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LABORATORIES

HPLC Post-Column Derivatization

Method Abstracts

Amino Acids / Glyphosate / Supplements / Contaminants & Residues / Antibiotics / Pharmaceuticals / Mycotoxins / Cannabis & Hemp / Sugars & Sweeteners

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Analysis of Amino Acids

30-minute Amino Acids Analysis of Hydrolyzed Samples Hydrolyzed Samples (MA-380)

Pickering Laboratories specializes in the manufacturing of cation-exchange columns and eluants for Amino Acids analysis. No other technique has been shown to match the reproducibility and selectivity of ion-exchange analysis with post-column Ninhydrin detection. Nor is there a chromatography technique that provides as much information; the 570/440 nm signal ratio for each amino acid is a constant and so offers information about peak purity.

Onyx PCX post-column derivatization system provides a unique opportunity to combine eluant gradient capabilities of modern HPLC instruments with column temperature gradients. As might be expected, this capability also reduces the analysis time.

We introduce an accelerated Amino Acids analysis method for hydrolyzed samples that utilizes temperature and eluant gradients.

Method

Analytical Conditions

Column: High-efficiency Sodium cation-exchange column, 4.6 x 110 mm, Catalog Number 1154110T

Guard: Cation-exchange GARD™, Catalog Number 1700-3102

Flow Rate: 0.6 mL/min

Mobile Phase: See method

Post-Column Conditions

Post-Column System: Onyx PCX or Pinnacle PCX

Reactor Volume: 0.5 mL

Temperature: 130 ˚C

Reagent: Trione Ninhydrin reagent

Flow Rate: 0.3 mL/min

Detection: UV/VIS 570 nm for primary amino acids
440 nm for secondary amino acids

Method for Oxidized Feeds Hydrolysate Samples				
Time	**Na270 %**	**Na425 %**	**Na640 %**	**RG011 %**
0	100	0	0	0
4.0	100	0	0	0
15.0	0	100	0	0
16.0	0	0	100	0
31.0	0	0	100	0
31.1	0	0	0	100
33.0	0	0	0	100
33.1	100	0	0	0
40.0	100	0	0	0

Column Oven Program	
Time	**Temp °C**
0	55
12	55
17	70
32	70
33	55

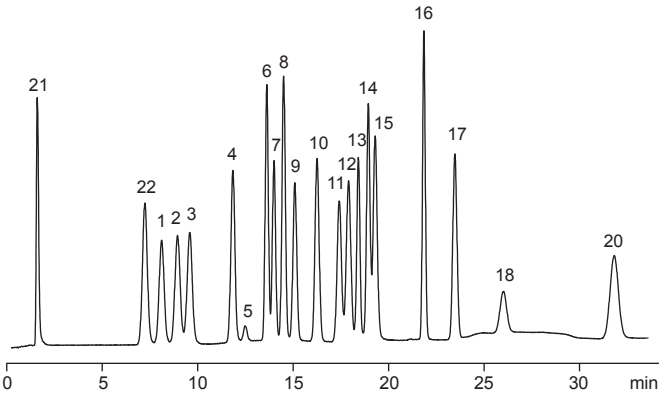


Fig 1. Chromatogram of oxidized feeds hydrolysate standard

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

Method for Protein Hydrolysate Samples				
Time	**Na315 %**	**Na425 %**	**Na640 %**	**RG011 %**
0	100	0	0	0
4.0	100	0	0	0
15.0	0	100	0	0
16.0	0	0	100	0
31.0	0	0	100	0
31.1	0	0	0	100
33.0	0	0	0	100
33.1	100	0	0	0
40	100	0	0	0

Column Oven Program	
Time	**Temp °C**
0	46
4	46
9	70
32	70
33	46

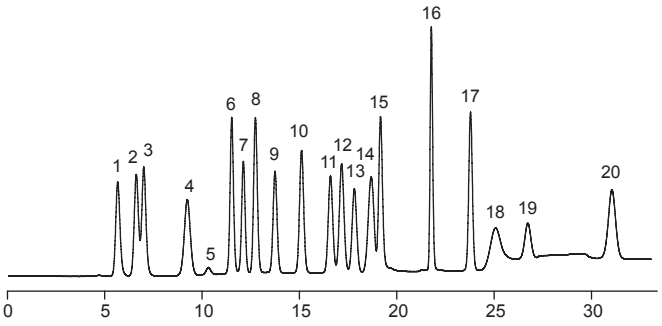


Fig 2. *Chromatogram of protein hydrolysate standard*

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

Amino Acids According to European Pharmacopeia (MA-391)

The **European Pharmacopoeia (Ph. Eur.)** defines requirements for the qualitative and quantitative composition of medicines, as well as the tests to be carried out on medicines and on substances and materials used in their production.

It covers active substances, excipients and preparations of chemical, animal, human or herbal origin, homoeopathic preparations and homoeopathic stocks, antibiotics, as well as dosage forms and containers. It also includes tests on biologicals, blood and plasma derivatives, vaccines and radiopharmaceutical preparations. The European Pharmacopoeia and its requirements are legally binding in the member states of the European Pharmacopoeia Convention and the European Union.

All manufacturers of medicines or substances for pharmaceutical use therefore must apply the Ph. Eur. quality standards in order to be able to market and use these products in Europe.

Amino Acids analysis can be used for:

- Identification tests on biopharmaceutical active ingredients (e.g. peptides, proteins) by means of amino acids composition analysis;
- Impurities and related substances determination on APIs (Active Pharmaceutical Ingredients, e.g. free amino acids) and intermediates;
- Single or total amino acids quantification in drug products, including markers determination in complex matrixes (e. g. phytopharmaceuticals).

The following Ph. Eur. monographs have already officially introduced the Amino Acids analysis method with post-column Ninhydrin derivatization as the analytical procedure required for the determination of the Ninhydrin-positive substances, and additional papers are expected to be published in upcoming months:

- Cysteine HCl Monohydrate 01/2014:0895
- Isoleucine 07/2013:0770
- Leucine 07/2013:0771
- Lysine HCl 07/2013:0930
- Serine 01/2014:0788
- Proline 01/2014:0785
- Threonine 01/2014:1049
- Valine 01/2014:0796
- Arginine 07/2014:0806

Pickering Laboratories, Inc. offers a complete solution for Amino Acids Analysis according to European Pharmacopoeia. This includes the Onyx PCX post-column derivatization instrument, analytical columns and GARD, buffers and Trione® Ninhydrin reagent. The Onyx PCX is capable of performing column temperature gradients that allow easily modified conditions and improved run times and amino acids separations. The methods presented in this application note were optimized to comply with system suitability requirements of Pharmacopoeia methods.

Each Pharmacopoeia monograph describes the preparation of the test and reference solutions specific for each amino acid. The solutions are used for calculations of percentage contents, impurity levels as well as parameters of system suitability. Resolution of 1.5 is required between Leucine and Isoleucine peaks.

Table 1 summarizes the solutions used in each monograph.

Table 1. *Reference guide for Pharmacopoeia methods*

		Reference Solutions, ug/mL												
Amino acids	**Test solutions ug/mL**	**Cys**	**CSSC**	**Lys**	**Ser**	**Thr**	**Val**		**Arg**	**Leu**	**Ile**	**Ile and Leu**	**Pro**	**NH4**
Valine	600						1.2				1.2	3 each	1.2	0.12
Proline	600							0.6				3 each	1.2	0.12
Leucine	600 and 24									1.2	0.12	3 each	1.2	0.12
Threonine	600					1.2						3 each	1.2	0.12
Serine	600				1.2							3 each	1.2	0.12
Lysine	600			1.2								3 each	1.2	0.12
Isoleucine	600						1.2			1.2	1.2	3 each	1.2	0.12
Arginine	600							1.2	1.2			3 each	1.2	0.12
Cysteine	600	1.2	1.2									3 each	1.2	0.12

Cys = Cysteine, CSSC = Cystine, Lys = Lysine, Ser = Serine, Thr = Threonine, Val = Valine, Ala = Alanine, Arg = Arginine, Ile = Isoleucine, Leu = Leucine, Pro = Proline, NH4 = ammonia

For all amino acids, except Cysteine, Sodium-based and Lithium-based methods are available. For Cysteine analysis, only Lithium-based methods are suitable. Sodium-based methods have shorter run times and are preferable for all amino acids except Cysteine.

Methods using High-efficiency Sodium column for analysis of following Amino Acids:

Valine, Proline, Leucine, Isoleucine, Serine, Threonine, Lysine, Arginine

Analytical Conditions

Column: High-efficiency Sodium cation-exchange column, 4.6 x 110 mm, Catalog Number 1154110T

Guard: Cation-exchange GARD™, Catalog Number 1700-3102

Flow Rate: 0.6 mL/min

Mobile Phase: See method in Table 2

Injection Volume: 50 uL

Post-Column Conditions

Post-Column System: Onyx PCX or Pinnacle PCX

Reactor Volume: 0.5 mL

Reagent: Trione®

Reagent Temperature: 130 °C

Column Temperature: See method in Table 3

Flow Rate: 0.3 mL/min

Detection: UV/VIS 570 nm for primary amino acids, 440 nm for secondary amino acids

Table 2. *HPLC program for column 1154110T*

Time	Na315 %	Na425 %	Na640 %	RG011 %
0	100	0	0	0
4	100	0	0	0
23	10	90	0	0
24	0	0	100	0
42	0	0	100	0
42.1	0	0	0	100
45	0	0	0	100
45.1	100	0	0	0
55	100	0	0	0

Table 3. *Column oven program*

Time	Temp °C
0	42
4	42
12	60
23	60
35	70
43	70
44	42

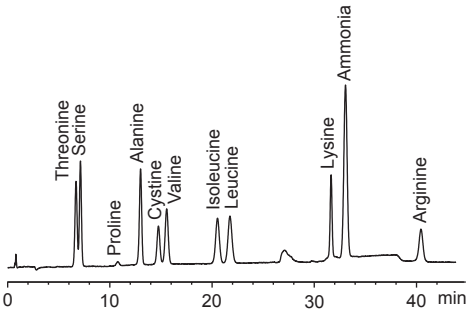


Fig 1. *Sodium chromatogram of amino acids analyzed using Pharmacopeia methods (3 ug/mL each, 50 uL injection).*

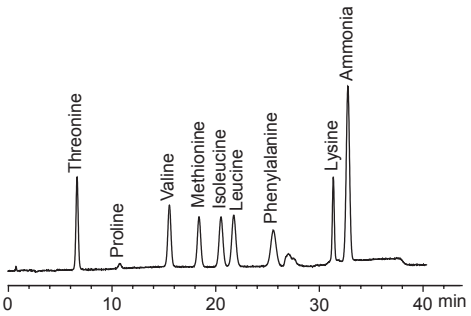


Fig 2. *Sodium chromatogram of alternative amino acids analyzed using Pharmacopeia methods (3 ug/mL each, 50 uL injection).*

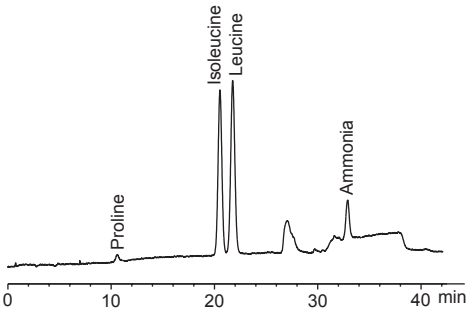


Fig 3. *Sodium chromatogram of amino acids used for calculations and system suitability check in Pharmacopeia methods (refer to Table 1). Proline – 1.2 ug/mL; Isoleucine – 3 ug/mL; Leucine – 3 ug/mL; Ammonia – 0.12 ug/mL. Injection volume – 50 uL.*

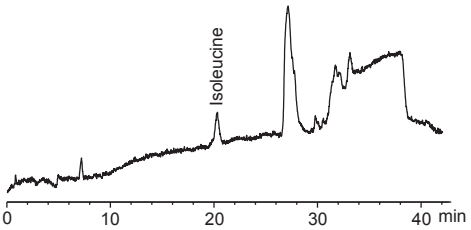


Fig 4. *Sodium chromatogram of low level Isoleucine reference solution – 0.12 ug/mL. Injection volume 50 uL*

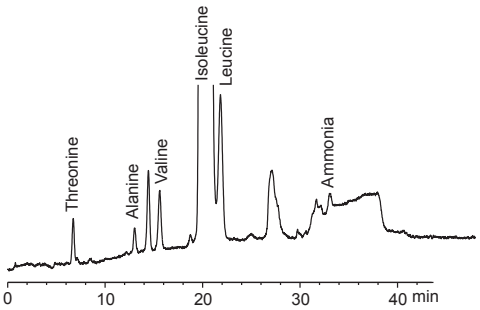


Fig 5. *Sodium chromatogram of Isoleucine test solution 600 ug/mL (zoomed). Injection volume 50 uL*

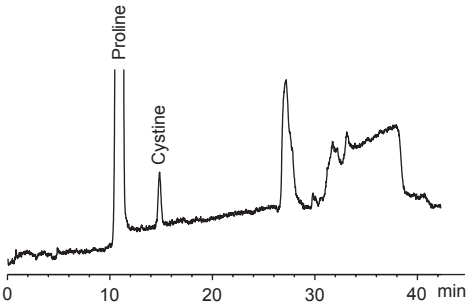


Fig 6. *Sodium chromatogram of Proline test solution 600 ug/mL (zoomed). Injection volume 50 uL*

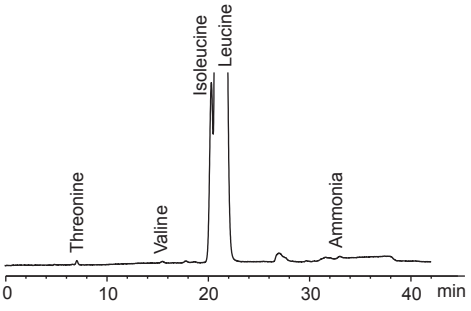


Fig 7. *Sodium chromatogram of Leucine test solution 600 ug/mL (zoomed). Injection volume 50 uL*

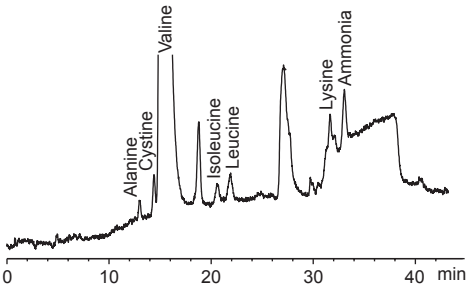


Fig 8. *Sodium chromatogram of Valine test solution 600 ug/mL (zoomed). Injection volume 50 uL*

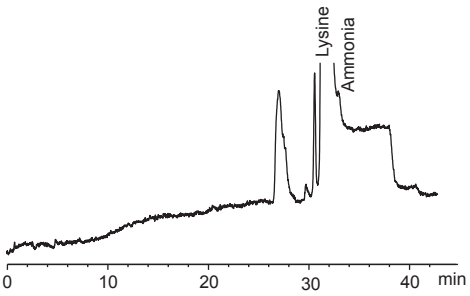


Fig 9. *Sodium chromatogram of Lysine test solution 600 ug/mL (zoomed). Injection volume 50 uL*

Due to the poor separation of Cysteine and Proline peaks with the Sodium-based method, the Lithium High-efficiency column needs to be used for Cysteine analysis according to Pharmacopeia. The same Lithium method can be used for all other amino acids, though analysis time is longer than with the Sodium method.

