procedure via the GBC 1650 Autosampler is described.

'...concern for the health of workers exposed to toxic vapours in industries where sodium azide is processed...'

Keywords:
Sodium azide, Hydrazoic acid, Automated pre-column derivatisation, Environmental pollution

Conditions

Column:	Spherisorb S5 ODS2, 250 x 4.6 mm ID
Guard:	Spherisorb S5 ODS2, 50 x 4.6 mm ID
Mobile Phase:	50% Acetonitrile in water (v/v)
Flow Rate:	1 ml/min
Temperature:	35°C
Wavelength:	240 nm
Injection Vol.:	25 µl

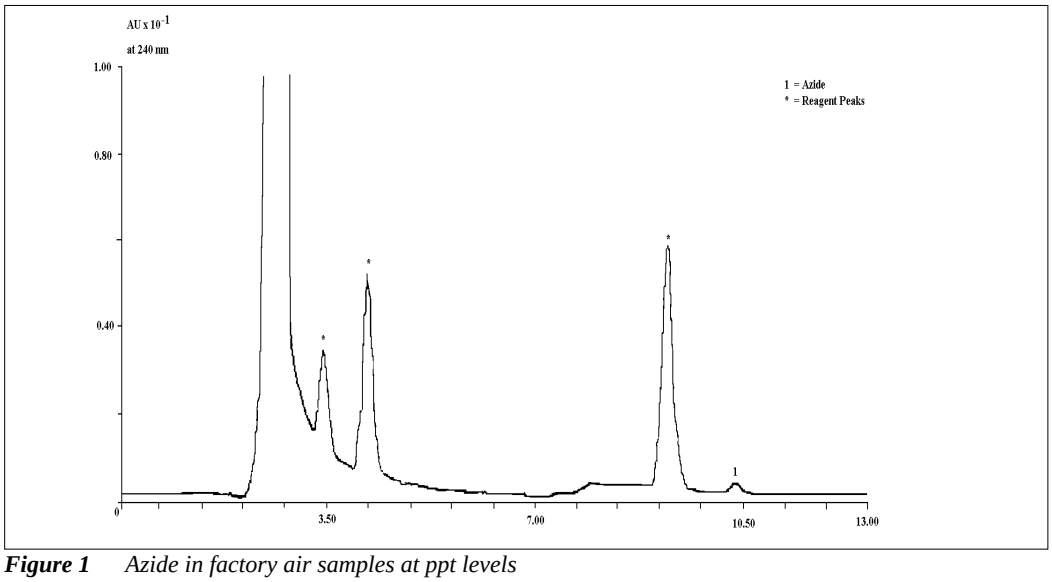

Figure 1 *Azide in factory air samples at ppt levels*

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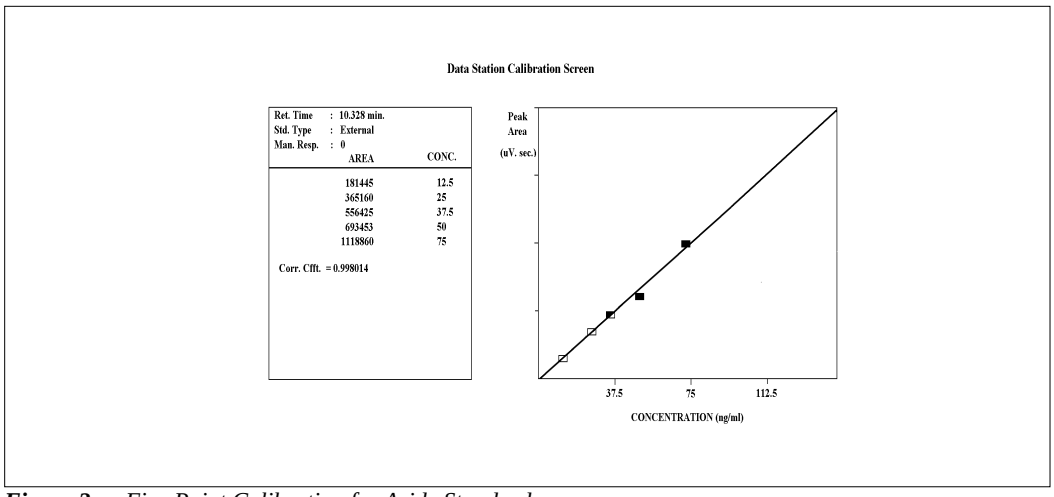


Figure 2 *Five Point Calibration for Azide Standards*

Procedure

The method is based on that of Swarin and Waldo. Known volumes of air were passed through midget impingers containing 0.01 M sodium carbonate solution. The pH of the solution was adjusted to pH 5 with 0.2 M hydrochloric acid and samples were loaded into the autosampler carousel. 50 µl of sample was mixed with 50 µl of a solution of 3,5-dinitrobenzoyl chloride in acetonitrile (1 mg/ml) using the robotic functions of the LC1650 autosampler. After three minutes, 25 µl of the reaction mixture was injected. 3,5-Dinitrobenzoyl azide eluted at 10.3 minutes and was quantitated by peak area using a five-point external standard calibration generated by the Data Station (Figure 2).

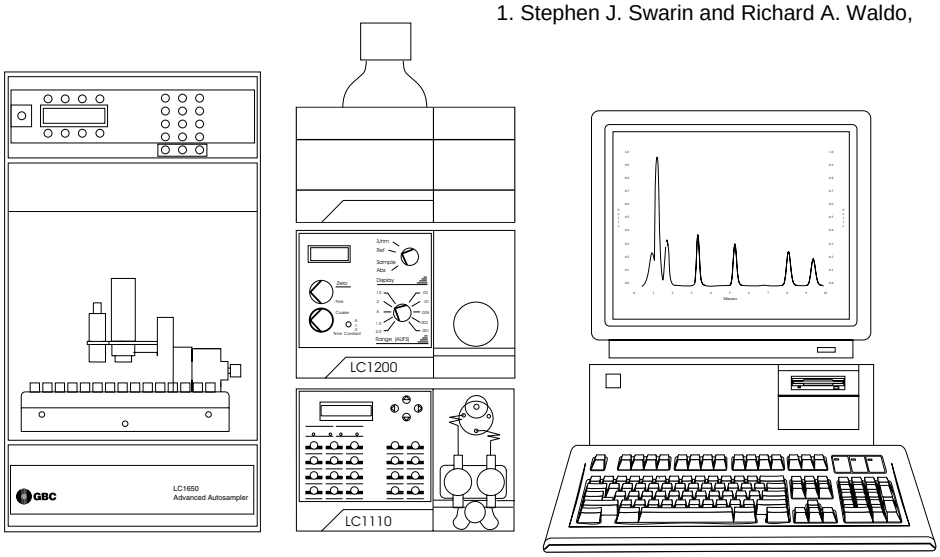
Unlike ion chromatography procedures, this method is not subject to interference by bromide, nitrate and nitrite ions. The method is fast, specific and highly sensitive (ppt detection) and may be run on a simple, isocratic HPLC system. Furthermore, the operation may be fully automated.

GBC HPLC Instrumentation

LC1110 Dual Piston HPLC Pump
LC1200 Variable Wavelength UV/Vis Detector
LC1650 Advanced Autosampler
GBC Column Heater
WinChrom Chromatography Data Management System

Reference

1. Stephen J. Swarin and Richard A. Waldo, 'Journal of Liquid Chromatography', 1982, 5(4), 597.



E4
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GBC publication number 01-0350-00
September, 1995

HPLC application note

Explosives Residues in Soil

'...factory sites have been grossly contaminated with toxic chemicals...'

The increasing demand for public housing in metropolitan areas has led to the closure of a number of factories which have subsequently been re-zoned as residential sites. Unfortunately, many of these sites have been grossly contaminated with toxic chemicals *e.g.*, heavy metals and potential carcinogens. Extensive investigation of the degree of contamination of both soil and ground water and the effectiveness of the site clean-up procedures has become mandatory.

This note outlines the HPLC analysis of contaminations due to explosives residues on the site of a former explosives factory where trinitrotoluene, isomeric dinitrotoluenes and cyclonite (RDX) were manufactured. Such compounds are considered to be toxic by all exposure routes and can cause headaches, nausea, fatigue, faintness, cyanosis, shortness of breath, CNS depression and respiratory depression. Evidence from available animal tests indicates that repeated or prolonged exposure to these chemicals could result in liver, blood and reproductive system disorders. The HPLC method employs simple, isocratic elution with UV detection at 230 nm (see Figure 1).

Keywords:
TNT, DNT, RDX, Cyclonite, Soil Residues, Environmental Pollution

Analysis is rapid (8 minutes to 2,4-DNT) and highly reproducible, especially when sample injection is effected under microprocessor control via the LC1600 Autosampler.

Conditions

Column:	Spherisorb S5 ODS2, 250 x 4.6 mm ID
Guard:	Spherisorb S5 ODS2, 50 x 4.6 mm ID
Mobile Phase:	Water:MeOH:THF (26:72:2)
Temperature:	35°C
Wavelength:	230 nm
Injection Vol:	10 µl

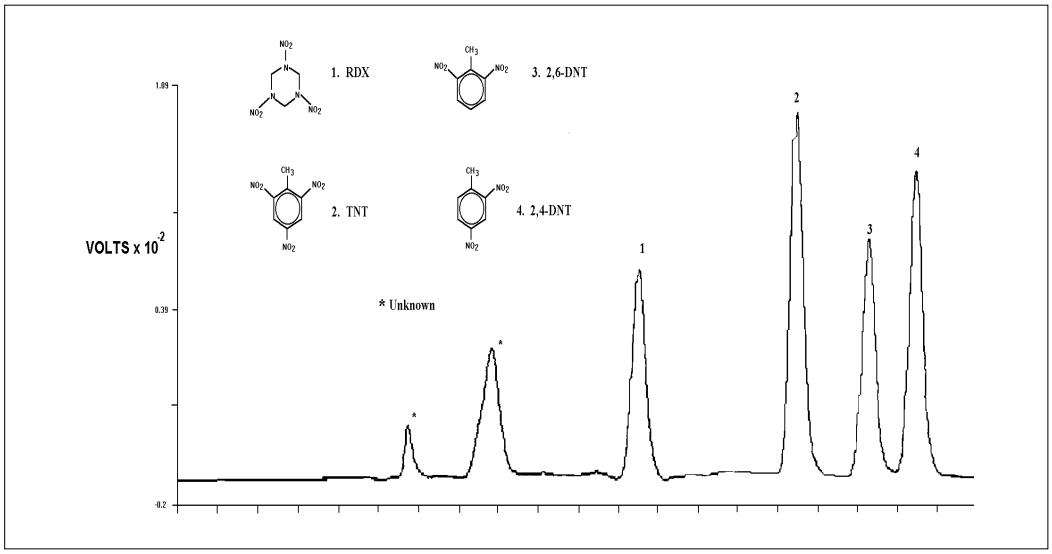

Figure 1 *Acetonitrile extract of soil spiked at 100 ng level.*

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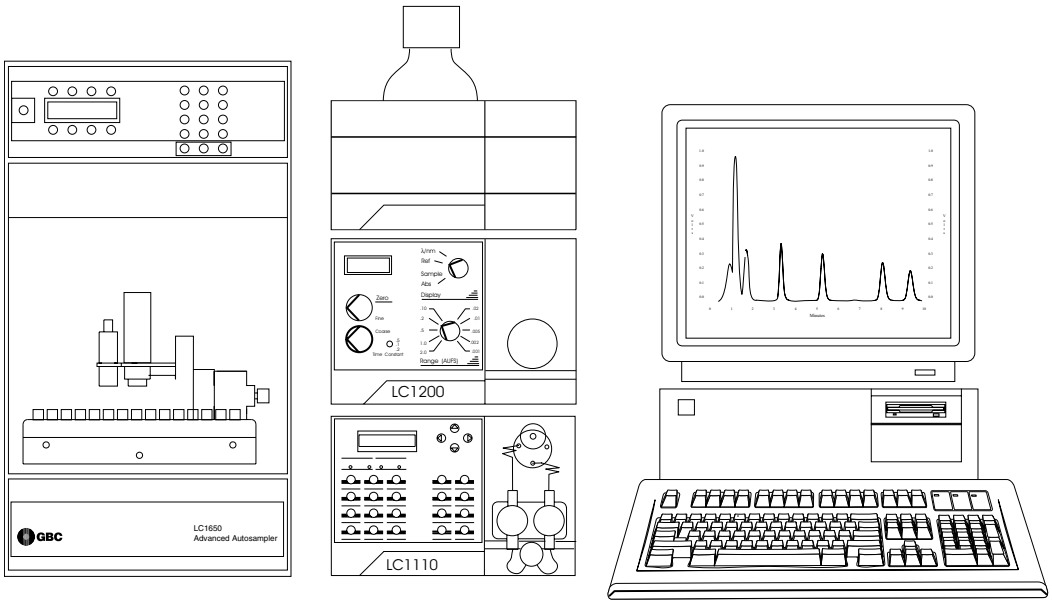
GBC HPLC Instrumentation

LC1110 Dual Piston HPLC Pump
LC1200 Variable Wavelength UV/Vis Detector
LC1650 Advanced Autosampler
WinChrom Chromatography Data Management System

'...analysis is rapid (8 minutes to 2,4-DNT) and highly reproducible...'



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GBC publication number 01-0351-00
September, 1995

HPLC application note

Polynuclear Aromatic Hydrocarbons with UV Detection

'...HPLC with isocratic elution and UV detection offers a simple but effective alternative...'

The analysis of organic pollutants in a range of environmental samples has become a priority in recent years. Persistent toxic compounds with known or suspected carcinogenic properties such as the polycyclic aromatic hydrocarbons, commonly called PAHs or PNAs, have received special attention. PAHs are widely distributed and have been detected in numerous situations, including sea water, drinking water, cigarette smoke, cooking oils and soil samples from industrial sites.

High sensitivity detection is required for the analysis of PAH pollutants as levels are usually low, especially in aqueous samples. HPLC with fluorescence detection is the method of choice for such analyses because it provides both the high sensitivity and high specificity needed, and is considered to be superior to gas chromatography for this application (Reference 1). For most purposes, however, HPLC with isocratic elution and UV detection offers a simple but effective alternative, as shown in Figure 1, where the separation of 15 EPA Priority Standards is displayed.

Keywords:
Carcinogens, Environmental Pollution, Polynuclear Aromatic Hydrocarbons, PAHs, PNAs, UV Detection

Conditions

Column:	Chromspher PAH 5 µm, 200 x 3.0 mm ID
Guard:	Spherisorb S5 ODS2, 50 x 4.6 mm ID
Mobile Phase:	Acetonitrile/Water (78:22)
Flow Rate:	1 ml/min
Detection:	UV at 280 nm
Temperature:	30°C

Reference

1. P. Jandera and J. Churacek, in 'Gradient Elution in Column Liquid Chromatography' (Elsevier, 1985), p.293.

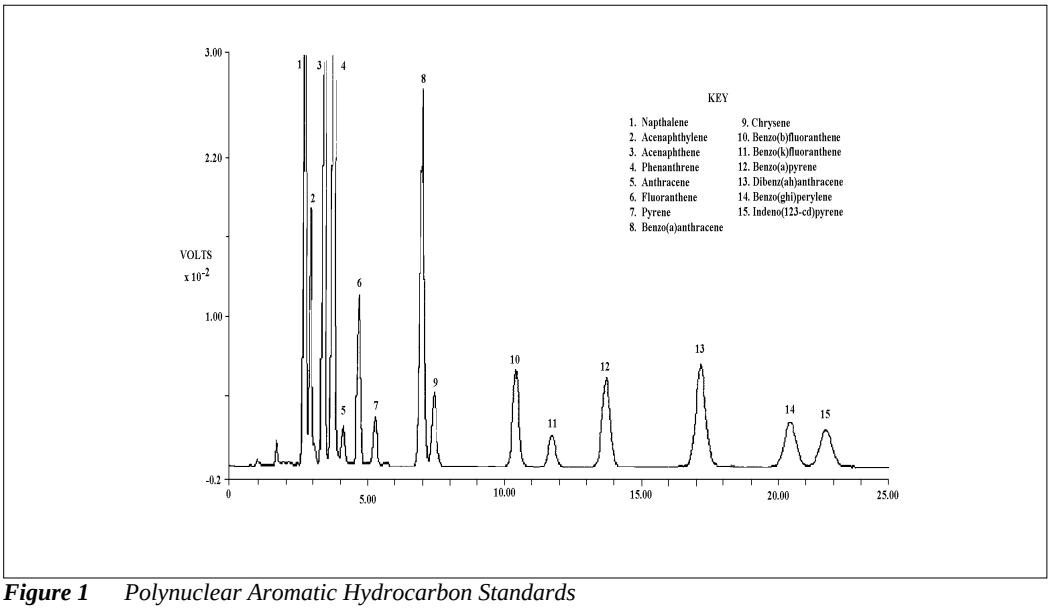

Figure 1 *Polynuclear Aromatic Hydrocarbon Standards*

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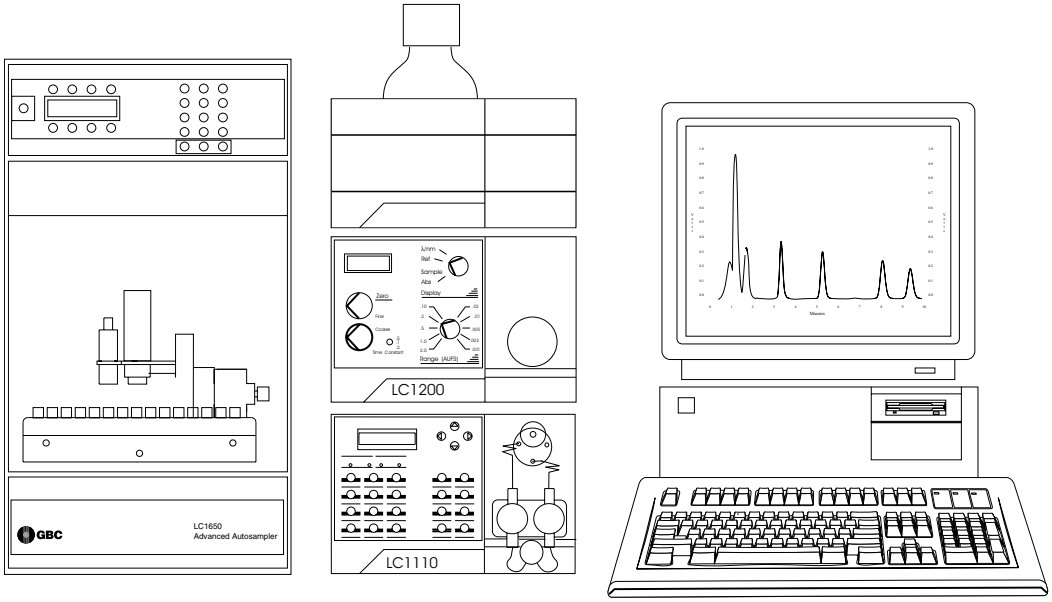
GBC HPLC Instrumentation

LC1110 Dual Piston HPLC Pump
LC1200 Variable Wavelength UV/Vis Detector
LC1445 System Organiser
LC1650 Advanced Autosampler
GBC Column Heater
WinChrom Chromatography Data Management System



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GBC publication number 01-0352-00
September, 1995

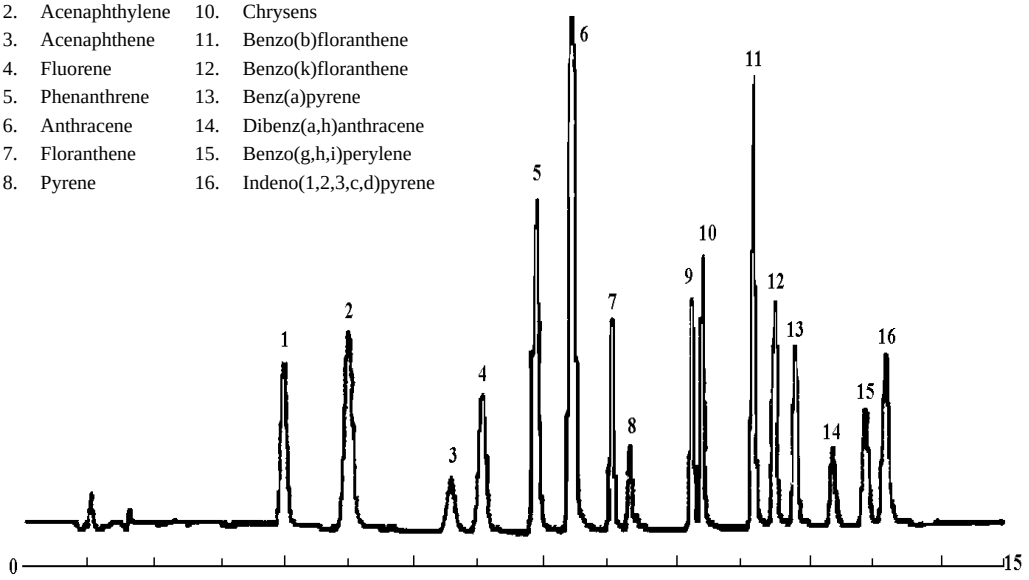
HPLC

application note

Determination of PAHs by Gradient Separation and UV Detection

'...determination of PAHs by gradient separation...'

1. Naphthalene
2. Acenaphthylene
3. Acenaphthene
4. Fluorene
5. Phenanthrene
6. Anthracene
7. Floranthene
8. Pyrene
9. Benz(a)anthracene
10. Chrysens
11. Benzo(b)floranthene
12. Benzo(k)floranthene
13. Benz(a)pyrene
14. Dibenz(a,h)anthracene
15. Benzo(g,h,i)perylene
16. Indeno(1,2,3,c,d)pyrene



Sample

16 PAHs according to US EPA Priority Pollutant List.

Conditions

Column:	Vydac 201TP C18 Column 5 µM (150 x 4.6 mm ID)
Mobile Phase:	Solvent A: Water, Solvent B: Acetonitrile
Gradient:	50% B for 3 min, linear to 100% B in 7 min.
Flow Rate:	1.5 ml/min
Injection Vol:	10 µl
Temperature:	Ambient
Detection:	254 nm

PAHs

This application illustrates the use of the low pressure quaternary solvent delivery system developed by GBC for the burgeoning environmental marketplace. Around the world dated environmental applications, which rely heavily on labor intensive extractions and expensive equipment and sample, are being replaced by simple HPLC methods. This change has already occurred in Europe and is rapidly becoming acceptable in the US.