Methods using High-efficiency Lithium column for analysis of following amino acids:

Cysteine, Valine, Proline, Leucine, Isoleucine, Serine, Threonine, Lysine, Arginine

Analytical Conditions

Column: High-efficiency Lithium cation-exchange column, 4.6 x 75 mm, Catalog Number 0354675T

Guard: Cation-exchange GARD™, Catalog Number 1700-3102

Flow Rate: 0.55 mL/min

Mobile Phase: See method in Table 4

Injection Volume: 50 uL

Post-Column Conditions

Post-Column System: Onyx PCX or Pinnacle PCX

Reactor Volume: 0.5 mL

Reagent: Trione®

Reagent Temperature: 130 °C

Column Temperature: See method in Table 5

Flow Rate: 0.3 mL/min

Detection: UV/VIS 570 nm for primary amino acids, 440 nm for secondary amino acids

Table 4. *HPLC Program for column 0354675T*

Time	1700-1125 %	Li365 %	Li375 %	RG003 %
0	100	0	0	0
15	100	0	0	0
35	40	60	0	0
38	0	100	0	0
43	0	100	0	0
43.1	0	0	100	0
57	0	0	100	0
57.1	0	0	0	100
60	0	0	0	100
60.1	100	0	0	0
72	100	0	0	0

Table 5. *Column oven program*

Time	Temp °C
0	34
6	34
30	45
32	70
59	70
60	34

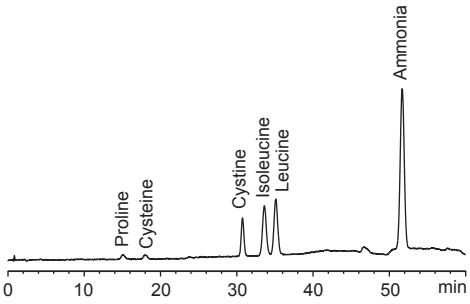


Fig 10. *Lithium chromatogram of amino acids used as reference solutions for Cysteine analysis (3 ug/mL each, 50 uL injection).*

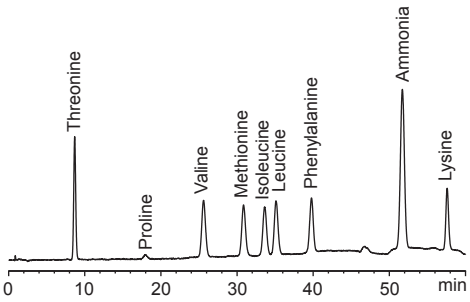


Fig 11. *Lithium chromatogram of amino acids analyzed using Pharmacopeia methods (3 ug/mL each, 50 uL injection).*

To make it easier to start using Pickering Laboratories methods, we offer chemistry kits that include: analytical column, GARD, buffers, reagents and analytical standards for Amino Acids analysis. All items in the kit could be ordered individually if needed. Please contact Pickering Laboratories if you have any questions regarding this application.

Part No.	Description
0352-0057	**30-Minute High Efficiency Protein Hydrolysate Kit (for Sodium methods):**
1154110T	30-Minute Sodium Cation-exchange Column 4.6 x 110 mm & 1700-0070 amino acid test mixture
1700-3102	Cation-exchange GARD™ assembly: Holder w/ 2 replaceable GARD™
Na220L	Sodium Diluent, pH 2.20, pH 2.20, 950 mL
Na315	Sodium Eluant, pH 3.15, 4 x 950 mL
Na425	Sodium Eluant, pH 4.25
Na640	Sodium Eluant, pH 6.40
RG011	Sodium Column Regenerant, 950 mL
T100C	TRIONE® Ninhydrin Reagent, 4 x 950 mL (4-month shelf life)
012506H	Sodium Calibration Standard, for protein hydrolysate, 0.25 µmole/mL, 5 mL
0352-0058	**Kit Identical to 0352-0057 with T200 replacing T100C:**
T200	TRIONE® Two-part Ninhydrin Reagent, prepares 4 x 900 mL (12-month shelf life)
0352-0006	**70-minute Physiologic Fluid/Native Sample Kit (for Lithium methods):**
0354675T	Lithium Ion-exchange Column, 4.6 x 75 mm (with test mixture 1700-0070)
1700-3102	Cation-exchange GARD™ assembly: Holder w/ 2 replaceable GARD™
1700-1125	Lithium Eluant, pH 2.80, 4 x 950 mL
Li220	Lithium Diluent, pH 2.20, 4 x 250 mL
Li365	Lithium Diluent, pH 3.65, 4 x 950 mL
Li375	Lithium Diluent, pH 3.75, 4 x 950 mL
RG003	Lithium Column Regenerant, 950 mL
1700-0170	Lithium Calibration Standard, without Norleucine and AGPA, 0.25 µmole/mL, 5 mL
SP100	SERAPREP™, 250 mL
UP100	URIPREP™, 250 mL
T100C	TRIONE® Ninhydrin Reagent, 4 x 950 mL (3-month shelf life)
0352-0007	**Kit Identical to 0352-0006 with T200 replacing T100C:**
T200	TRIONE® Two-part Ninhydrin Reagent, prepares 4 x 900 mL (12-month shelf life)

Pickering Laboratories will keep updating its Pharmacopeia 8.0 methods as new monographs are released. Please contact support@pickeringlabs.com for the latest methods and chromatograms.

Amino Acids Analysis of Cell Culture Media (MA-371)

Cell cultures are widely used to produce biopharmaceuticals and other biologically active compounds. The composition of the cell culture media affects the yield and structure of the desired products and must be carefully optimized. Cell culture media is typically composed of mixtures of amino acids, vitamins, carbohydrates, inorganic salts as well as different peptides, proteins and other compounds. As the cells grow, they consume nutrients and release target biopharmaceuticals as well as waste products.

Amino acids serve as the building blocks of proteins, as well as intermediates in many metabolic pathways. Amino acids are typically added to cell culture media to provide nutritional requirements for the cells. Monitoring and adjusting amino acids composition is an essential part of optimizing the manufacturing process to ensure high quality and optimum yield of the final product.

Amino Acids analysis using cation-exchange chromatography with post-column Ninhydrin derivatization allows for easy determination of amino acids concentrations in many complex matrices, including cell culture media. The post-column method is very sensitive, reproducible and rugged. It has been and continues to be a method of choice for laboratories running biological samples, protein, peptides and foods analysis. Most chemical compounds present in the media do not interfere with analysis, so the majority of samples only need diluting with citric buffer and filtering before analysis. If serum is added to the media, then the proteins need to be precipitated using either Seraprep™ solution or ultrafiltration.

Pickering Laboratories, Inc. offers the complete solution for Amino Acids analysis, including post-column derivatization instruments, columns, eluants, reagents and standards. The Onyx PCX derivatization system has a programmable column oven to allow for shorter run times and easy method optimization.

Method

Analytical Conditions

Column: High-efficiency Lithium cation-exchange column, 4.6 x 75 mm, Catalog Number 0354675T

Guard: Cation-exchange GARD™, Catalog Number 1700-3102

Flow Rate: 0.55 mL/min

Mobile Phase: See method in Table 1

Injection Volume: 10-50 uL

Post-Column Conditions

Post-Column System: Onyx PCX or Pinnacle PCX

Reactor Volume: 0.5 mL

Reactor Temperature: 130 °C

Flow Rate: 0.3 mL/min

Detection: UV/VIS 570 nm for primary amino acids, 440 nm for secondary amino acids

Table 1. HPLC Program

Time	1700-1125 %	Li365 %	Li375 %	RG003 %
0	100	0	0	0
10	100	0	0	0
19.0	40	60	0	0
32.0	0	100	0	0
43.0	0	100	0	0
43.1	0	0	100	0
57.0	0	0	100	0
57.1	0	0	70	30
72.0	0	0	70	30
72.1	100	0	0	0
84.0	100	0	0	0

Table 2. Column Oven Program

Time	Temp °C
0	34
6	34
17	65
25	70
70	70
71	34

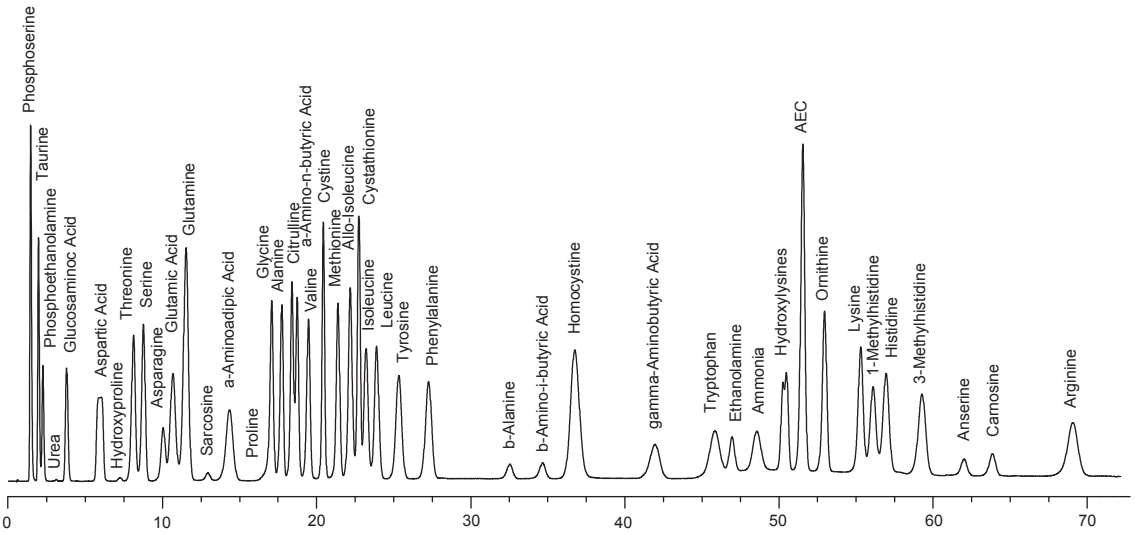


Fig 1. *Chromatogram of Amino Acids standard*

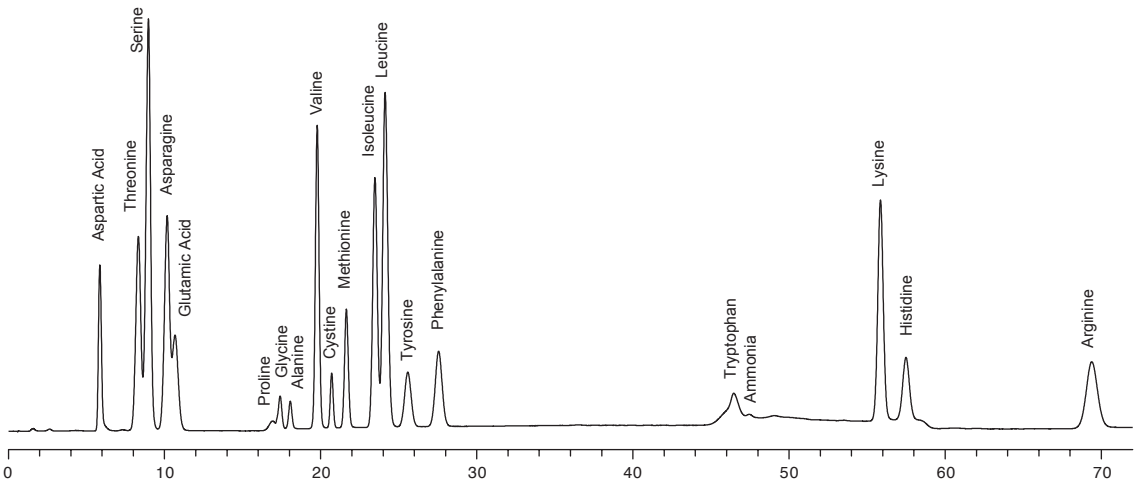


Fig 2. *Chromatogram of cell culture media sample*

Amino Acids Analysis of Monoclonal Antibodies (MA-373)

The peptide and protein based pharmaceuticals are a rapidly expanding class of therapeutical agents that are used to treat a wide variety of health conditions, including cancer, metabolic and auto-immune diseases, HIV and more. Biologic drugs, such as monoclonal antibodies, are derived from living organisms and are usually very expensive. As many biologics are coming off of patents, the market is ready for cost-saving biogenerics. But all proteins, including monoclonal antibodies, have complex structures that determine their function. Differences in structure would alter biological activity leading to changes in safety and efficacy of the drug.

ICH Q6B is a guidance document that provides a set of internationally accepted specifications for biotechnological and biological products to support new marketing applications. It establishes the set of criteria to which a drug substance, drug product or material should conform to be considered acceptable for intended use.

Determining amino acid composition following hydrolysis is listed in ICH Q6B as a way to characterize the protein and to confirm its identity by comparing with Amino Acid composition deduced from the gene sequence of the desired product. Amino Acids analysis data is also used to accurately determine the protein content.