This application is particularly useful in laboratories where cost reduction and reduction in the level of expertise required to perform analyses, is important.

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Key Features

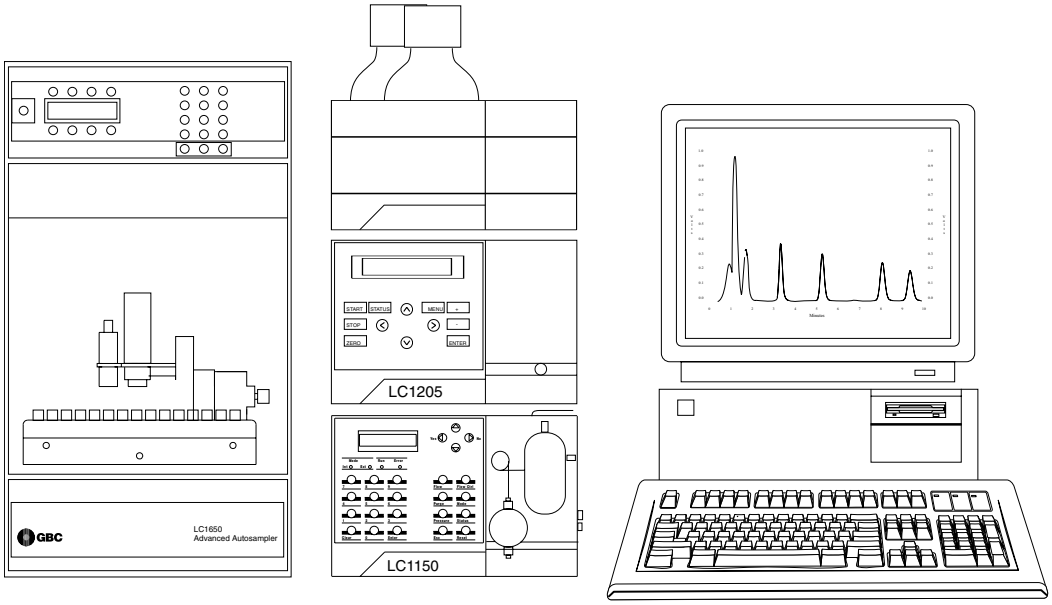
- Sensitivity
- Cost savings
- Selectivity
- Limited technical expertise required
- Gradient performance
- Precision and accuracy of mixing
- Excellent flow characteristics
- Excellent chromatography

Relevant Industries

- Commercial environmental laboratories
- EPA laboratories
- Power plants
- Light electronics manufacture
- Wastewater treatment
- Effluent monitoring
- Air quality monitoring
- Drinking water analysis

GBC HPLC Instrumentation

LC1150 Quaternary Gradient HPLC Pump
LC1205 Programmable UV/Vis Detector
LC1445 System Organiser
LC1650 Advanced Autosampler
WinChrom Chromatography Data Management System



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GBC publication number 01-0353-00
September, 1995

HPLC application note

Formaldehyde in Air

'...the use of UV detection at 340 nm provides sensitivity in the parts per trillion range...'

Formaldehyde is a powerful antiseptic widely used as a fumigant for dwellings, ships, warehouses and clothing. It is also employed in the manufacture of phenolic resins, dyes, organic chemicals, glass mirrors, explosives, leather goods and latex rubber products. Formaldehyde vapours present in the workplace are lachrymatory and intensely irritating to mucous membranes even at low concentrations. As a result, responsible employers frequently monitor the level of formaldehyde in factory air.

There are a number of methods available for such purposes, but trapping the aldehyde in impingers containing a solution of 2,4-dinitrophenylhydrazine is perhaps the most simple. Formation of the corresponding phenylhydrazone is rapid and the product is quite stable. Reversed phase HPLC separates the formaldehyde-2,4-dinitrophenylhydrazone from the other species present, while the use of UV detection at 340 nm provides sensitivity in the parts per trillion range. Chromatograms obtained for a formaldehyde 2,4-DNPH standard and an air sample are present in Figures 1 and 2 respectively.

Keywords:
Air Pollution, Formaldehyde, 2,4-Dinitrophenylhydrazine (2,4-DNPH)

Conditions

Column:	Spherisorb S5 ODS2, 250 x 4.6 mm ID
Guard:	Spherisorb S5 ODS2, 50 x 4.6 mm ID
Mobile Phase:	Acetonitrile:Water (50:50)
Flow Rate:	1 ml/min
Detection:	UV at 340 nm

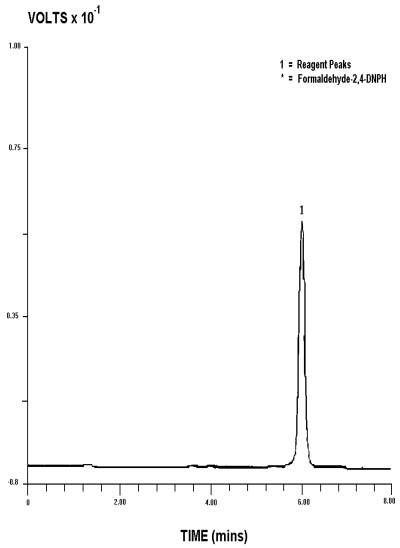

Figure 1 *Formaldehyde-2,4-DNPH*

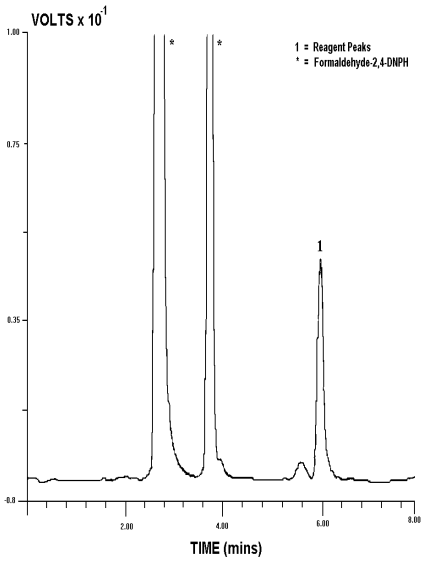

Figure 2 *Factory air sample*

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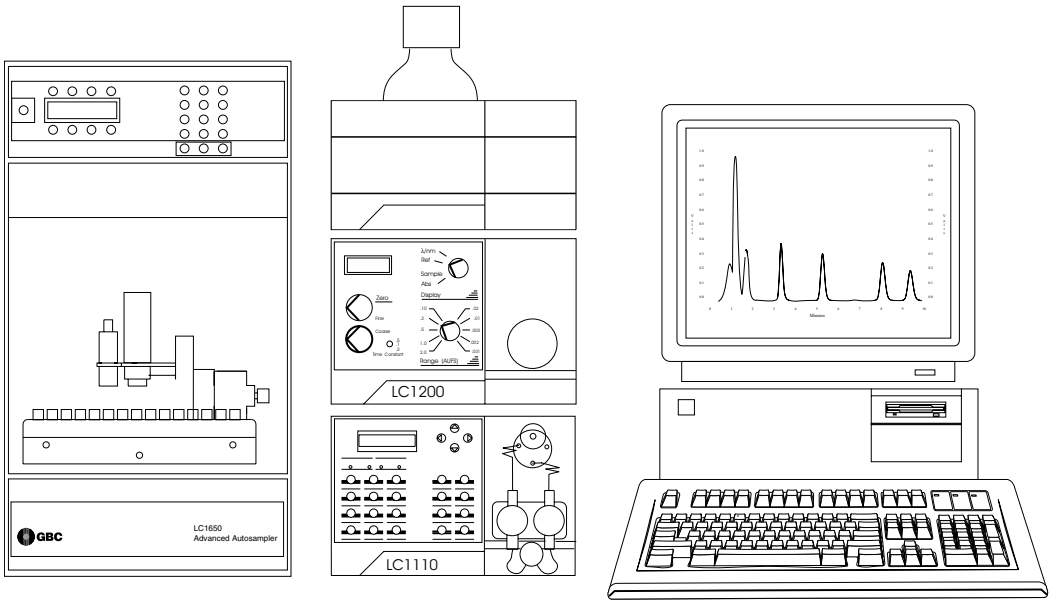
LC1110 Dual Piston HPLC Piston
LC1200 Variable Wavelength UV/Vis Detector
LC1445 System Organiser
LC1650 Advanced Autosampler
WinChrom Chromatography Data Management System



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GBC publication number 01-0354-00
September, 1995

HPLC application note

Determination of Formaldehyde in Waste Water by Pre-column DNP Derivatisation and UV Detection with Sub-ppb Sensitivity

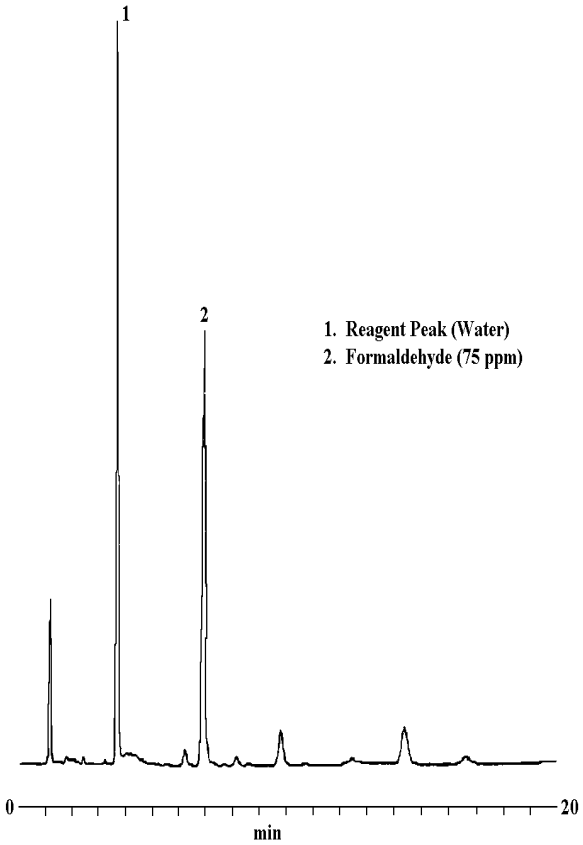
Conditions

Column:	Spherisorb 5 µm O S2 (C18) Column (150 x 4.6 mm ID)
Mobile Phase:	Water (50%) and Acetonitrile (50%)
Flow Rate:	1.0 ml/min
Injection Vol:	10 µl
Temperature:	Ambient
Detection:	340 nm

Keywords:
Waste Water, DNP

Sample

Waste water from treatment plant before treatment.



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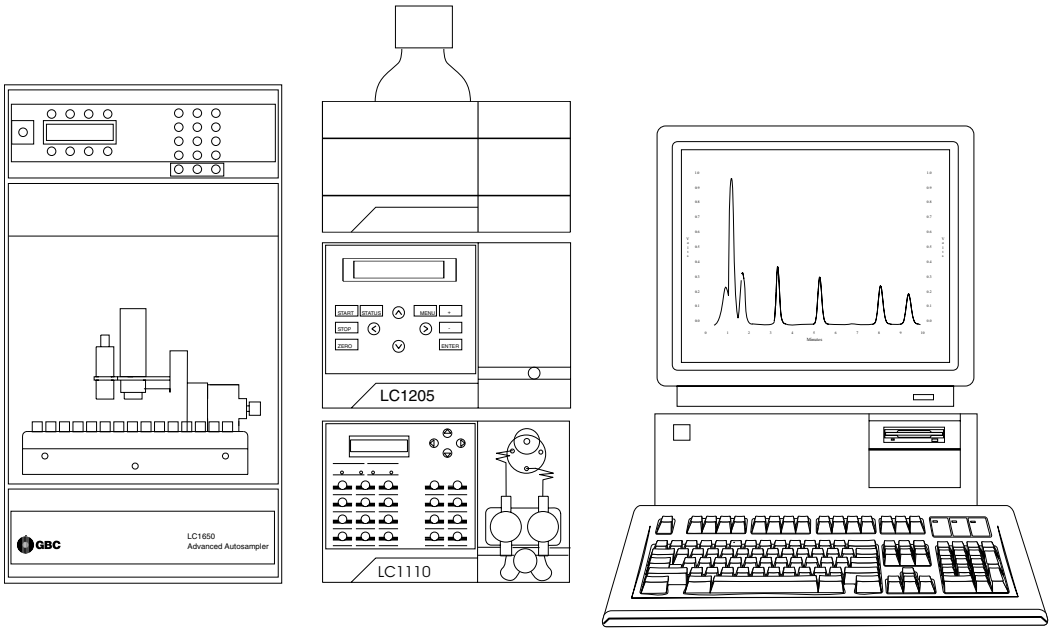
E9
01-0355-00

GBC HPLC Instrumentation

LC1110 Dual Piston HPLC Pump
LC1205 Programmable UV/Vis Detector
LC1650 Advanced Autosampler
WinChrom Chromatography Data
Management System



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GBC publication number 01-0355-00
September, 1995

HPLC application note

Phthalate Esters

'... difficulties may arise when samples contain a mixture of phthalate esters, especially isomers...'

Phthalate esters are used in diverse applications throughout the chemical industry, e.g., as plasticisers, solvents for substances like cellulose esters, insect repellents, fixatives in perfumes and in the denaturation of ethyl alcohol. The widespread use of phthalates has resulted in their current environmental status as 'priority pollutants', even though their toxicity is low. Consequently, numerous methods for the analysis of various phthalates have been developed.

Chromatographic separation of individual phthalate esters from other components in complex samples is often a trivial problem, however difficulties may arise when samples contain a mixture of phthalate esters, especially isomers. As shown in the accompanying chromatogram, Figure 1, reversed phase HPLC with gradient elution is successful under such circumstances.

Conditions

	Column:
Guard Column:	Spherisorb S5 ODS2, 250 x 4.6 mm ID Spherisorb S5 ODS2, 50 x 4.6 mm ID
Temperature:	35°C
Mobile Phase:	Solvent A:Acetonitrile/Water/ Tetrahydrofuran (50:48:2), Solvent B: Acetonitrile/ Tetrahydrofuran (98:2), Linear gradient, 20% B – 100% B over 6 minutes
Flow Rate:	1.5 ml/min
Detection:	UV at 230 nm

Keywords:
Butylbenzyl Phthalate, Dibutyl Phthalate, Diethyl Phthalate, Di-(2-ethylhexyl)phthalate, Dimethyl Phthalate, Dioctyl Phthalate

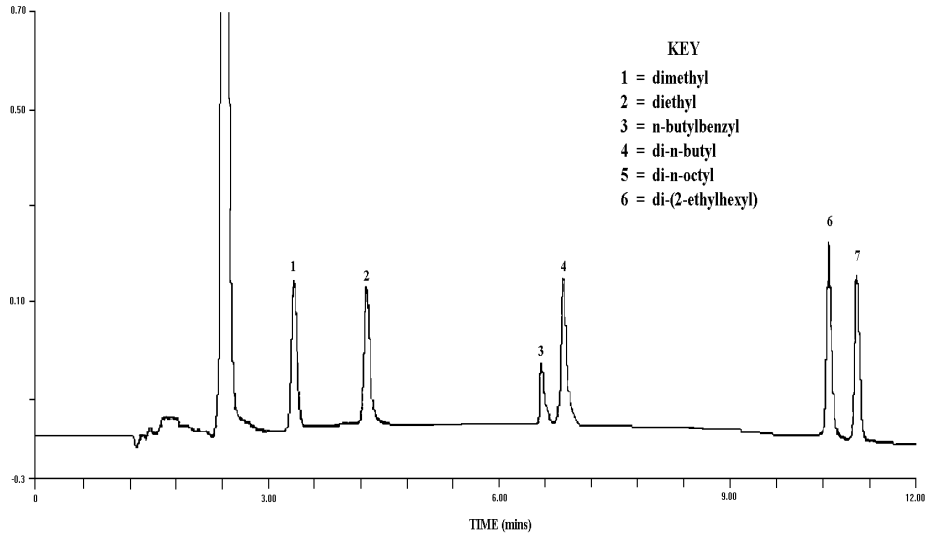

Figure 1 *Separation of EPA Priority Pollutant Phthalate Esters*

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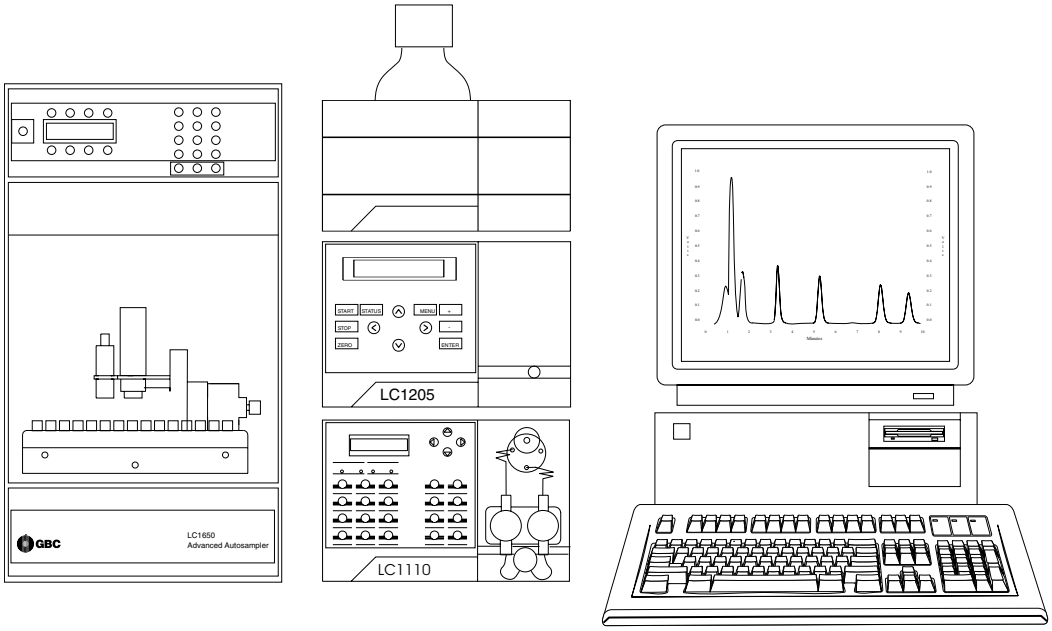
01-0356-00

GBC HPLC Instrumentation

LC1110 Dual Piston HPLC Pump (x2)
LC1205 Programmable UV/Vis Detector
LC1431 System Organiser
LC1650 Advanced Autosampler
GBC Column Heater
WinChrom Chromatography Data
Management System



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GBC publication number 01-0356-00
September, 1995

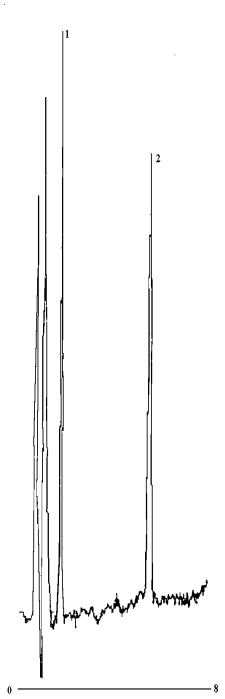
HPLC

application note

Benzidine Separation Using Glassy Carbon Electrode (3 mm) with ppb Sensitivity

'...the sample preparation in this case consists of filtering of the sample, followed by injection...'

Peak 1: Benzidine (10 ppb)
Peak 2: 3,3'-Dichlorobenzidine (25 ppb)



Conditions

Column:	Spherisorb 5 µ OD S2 (C18), 150 x 4.6 mm ID
Temperature:	Ambient
Mobile Phase:	50% 0.1 M Sodium Acetate (pH 4.7) with 50% Acetonitrile
Flow Rate:	1.5 ml/min
Electrode:	3 mm Glassy Carbon
Potential	800 mV (Ag/Ag/Cl)

This application illustrates the use of the patented impinging wall jet electrochemical detector. Most environmental applications require extractions and derivative formation to ensure sensitivity and selectivity. This detector removes labour intensive sample preparation required for other electrochemical detectors. This can mean up to a days labour saving. The sample preparation in this case consists of filtering of the sample, followed by injection. When sample throughput is required, this application can save the commercial clinical laboratory hundreds of dollars per run.