The Amino Acids analysis with post-column derivatization is a very sensitive, reproducible and rugged method and it has been a preferred approach for laboratories running biological samples, protein, peptides and foods analysis. Pickering Laboratories Inc. offers many Amino Acids analysis products including post-column derivatization instruments, columns, eluants, reagents and standards. All products are designed to work together to deliver optimum results for any chosen sample.

Method

Analytical Conditions

Column: High-efficiency Sodium cation-exchange column, 4.6 x 110 mm, Catalog Number 1154110T

Guard: Cation-exchange GARD™, Catalog Number 1700-3102

Flow Rate: 0.6 mL/min

Mobile Phase: See method in Table 1

Injection Volume: 10-50 uL

Post-Column Conditions

Post-Column System: Onyx PCX or Pinnacle PCX

Reactor Volume: 0.5 mL

Reactor Temperature: 130 °C

Flow Rate: 0.3 mL/min

Detection: UV/VIS 570 nm for primary amino acids, 440 nm for secondary amino acids

Table 1. HPLC Program

Time	Na315 %	Na425 %	Na640 %	RG011 %
0	100	0	0	0
4.0	100	0	0	0
15.0	0	100	0	0
16.0	0	0	100	0
31.0	0	0	100	0
31.1	0	0	0	100
33.0	0	0	0	100
33.1	100	0	0	0
40.0	100	0	0	0

Table 2. Column Oven Program

Time	Temp °C
0	46
4	46
9	70
32	70
33	46

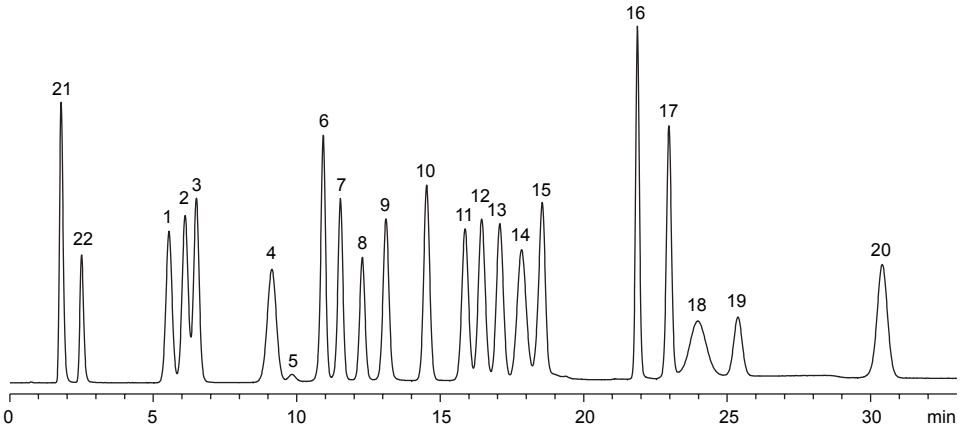


Fig 1. *Chromatogram of Amino Acids standard*

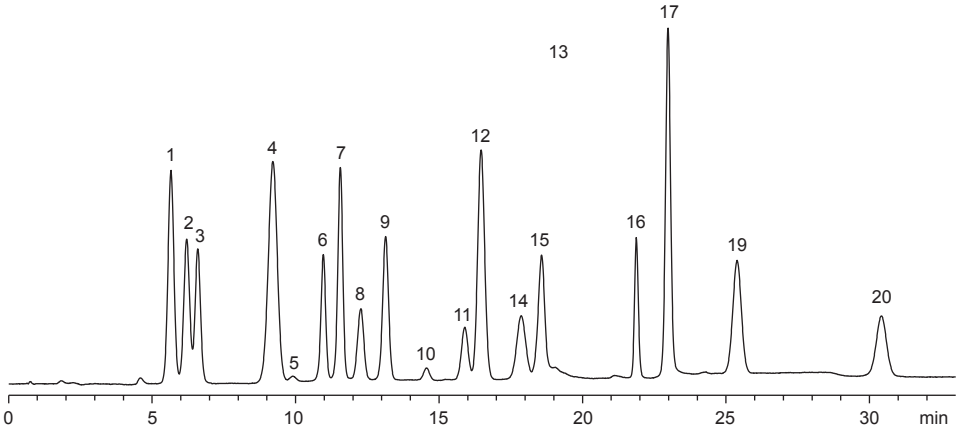


Fig 2. *Chromatogram of hydrolyzed sample of IL-17F monoclonal antibody*

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

Acknowledgements

We would like to thank Frank N. Konstantinides and AminoAcids.com for their contribution to this project.

Amino Acids in Oxidized and Unoxidized Feed Samples (MA-374)

Commission Regulation (EC) No 152-2009 published in Official Journal of European Union laid down the methods of sampling and analysis for the official control of feed. The Regulation describes methods of analysis to control the composition of feed materials and compound feed products. Establishing the amino acids profile is an important way to control quality and nutritional value of feeds. This regulation specifies HPLC with post-column derivatization with Ninhydrin reagent as the method of analysis for total and free amino acids. Pickering Laboratories developed the analytical method to comply with all the chromatographic requirements of Commission Regulation (EC) No 152-2009. The same method is used to analyze oxidized and unoxidized feed samples.

Method

Analytical Conditions

Column: High-efficiency Sodium cation-exchange column, 4.0 x 150 mm, Catalog Number 1154150T

Guard: Cation-exchange GARD™, Catalog Number 1700-3102

Flow Rate: 0.4 mL/min

Mobile Phase: Na270, Na740, RG011. See method in Table 1

Injection Volume: 10 uL

Post-Column Conditions

Post-Column System: Onyx PCX or Pinnacle PCX

Reactor Volume: 0.5 mL

Reagent: Trione®

Column Temperature: See Method in Table 2

Reactor Temperature: 130 °C

Flow Rate: 0.25 mL/min

Detection: UV/VIS 570 nm for primary amino acids, 440 nm for secondary amino acidsCalibration

Table 1. HPLC Program

Time	Na270 %	Na740 %	RG011 %
0	100	0	0
16	100	0	0
40	54	46	0
45	0	100	0
66	0	100	0
66.1	0	0	100
70	0	0	100
70.1	100	0	0
80	100	0	0

Table 2. Column Oven Program

Time	Temp °C
0	55
32	55
33	65
41	65
42	55

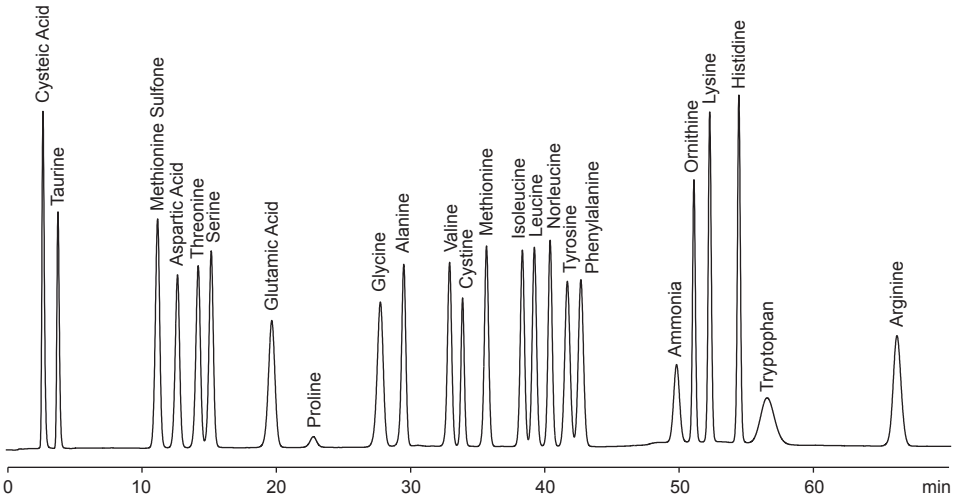


Fig 1. *Chromatogram of a standard solution of amino acids*

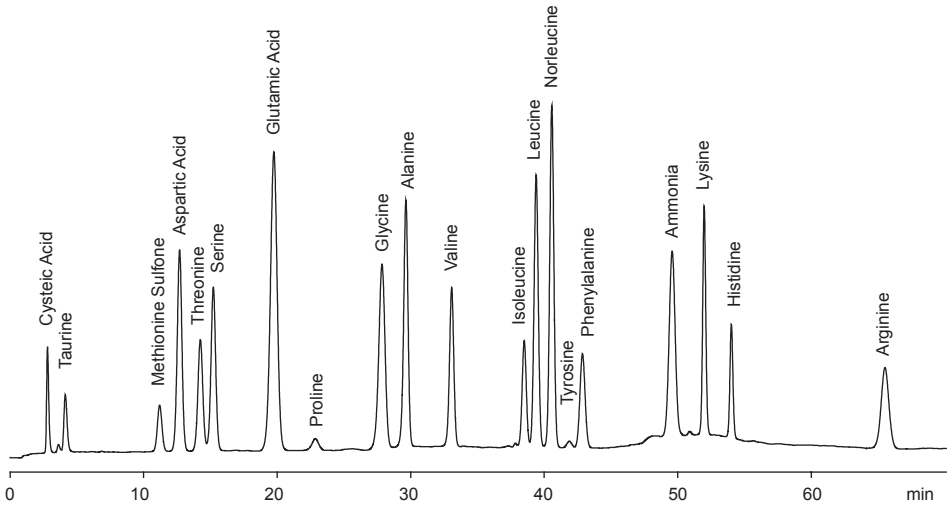


Fig 2. *Chromatogram of an oxidized feed sample*

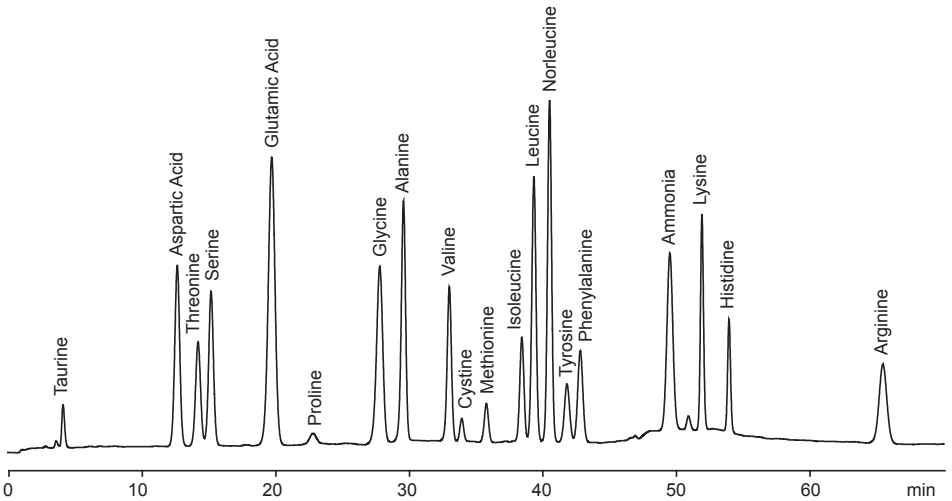


Fig 3. *Chromatogram of a non-oxidized feed sample*

Amino Acids in Supplements (MA-393)

Amino acids are building blocks of proteins and essential amino acids need to be obtained from diet as they cannot be synthesized by our bodies. Amino acids supplements are popular among athletes and bodybuilders and are sold in the form of pills, powders and drinks. They usually also contain vitamins, electrolytes, herbs, flavorings and other additives.

Analysis of these supplements is important to confirm the label claims and to maintain industry standards.

Amino Acids analysis with post-column derivatization is a very sensitive, reproducible and rugged method and it has been a preferred approach for laboratories running biological samples, protein, peptides and foods analysis. Pickering Laboratories' products for Amino Acids analysis include post-column derivatization instruments, columns, eluants, reagents and standards. All products are designed to work together to deliver optimum results for any chosen sample.

The amino acids present in the product determine if Sodium or Lithium columns and buffers need to be used. Lithium columns have longer run time but have higher selectivity and are able to separate more amino acids than Sodium columns. Pickering Laboratories will help you to identify the best method for your Amino Acids analysis.

Sample Preparation

Grind 10 pills to fine powder before taking a sample for analysis. Mix all the powdered supplements thoroughly. Sample size depends on concentrations of amino acids present.

To finely ground sample (0.05 g – 0.2 g) add 100 mL of Na220L. Mix well and sonicate for 10 min. Filter through 0.45 um filter and inject.

Method

Analytical Conditions

Column: High-efficiency Sodium cation-exchange column, 4.6 x 110 mm, Catalog Number 1154110T

Guard: Cation-exchange GARD™, Catalog Number 1700-3102

Flow Rate: 0.6 mL/min

Mobile Phase: See method in Table 1

Injection Volume: 10-50 uL

Post-Column Conditions

Post-Column System: Onyx PCX or Pinnacle PCX

Reactor Volume: 0.5 mL

Reactor Temperature: 130 °C

Column Temperature: See method in Table 2

Flow Rate: 0.3 mL/min

Table 1. HPLC Program for Column 1154110T

Time	Na315 %	Na425 %	Na640 %	RG011 %
0	100	0	0	0
4.0	100	0	0	0
15.0	0	100	0	0
16.0	0	0	100	0
31.0	0	0	100	0
31.1	0	0	0	100
33.0	0	0	0	100
33.1	100	0	0	0
40	100	0	0	0

Table 2. Column Oven Program

Time	Temp °C
0	46
4	46
9	70
32	70
33	46

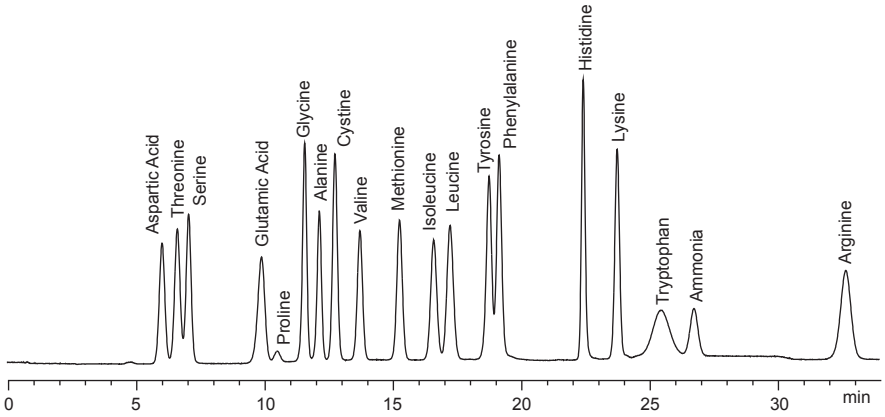


Fig 1. *Chromatogram of amino acids standard using sodium column 1154110T*

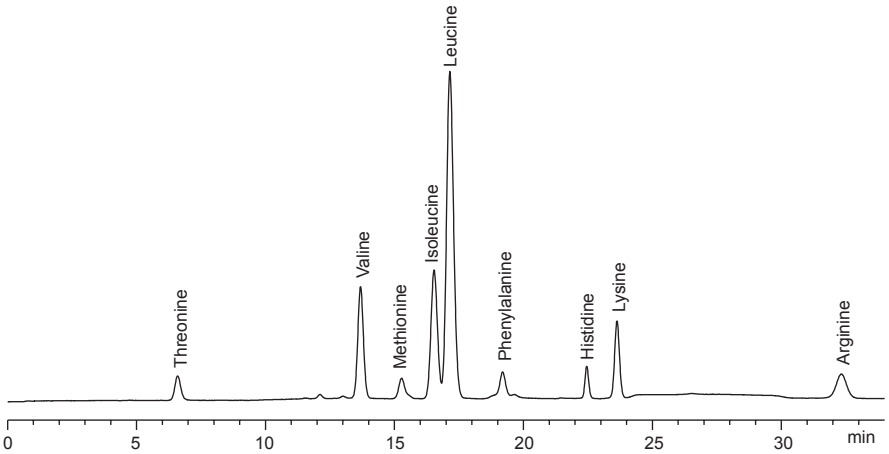


Fig 2. *Chromatogram of a sample of commercial training supplement using sodium column 1154110T*

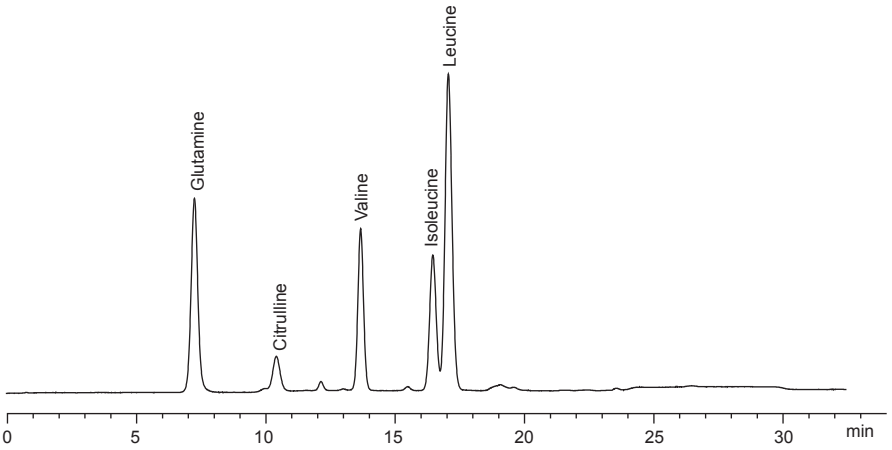


Fig 3. *Chromatogram of the a sample of sports recovery drink using sodium column 1154110T*

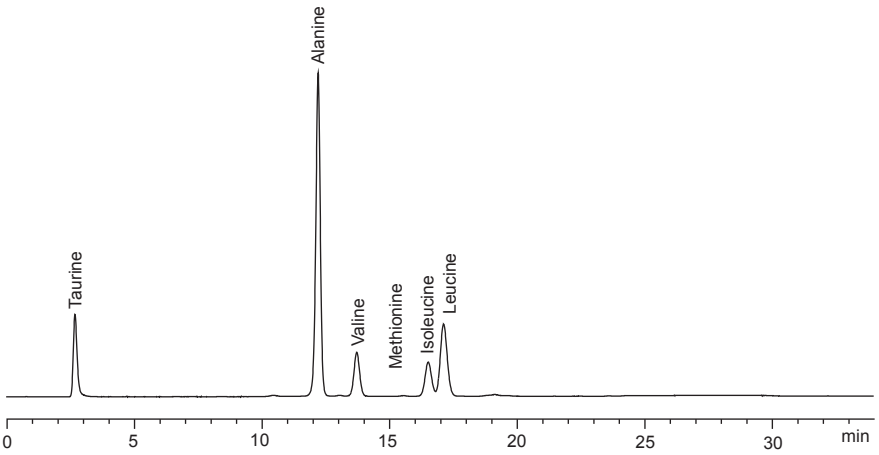


Fig 4. *Chromatogram of a commercial sports performance drink using sodium column 1154110T*

Method for Lithium Column

Analytical Conditions

Column: High-efficiency Lithium cation-exchange column, 4.6 x 75 mm, Catalog Number 0354675T

Guard: Cation-exchange GARD™, Catalog Number 1700-3102

Flow Rate: 0.55 mL/min

Mobile Phase: See method in Table 3

Injection Volume: 50 uL

Post-Column Conditions

Post-Column System: Onyx PCX or Pinnacle PCX

Reactor Volume: 0.5 mL

Reagent: Trione®

Reactor Temperature: 130 °C

Column Temperature: See method in Table 4

Flow Rate: 0.3 mL/min

Detection: UV/VIS 570 nm for primary amino acids, 440 nm for secondary amino acids

Table 3. HPLC Program for Column 0354675T

Time	1700-1125 %	Li365 %	Li375 %	RG003 %
0	100	0	0	0
15	100	0	0	0
35	40	60	0	0
38	0	100	0	0
43	0	100	0	0
43.1	0	0	100	0
57	0	0	100	0
57.1	0	0	0	100
60	0	0	0	100
60.1	100	0	0	0
72	100	0	0	0

Table 4. Column Oven Program

Time	Temp °C
0	34
6	34
30	45
32	70
59	70
60	34

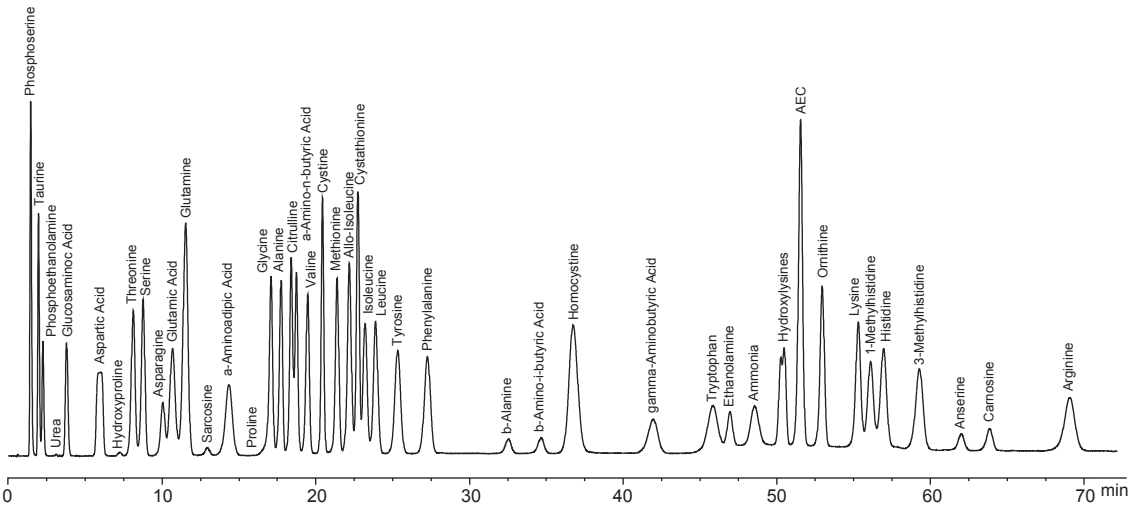


Fig 5. *Chromatogram of amino acids standard using lithium column 0354675T*

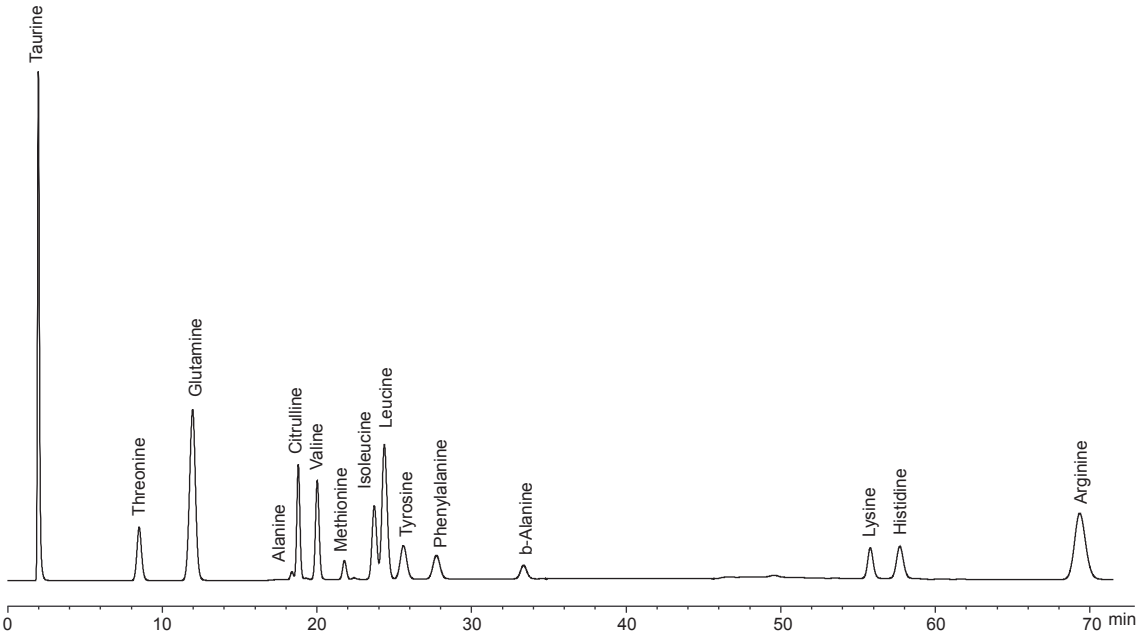


Fig 6. *Chromatogram of a pre-workout amino acids supplement drink using lithium column 0354675T*

Amino Acids in Dietary Ingredients and Supplements (MA-397)

Supplements containing Amino Acids are widely used as part of the treatment of a number of medical conditions, including ALS, some brain conditions caused by liver disease, muscle degeneration and movement disorders. Amino Acids supplements are also used by athletes to improve performance and prevent muscle breakdown. These supplements are available in variety of forms and dosages, and they often contain other ingredients like vitamins, minerals, herbal extracts and flavorings.

Manufacturers are responsible for supporting label claims. The testing of dietary ingredients as well as finished products for active components is an important part of any quality assurance program.

Analysis of amino acids using cation-exchange columns and Ninhydrin post-column reagent is a well-establish methodology that is recommended by European Pharmacopeia. Pickering Laboratories developed and validated a post-column method for amino acids analysis of supplements that is sensitive, selective and can be used without modification to analyze capsules, tablets, drinks and other samples. Accelerated methods are available for samples with a limited number of amino acids.

Method

Analytical Conditions

Column: High-efficiency Lithium cation-exchange column, 4.6 x 75 mm (Catalog Number 0354675T)

Guard: Cation-exchange GARD™, Catalog Number 1700-3102

Flow Rate: 0.55 mL/min

Mobile Phase: See method in Table 1

Post-Column Conditions

Post-Column System: Onyx PCX or Pinnacle PCX

Reactor Volume: 0.5 mL

Reactor Temperature: 130 °C

Flow Rate: 0.3 mL/min

Detection: UV/VIS 570 nm for primary amino acids, 440 nm for secondary amino acids

Injection Volume: 10–50 µL

Sample Preparation

Place 100 mg of homogenized sample into a 25 mL volumetric flask. Bring to volume with Li220 Diluent. Sonicate for 30 min and filter through a glass microfiber filter if the solution is cloudy. Dilute the sample to fit within calibration curve as necessary. More than one dilution may be needed if concentrations of amino acids differ by more than 10 times. Filter the final solution though 0.45 um Nylon filter before injecting.