Key Features

- Sensitivity
- Cost savings
- Selectivity
- New patented technology
- Limited technical expertise

Relevant Industries

- Commercial environmental laboratories
- EPA laboratories
- CLP
- Industrial monitoring laboratories
- Water quality laboratories
- Water treatment laboratories
- Landfill monitoring laboratories

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E11
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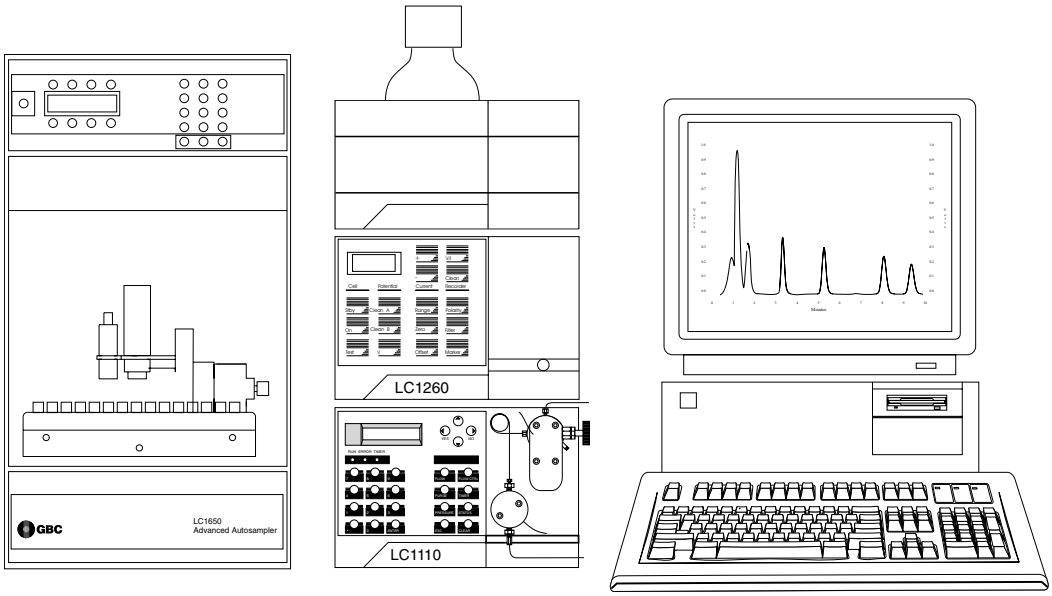
GBC HPLC Instrumentation

LC1110 Dual Piston HPLC Pump
LC1260 Electrochemical Detector
LC1445 System Organiser
LC1650 Advanced Autosampler
WinChrom Chromatography Data Management System



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GBC publication number 01-0357-00
September, 1995

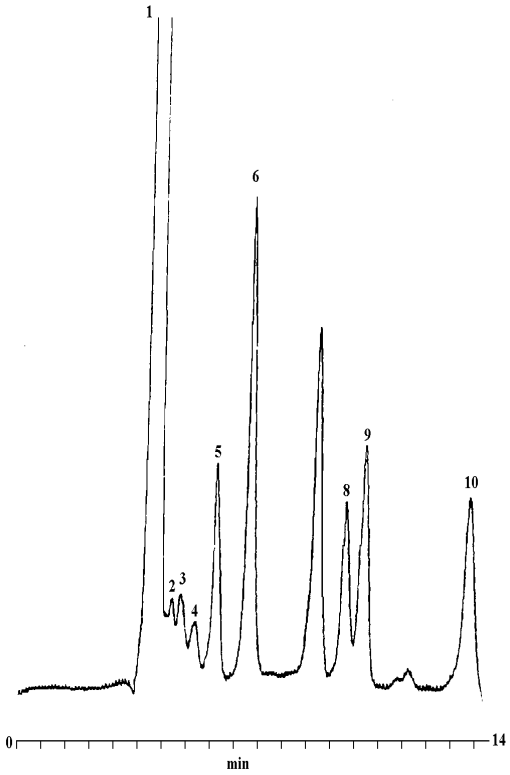
HPLC

application note

Analysis of Sugars (Saccharides) and Sugar Alcohols in Fermentation Liquor

'...sugars and sugar alcohols in fermentation liquor...'

1. Oligosaccharides (DP6)
2. DP6
3. DP
4. DP4
5. Raffinose (DP3)
6. Sucrose
7. Glucose
8. Galacose
9. Fructose
10. Sorbitol/Ethanol



GBC HPLC Instrumentation

LC1110 Dual Piston HPLC Pump
LC1240 Refractive Index Detector
LC1650 Advanced Autosampler
Timberline Column Heater
WinChrom Chromatography Data Management System

HPLC Conditions

Column:	Interaction CHO-620 Carbohydrate Column (30 cm)
Mobile Phase:	Water
Flow Rate:	0.5 ml/min
Injection Vol:	20 µl
Column Temp:	90°C
RI Temp:	50°C

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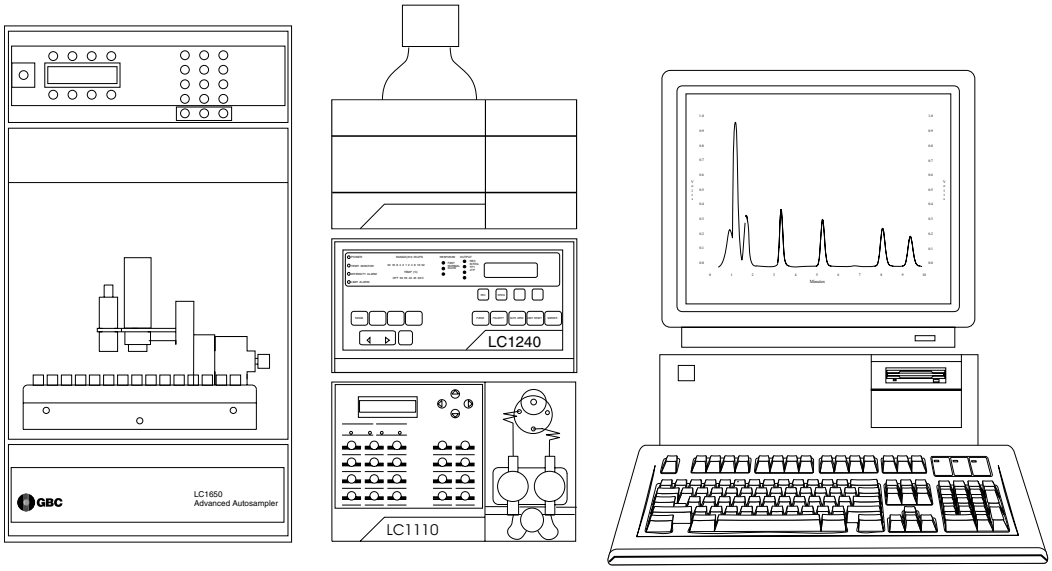

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GBC publication number 01-0358-00
September, 1995

HPLC

application note

Analysis of Sugars (Saccharides) and Sugar Alcohols by Ligand Exchange Chromatography and RI Detection

'...sugars and sugar alcohols in fermentation liquor...'

1. Raffinose
2. Sucrose
3. Glucose
4. Galactose
5. Fructose
6. Sorbitol

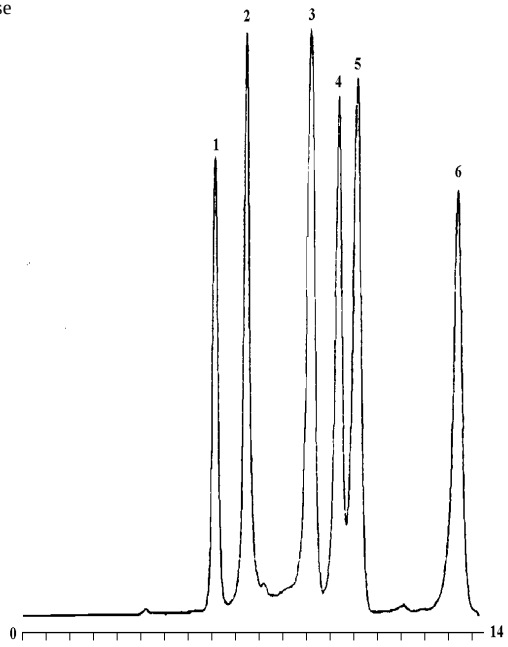

Figure 1 *Standard (Overspike) Analysis (7 ppm)*

GBC HPLC Instrumentation

LC1110 Dual Piston HPLC Pump
LC1240 Refractive Index Detector
Timberline Column Heater
LC1650 Advanced Autosampler
WinChrom Chromatography Data Management System

Conditions

Column:	Interaction CHO-620 Carbohydrate Column
Mobile Phase:	Water
Flow Rate:	0.5 ml/min
Injection Vol:	20 µl
Column Temp.:	90°C
RI Temp.:	50°C

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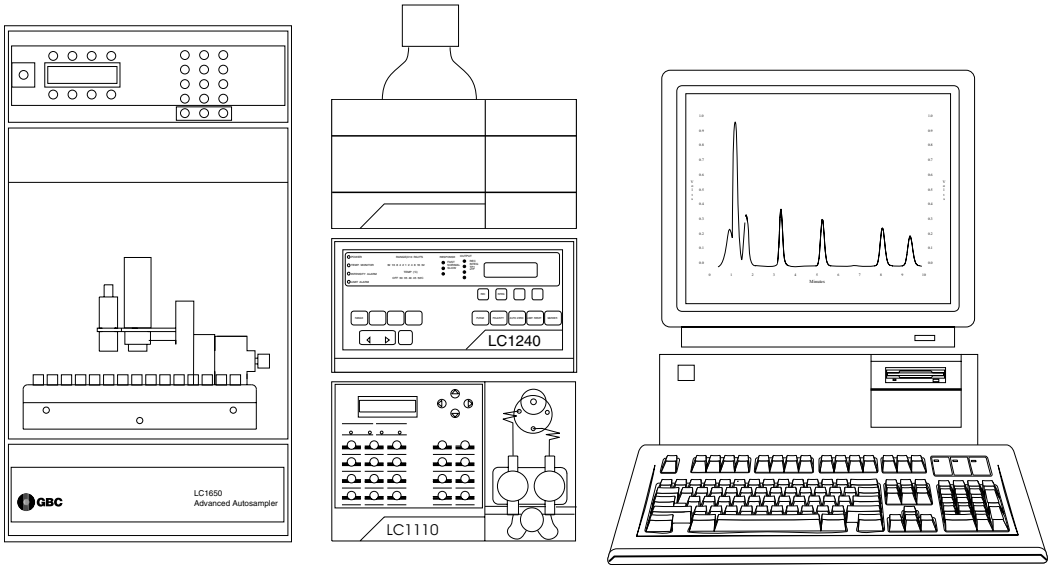

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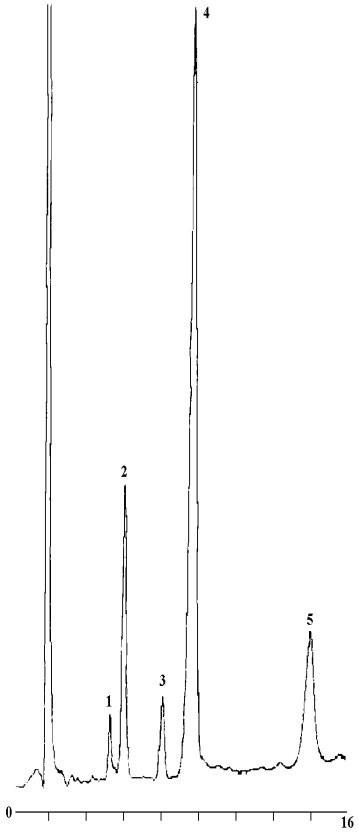
GBC publication number 01-0359-00
September, 1995

HPLC

application note

Analysis of Sugars (Saccharides) in Brewery Malt Extract

1. Fructose (1.02 ppm)
2. Glucose (6.09 ppm)
3. Sucrose (1.66 ppm)
4. Maltose (30.6 ppm)
5. Maltotriose (7.47 ppm)



GBC HPLC Instrumentation

LC1110 Dual Piston HPLC Pump
LC1240 Refractive Index Detector
GBC Column Heater
LC1650 Advanced Autosampler
WinChrom Chromatography Data Management System

Conditions

Column:	Spherisorb 5 µM Amino Column (250 x 4.6 mm ID)
Mobile Phase:	Acetonitrile (75%) and Water (25%)
Flow Rate:	1.5 ml/min
Injection Vol:	20 µl
Column Temp.:	32°C
RI Temp.:	50°C

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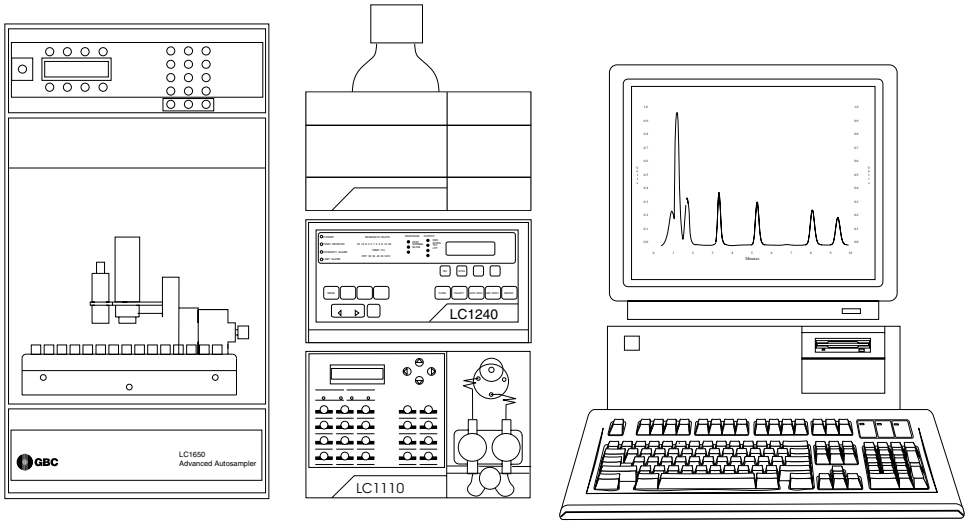

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GBC publication number 01-0360-00
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HPLC application note

Analysis of Sugars (Saccharides) Using Isocratic Normal Phase Chromatography and RI Detection

1. Fructose
2. Glucose
3. Sucrose
4. Maltose
5. Maltotriose

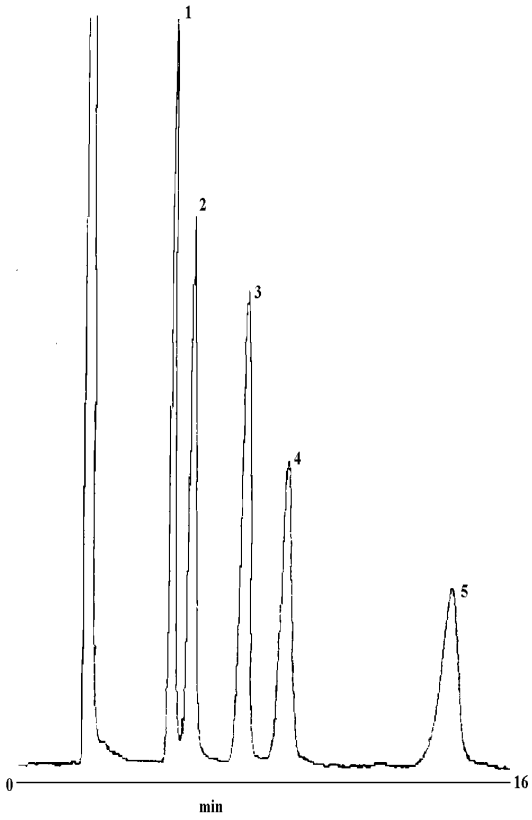

Figure 1 *Standard (Overspike) Analysis (10 ppm)*

GBC HPLC Instrumentation

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LC1240 Refractive Index Detector
LC1650 Advanced Autosampler
WinChrom Chromatography Data Management System

Conditions

Column:	Spherisorb 5 µM Amino Column (250 x 4.6 mm ID)
Mobile Phase:	Acetonitrile (75%) and Water (25%)
Flow Rate:	1.5 ml/min
Injection Vol:	20 µl
Column Temp.:	32°C
RI Temp.:	32°C

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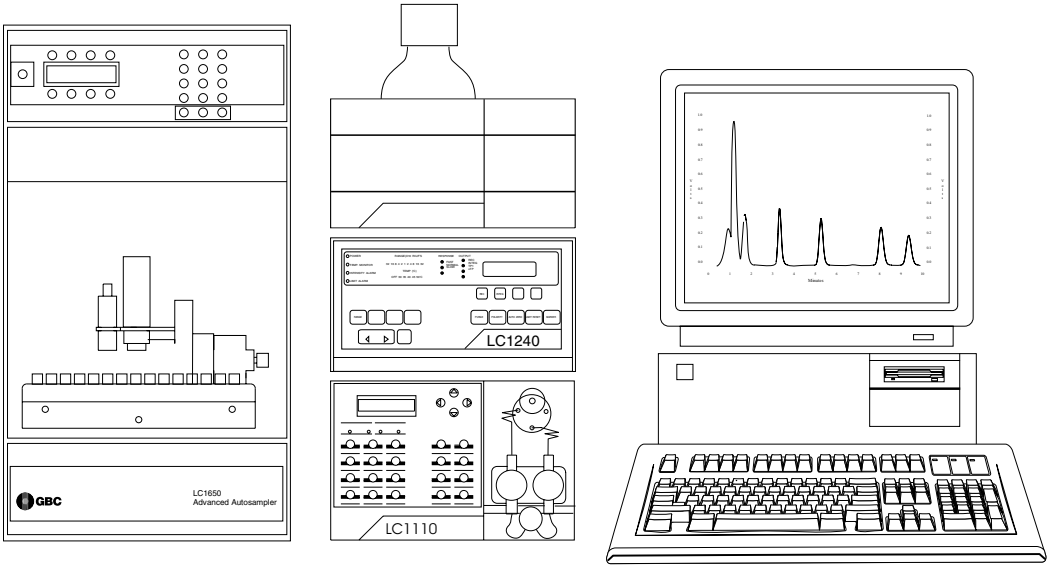

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GBC publication number 01-0361-00
September, 1995

HPLC application note

Sugars and Alcohol in Food and Beverages

'...the resultant derivatives are very stable and highly fluorescent, offering assay sensitivity in the fmol range...'

Keywords:
Ethanol, Fructose, Glucose, Sucrose, Beer, Coca-Cola™, Honey, Liqueur, Molasses, Orange Juice, Whisky, Wine

Selection of the most appropriate method for the analysis of carbohydrates and their fermentation products usually depends on several factors:-

(i) Sample matrix — the presence of high MW species, *e.g.*, proteins and polysaccharides, may demand extensive sample pre-treatment prior to chromatographic analysis.

(ii) Detection sensitivity required - this is generally not a problem as the level of sugars in food and beverage samples are high.

(iii) Resolution desired — the performance of the various columns commercially available is optimised for specific applications.

(iv) Frequency of analysis — the run time will vary, depending on the type of chromatographic column selected.

The most popular HPLC method for the analysis of sugars and alcohols uses a column packed with a cation exchange resin in the calcium form, with water as the mobile phase. Separations are effected via a combination of mechanisms involving ligand exchange and size exclusion phenomena.

Complex samples containing polysaccharides as well as lower MW sugars and alcohols may be analysed in a single, isocratic run with excellent resolution. As the mobile phase is water, differential refractometers provide adequate sensitivity for detection, while safety and environmental concerns are minimal.

In Figures 1-10, typical separations are presented for a variety of food and beverage samples. Sample pre-treatment in most cases simply involved dilution and filtration. Carbonated beverages were digested prior to filtering.

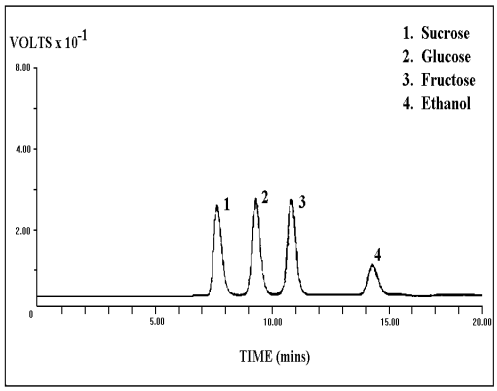

Figure 1 *Sugar amd Ethanol Standards*

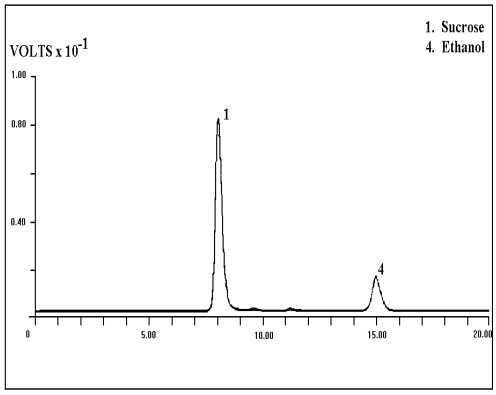

Figure 2 *Suntory Midori Melon Liqueur*

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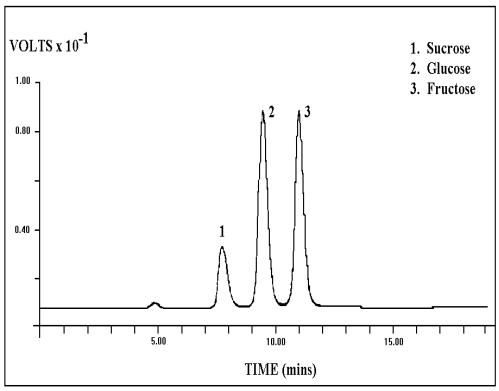