Table 1. HPLC Program

Time	1700-1125 %	Li365 %	Li375 %	RG003 %
0	100	0	0	0
10	100	0	0	0
19	40	60	0	0
32	0	100	0	0
43	0	100	0	0
43.1	0	0	100	0
57	0	0	100	0
57.1	0	0	70	30
72	0	0	70	30
72.1	100	0	0	0

Table 2. Column Oven Program

Time	Temp °C
0	34
6	34
17	65
25	70
70	70
71	34

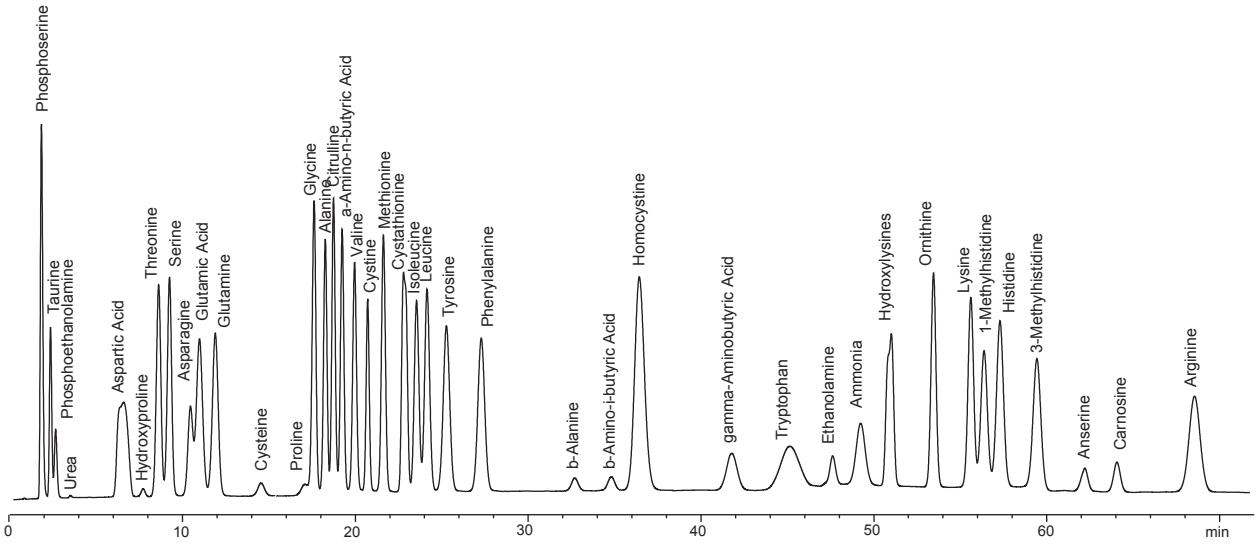


Fig 1. *Amino Acids Standard*

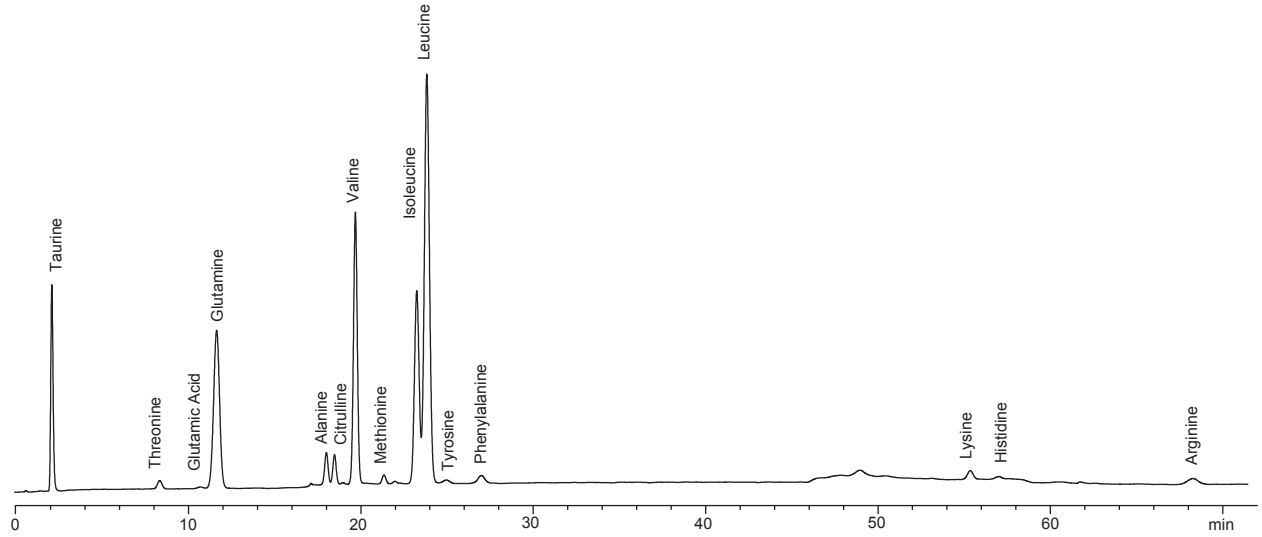


Fig 2. *Chromatogram of powdered drink mix*

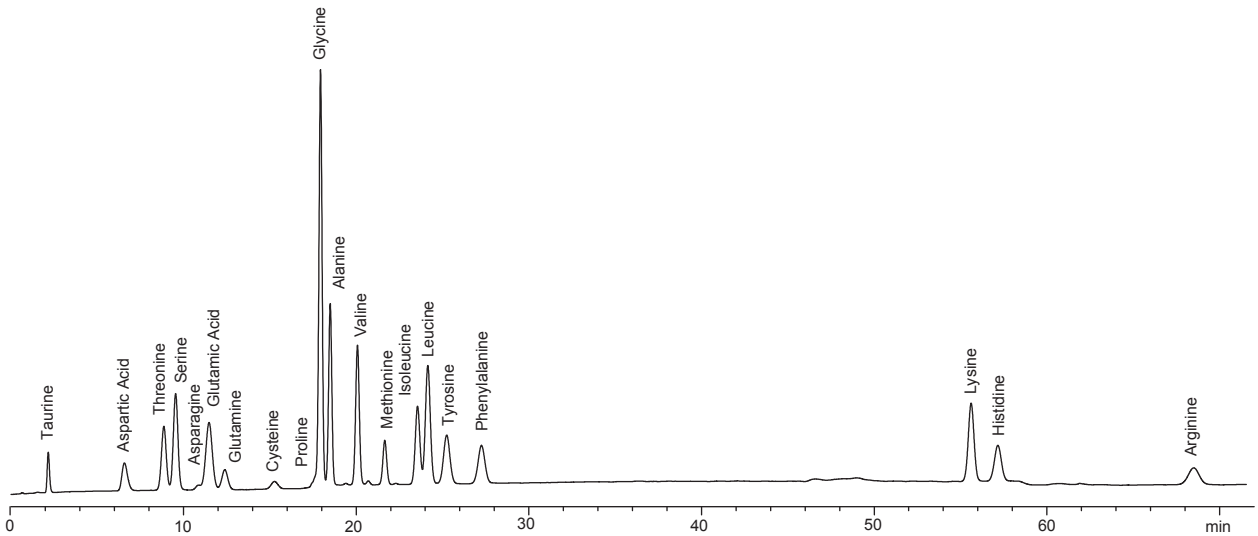


Fig 3. *Chromatogram of encapsulated amino acids supplement*

PKU and MSUD Screening Methods (MA-385)

Phenylketonurea (PKU) and Maple Syrup Urine Disease (MSUD) are amino acid disorders – rare inherited conditions that prevent people from digesting certain amino acids in food proteins. Untreated, these serious conditions lead to mental and physical disability and even death. PKU and MSUD are part of newborn screening program and are detected and monitored through Amino Acids analysis of blood samples.

Pickering Laboratories offers full range of products for post-column Amino Acids analysis of physiological fluids. The same column and buffers can be used for running full amino acid profile as well as accelerated screening methods for specific medical conditions. This application note describes PKU and MSUD screening methods for Pickering 0354100T and 0354675T columns.

PKU MSUD Screening Method for Column 0354100T

Analytical Conditions

Column: High-efficiency Lithium cation-exchange column, 4x100 mm, Catalog Number 0354100T

Guard: Cation-exchange GARD™, Catalog Number 1700-3102

Flow Rate: 0.4 mL/min

Mobile Phase: 1700-1125, Li265, Li375, RG003

Column Oven Program	
Time	Temp °C
0	50
20	61
30	61
30.1	50

HPLC Program				
Time	1700-1125 %	Li365 %	Li375 %	RG003 %
0	70	30	0	0
20	0	100	0	0
30	0	0	100	0
30.1	0	0	0	100
33	0	0	0	100
33.1	70	30	0	0

Post-Column Conditions

Post-Column System: Onyx PCX or Pinnacle PCX

Reactor Volume: 0.5 mL

Reactor Temperature: 130 °C

Reagent: Trione Ninhydrin reagent

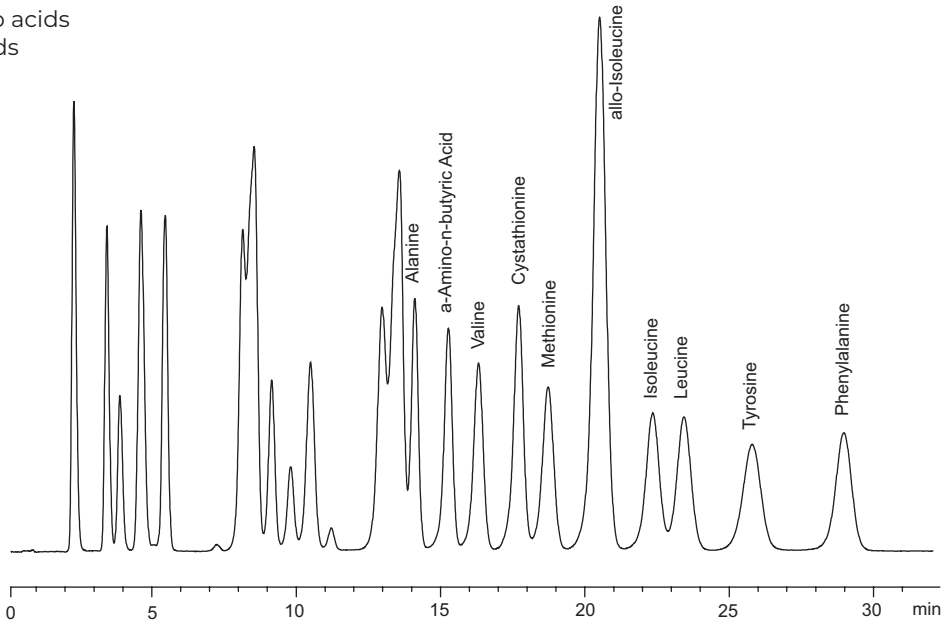
Reagent Flow Rate: 0.3 mL/min

Detection: UV/VIS 570 nm for primary amino acids
440 nm for secondary amino acids

Run Time: 33 min

Equilibration Time: 10 min

Contact Pickering Laboratories for information about full amino acid profile method for this column.



PKU MSUD Screening Method for Column 0354675T

Analytical Conditions

Column: High-efficiency Lithium cation-exchange column, 4.6x75 mm, Catalog Number 0354675T

Guard: Cation-exchange GARD™, Catalog Number 1700-3102

Flow Rate: 0.55 mL/min

Mobile Phase: 1700-1125, Li265, Li375, RG003

Post-Column Conditions

Post-Column System: Onyx PCX or Pinnacle PCX

Reactor Volume: 0.5 mL

Reactor Temperature: 130 ˚C

Reagent: Trione Ninhydrin reagent

Reagent Flow Rate: 0.5 mL/min

Detection: UV/VIS 570 nm for primary amino acids
440 nm for secondary amino acids

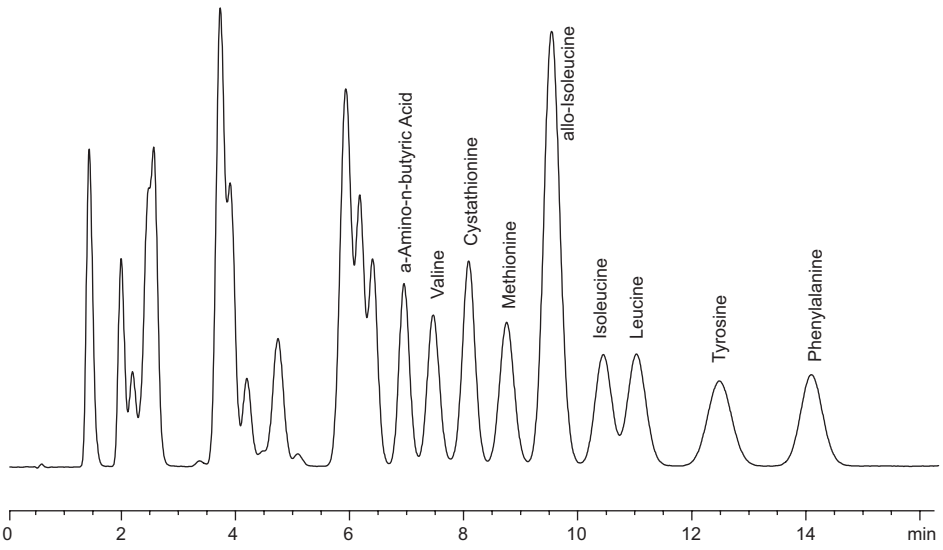
Run Time: 16 min

Equilibration Time: 10 min

Contact Pickering Laboratories for information about full amino acid profile method for this column.

Column Oven Program	
Time	**Temp °C**
0	50
14	61
15	50
30.1	50

HPLC Program				
Time	**1700-1125 %**	**Li365 %**	**Li375 %**	**RG003 %**
0	60	40	0	0
9	0	100	0	0
14	0	30	70	0
14.1	0	0	0	100
16	0	0	0	100
16.1	60	40	0	0



70-min Amino Acids Analysis of Physiological Samples (MA-382)

Pickering Laboratories specializes in the manufacturing of cation-exchange columns and eluants for Amino Acids analysis. Post-column derivatization with Ninhydrin offers unmatched selectivity and reproducibility of the analysis of the most challenging matrices.

Onyx PCX post-column derivatization system allows analysts to combine eluant gradient capabilities of modern HPLC instruments with column temperature gradients. We introduce the new accelerated Amino Acids analysis method for physiological samples that utilizes temperature and eluant gradients.

Method

Analytical Conditions

Column: High-efficiency Lithium cation-exchange column, 4.6 x 75 mm, 5 µm
Catalog Number 0354675T

Flow Rate: 0.55 mL/min

Mobile Phase: 1700-1125, Li365, Li375, RG003

Post-Column Conditions

Post-Column System: Onyx PCX or Pinnacle PCX

Reactor Volume: 0.5 mL

Temperature: 130 ˚C

Reagent: Trione

Flow Rate: 0.5 mL/min

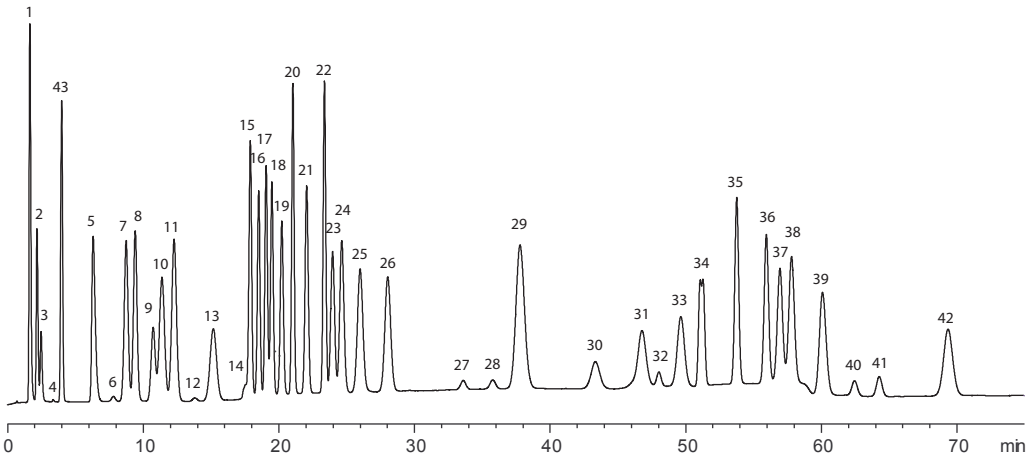
Detection: UV/VIS 570 nm for primary amino acids
440 nm for secondary amino acids

Runtime: 72 min

Equilibration Time: 15 min

Column Oven Program	
Time	**Temp °C**
0	34
6	34
17	65
25	70
70	70
71	34

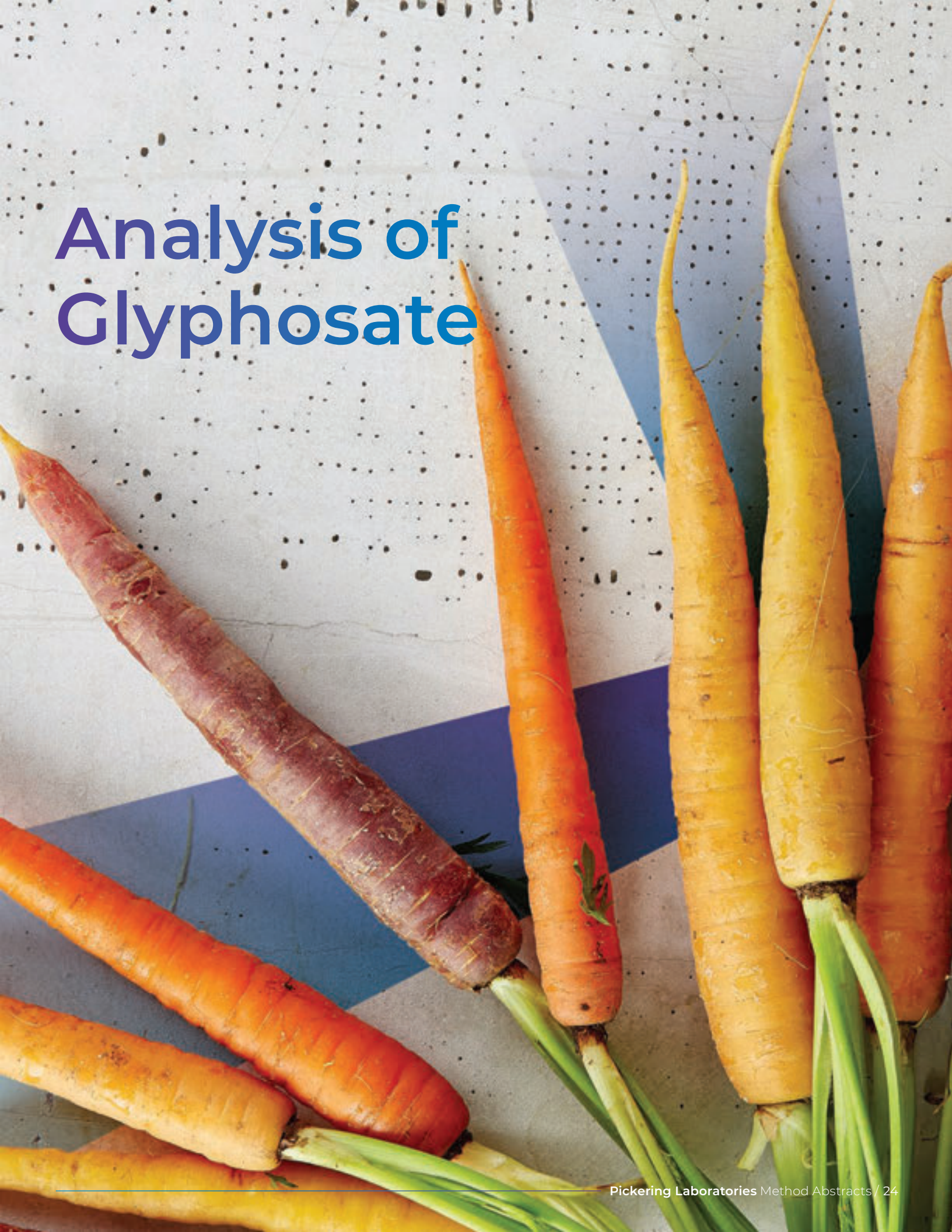
HPLC Program				
Time	**1700-1125 %**	**Li365 %**	**Li375 %**	**RG003 %**
0	100	0	0	0
10	100	0	0	0
19	40	60	0	0
32	0	100	0	0
43	0	100	0	0
43.1	0	0	100	0
57	0	0	100	0
57.1	0	0	70	30
72	0	0	70	30
72.1	100	0	0	0



1 Phosphoserine
2 Taurine
3 Phosphoethanolamine
4 Urea
5 Aspartic acid
6 Hydroxyproline
7 Threonine
8 Serine
9 Asparagine
10 Glutamic acid
11 Glutamine
12 Sarcosine
13 α-Aminoadipic acid
14 Proline
15 Glycine
16 Alanine
17 Citrulline
18 α-Amino-n-butyric acid
19 Valine
20 Cystine
21 Methionine
22 Cystathionine
23 Isoleucine
24 Leucine
25 Tyrosine
26 Phenylalanine
27 β-Alanine
28 β-Amino-i-butyric acid
29 Homocystine
30 γ-Aminobutyric acid
31 Tryptophan
32 Ethanolamine
33 Ammonia
34 Hydroxylysines
35 Ornitine
36 Lysine
37 1-Methylhistidine
38 Histidine
39 3-Methylhistidine
40 Anserine
41 Carnosine
42 Arginine
43 Glucosaminic Acid*

*Internal Standard

Analysis of Glyphosate



Analysis of Glufosinate and Glyphosate in Water by HPLC With Post-Column Derivatization (MA-296)

Glyphosate is a broad-spectrum herbicide that has been used for several decades to protect a variety of crops. Due to its heavy use in agriculture many weeds have developed resistance to Glyphosate causing increased interest in finding alternative herbicides. Glufosinate also provides broad-spectrum weed control and can be successfully used against many Glyphosate-resistant plants and grasses. Glufosinate has become increasingly popular creating a need for analytical methods to monitor food and water to ensure protection of human health and environment.

EPA mandates maximum contamination level (MCL) for Glyphosate in water at 700 ug/L. EPA Method 547 describes Glyphosate analysis in drinking water by direct-injection HPLC with post-column derivatization. Glufosinate contains a primary amino group and is also capable of reacting with o-Phthalaldehyde (OPA) reagent. Utilizing mixed-mode analytical column allowed us to develop a simple method capable of separating Glufosinate and Glyphosate in water with direct injection. This method eliminates complicated and labor-intensive sample pretreatment steps, required by LC/MS analysis. Post-column derivatization with OPA reagent ensures high sensitivity of analysis without matrix interferences or signal suppression.

Method

Calibration

Calibration range for Glyphosate and Glufosinate is from 25 ug/L to 1000 ug/L. A quadratic calibration curve with R^2=0.9998 is observed for Glufosinate. A linear calibration curve with R^2=0.9998 is observed for Glyphosate.

Sample Preparation

Filter water samples though 0.45 um Nylon filter.

Analytical Conditions

Column: Acclaim Mixed-Mode WAX-1, 4.6x150 mm (Thermo Scentific)

Column Temperature: 55 °C

Flow Rate: 1.0 mL/min

Mobile Phase: 85% of K600, 15% of ACN

Injection Volume: 100 uL

Post-Column Conditions

Post-Column System: Onyx PCX, Pinnacle PCX or Vector PCX

Heated Reactor Volume: 0.5 mL

Temperature: 36 °C

Ambient Reactor: 0.1 mL

Reagent 1: 90 uL of 5% NaOCl (Bleach) in 950 mL of GA116 Diluent

Reagent 2: 100 mg of OPA and 2 g of Thiofluor in 950 mL of GA104 Diluent

Reagent Flow Rate: 0.3 mL/min each reagent

Detection: λ_{EX}:330 nm, λ_{EM}:465 nm

Table 1. Glufosinate and Glyphosate Analysis in water

Analyte	Surface Water		Bottled Water	
	Glufosinate	Glyphosate	Glufosinate	Glyphosate
Spike	100 ug/L	100 ug/L	100 ug/L	100 ug/L
RSD, N=3	3.7%	1.0%	4.2%	0.8%
Recoveries	96%	94%	85%	92%
Spike	300 ug/L	300 ug/L	300 ug/L	300 ug/L
RSD, N=3	2.5%	0.6%	2.1%	0.5%
Recoveries	87%	93%	87%	92%

Conclusion

This simple, fast and sensitive method allows simultaneous detection of Glufosinate and Glyphosate in drinking and surface waters. The method utilizes common HPLC equipment and doesn't require complex extraction and derivatization of the samples prior to injection. Avoiding sample pretreatment steps reduces analysis time and costs as well as minimizes errors. This promising protocol can be easily adopted by laboratories, especially the ones already set up to run Glyphosate analysis according to EPA Method 547.

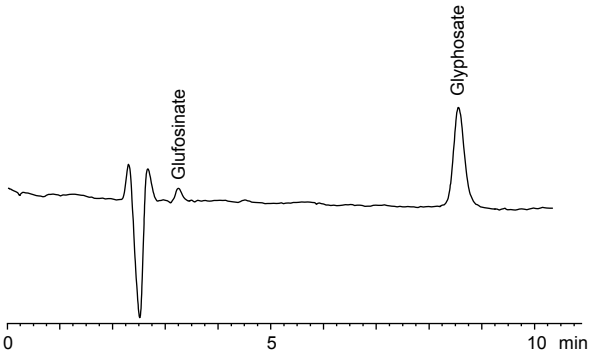


Fig 1. *Chromatogram of 50 ug/L calibration standard of Glufosinate and Glyphosate*

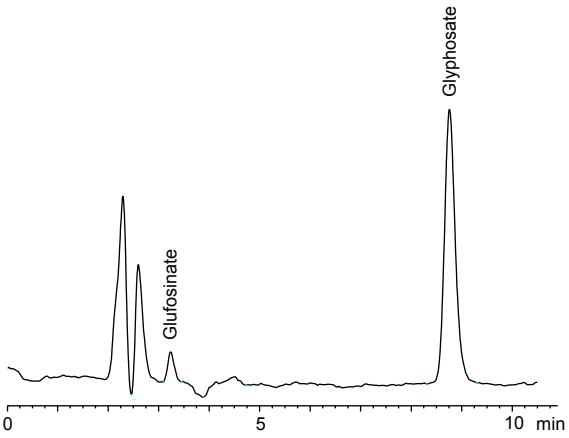


Fig 3. *Chromatogram of surface water spiked with Glufosinate and Glyphosate at 100 ug/L*

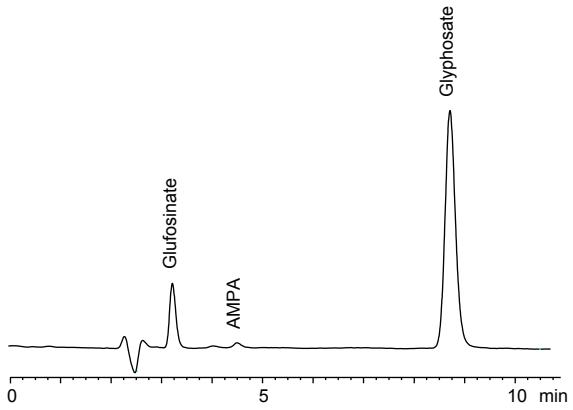


Fig 2. *Chromatogram of 500 ug/L calibration standard of Glufosinate, Glyphosate and AMPA*

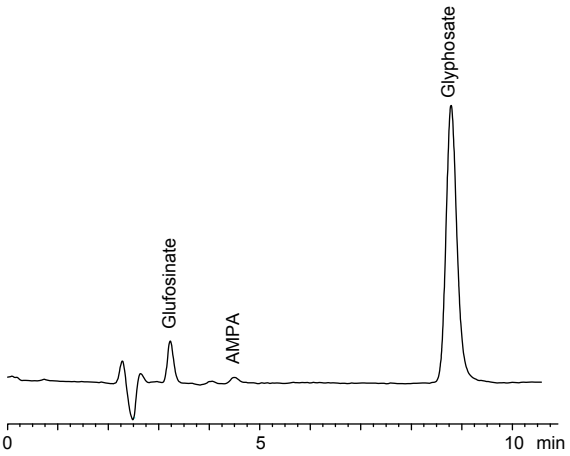


Fig 4. *Chromatogram of bottled water spiked with Glufosinate, Glyphosate and AMPA at 300 ug/L*

Glyphosate Analysis in Foods by HPLC With Post-Column Derivatization and Fluorescence Detection (MA-211)

Glyphosate, the active ingredient in Roundup®, is the most widely used herbicide in the world. In the United States, the Environmental Protection Agency (EPA) regulates Glyphosate and sets the maximum amount of herbicide allowed to be present in assorted crops as well as drinking water. Recent research, however, has raised concerns about Glyphosate safety and its prevalence in the environment. In response to the evidence of increased human exposure to this herbicide, the Food and Drug Administration (FDA) announced it will begin testing Glyphosate in foods including soybeans, corn, milk, and eggs.

Pickering Laboratories has over 35 years of experience manufacturing and selling instruments and reagents for Glyphosate analysis in accordance to the US-EPA Method 547 for Glyphosate Analysis in Drinking Water as well as the AOAC Method 991.08 for Glyphosate Analysis in Environmental Waters. These HPLC methods are based on post-column derivatization technology with florescence detection. The AOAC Official Method 2000.05 for Analysis of Glyphosate in Crops describes an easy clean-up procedure using cation-exchange cartridges that was successfully combined with Pickering Laboratories' post-column derivatization for analysis of Glyphosate in crops such as soy, corn, alfalfa, and sunflower seeds as well as vegetables such as tomatoes and broccoli (1, 2). The downside of this sample preparation technique is the fairly high volume of the water-based solution used to elute Glyphosate from the clean-up cartridge and consequently long evaporation times.

The presented post-column derivatization method for Glyphosate analysis utilizes an accelerated sample preparation procedure to quickly and efficiently analyze Glyphosate in a wide range of foods. The analytical method is sensitive and selective, and it can be easily implemented in any laboratory.