Figure 3 *Coca Cola*

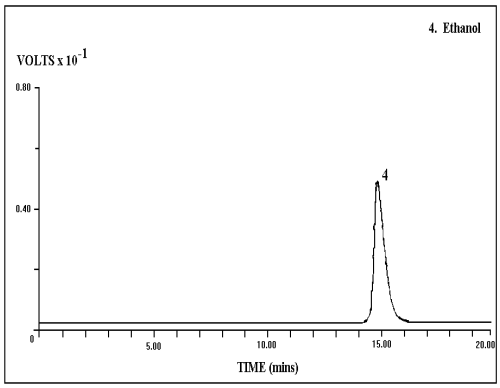


Figure 4 *Scotch Whisky*

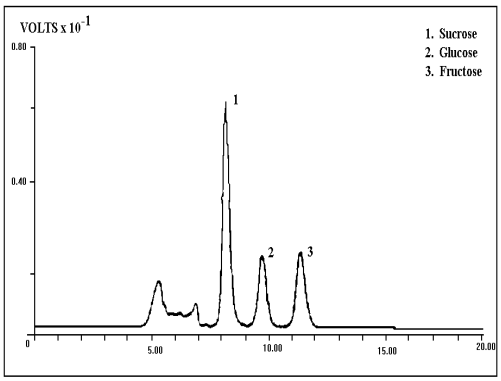


Figure 5 *Orange Juice, freshly squeezed*

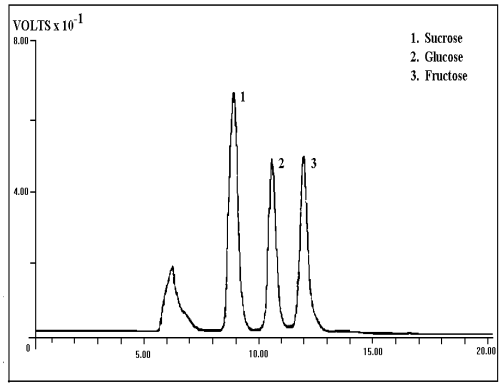


Figure 6 *Orange Juice, commercial brand*

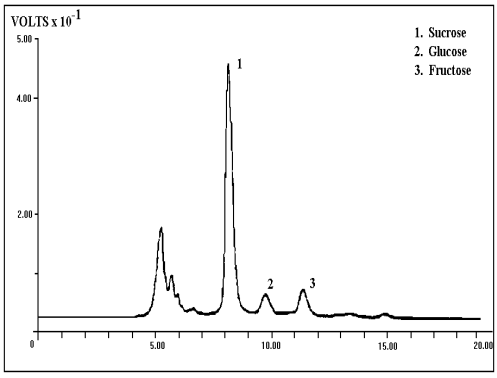


Figure 7 *'C Molasses' from Sugarcane Mill*

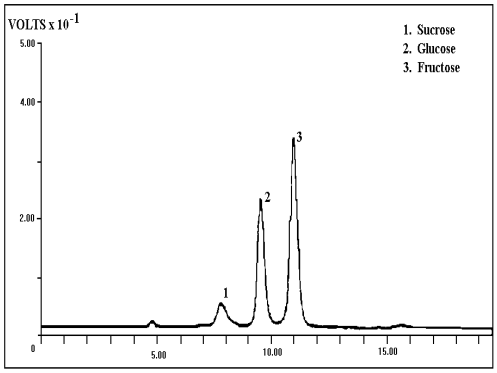


Figure 8 *Honey*

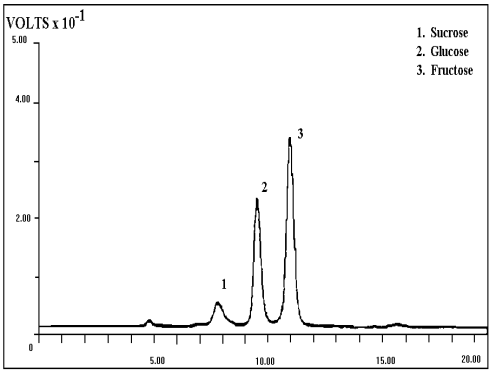


Figure 9 *Australian Beer*

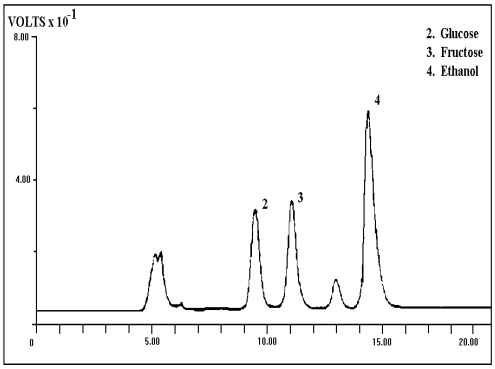


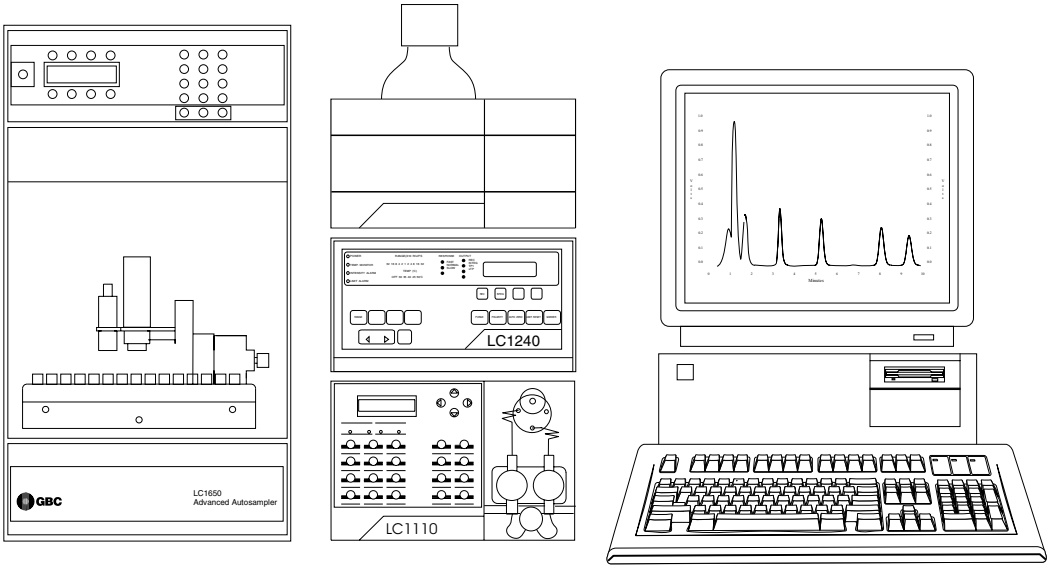
Figure 10 *Australian White Wine (Moselle)*

GBC HPLC Instrumentation

LC1110 Dual Piston HPLC Pump
Timberline Column Heater
LC1240 Refractive Index Detector
LC1650 Advanced Autosampler
WinChrom Chromatography Data Management System

Conditions

Column:	Intesaltion CHO-620 Carbohydrate Column
Guard Column:	Spherisorb S5 ODS2, 50 x 4.6 mm ID
Mobile Phase:	Water (distilled)
Flow Rate:	0.5 ml/min
Column Temp.:	80°C
RI Temp.:	50°C



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GBC publication number 01-0362-00
September, 1995

HPLC application note

Ascorbic Acid in Orange Juice

Ascorbic acid, commonly known as Vitamin C, is a dietary essential for humans, other primates and the guinea pig, but can be synthesised by all species of animals which have been investigated. The most prominent chemical property of the vitamin is its ability to oxidise to dehydroascorbic acid, which is catalysed by metal ions (Reference 1). This feature also renders Vitamin C suitable for anlaysis by HPLC using electrochemical detection, which is highly selective for ascorbic acid even in complex samples, and is highly sensitive, with detection limits in the picogram range. However, due consideration must be given to sample preparation procedures in order to avoid the loss of ascorbic acid by oxidative degradation prior to the chromatographic analysis. The HPLC instrumentation and sample preparation required for the analysis of ascorbic acid in orange juice are detailed below. The perchloric acid extraction technique is applicable to other sample matrices, *e.g.*, milk products, blood and tissue, with minor modification.

Figures 1 and 2 show ascorbic acid standard (ca. 10 ng on column) and a commercial orange juice sample, respectively.

Keywords:
Ascorbic Acid, Electrochemical Detection, Vitamin C

Conditions

Column:	Spherisorb S5 ODS2, 250 x 4.6 mm ID
Guard:	50 x 4.6 mm ID
Temperature:	30°C
Mobile Phase:	0.1 M sodium acetate containing 0.4 mM disodium DETA and 1 mM octylamine:methanol (90:10) - degassed
Flow Rate:	0.8 ml/min

Sample Preparation

Dilute juice 1:100 with 0.05 M perchloric acid
Ultrasonicate solution for five minutes
Filter solution (0.2 micron)
Further dilutions should be made with mobile phase, if necessary.
Inject 20 µl.

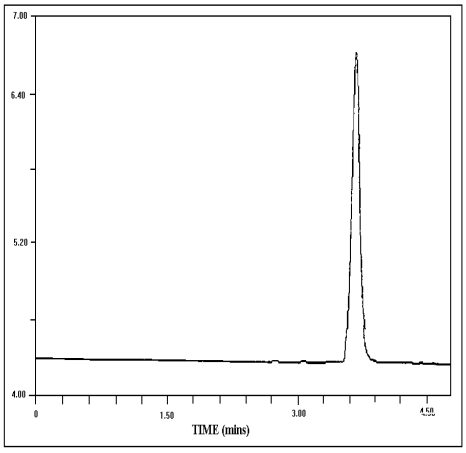

Figure 1 *Ascorbic acid standard*

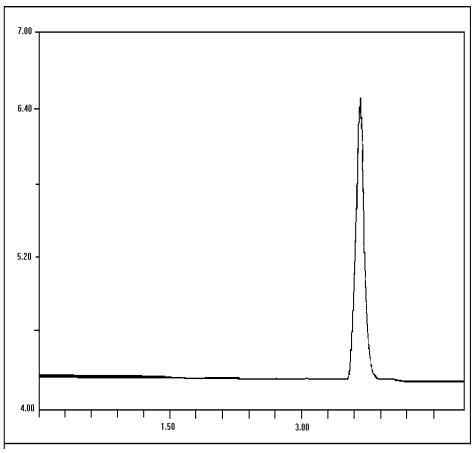

Figure 2 *Commercial orange juice*

'...due consideration must be given to sample preparation procedures in order to avoid the loss of ascorbic acid by oxidative degradation prior to the chromatographic analysis...'

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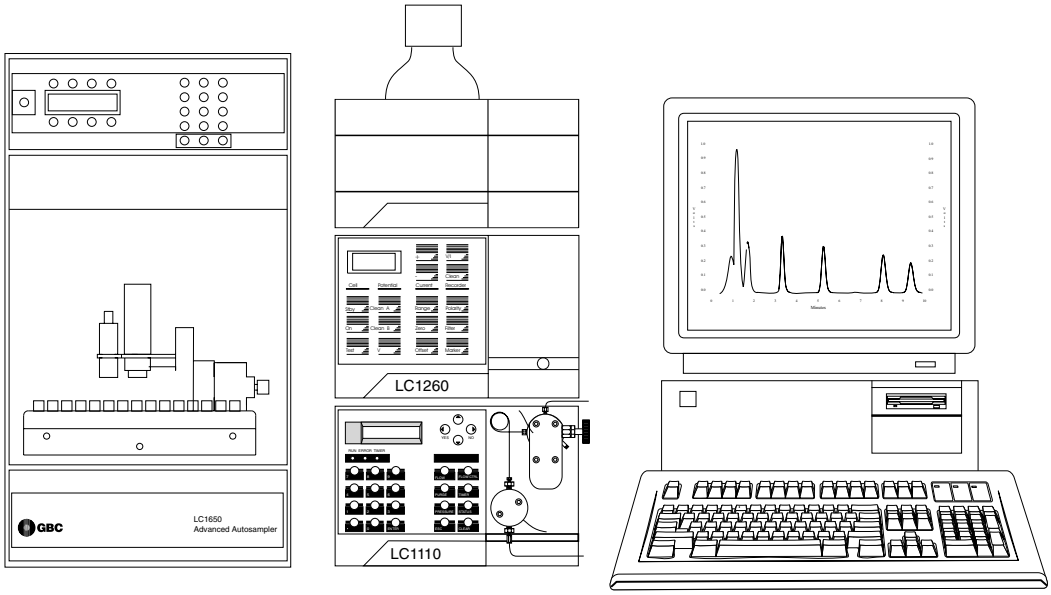
GBC HPLC Instrumentation

LC1110 Dual Piston HPLC Pump
LC1260 Electrochemical Detector
LC1445 System Organiser
LC1650 Advanced Autosampler
LC1120/LC1150 HPLC Column Oven Option
WinChrom Chromatography Data
Management System
Online Degasser

Reference

1. 'Principles of Biochemistry', by White, Handler and Smith, p. 973 (McGraw-Hill, 1964).



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01-0363-00

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GBC publication number 01-0363-00
September, 1995

HPLC application note

Anaesthesia of the Southern Elephant Seal

'...a study designed to assess the appropriate anaesthetic dosage for these marine giants using Zoletil...'

There is some evidence to suggest that during the past forty years the population of Elephant Seals in the Southern Ocean has been halved. Whether increased predation or diminished food supply is responsible for limiting the number of seals is not known (Reference 1). Intense fishing by commercial fleets in Southern waters, in particular those using 'drift-net' techniques, may also be a significant factor.

As part of a continuing international program aimed at monitoring the population of the Southern Elephant Seal, scientists from the Australian Antarctic Division (Kingston, Tasmania) attach sensors to the hides of anaesthetised seals in order to record variations in temperature and pressure experienced by individual members of the species.

The chromatograms displayed in Figure 1 resulted from a study designed to assess the appropriate anaesthetic dosage for these marine giants using Zoletil™, a combination of tiletamine (1) and zolazepam (2).

Keywords:
Zoletil, tiletamine, anaesthetics, tranquilisers, conservation studies

Hydrophobic bases such as the anaesthetic, tiletamine (1), usually exhibit poor peak symmetry owing to excessive tailing when chromatographed on octadecylsilyl - modified silica, i.e., on an ODS or C18 column. This undesirable asymmetry may be remedied by the addition of alkylamine salts, *e.g.*, triethylammonium phosphate, to the mobile phase. However, in this instance, a column packed with cyanopropylsilyl - modified silica, i.e., a cyano (CN) or nitrile column, which provided suitable peak symmetry throughout the simple, isocratic analysis was chosen.

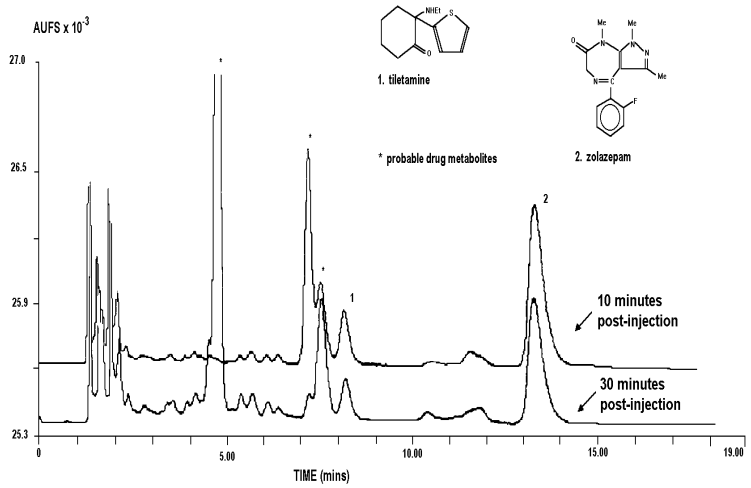

Figure 1 *Serum levels of tiletamine and zolazepam at 10 and 30 minutes post-injections*

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HPLC Conditions

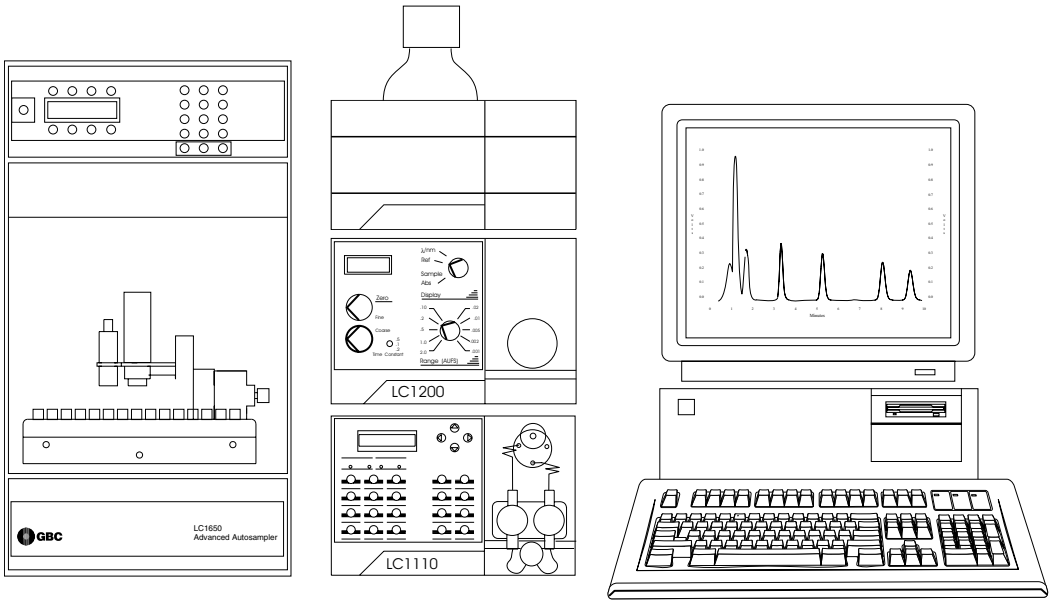
Column:	Spherisorb S5 CN, 250 mm x 4.6 mm ID
Guard:	Spherisorb S5 CN, 50 mm x 4.6 mm ID
Mobile Phase:	Acetonitrile: 0.1 M NH_4 C104 aq (8.92)
Wavelength:	227 nm
Injection Vol.:	20 µl

GBC HPLC Instrumentation

LC1110 Dual Piston HPLC Pump
LC1650 Advanced Autosampler
LC1200 Variable Wavelength UV/Vis Detector
WinChrom Chromatography Data Management System

Reference

1. Harry Burton, 'The Tasmanian Naturalist', 1986, No.86 (July), 4.



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GBC publication number 01-036400
September, 1995

HPLC application note

Inorganic Anions in Groundwater

Analysis of inorganic anions using conventional HPLC instrumentation is now considered routine. Such analyses may be conveniently classified as follows:

pm; or

(ii)ppm and higher.

Keywords:
Fluoride, carbonate, chloride, nitrite, bromide, nitrate, phosphate, sulphate, ion chromatography, groundwater salinity studies

For sub-ppm levels of inorganic anions, sophisticated approaches are usually necessary, e.g., sample pre-concentration, suppression of eluent conductivity or specific detection techniques, as in the electrochemical detection of cyanide and sulphide. Correspondingly expensive instrumentation may be required.

The purpose of this application note is to indicate that for the majority of inorganic anion analyses, at ppm or higher levels, a simple isocratic HPLC system equipped with a UV detector will provide the desired results.

In Figure 1, the resolution of seven common anion standards is shown. Fluoride, carbonate, chloride and sulphate in a groundwater sample from a mining site in Queensland are displayed in Figure 2. These separations were performed on a Hamilton PRP-X100 column, which is packed with a polymeric stationary phase stable throughout the pH ranges 1-13.

Spectrophotometric detectors are much less affected by temperature changes than are conductivity detectors, and as a result this analysis can be carried out at room temperature.

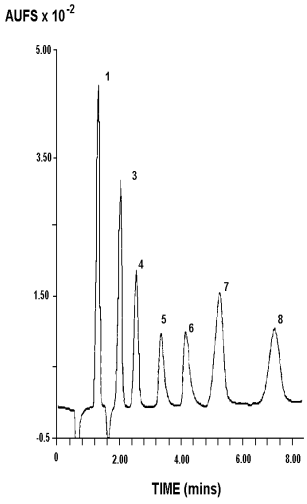

Figure 1 Separation of Anion Standards

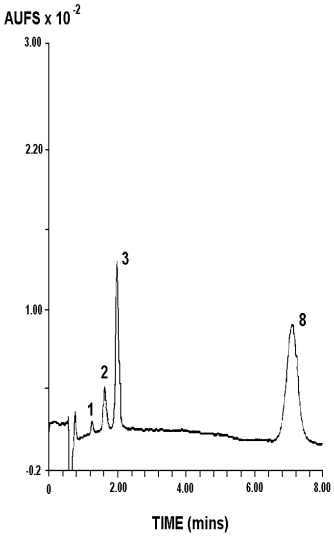

Figure 2 Anions in Groundwater Sample

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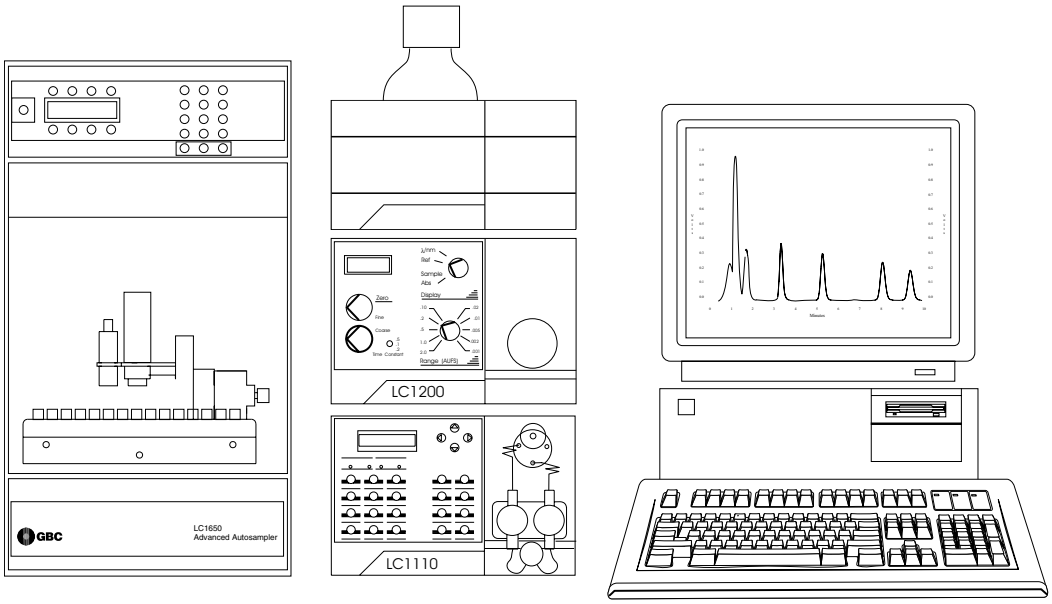
GBC HPLC Instrumentation

LC1110 Dual Piston HPLC Pump
LC1650 Advanced Autosampler
LC1200 Variable Wavelength UV/Vis Detector
WinChrom Chromatography Data Management System

Conditions

Column:	Hamilton PRP-X100 with corresponding Guard Column
Mobile Phase:	4 mM p-hydroxybenzoate (pH 8.5)
Wavelength:	310 nm



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GBC publication number 01-0365-00
September, 1995

HPLC application note

UV Absorbing Inorganic Anions

'...this demonstrates the ease with which the analysis of UV absorbing ions such as nitrite and nitrate may be accomplished in the presence of a other ions which show little appreciable UV absorbance...'

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01-0366-00

The commonly encountered inorganic anions which absorb UV radiation, indicated above, may be analysed by HPLC by either:

(i) Ion exchange chromatrography on an anion exchange column or

(ii) Paired ion chromatography on a reversed phase column.