Method

Analytical Conditions

Column: Cation-exchange Column for Glyphosate analysis, 4 x 150 mm, Catalog Number 1954150

Guard: Cation-exchange GARD™, Catalog Number 1700-3102

Column Temperature: 55 °C

Flow Rate: 0.4 mL/min

Mobile Phase: K200, RG019

Injection Volume: 100 uL

HPLC Gradient		
Time	**K200 %**	**RG019 %**
0	100	0
15	100	0
15.1	0	100
17	0	100
17.1	100	0
25	100	0

Post-Column Conditions

Post-Column System: Onyx PCX, Pinnacle PCX or Vector PCX

Heated Reactor Volume: 0.5 mL

Temperature: 36 °C

Ambient Reactor: 0.1 mL

Reagent 1: 100 uL of 5% NaOCl (Bleach) in 950 mL of GA116 Diluent

Reagent 2: 100 mg of OPA and 2 g of Thiofluor in 950 mL of GA104 Diluent

Reagent Flow Rate: 0.3 mL/min each reagent

Detection: λ_{ex} : 330 nm, λ_{em} : 465 nm

Supplies for Sample Preparation

Methylene Chloride, HPLC Grade

Methanol, HPLC Grade

Elution Solution (90% Methanol – 10% 1N HCl)

RESTORE™ solution (P/N 1700-0140)

SAX cartridges, 500 mg, 6 mL (Agilent Bond Elut, P/N 12102144)

Sample Preparation

Extraction

To 25 g of homogenized sample, add enough water (after estimating moisture content) such that the total volume of water is 125 mL. Blend at high speed for 3-5 min and centrifuge for 10 min. Transfer 20 mL of the aqueous extract into a centrifuge tube and add 15 mL of Methylene Chloride. Shake for 2-3 min and centrifuge for 10 min. Use the upper layer for SPE clean-up.

Matrix-Specific Modifications

1) For samples that absorb large amounts of water, reduce test portion to 12.5 g while keeping water volume the same.
2) For samples with high fat content, do the Methylene Chloride partitioning twice.

SPE Cleanup

Condition the SAX cartridge with 5 mL of Methanol followed by 5 mL of D.I. water draining the liquid to the top of the sorbent bed. Transfer 1 mL of extract and elute to the top of the sorbent bed. Wash with 5 mL of Methanol. Elute Glyphosate with 5 mL of Elution Solution. Evaporate to dryness at 55 °C. Dissolve the residue in 1.5 mL of a solution of 10% RESTORE™ in water, filter through a 0.45 um syringe filter and inject onto the HPLC column.

Recoveries for Glyphosate							
Matrix	**EPA Tolerances**	**Spike Level 1**	**Recoveries**	**RSDr**	**Spike Level 2**	**Recoveries**	**RSDr**
Rolled Oats	20 ug/g	0.1 ug/g	85%	1.6%	0.2 ug/g	87%	4.9%
Wheat Flour	NA	0.1 ug/g	88%	4.6%	0.2 ug/g	87%	1.3%
Whole Milk	NA	0.025 ug/g	99%	3.9%	0.05 ug/g	92%	2.9%
Eggs	0.05 ug/g	0.025 ug/g	75%	2.8%	0.05 ug/g	77%	5.1%
Soybeans	20 ug/g	0.1 ug/g	77%	6.7%	0.2 ug/g	74%	5.5%
Corn	5 ug/g	0.1 ug/g	94%	3.7%	0.2 ug/g	99%	3.6%
Beer	NA	0.025 ug/g	78%	6.9%	0.05 ug/g	73%	3.6%

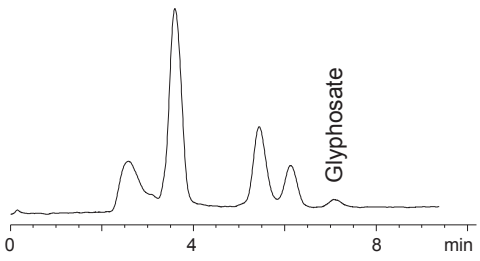


Fig 1. *Chromatogram of whole milk sample spiked with Glyphosate at 0.025 ppm level*

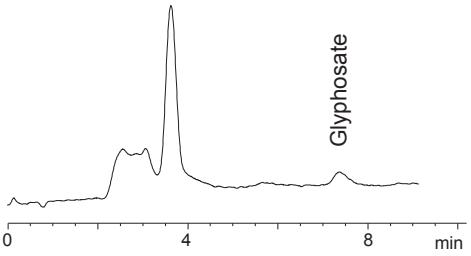


Fig 2. *Chromatogram of egg sample spiked with Glyphosate at 0.025 ppm level*

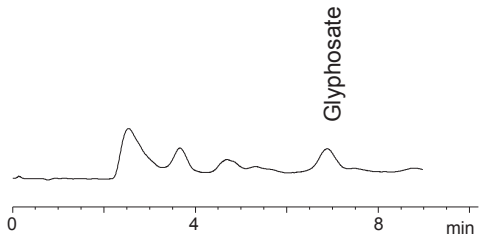


Fig 3. *Chromatogram of corn sample spiked with Glyphosate at 0.1 ppm level*

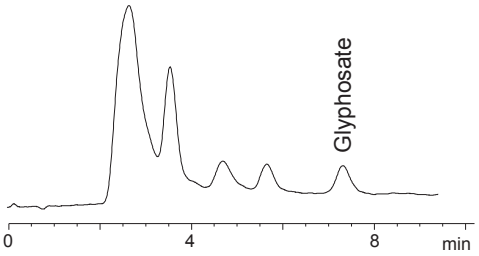


Fig 4. *Chromatogram of wheat flour samples spiked with Glyphosate at 0.1 ppm level*

Conclusion

The simple and robust post-column method was shown to be suitable for analysis of Glyphosate on sub-ppm levels in many types of foods. Accelerated sample preparation improves throughput of samples and reduces cost of testing for busy laboratories. The sensitivity of the method allows for detection of Glyphosate well within residue tolerances set by regulatory agencies.

References

1. Pickering Laboratories method abstract MA207 “Glyphosate Analysis in soy beans, corn and sunflower seeds”
2. Pickering Laboratories method abstract MA206 “Glyphosate and AMPA Analysis in crops”

Glyphosate in Soy Beans, Corn and Sunflower Seeds (MA-207)

By HPLC With Post-Column Derivatization and Fluorescence Detection

Glyphosate is a broad spectrum herbicide widely used around the world. Monitoring of Glyphosate in crops and water is mandated in many countries. We describe a sensitive and robust HPLC method for analysis of Glyphosate in soy beans, corn and sunflower seeds. This method utilizes a simplified sample preparation procedure that has proven to be effective even for challenging matrices.

Method

Analytical Conditions

Column: Cation-exchange Column for Glyphosate analysis, 4 x 150 mm, Catalog Number 1954150

Guard: Cation-exchange GARD™, Catalog Number 1700-3102

Column Temperature: 55 °C

Flow Rate: 0.4 mL/min

Mobile Phase: K200, RG019

Injection Volume: 100 uL

HPLC Gradient		
TIME	K200 %	RG019 %
0	100	0
15	100	0
15.1	0	100
17	0	100
17.1	100	0
25	100	0

Post-Column Conditions

Post-Column System: Onyx PCX, Pinnacle PCX or Vector PCX

Heated Reactor Volume: 0.5 mL

Temperature: 36 °C

Ambient Reactor: 0.1 mL

Reagent 1: 100 uL of 5% NaOCl (Bleach) in 950 mL of GA116 Diluent

Reagent 2: 100 mg of OPA and 2 g of Thiofluor in 950 mL of GA104 Diluent

Reagent Flow Rate: 0.3 mL/min each reagent

Detection: FLD Detector
λ_{ex}: 330 nm, λ_{em}: 465 nm

Supplies for Sample Preparation

- Methylene Chloride, HPLC Grade
- Acidic Modifier Solution (16 g KH2PO4, 160 mL of water, 40 mL of Methanol, 13.4 mL of conc. HCl)
- Elution Solution (160 mL of water, 40 mL of Methanol, 2.7 mL of HCl)
- RESTORE™
- SPE sample clean-up cartridges P/N 1705-0001

Sample Preparation

Extraction

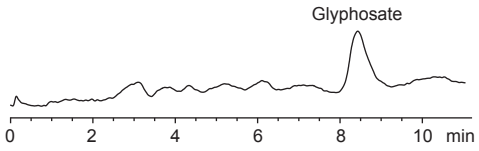
To 25 g of homogenized sample, add enough water (after estimating moisture content) such that the total volume of water is 125 mL. Blend at high speed for 3-5 min and centrifuge for 10 min. Transfer 20 mL of the aqueous extract into a centrifuge tube and add 15 mL of Methylene Chloride. Shake for 2-3 min and centrifuge for 10 min. Transfer 4.5 mL of aqueous layer to another centrifuge tube and add 0.5 mL of Acidic Modifier Solution. Shake and centrifuge for 10 min. Filter through a 0.45 um filter.

Matrix-Specific Modifications

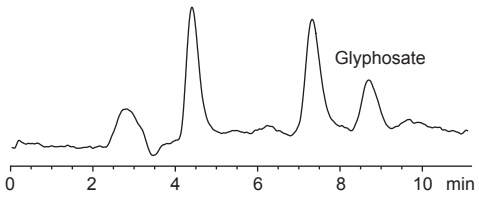
1) For samples that absorb large amounts of water, reduce test portion to 12.5 g while keeping water volume the same.
2) For samples with high protein content, add 100 uL of concentrated HCl to 20 mL of crude extract. Shake and centrifuge for 10 min.
3) For samples with high fat content, do the Methylene Chloride partitioning twice.

SPE Cleanup
Remove the top cap first, then the bottom cap of the SPE columns and place them into the manifold. Drain the solution to the top of the resin bed. Transfer 1 mL of extract into the column and elute to the top of the resin bed. Add 0.7 mL of the Elution Solution and discard the effluent. Repeat with a second 0.7 mL portion of the Elution Solution and discard the effluent. Elute Glyphosate with 12 mL of the Elution Solution and collect the effluent in a round bottom flask. Evaporate to dryness at 40 °C using a rotary evaporator. Dissolve the residue in 2.0 mL of a solution of 10% RESTORE™ in water (use 1.5 mL for dry samples), filter through a 0.45 um syringe filter and inject onto the HPLC column. Extracts can be stored refrigerated for up to 7 days before the evaporation step.

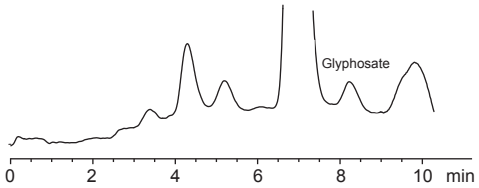
Recoveries for Glyphosate			
Spike Level	**Soy Beans**	**Corn**	**Sunflower Seeds**
0.2 ug/g	109%	102%	70%
0.1 ug/g	90%	93%	82%
0.05 ug/g	93%	93%	71%



Chromatogram of soy beans sample spiked with Glyphosate at 0.1 ppm level



Chromatogram of corn sample spiked with Glyphosate at 0.1 ppm level



Chromatogram of sunflower seeds sample spiked with Glyphosate at 0.1 ppm level

Glyphosate and AMPA Analysis in Crops (MA-206)

A Simple and Reproducible Extraction and Clean-up for HPLC Post-Column Derivatization

The recently practiced method[1] for analysis of Glyphosate and AMPA in crops suffers from an expensive, time consuming clean-up procedure that has less than ideal recoveries. Although the analysis (after clean up) by ion-exchange chromatography with post-column derivatization is rugged and sensitive, a new method was sought to improve the sample preparation. This resulted in AOAC Method 2000.52[2] which has a streamlined cleanup followed by pre-column derivatization and GC/MS analysis. We show how this simplified sample preparation is suitable for the classic ion-exchange/post-column analytical protocol.

Method

Analytical Conditions

Column: Cation-exchange Column for Glyphosate analysis, 4 x 150 mm, Catalog Number 1954150

Guard: Cation-exchange GARD™, Catalog Number 1700-3102

Column Temperature: 55 °C

Flow Rate: 0.4 mL/min

Mobile Phase: K200, RG019

Injection Volume: 100 uL

Post-Column Conditions

Post-Column System: Onyx PCX, Pinnacle PCX or Vector PCX

Heated Reactor Volume: 0.5 mL

Temperature: 36 °C

Ambient Reactor: 0.1 mL

Reagent 1: 100 uL of 5% NaOCl (Bleach) in 950 mL of GA116 Diluent

Reagent 2: 100 mg of OPA and 2 g of Thiofluor in 950 mL of GA104 Diluent

Reagent Flow Rate: 0.3 mL/min each reagent

Detection: Fluorescence detector
λ_{ex} : 330 nm, λ_{em} : 465 nm

HPLC Gradient		
TIME	**K200 %**	**RG019 %**
0	100	0
15	100	0
15.1	0	100
17	0	100
17.1	100	0
25	100	0

Sample Preparation

Extraction

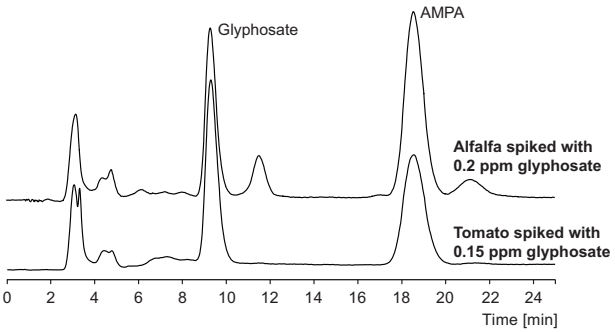
To 25 g of a homogenous sample add enough water (after estimation of moisture content) to make the total volume of water 125 mL. Blend at high speed for 3–5 min. and centrifuge for 10 min. Transfer 20 mL of the aqueous extract into a centrifuge tube and add 15 mL of methylene chloride (to remove nonpolar co-extractives). Shake for 2–3 min. and centrifuge for 10 min. Transfer 4.5 mL of the aqueous layer into a vial and add 0.50 mL acidic modifier solution (16g KH_2PO_4, 160 mL H_2O, 40 mL Methanol, 13.4 mL HCl). Shake and centrifuge for 10 min.