Mobile phases which have low UV absorption, such as phosphate buffers and alkyl sulphonate solutions, are employed in order to optimise sensitivity and linearity of detection. UV detection at lower wavelength , *e.g.*, 210 nm, is preferred for the same reason. Where interferences due to the presence of other UV absorbing sample components are observed, sample pre-treatment may be necessary.

A major advantage of the direct spectrophotometric detection of suitable inorganic anions over conductometric detection is selectivity. In Figure 1, the separation of a standard solution of anions containing 7 ppm nitrite and 9 ppm nitrrate together with similar levels of fluoride, chloride, phosphate and sulphate is shown.

Keywords:
Inorganic anions, ion chromatography, azide, bromate, bromide, chromate, iodate, iodide, molybdate, nitrate, nitrite, sulphite, thiosulphate

This demonstrates the ease with which the analysis of UV absorbing ions such as nitrite and nitrate may be accomplished in the presence of a other ions which show little appreciable UV absorbance.

A further example of this is given in Figure 2, where the levels of nitrite and nitrate in a groundwater sample from a mining site were found to be 24 and 60 ppm respectively. The results from this groundwater sample, known to contain approximately 1,700 ppm chloride and 600 ppm sulphate, indicates that this approach may be successful even where there is a large excess of ions which could interfere with conductometric detection techniques.

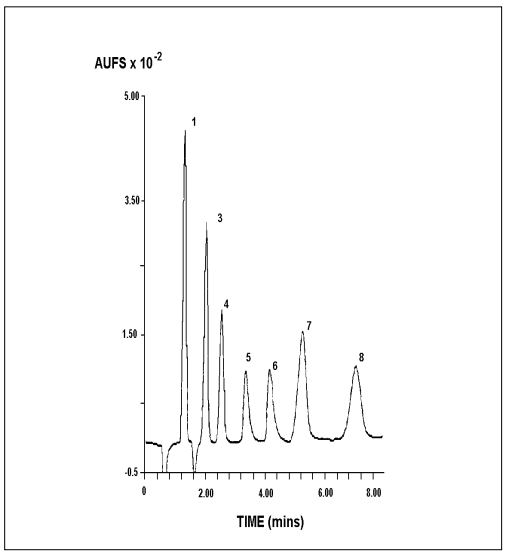


Figure 1 *Standards solution*

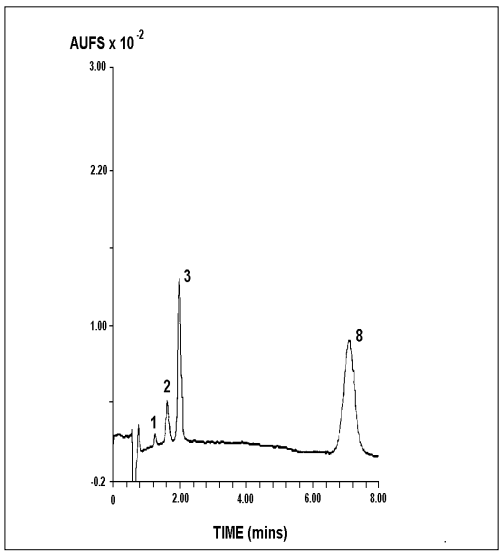


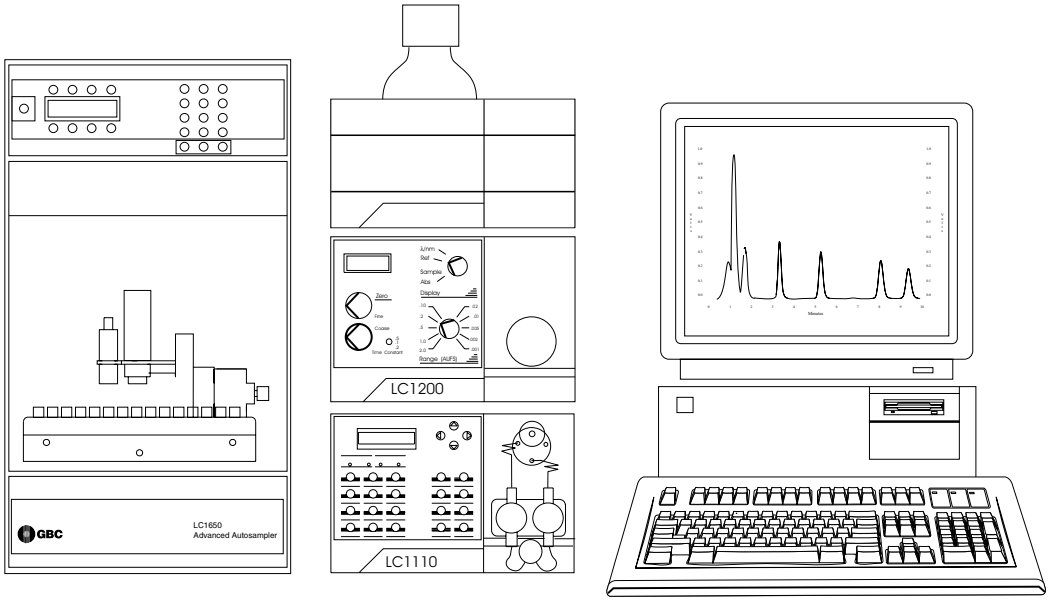
Figure 2 *Groundwater Sample*

GBC HPLC Instrumentation

LC1110 Dual Piston HPLC Pump
LC1650 Advanced Autosampler
LC1200 Variable Wavelength UV/Vis Detector
WinChrom Chromatography Data Management System

Conditions

Column:	Spherisorb S5 ODS2, 25 cm x 4.6 mm ID
Guard Column:	Spherisorb S5 ODS2, 5 cm x 4.6 mm ID
Mobile Phase:	50 mM ammonium phosphate with 2.5 mM tetrabutyl ammonium phosphate, pH 6
Wavelength:	210 nm



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01-0366-00

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GBC publication number 01-0366-00
September, 1995

HPLC application note

Antioxidants in Aviation fuel

'...the increase in detection sensitivity at 210 nm (vs. 254 nm), made possible by the selection of solvents which may be used at low-UV wavelengths, is demonstrated...'

Keywords:
Antioxidants, alkylated t-butylphenols, petroleum products

HPLC is often the method of choice for the analysis of additives in petroleum products for the following reasons:

(i) Analyses may be carried out at room temperature, avoiding degradation of thermally sensitive molecules.

(ii) Derivatisation is usually unnecessary and therefore confidence in analytical results is higher.

(iii) Instrumentation required is a simple, isocratic system capable of being operated by relatively unskilled technicians.

(iv) The system may be fully automated.

(v) Analysis is rapid, permitting high frequency monitoring if required.

Owing to the incompatibility of many petroleum products with the usual reversed phase solvents such as methanol and water, the selection of the appropriate column and mobile phase required some thought. In the set of chromatograms shown in Figures 1–3, non-aqueous reversed phase chromatography has been used for the separation of a commercial preparation of an homologous series of alkylated t-butylphenols, together with geometrical isomers.

The mobile phase chosen, 5% isopropanol in hexane, will tolerate small amounts of water in samples and the stationary phase, Spherisorb CN, is exceptionally robust under these conditions. In Figure 3, the increase in detection sensitivity at 210 nm (vs. 254 nm), which is made possible by the selection of solvents that are used at low-UV wavelengths, is demonstrated.

Conditions

Column:	Spherisorb S5 CN, 250 x 4.6 mm ID
Guard:	Spherisorb S5 CN, 50 x 4.6 mm ID
Mobile Phase:	5% Isopropanol in hexane
Flow Rate:	1 ml/min
Detection:	UV at 254 and 210 nm

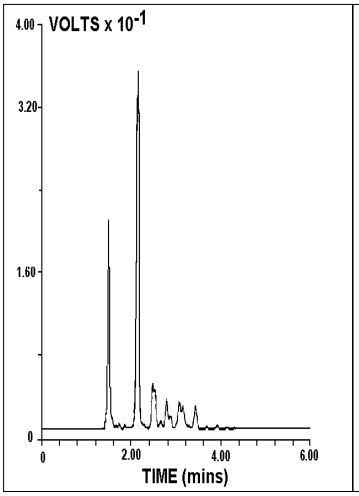

Figure 1 *Alkylated t-butylphenols at 254 nm*

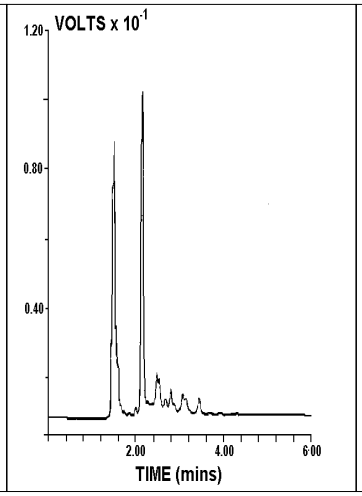

Figure 2 *Aviation fuel sample at 254 nm*

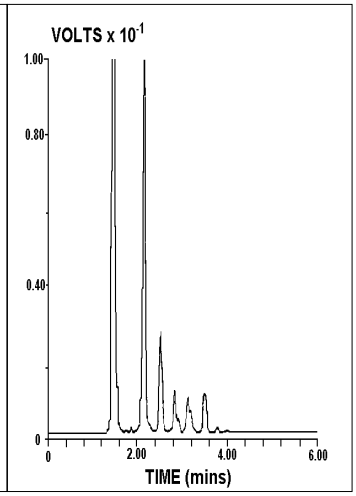

Figure 3 *Aviation fuel sample at 210 nm*

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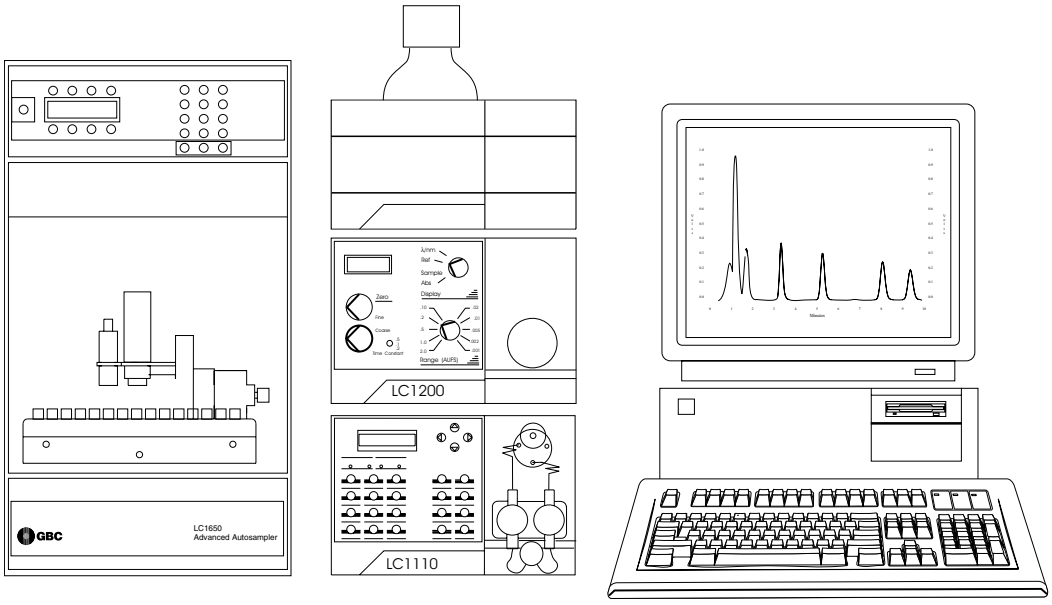

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GBC publication number 01-0367-00
September, 1995

HPLC application note

Pthalamic Acid

'..alkylammonium salts of phthalamic acid, the monoamide of phthalic acid, are used as corrosion inhibitors in metal finishing baths...'

Alkylammonium salts of phthalamic acid, the monoamide of phthalic acid, are used as corrosion inhibitors in metal finishing baths. The concentration of the inhibitor in the bath is crucial for the success of the process. Owing to the complexity of the chemical composition of metal finishing fluids, monitoring the phthalamic acid level by most analytical procedures is an arduous task.

HPLC analysis, on the other hand, is quite straightforward. In Figure 1, the reversed phase separation of a phthalamic acid standard is shown. Elution was effected under isocratic conditions with the UV detection at 230 nm, using a 5 cm analytical column enabling rapid analysis. Phthalamic acid levels in freshly constituted and inhibitor depleted bath samples were determined from the chromatograms displayed in Figures 2 and 3, respectively.

Keywords:
Corrosion inhibitors, metal finishing, phthalamic acid

Conditions

Column:	Spherisorb S5 ODS2, 50 mm x 4.6 mm ID
Mobile Phase:	0.1% Aqueous phosphoric acid:methanol (80:20)
Flow Rate:	1 ml/min
Detection:	UV at 230 nm

1 = Phthalamic Acid

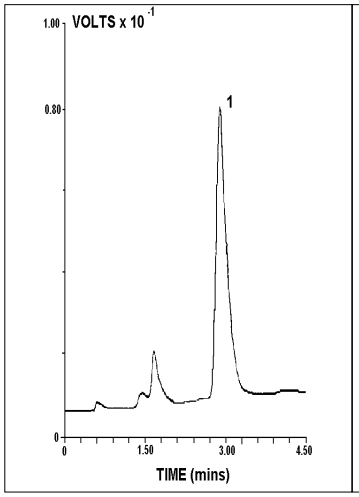

Figure 1 *Phthalamic Acid Standard*

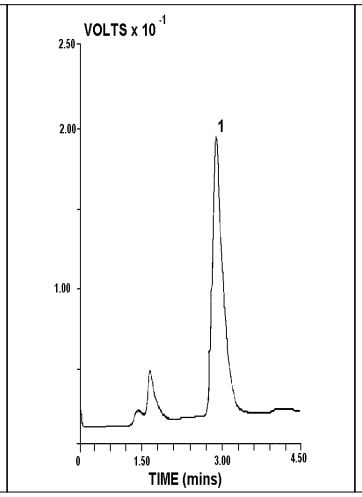

Figure 2 *Fresh bath*

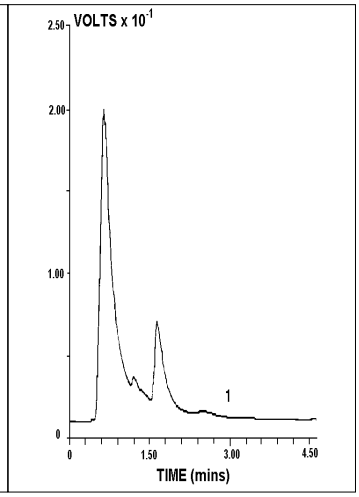

Figure 3 *Depleted bath*

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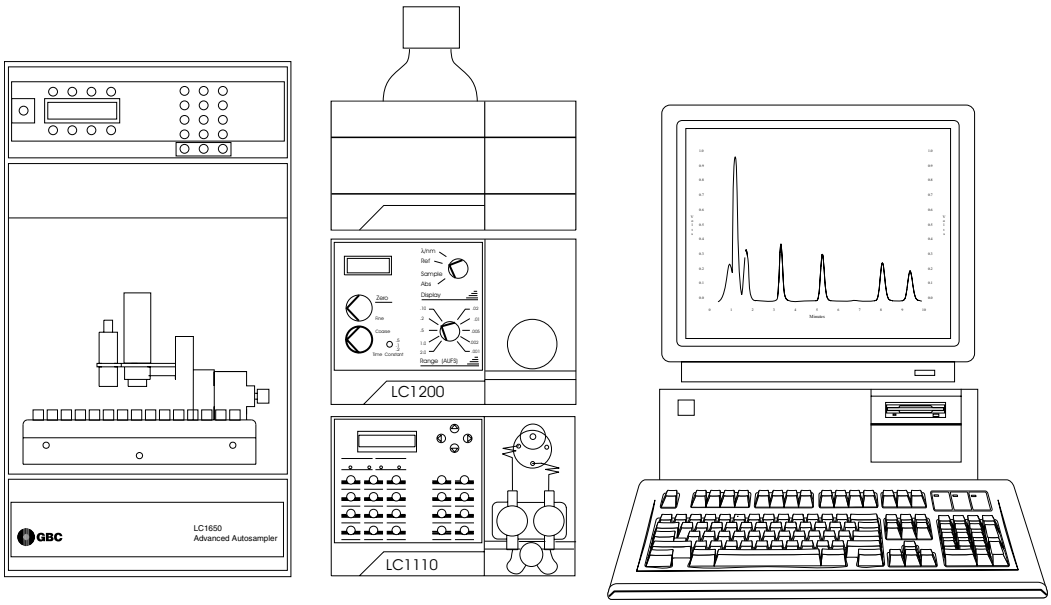

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HPLC application note

Non-Ionic Surfactants

'...normal phase chromatography using UV transparent eluents with cyano (CN) columns has been successfully employed for profiling various surfactant formulas...'

Non-ionic surface active agents are used in the preparation of a wide range of foods, cosmetics and pharmaceutical products including emulsions, creams, ointments and suppository bases. HPLC analysis of formulations containing non-ionic surfacants may be complicated by a number of factors:

(i) Lack of pure standards for chromatographic analysis;
(ii) Insolubility of some samples in reversed phase solvents;
(iii) Detection problems, *e.g.*, poor UV absorbance;
(iv) Selection of an appropriate column for the MW range of samples;
(v) Complexity of surfactant samples, owing to differing degrees of esterification and ethoxylation.

Identification of individual components of surfactant mixtures is seldom required for quality control purposes. Instead, chromatographic 'fingerprints' are compared in order to detect batch to batch variations. Normal phase chromatography using UV transparent eluents with cyano (CN) columns has been successfully employed for profiling various surfactant formulas.

Keywords:
Non-Ionic surfactants, normal phase, CN columns

In Figures 1 and 2, the analyses of two different samples of commercially available non-ionic surfacants are shown. Although baseline resolution of all peaks is not observed under the isocratic elution conditions chosen, separations are relatively fast and the resulting fingerprints adequately characterise the respective samples.

Conditions

Column:	Spherisorb S5 CN, 250 mm x 4.6 mm ID
Guard:	Spherisorb S5 CN, 50 mm x 4.6 mm ID
Mobile Phase:	Isopropanol, hexane (15:85)
Flow Rate:	2 ml/min
Detection:	UV at 215 nm

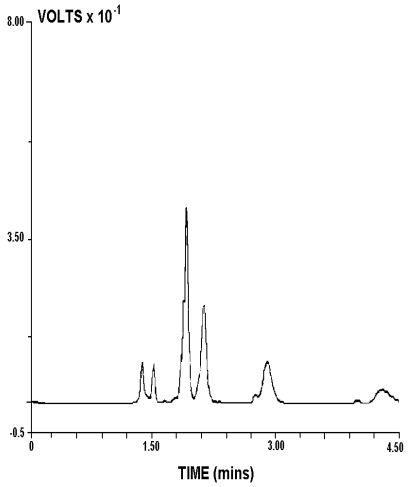

Figure 1 *Sample A*

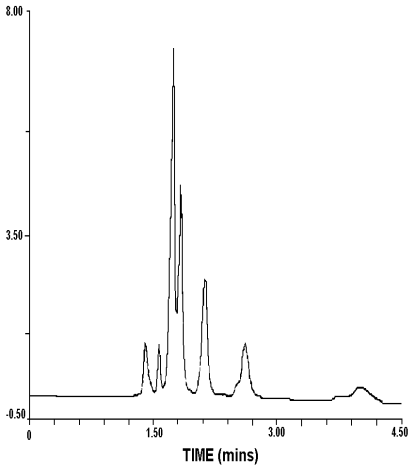

Figure 2 *Sample B*

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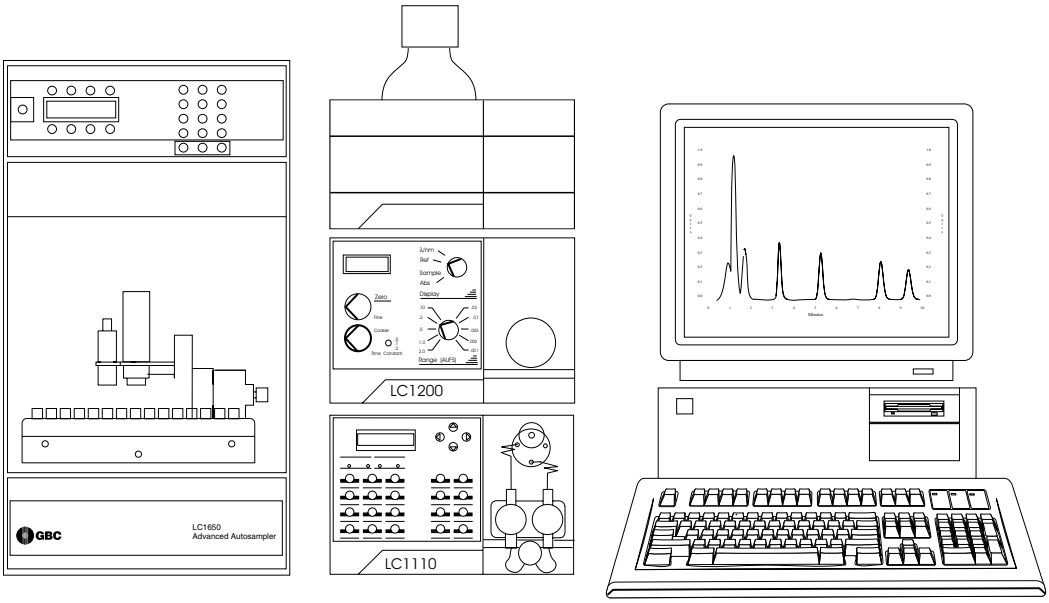

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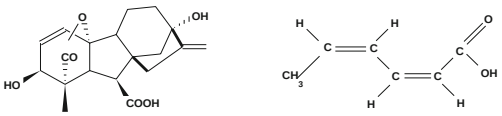
HPLC application note

Gibberellic Acid

'...the most prominent member of the group is gibberellic acid, used commercially to promote the growth of seedlings and ensure uniform ripening of crops, e.g., grapes...'

The gibberellins are a group of tetracarbocyclic lactonic carboxylic acids first isolated in Japan from the fungus 'Gibberella fujikuroi' the organism causing Bakanae disease in rice. They promote many of the normal processes of plant growth and development and are now known to be widely distributed in higher plants. The chemical structures of the gibberellins were established mainly by B.E. Cross, J.F. Grove, J.MacMillan and T.P.C. Mulholland (Reference 1).

The most prominent member of the group is



gibberellic acid (1), used commercially to promote the growth of seedlings and ensure uniform ripening of crops, *e.g.*, grapes.

Keywords:
Gibberellic acid, gibberellins, plant growth hormone

A convienent method of analysis of formulations containing gibberellic acid involves reversed phase HPLC with UV detection at 206 nm. In Figure 1, a typical chromatogram for gibberellic acid is presented. Sobic acid (2) was used as an internal standard. Comparison of commercial batches is made easy with the WinChrom SuperCompare feature, which enables different samples to be overlaid either on a VDU screen or hardcopy, as shown in Figure 2.

Conditions

Column:	Spherisorb S5 ODS2, 250 mm x 4.6 mm ID
Guard:	Spherisorb S5 ODS2, 50 mm x 4.6 mm ID
Mobile Phase:	Methanol, aqueous 0.1% Phosphoric acid (35:65)
Flow Rate:	1.5 ml/min
Temperature:	35°C
Detection:	UV at 206 nm

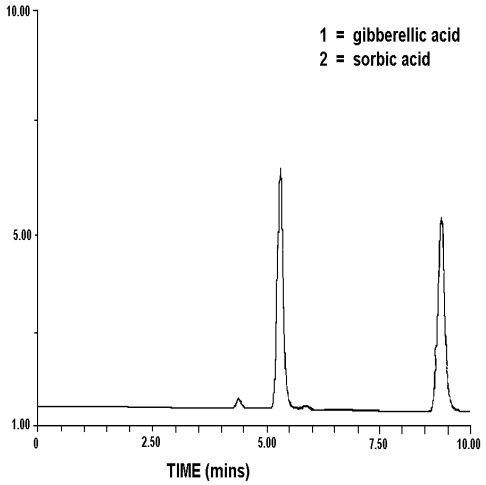


Figure 1 *Gibberellic Acid Standard*

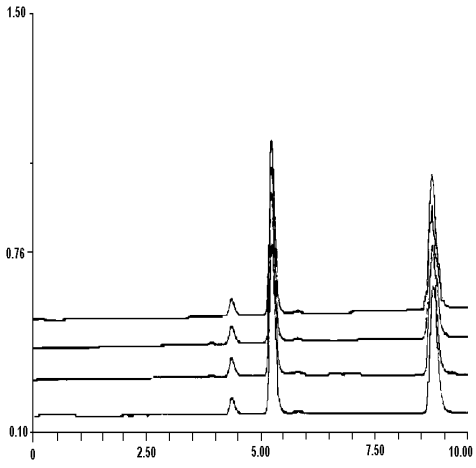


Figure 2 *Batch Comparison*

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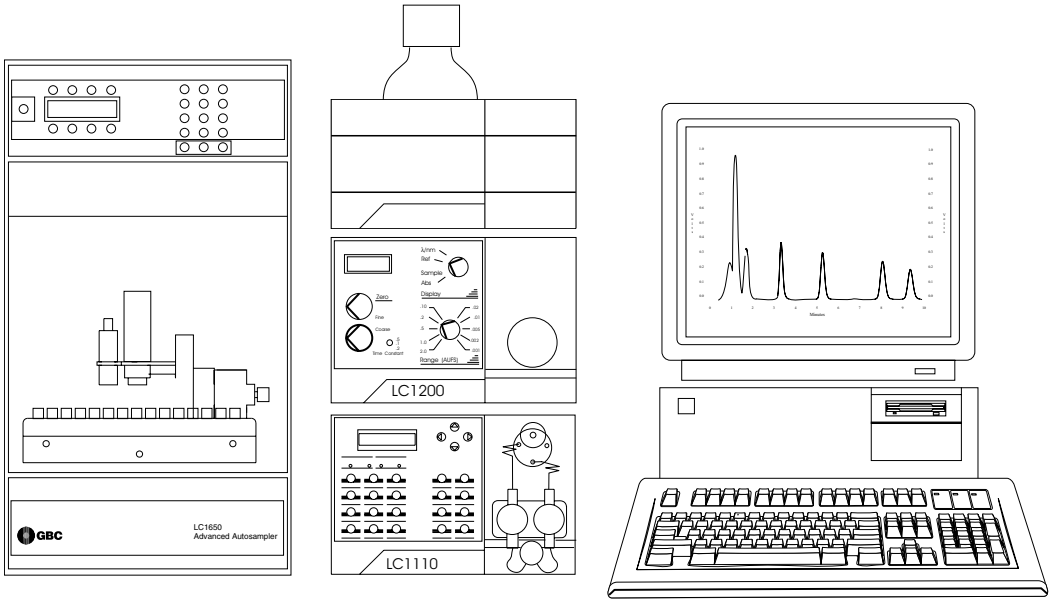
Reference

1. Fieser, L.L. and Fieser, M., 'Topics in Organic Chemistry' (Reinhold, 1963), p. 200.

Acknowledgement

This method was developed by Caroline Cairncross at ICI Central Research Laboratories, Ascot Vale, Australia.



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HPLC application note

Determination of Tetracycline Antibiotics by C18 RP-HPLC

'...tetracycline, oxytetracycline and chlortetracycline are three of the most frequently applied antibiotics in animal nutrition...'

Abstract

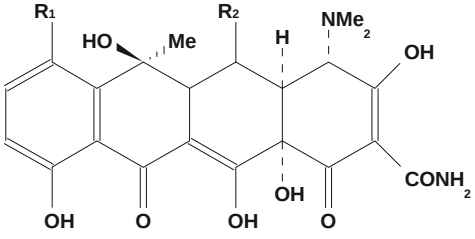
A method is described for the determination of basic antibiotics, Chlortetracycline, Tetracycline and Oxytetracycline by RP-HPLC on a C18 column with UV detection. An oxalic acid buffer, tetramethylammonium chloride (TMA) and EDTA, was used. The use of TMA effectively eliminates the undesirable secondary interactions due to free silanols on the bonded silica column, providing effective and reproducible separation within 13 minutes.

Keywords:
Tetracycline, Oxytetracycline, Chlortetractycline, Antibiotic, Pharmaceutical, Veterinary Medicine, Animal Nutrition, Meat, Silanol blocking Agent, RP-HPLC

Tetracycline antibiotics are widely used in modern medicine[1] and agricultural practice.[2] Chlortetracycline, oxytetracycline and tetracycline are three of the most frequently applied antibiotics in animal nutrition. Residual tetracyclines are found in muscle tissues and the organs of slaughtered animals.[3] Traditionally, microbiological[4] and fluorimetric[5] methods have been employed for their monitoring. These methods suffer from their inherent lack of selectivity. However HPLC offers high selectivity and sensitivity, and as a result has become the method of choice for the routine analysis of tetracyclines.

Bonded reversed phase columns have been reported to vary greatly in their suitability for the chromatography of tetracyclines. Unreacted silanols on the column interact with basic compounds, such as tetracyclines, giving poor chromatrographic results.[6] As an alternative, more expensive polymeric RP columns have been used.[7]

By employing oxalic acid as a buffer, which also serves the dual purpose as a chelating agent, a method has been developed for the routine analysis of chlortetracycline, oxytetracycline and tetracycline using C18 columns. Depending on the acidity of the column, tetramethylammonium chloride could be added to further eliminate the secondary interactions of free silanols. These interactions are usually manifested as severe peak broadening and tailing.



Chlortetracycline	R_1 = Cl,	R_2 = H
Tetracycline	R_1 = H,	R_2 = H
Oxytetracycline	R_1 = H,	R_2 = OH

Conditions

Column:	Spherisorb S5 ODS2, 250 mm x 4.6 mm ID
Mobile Phase:	0.01 M oxalic acid, 0.01 M tetramythlammonium chloride, 3 mM EDTA (ph 2.5)/ Acetonitrile (75:25)
Flow Rate:	1.0 ml/min
Temperature:	30°C
Detection:	UV at 280 nm
Injection Vol.:	20 µl

Preparation of Standard

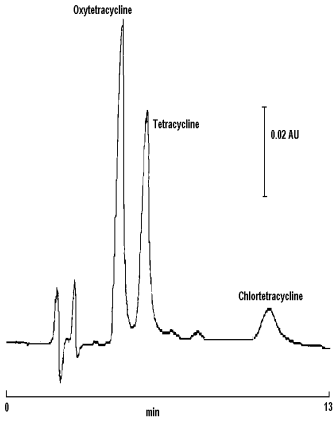


Figure 1 *HPLC Separation of Tetracyclines*

Chloretetracline (1.0 mg), oxytetracycline (1.0 mg) and tetracycline (1.0 mg) were dissolved in 0.01 M HCl (10 ml).

Acknowledgement

We are grateful to Mr John Walsh, Department of Agriculture and Rural Affairs (Victoria) for his technical advice and to his department for the kind gift of oxytetracycline and chloretetracycline.

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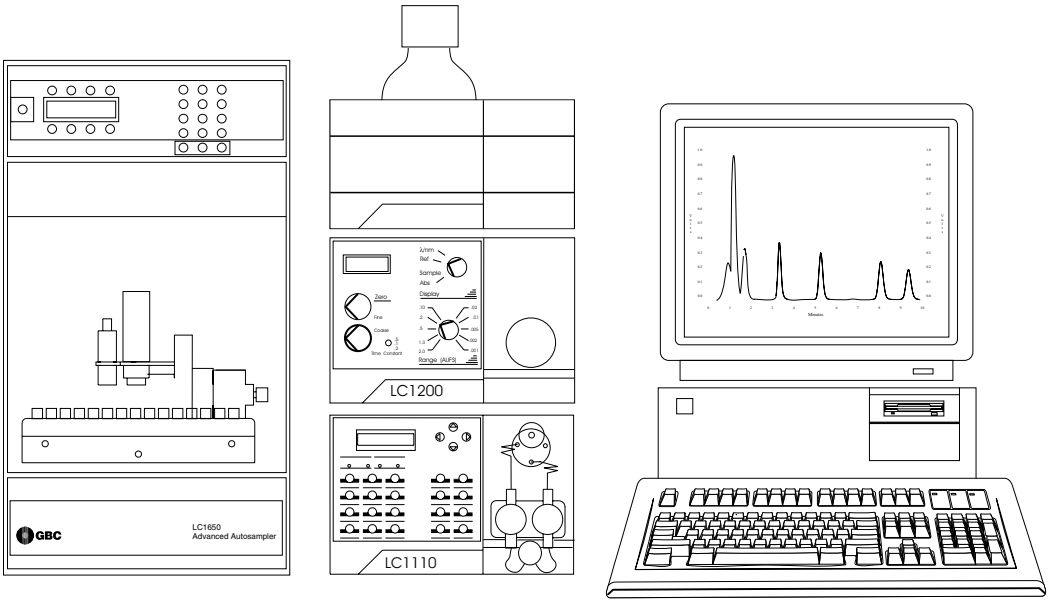

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September, 1995

HPLC application note

Determination of Anticonvulsants by Isocratic Separation and UV Detection

1. Primidone (12 ppm)
2. Phenobarbitone (7 ppm)
3. Phenytoin (2.3 ppm)
4. Carbamazepine (1.5 ppm)

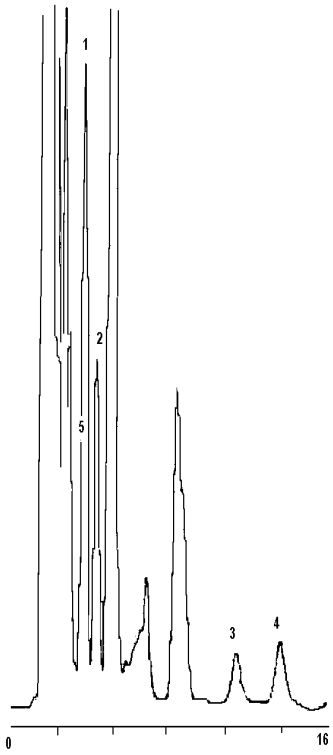

Figure 1 *Sample: Reconstituted human serum containing 45 theraputic drugs**

*Acetaminophen, Amikacin, Caffeine, Carbamazepine, Carbamazepine (free), Chloramphenicol, Cortisol, Digoxin, Disopyramide, Estriol (total), Thosuximide, Gentamicin, Kanamycin, Lidocaine, Lithium, Methotrexate, NAPA, Netilmicin, Phenobarbital, Phenytoin, Phenytoin (free), Primidone, Procainamide, Propranolol, Quinidine, Salicylate, Streptomycin, T3 (total), T3 Uptake, T4 (total), T4 (free), Theophylline, Tobramycin, TSH, Valproic Acid, Valproic Acid (free), Vancomycin.

Conditions

Column:	Spherisorb OD S2 (C18) Column 5 µM (250 x 4.6 mm ID)
Mobile Phase:	10 mM Dipotassium Phosphate, 10 mM Tetramethyl Ammonium Chloride (pH 7.3) (70%) and Acetonitrile (30%)
Flow Rate:	1.0 ml/min
Temperature:	30°C
Detector:	205 nm

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LC1110 Dual Piston HPLC Pump (x2)
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GBC Column Oven
LC1650 Advanced Autosampler
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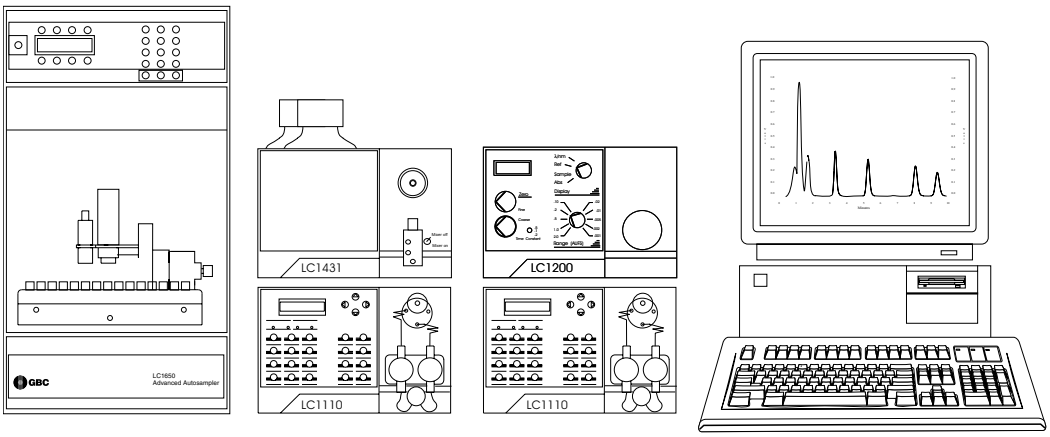

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HPLC application note

Determination of Penicillins by C18 RP-HPLC

Abstract

A method is described for the determination of Amoxicillin, ampicillin and Penicillin-G by reversed phase HPLC on a C18 column with UV detection. An oxalic acid buffer containing tetramethylammonium chloride (TMA) and DETA was employed. The use of this mobile phase has eliminated the undesirable secondary interactions between the acidic silanols on the column and the basic functionalities of the analytes, providing effective separation of the three penicillins in less than 8 minutes.

'...in the treatment of mastitis and other animal diseases, methods capable of distinguishing individual penicillins have been important from a regulatory point of view...'

Penicillins are one of the most widely used antibiotics in modern mecidine. Traditionally the detection of penicillins in biological samples, *e.g.*, milk and tissues, has been carried out by bioassay techniques.[1] However, these methods lack the selectivity in determining one penicillin from another analogue. With a variety of penicillins other than Penicillin-G being used today in the treatment of mastitis and other animal diseases, methods capable of distinguishing individual penicillins have been important from a regulatory point of view. HPLC offers the versatility necesssary, in that, standard LC procedures can be easily modified to accomodate the specific requirements of different analyses.

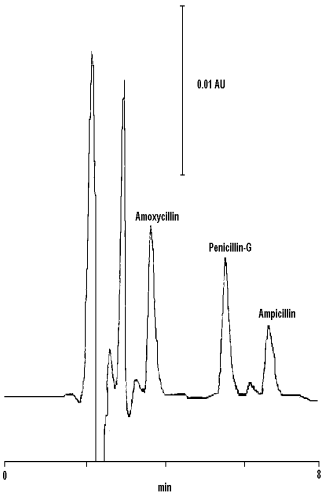
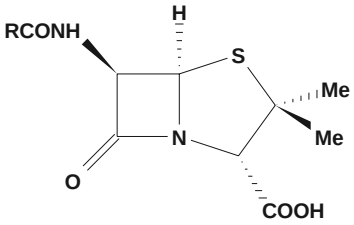


Figure 1 *HPLC Separation of Penicillins*

One of the inherent problems of the analysis of penicillins with basic functionalities on reversed-phase packings is the interactions of these moieties with the free silanols on the silica support.[2] Binding to these silanols causes broadening and distortion of chromatographic peaks of basic penicillins like Amoxicillin and Ampicillin. By employing an oxalic acid as a buffer, which also acts as a chelating agent, a method has been developed for the routine analyis of Amoxicillin, Ampicillin and Penicillin-G using C18 columns. Depending on the acidity of the column, tetramethylammonium chloride (TMA) could be added to further eliminate the secondary interactions of free silanols.

Keywords:
Penicillin, Amoxycillin, Ampicillin, Antibiotic, Silanol Blocking Agent, Pharmaceutical, Veterinary Medicine, Meat, Animal Nutrition, RP-HPLC



Amoxicillin R = -4OH-C$_6$H$_4$CH(NH$_2$)-

Penicillin-G R = -4OH-C$_6$H$_4$OCH$_2$-

Ampicillin R = -C$_6$H$_5$CH(NH$_2$)-

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Conditions

Column:	Spherisorb S5 ODS2, 250x4.6mmID
Mobile Phase:	0.01 M Oxalic Acid, 0.01 M, Tetramethylammonium Chloride, 3 mM EDTA, pH 2.5/Acetonitrile(80:20)
Flow Rate:	1.0 ml/min
Temperature:	30°C
Detection:	UV at 265 nm
Injection Vol:	20 µl
Standard Prep.:	1.0 mg in 10 ml of 0.01

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LC1120/LC1150 HPLC Column Oven Option

Acknowledgement

We are grateful to the Department of Agriculture and Rural Affairs, Victoria, for the kind gift of Amoxycilin, Ampicillin and Penicillin-G.

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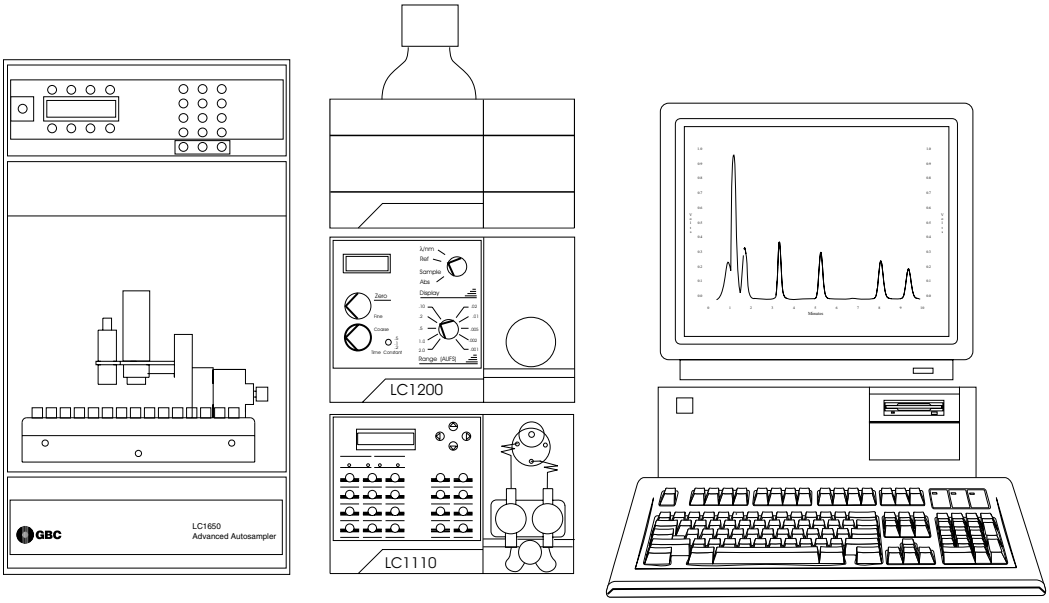

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September, 1995

HPLC application note

Determination of Erythromycin A by LCEC

Abstract

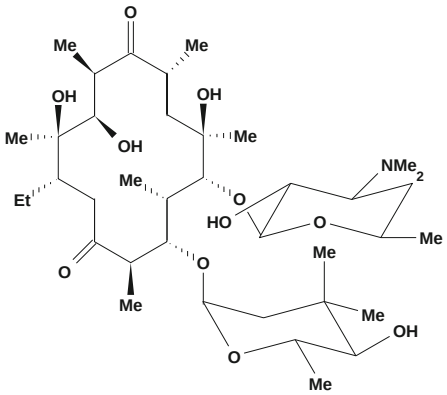
A method is described for the determination of Erythromycin A by RP-LCEC (Reversed Phase-Liquid Chromatography with Electrochemical Detection) on silica-based columns. Sensitivity of a method is at sub-ppm levels. The combination of a micro-composite glassy carbon electrode with a wall jet cell offers a LCEC system which is reliable and user friendly.

'...erythromycin is a widely used macrolide antibiotic produced by fermentation...'

Drugs are used extensively in food-producing animals to maintain optimal health and promote growth. Of all the types of drugs used in animal nutrition, antibiotics have the largest sales by dollar value and volume.[1] The use of these drugs has the potential to leave residues in meat, milk and eggs. For those drugs that require a withholding period, methods of analysis are required.

Erythromycin is a widely used macrolide antibiotic produced by fermentation. Traditional microbiological methods,[2] although suitable for residual screening, suffer from a lack of sensitivity,[3] while HPLC with fluorescent detection, although very sensitive, requires complex postcolumn derivatisation.[4] By comparison, LCEC offers simplicity in sample clean-up, no derivatisation requirement and excellent selectivity. High selectivity is achieved as analytes must be electroactive at the applied potential in order to be detected.

Keywords:
Erythromycin A, Electrochemical, RP-LCEC, Macrolide Antibiotic, Pharmaceutical, Vetrinary Medicine, Animal Nutrition



Erythromycin A

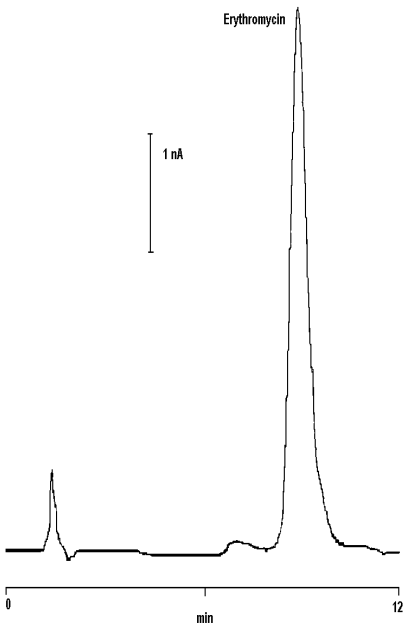


Figure 1 *Erythromycin Standard*

The LC1260 Electrochemical Detector, with its unique 'Wall Jet' design allows shorter equilibration time and increased reliability. The use of a micro-composite glassy carbon electrode results in increased sensitivity when compared with conventional 3 mm glassy carbon electrodes. Sensitivity is enhanced through the used of low noise electronic circuitry with active and digital filtering.

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Conditions

Column:	Spherisorb S5 CN, 150 mm x 4.6 mm ID
Mobile Phase:	0.06 M sodium acetate, 0.1 mM EDTA disodium salt, (pH 6.8):Acetronitrile (55:45) (Helium sparging)
Flow Rate:	1.0 ml/min
Temperature:	Ambient
Detection:	
Working Electrode:	3 mm MicroComposite Glassy Carbon
Reference Electrode:	Ag/AgCl (3 M KCl)
Auxiliary Eelctrode:	Cell Body
Applied Potentail:	900 mV

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LC1650 Advanced Autosampler
WinChrom Chromatography Data Management System
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Acknowledgement

We are grateful to the Department of Agricultural and Rural Affairs, Victoria, for the kind gift of Erythromycin.

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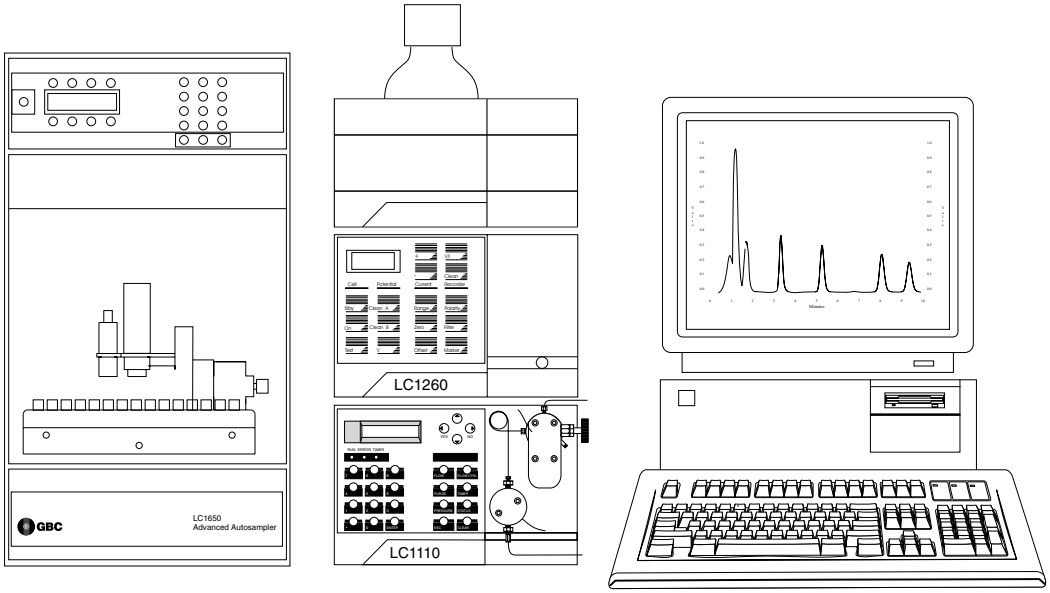

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GBC publication number 01-0375-00
September, 1995

HPLC application note

Determination of Chloramphenicol by C18 RP-HPLC

'...HPLC offers high selectivity and sensitivity for CAP quantitation when coupled with the appropppriate sample preparation procedures...'

Abstract

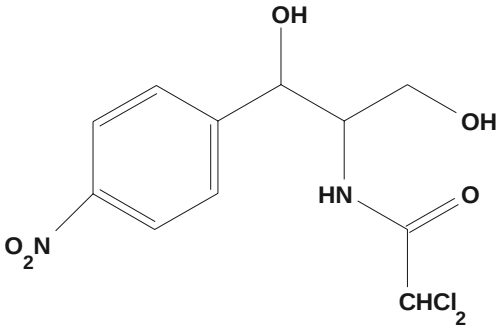
A method is described for the determination of Chloramphenicol by C18 reversed-phase HPLC with UV detection. The method is straightforward and sensitivity is at the sub-ppm level with the retention time of chloramphenicol being approximately 4 minutes.

Keywords:
Chloramphenicol, CAP, Antibiotic, Veterinary Medicine, Meat, RP-HPLC

Chloramphenicol (CAP) is a broad spectrum antibiotic which has been widely used for the treatment of the microbial infections in humans between 1950 and 1980.[1] Considerable evidence has now been accumulated detailing its toxic effect on humans.[1] To date, the clinical use of CAP has been reserved as a last resort. Two potentially fatal adverse reactions to CAP treatment are Gray Syndrome, which is dose related, and Aplastic Anaemia, which is not.

A Joint FAO/WHO (Food and Agriculture Organisation/World Health Organisation) Expert Committee on Food Additives has proclaimed that CAP residues in human food supply are unacceptable.[2] Despite the ban, CAP is still being used to treat food-producing animals for such diseases as mastitis in dairy cattle,[3] shipping fever in calves[4] and other caused by gram-negative bacteria.[5] As long as CAP products are still available, there exists the potential for their use in food-producing animals. Appropriate methods to monitor meat, milk and eggs for CAP residues will be required.

Microbiological assays could be employed for preliminary screening of antibiotic residues, however they lack the selectivity for specific quantitation. HPLC on the other hand offers high selectivity and sensitivity for CAP quantitiation when coupled with the appropppriate sample preparation procedures.[6]



Chloramphenicol

Conditions

Column:	Spherisorb S5 ODS2, 250 x 4.6 MM ID
Mobile Phase:	Water/Methanol (40:60) (Helium Sparging)
Flow Rate:	1.0 ml/min
Temperature:	30°C
Detection:	UV at 280 nm
Injection Vol:	20 µl
Standard Prep.:	1.0 mg of chloramphenicol in 10 ml of methanol.

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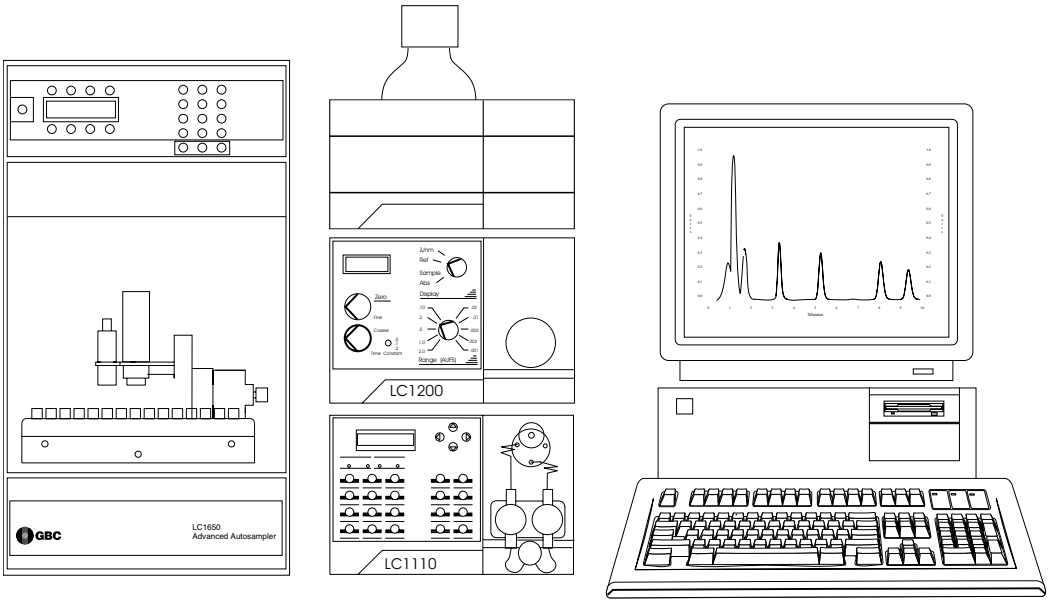

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GBC publication number 01-0376-00
September, 1995

HPLC application note

Determination of Zeranol by C18 RP-HPLC

Abstract

A method is described for the determination of the anabolic agent, Zeranol, by the C18 reversed phase HPLC with UV detection. The method is straightforward, with sensitivity in the sub-ppm range. Retention time for Zeranol is approximately 14 minutes.

Keywords:
Zeranol, Zearalonaol, Anabolic, Growth Stimulant, Veterinary Medicine, RP-HPLC

'...a standard LC procedure when coupled with the appropriate sample preparation procedures can deliver rapid methods for the analysis of zeranol in urine and tissues...'

Zeranol (zearalanol) has been adopted as a growth stimulant and also employed to reduce stress in cattle.[1] The radioactive study, using tritium-labelled zeranol pellets in cattle indicated that while the majority of zeranol was found in faeces (45%) and urine (10%), 10% remained in the site of implant.[2] The use of this chemical agent therefore has the potential to leave residues in food-producing animal tissues and organs, and methods for its analysis are required.

HPLC offers high selectivity and sensitivity. In addition, it also provides the instrumental flexibility to allow a standard LC procedure to be readily modified to suit the requirements of specific assays. This, when coupled with the appropriate sample preparation procedures, can deliver rapid methods for the analysis of zeranol in urine[3] and tissues.

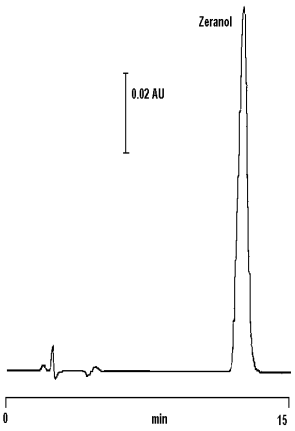
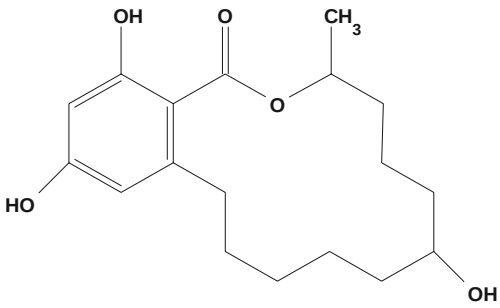


Figure 1 *Zeranol Standard*



Zeranol

Conditions

Column:	Sperisorb S5 ODS2, 250 x 4.6 mm ID
Mobile Phase:	Water/Methanol (40:60) (Helium Sparging)
Flow Rate:	1.0 ml/min
Temperature:	30°C
Detection:	UV at 265 nm
Injection Vol.:	20 µl
Standard Prep.:	1.0 Mg of Zeranol dissolved in 10 ml of methanol

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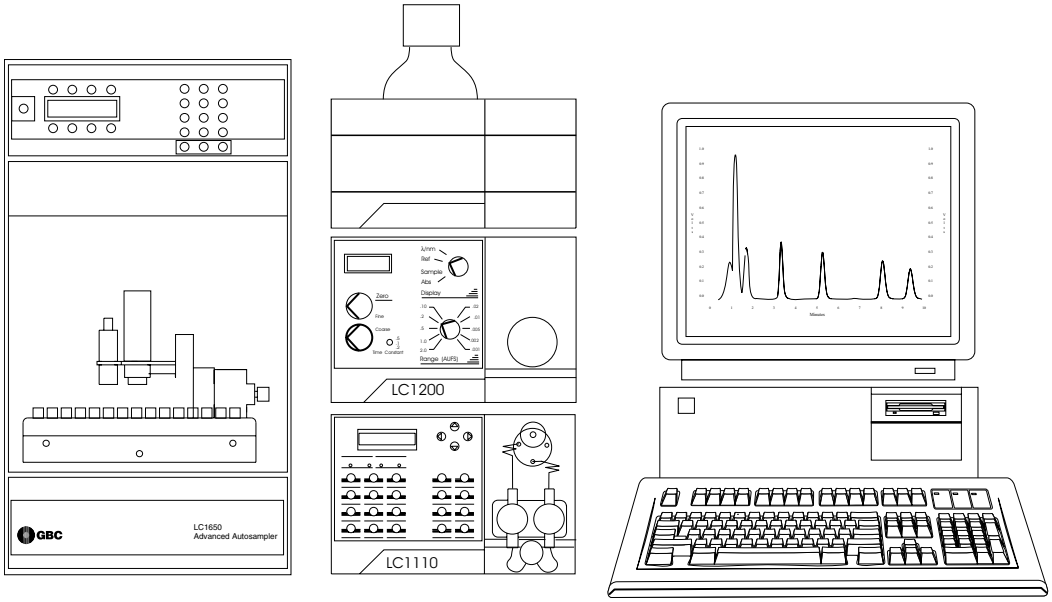

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GBC publication number 01-0377-00
September, 1995

HPLC application note

Determination of Diethylstilbestrol by C18 RP-HPLC

Abstract

A method is described for the determination of nonsteroidal estrogen, Diethylstilbestrol, using C18 reversed phase HPLC and UV detection. The procedure is straight forward with sensitivity at the sub-ppm level. The retention time for diethylstibestrol is approximately 4 minutes.

Keywords:
Diethylstilbestrol, Stilboestrol, Nonsteroidal Estrogen, Female Sex hormone, Pharmaceutical, Veterinary Medicine, RP-HPLC

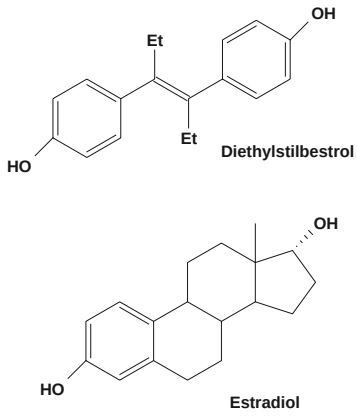
'...HPLC offers high selectivity and sensitivity for the determination of diethylstilbestrol when coupled with the appropriate sample preparation techniques...'

The two principal steroidal classes of female sex hormones are estrogens and progestins. Estrogens are substances that induce estrus in various mammalian species.[1] They are important becuase they bring about the secondary sex characteristics in females. The steroid nucleus is however not required for estrogenic activity. Many derivatives of stilbene, especially the transisomers, are potent estrogenic substances used therapeutically, and in the animal industry.[2]

One of the most important synthetic estrogens is diethylstilbestrol. This drug is significantly cheaper than naturally occuring estrogens and yet can produce all the same effects. The official diethylstilbestrol is the transisomer, which has 10 times the estrogenic potency of the cis-isomer because it resembles more closely the natural estrogen, estradiol.[2] HPLC offers high selectivity and sensitivity for the determination of diethylstilbestrol when coupled with the appropriate sample preparation techniques.

Conditions

Column:	Spherisorb S5 ODS2, 250 x 4.6 mm ID
Mobile Phase:	Water/Acetonitrile (25:75) (Helium Sparging)
Flow Rate:	1.0 ml/min
Temperature:	30°C
Detection:	UV at 254 nm
Injection Vol:	20 µl
Standard Prep.:	1.0 mg dissolved in 10 ml of methanol.



Diethylstilbestrol

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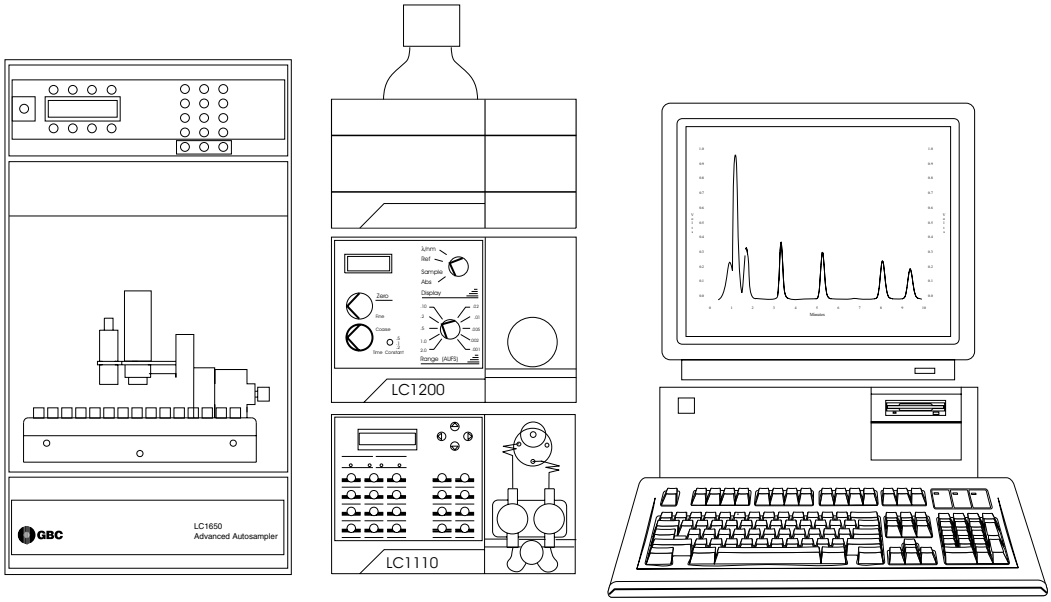

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GBC publication number 01-0378-00
September, 1995

HPLC application note

Determination of Sulfonamides by C18 Rp-HPLC Part 2

Abstract

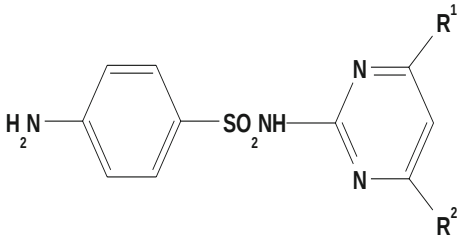
A method is described for the determination of three sulfonamides; Sulfadiazine, Sulfamerazine and Sulfamethazine, by reversed-phase HPLC on a C18 column with UV detection. Sensitivity of the method is at the sub-ppm level with separation achievable within 12 minutes.

Keywords:
Sulfonamide, Sulfadiazine, Sulfamerazine, antibiotic, Pharmaceutical, Veterinary Medicine, meat, RP-HPLC

Sulfonamides are used as antibacterials in domestic animals via medicated feeds. These agents increase the vigor and general well-being of food producing animals. In addition, they reduce the economic losses of the animal industry due to the incidences of parastic protozoan infections in species like poultry, cattle and sheep.[1] These commercially valuable animals are prone to coccidiosis infection and treatment consists of administering sulfonamides via drinking water.

Acceptable levels of sulfonamides in animal tissue, which are governed by the witholding and withdrawal periods prior to slaughter, have been established. However, these levels are subject to change based on new evidence of toxicological profiles of these drugs. For example, recent evidence[1] has implied sulfamethaxine as a possible carcinogen, and this has necessitated the re-evaluation of legal levels.

Sulfonamide residue levels have been monitored by various analytical techniques,[2-4] including colorimetry, TLC, GC and HPLC. Among the various methods, HPLC offers high selectivity and sensitivity when coupled with the appropriate sample preparation technique.[5,6]



Sulfadiazine	R^1 = H	R^2 = H
Sulfamerazine	R^1 = Me	R^2 = H
Sulfamethazine	R^1 = Me	R^2 = Me

Conditions

Column:	Sperhisorb S5 ODS2, 250 x 4.6 mm ID
Mobile Phase:	0.01 M Sodium Acetate, 1% Acetic Acid /Methanol (75:25) (HeliumSparging)
Flow Rate:	1.0 ml/min
Temperature:	30°C
Detection:	UV at 265 nm
Injection Vol:	20 µl
Standard Prep.:	1.0 mg of each standard is dissolved in 10 ml of methanol

Acknowledgements

We are grateful to Mr. John Walsh, Department of Agriculture and Rural Affairs (Victoria) for his technical advice, and to his department for the kind gift of the penicillin standards.

'...sulfonamide residue levels have been monitored by various analytical techniques... HPLC offers high selectivity and sensitivity when coupled with the appropriate sample preparation technique...'

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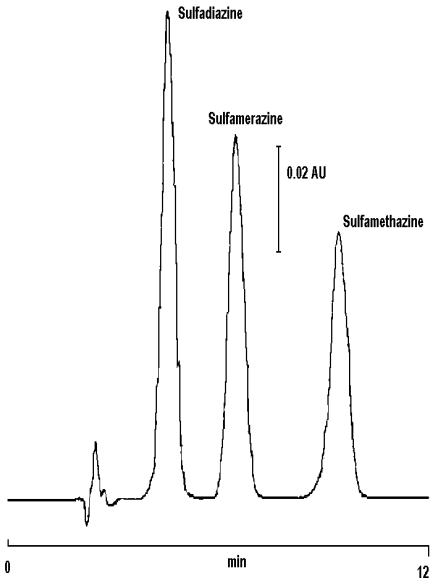

Figure 1 *HPLC Separation of Sulfonamides*

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References

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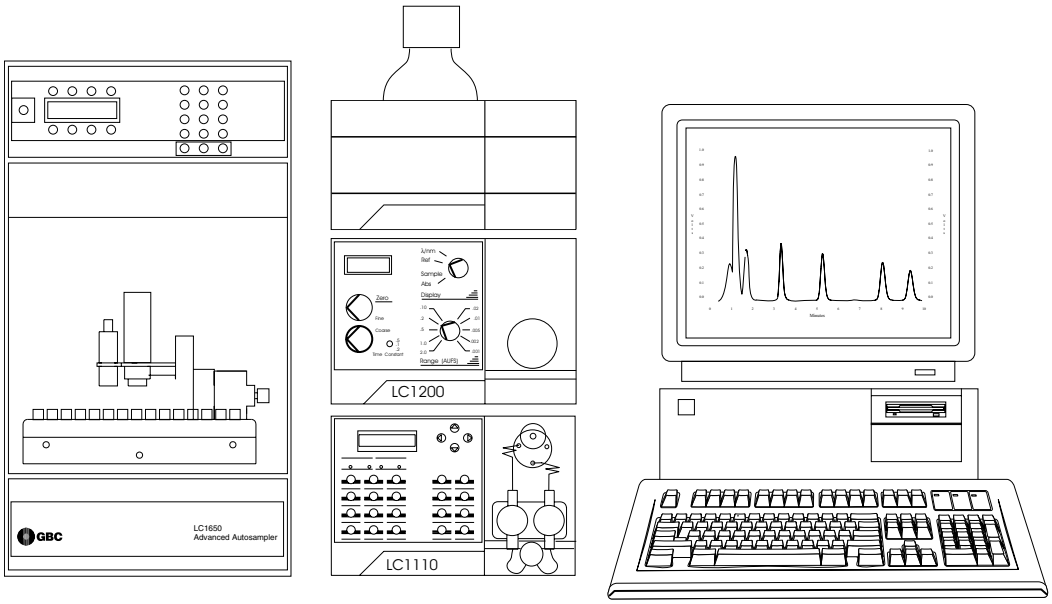

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GBC publication number 01-0379-00
September, 1995

HPLC application note

Determination of Streptomycin by Ion-Pair RP-HPLC

Abstract

An isocratic, ion-pair reversed phase HPLC method is described for the determination of Streptomycin. The procedure employes a C18 column with hexanesulfonic acid as the ion-pairing agent, and detection by UV absorbance at 195 nm. The sensitivity is at the sub-ppm level. The retention time for streptomycin is approximately 7 minutes.

'...it has serious side effects such as ototoxicity and nephrotoxicity... this has necessitated the therapeutic drug monitoring of streptomycin ...'

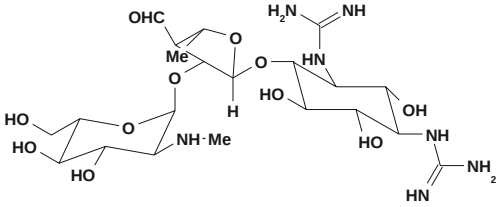
Keywords:
Streptomycin, Aminoglycoside Antibiotic Tuberculosis,Pharmaceutical, Veterinary Medicine,RP-Ion-Pair HPLC

Streptomycin, an aminoglycoside, was first isolated by Waksman and co-workers[1] in 1944 from a strain of Streptomyces griseus. It exhibits comparable anti-mirobial activity against a wide range of gram-negative and positive bacteria as well as mycobacteria. Streptomycin became the first clinically available drug for the treatment of tuberculosis. Despite its clinical usefulness, it has serious side effects such as ototoxicity and nephrotoxicity, which are related to the concentration in serum. This has necessitated the therapeutic drug monitoring of streptomycin.[2]

The traditional methods for the analysis of streptomycin are microbiological turbidimetric procedures and plarography. These methods have the disadvantages of being slow, with poor reproducibility and interferences from other co-administered antibiotics. HPLC, with its sensitivity, selectivity, ease-of-operation and speed, offers a powerful alternative for routine analyses over the traditional methods.[3]

Conditions

Column:	Spherisorb S5 ODS, 250 x 4.6 mm ID
Mobile Phase:	0.025 M Sodium Phosphate Dibasic, 0.01 M hexanesulfonic acid, pH 2.6 / Acetonitrile, (90:100) (Helium Sparging)
Flow Rate:	1.0 ml/min
Temperature:	40°C
Detection:	UV at 195 nm



Streptomycin

Acknowledgement

We are grateful to the Department of Agriculture and Rural Affairs (Victoria), for the kind gift of streptomycin.

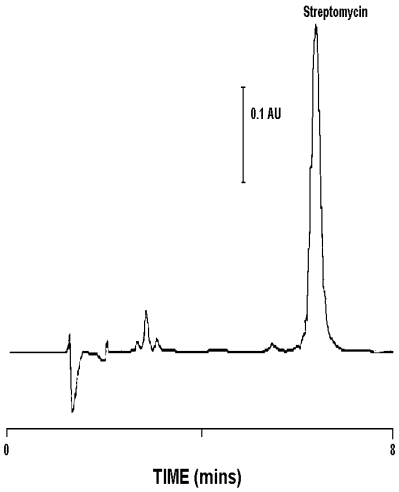


Figure 1 *Streptomycin Standard*

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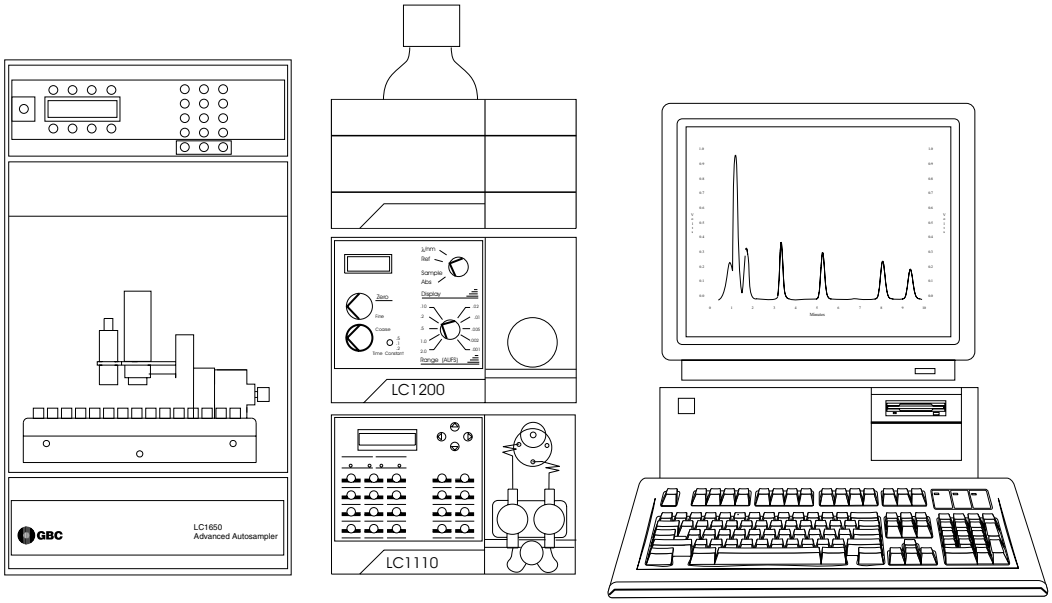

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GBC publication number 01-0380-00
September, 1995

HPLC application note

Morphine Determination by RP-LCEC

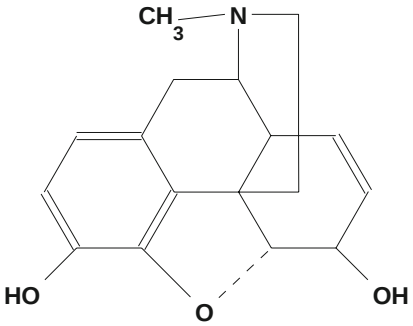
Abstract

A method is described for the determination of morphine by reversed phase LCEC (Liquid Chromatography with Electrochemical Detection). Sensitivity of the method is in the sub-ppm range and the retention time of morphine is approximately 7 minutes.

'White was the first to report a HPLC method... because of its sensitivity and specificity, this technique has become the method-of-choice for the determination of morphine in biological fluids...'

Morphine was first isolated in 1803 and since then a number of related compounds have been synthesised in an attempt to overcome the main drawbacks of the parent drug *i.e.*, tolerance and dependence on heroin and the 3,6-diacetate of morphine. In the body, heroin undergoes rapid enzymatic deacylation to 6-acetylmorphine which is further converted to morphine, presumably in the liver.

Immunological assays, specifically RIA, EIA and fluorescence polarisation immunoassays, have all been used for the analysis of morphine with varying degrees of success. TLC is simple and inexpensive but lacks sensitivity and specificity. GC methods require careful and time consuming sample preparation including derivatisation in order to achieve the same sensitivity as RIA.[1]



HPLC is the preferred method for morphine analysis due to its inherent features of specificity, reliability, sensitivity and reduced sample preparation requirements. White was the first to report a HPLC method for morphine use using electrochemical oxidation.[2] Because of its sensitivity and specificity, this technique has become the method-of-choice for the determination of morphine in biological fluids.[1]

Keywords:
Morphine, Electrochemical, LCEC, Pharmaceutical

Serum, plasma and blood preparation techniques for morphine analysis are well reviewed in reference 1.

Conditions

Column:	Sperhisorb S5 ODS2, 250 x 4.6 mm ID
Mobile Phase:	0.2 M Sodium Perchlorate, 0.005 M Sodium Citrate, 3 mM Triethylamine, (adjusted to pH 4.9 with conc.HCl)/ Acetonitrile (90:10) (Helium Sparging)
Flow Rate:	1.5 ml/min
Temperature:	30°C
Detection:	
Working Electrode:	Glassy Carbon
Reference Electrode:	Ag/AgC1(3 M KCl)
Auxiliary Electrode:	Cell Body
Applied Potential:	700 mV
Morphine Standard:	800 pg in column/ 150 mM (20 µl injection vol)

References

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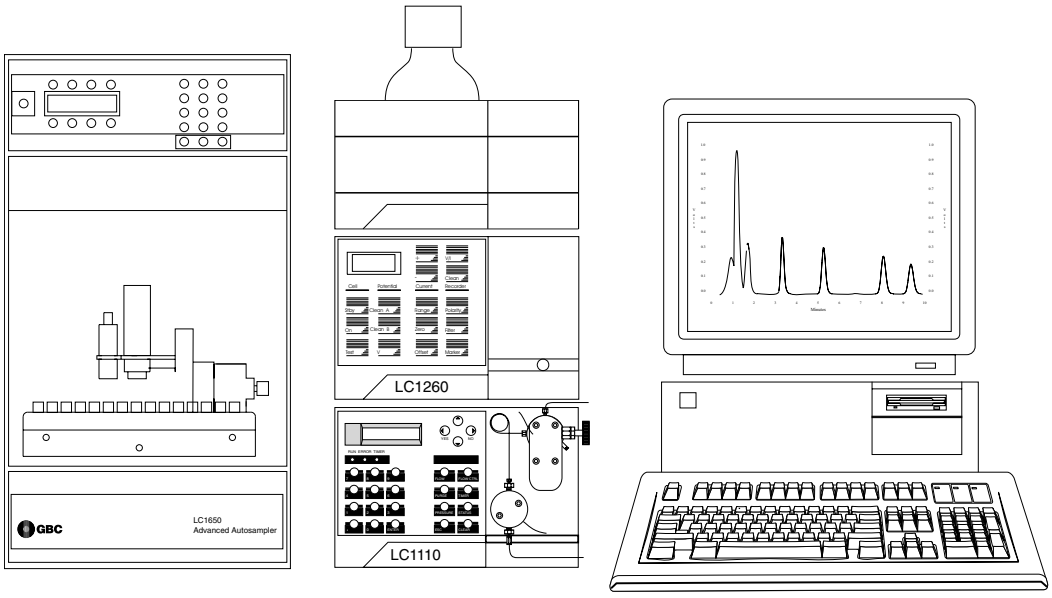

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GBC publication number 01-0381-00
September, 1995

HPLC

application note

Determination of Morphine Using Glassy Carbon Electrode (3 mm) with ppb Sensitivity

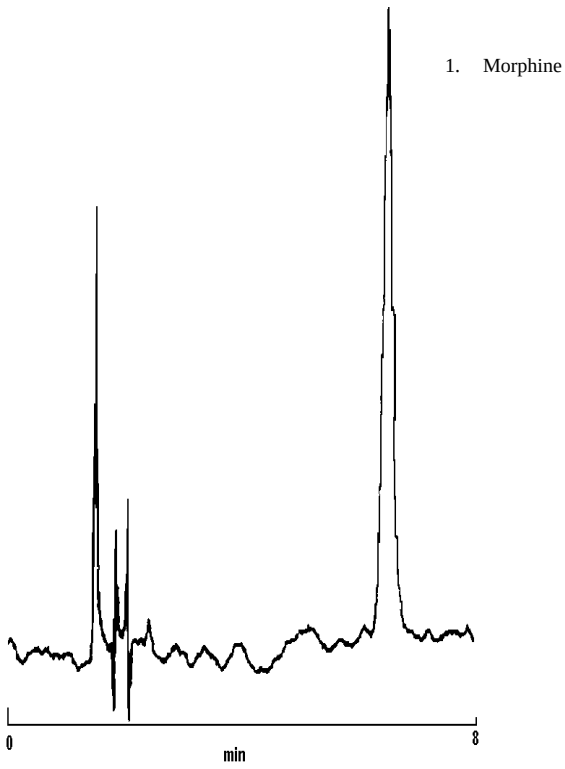

Figure 1 *Standard (Overspike) Analysis (40 ppb)*

Conditions

Column:	Sperhisorb OD S2 (C18), 5 µM Column (250 x 4.6 mm ID)
Mobile Phase:	0.2 M Sodium Perchlorate, 5 mM Sodium Citrate, 3 mM Triethylamine, (pH 4.9) (90%) and Acetonitrile (10%)
Flow Rate :	1.5 ml/min
Injection Vol.:	20 µl
Temperature:	30°C
Electrode:	3 mm Glassy Carbon
Potential:	700 mV (Ag/AgCl)

GBC HPLC Instrumentation

LC1110 Dual Piston HPLC Pump
LC1260 Electrochemical Detector
GBC Column Heater
LC1650 Advanced Autosampler
WinChrom Chromatography Data Management System
LC1445 System Organiser

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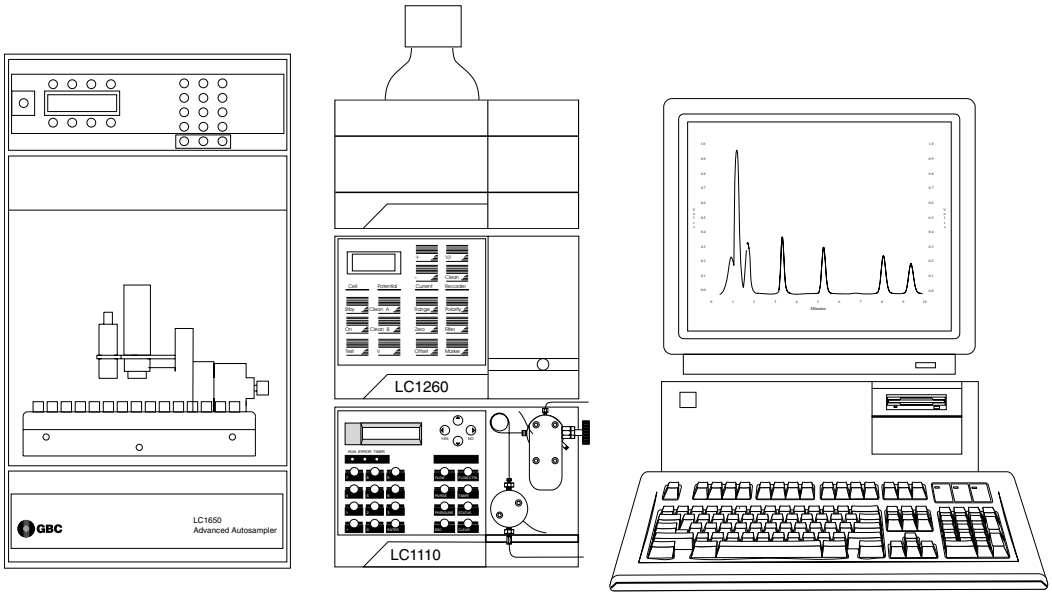

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HPLC application note

Sulpha Drugs

'...the separation of seven sulphonamide drugs under isocratic conditions with UV detection is shown...'

The antimicrobial drugs known as the sulphonamides, or sulpha drugs, were the first of the 'wonder drugs'. In the 1930's, chemists found that by appropriate substitution of the sulphanilamide molecule, a series of compounds was obtained each of which had slightly different antibacterial properties. The relative potencies of the sulphanamides used systematically appear to depend mainly on differences in solubility, absorption and excretion as a result of such substitution, rather than on any specificity. The mode of action of the sulphonamides is bacteriostatic rather than bacteriocidal, and is considered to be due to the similarity of their chemical structures to p-aminobenzoic acid, a substance that is essential for the synthesis of folic acid by bacterial cells. Without folic acid the bacterial cell cannot divide and is halted in metaphase (Reference 1).

Sulphonamides act on many species of bacteria, including streptococci and many gram-negative rods such as Escherichia coli and Proteus spp. Resistance to sulpha drugs has appeared rapidy in many strains and their main use now is against organisms causing urinary tract infections and in certain veterinary applications.

Keywords:
Sulphadiazine, sulphadimidine, sulphaguanidine, sulphamethoxydiazine, sulphanilamide, sulphapyridine, sulphathiazole

In the latter case, it is possible for sulphonamide residues to enter the food chain, especially via meat and dairy products. Sulphonamides are contra-indicated in the presence of renal or hepatic disease and in hypersensitive patients. Futhermore, because sulphonamides are secreted in milk, they should be used with caution in nursing mothers owing to the danger of developing permanent brain damage (kernicterus) in premature and newborn babies.

HPLC is the method of choice for trace level analysis of sulpha drug residues in a variety of samples. In Figure 1, the separation of seven sulphonamide drugs under isocratic conditions with UV detection is shown.

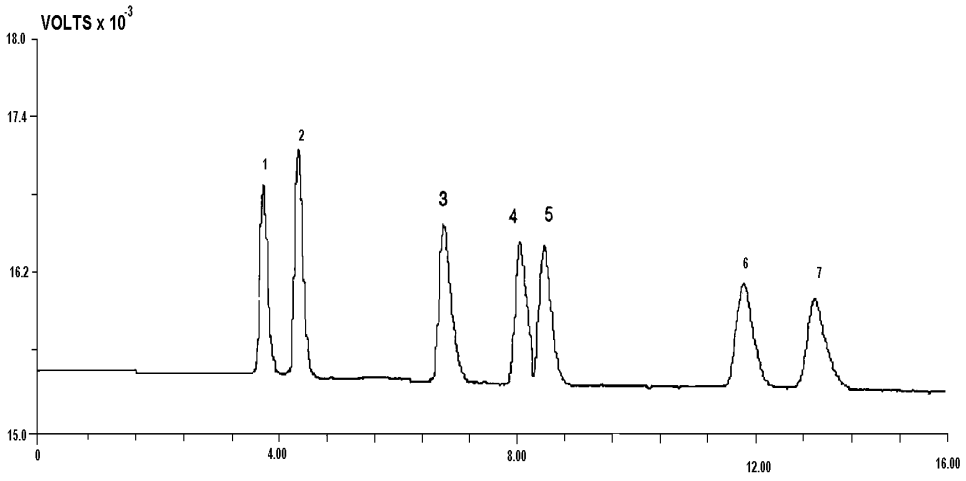

Figure 1 *Separation of Sulphonamide Standards*

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GBC HPLC Instrumentation

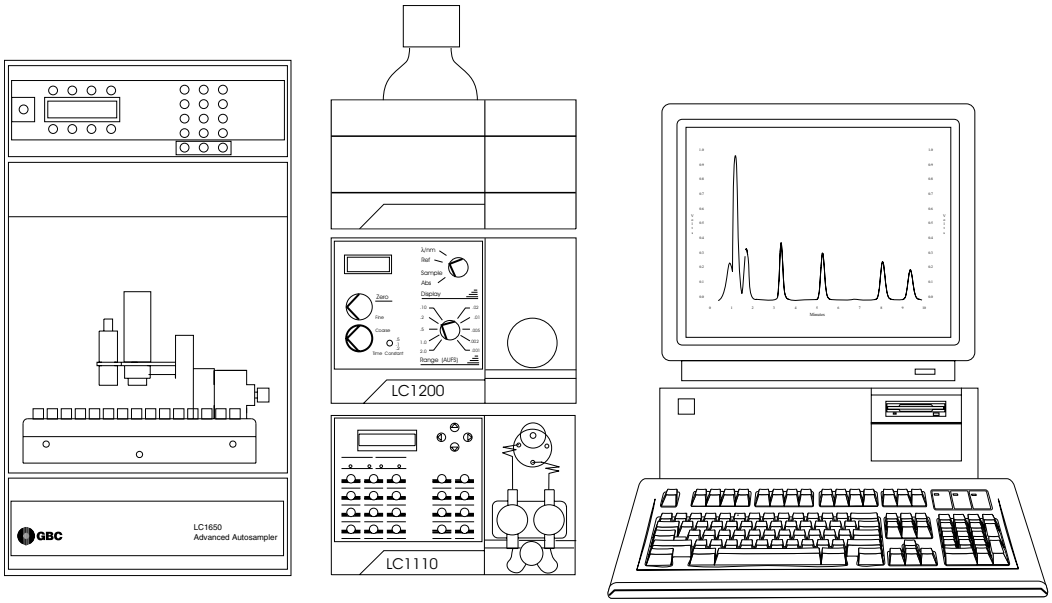
LC1110 Dual Piston HPLC Pump
LC1200 Variable Wavelength UV/Vis Detector
LC1650 Advanced Autosampler
WinChrom Chromatography Data Management System
LC1445 System Organiser

Conditions

Column:	Spherisorb S5 C8, 250 mm x 4.6 mm ID
Guard:	50 mm x 4.6 mm ID
Mobile Phase:	Acetonitrile:Water (17.5:82.5)
Flow Rate:	1 ml/min
Detection:	UV at 270 nm

Reference

1. 'The Pharmaceutical Codex' (11th Edition, 1979), p.867.



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