Matrix Specific Modification

1) For crops that absorb large amounts of water, reduce test portion to 12.5 g keeping water volume the same.
2) For crops that have high protein content add 100 µL HCl to 20 mL aliquot of crude extract. Cap, shake and centrifuge for 10 min.
3) For crops that have high oil content, do the methylene chloride partition twice.

Cation-Exchange Cleanup

Transfer 1 mL of extract (representing 0.18 g normal crop or 0.09 g dry crop) to the column reservoir and elute to the top of the resin bed. Add 0.70 mL of the elution solution (160 mL H_20, 2.7 mL HCl, 40 mL Methanol) and discard the effluent. Repeat with a second 0.70 mL portion and discard effluent. Elute with 12 mL of the elution solution and collect in a round-bottomed flask. Evaporate to dryness in a water bath set at 40 ˚C using a rotary evaporator. Or collect in a centrifuge tube and evaporate using a vacuum vortex evaporator. Dissolve residue in 2.0 mL of the elution solution (use 1.5 mL for dry crops). Extracts before evaporation can be stored refrigerated for up to 7 days.

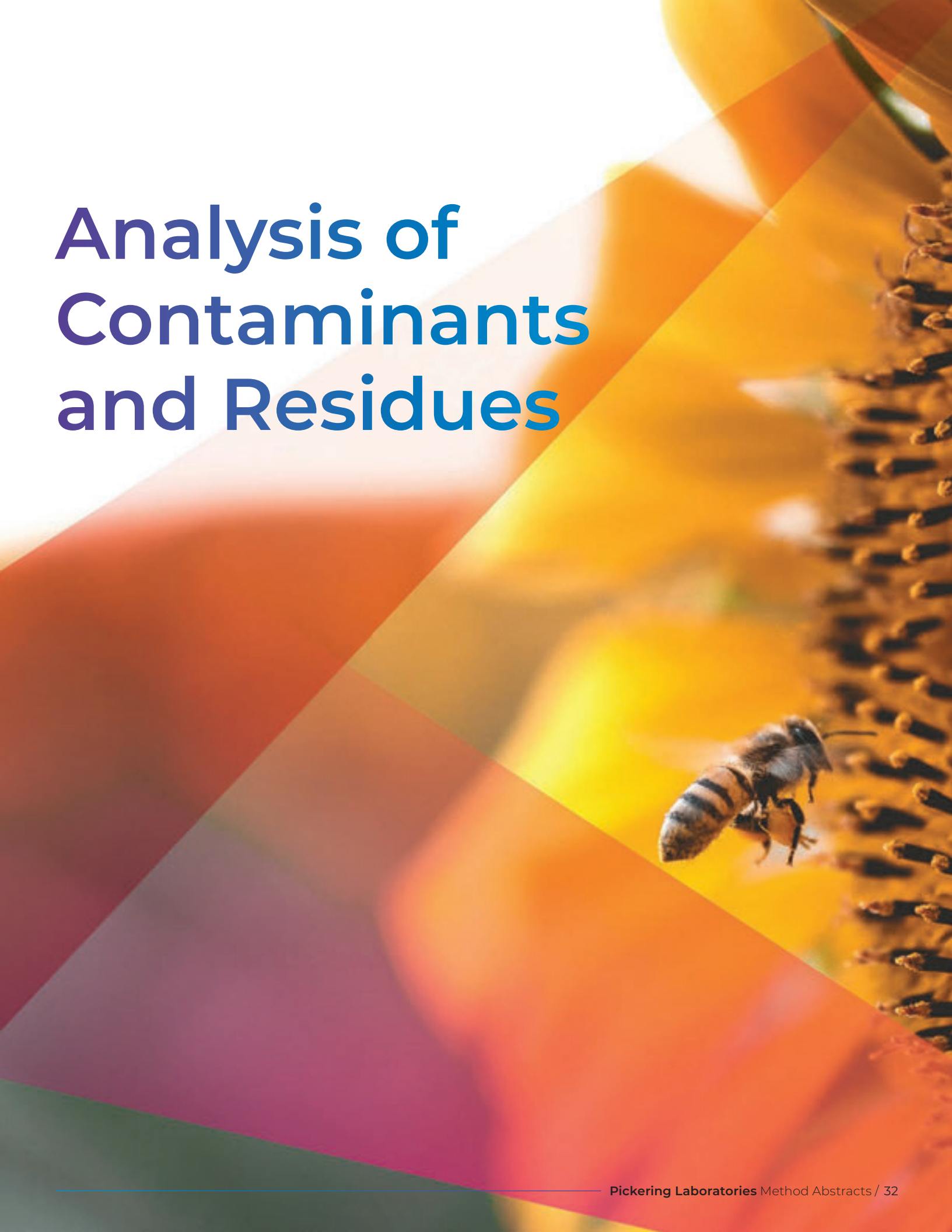


Chromatograms of Alfalfa and Tomato matrix spiked with glyphosate and AMPA

References:

4. "Validation of an Analytical Residue Method for Analysis of Glyphosate and Metabolite: An Interlaboratory Study." J. Agric. Food Chem. 34, (1986) 955–960.
5. P.L. Alferness and L.A. Wiebe, "Determination of Glyphosate and Aminomethylphos-phonic Acid in Crops by Capillary Gas Chromatography with Mass-Selective Detection: Collaborative Study." Journal of AOAC International, 2001 84, 823–846.

Analysis of Contaminants and Residues



Analysis of N-Methyl Carbamate Pesticides in Food (MA-472)

By HPLC With Post-Column Derivatization and Fluorescence Detection

Carbamate pesticides are widely used around the world to protect agricultural produce. The main route of exposure for people to N-Methyl Carbamates is through food pathways, so pesticide use in food crops is strictly regulated.

As part of FDA's pesticide monitoring program, individual lots of domestic and imported foods and feeds are sampled and tested for pesticide residues in order to enforce the tolerances set by the EPA. Methyl carbamates are separated using a reversed-phase column and then reacted with *o*-Phthalaldehyde and a mercaptan after hydrolysis to form a highly fluorescent derivative. This post-column reaction is the basis for EPA Method 531.2 and AOAC official Method 985.23.

The "QuEChERS" (Quick, Easy, Cheap, Effective, Rugged, and Safe) method is a single step sample extraction and salting out technique that is combined with dispersive SPE clean-up for multi-residue pesticide analysis. AOAC official Method 2007.01 utilizes QuEChERS extraction and clean-up for wide range of pesticides in food matrices. This method abstract demonstrates that dispersive SPE can be successfully used in combination with post-column derivatization and fluorescence detection for analysis of carbamates in food.

Method

Sample Preparation

Place 15 g (5 g for spices or other challenging matrices) of homogenized sample into 50 mL centrifuge tube and add 15 mL of 1% Acetic Acid in Acetonitrile (v/v). Mix well. Add one Q-sep packet (Cat. # 26238, Restek), containing 6.0 g Magnesium Sulfate and 1.5 g of Sodium Acetate, to the mixture and immediately shake or vortex for 1 min. Centrifuge for 1 min to separate solid material.

Take 1 mL of supernatant and place into Q-sep d-SPE tube and shake vigorously for 2 min.

To clean-up samples containing fats and waxes, 50 mg PSA, 150 mg $MgSO_4$, and 50 mg C_{18} (Q-sep Cat # 26125, Restek).

To clean-up intensely colored extracts, 50 mg PSA, 150 mg $MgSO_4$, 50 mg C_{18} and 50 mg graphitized carbon (Q-sep Cat # 26219, Restek). Centrifuge the tube for 1 min to separate the solid material, filter through 0.45 um filter and place into injection vial for HPLC analysis.

Table 1. HPLC Conditions

Time	Water %	Methanol %
0	100	0
1	100	0
1.1	82	18
36	30	70
39	30	70
39.1	0	100
41	0	100
41.1	100	0
55	100	0

Analytical Conditions

Column: Carbamate Column
P/N 0846250 (250 x 4.6 mm), C8, 5 um

Guard: Reversed-phase guard cartridge,
P/N 18ECG001

Flow Rate: 1 mL/min

Column Temperature: 42 °C

Mobile Phase: see Table 1

Injection Volume: 10-20 uL

Post-Column Conditions

Post-Column System: Onyx PCX, Pinnacle PCX or Vector PCX

Reactor 1: 100 °C, 0.5 mL

Reactor 2: Ambient, 0.1 mL

Reagent 1: Hydrolysis Reagent CB130 or CB130.2

Reagent 2: 100 mg of OPA, 2 g of Thiofluor
in 950 mL of CB910

Detection: FLD, λ_{ex} : 330 nm, λ_{em} : 465 nm

Table 2. Recoveries of N-Methyl Carbamates in Food Matrices					
Sample	**Apples**	**Banana**	**Ginger powder**	**Brown rice**	**Blueberries**
Spike Concentration	10 ng/g	10 ng/g	25 ng/g; sample is contaminated with 62 ng/g of Carbofuran	25 ng/g	10 ng/g
Aldicarb Sulfoxide	99.6%	109.4%	91.1%	87.1%	92.8%
Aldicarb Sulfone	107.0%	98.4%	79.8%	81.8%	92.5%
Oxamyl	73.1%	73.3%	76.8%	80.8%	68.7%
Methomyl	98.5%	84.2%	83.1%	86.9%	115.4%
3-Hydroxycarbofuran	84.1%	107.0%	87.4%	72.1%	80.8%
Aldicarb	89.7%	99.8%	77.8%	87.2%	87.5%
Propoxur	101.5%	91.0%	72.3%	88.4%	86.5%
Carbofuran	103.4%	100.3%	97.0%	84.1%	89.2%
Carbaryl	76.2%	111.8%	81.8%	83.4%	82.2%
Naphthol	61.6%	82.3%	75.0%	75.8%	37.2%
Methiocarb	86.8%	93.1%	49.8%	88.4%	83.5%

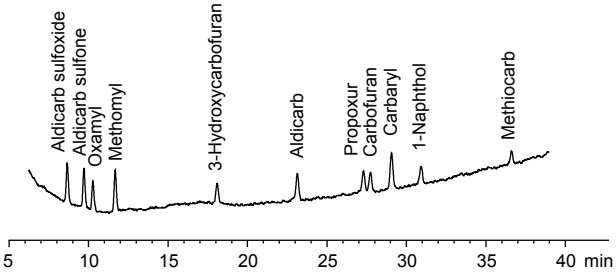


Fig 1. *Carbamates standard solution, 5 ng/mL*

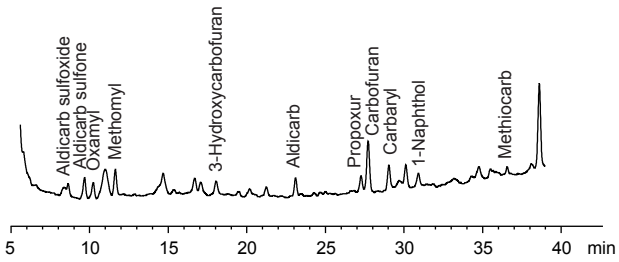


Fig 3. *Ginger powder spiked with 25 ng/g of carbamates*

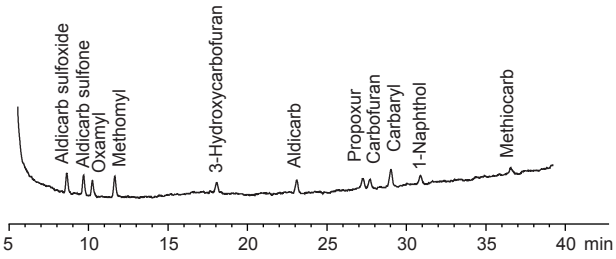


Fig 2. *Banana sample spiked with 10 ng/g of carbamates*

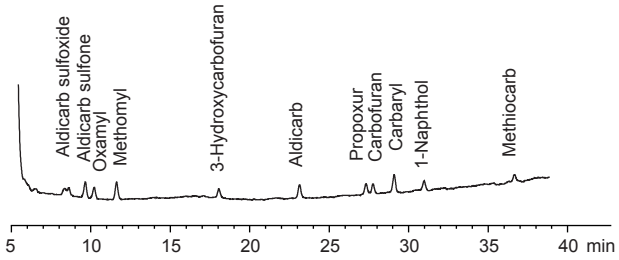


Fig 4. *Brown rice sample spiked with 25 ng/g of carbamates*

Highly Sensitive Analysis of Chromium (VI) in Drinking Water by Ion Chromatography (IC) with Post-Column Derivatization and UV/VIS Detection (MA-388)

Chromium is widely used in the manufacturing of metal alloys, surface coatings, pigments and other products. It is also present naturally in soil. Chromium (VI) is the most toxic form of Chromium and is a possible human carcinogen. Due to its high solubility, Hexavalent Chromium can easily contaminate drinking and ground water sources and its levels must be monitored.

In July 2011, the Office of Environmental Health Hazard Assessment (OEHHA) established a Public Health Goal (PHG) for Chromium (VI) at a concentration of 0.02 ug/L. Based on the PHG, the California Department of Public Health is developing a primary drinking water standard (Maximum Contaminant Level, MCL) that is specific for Chromium (VI).

EPA Method 218.7 uses post-column derivatization with diphenylcarbazide under highly acidic conditions and UV/VIS detection to analyze Chromium (VI) in drinking water. Injection volumes of 1000 uL and 1250 uL were used in the method but Pickering Laboratories was able to meet method requirements with as low as 100 uL injections. Modern IC systems and columns can successfully separate Chromium (VI) from other ions with short run times. Pickering Laboratories' post-column system is uniquely suitable for delivering aggressive derivatizing reagents with unmatched flow precision and low noise level in order to consistently provide highly sensitive detection of Hexavalent Chromium.

Method

Sample Preparation

Clean all glassware and containers with 1:1 H2O/HNO3. Rinse well with deionized water.

Adjust all samples and standards to pH 9.0-9.5 using Sample Adjustment Buffer (250 mM Ammonium Sulfate, 500 mM Ammonium Hydroxide in water). For most samples, no more than 1 mL of Adjustment Buffer is used per 100 mL of water sample. Correct final results for dilution. Store all samples and standards in the refrigerator and analyze within 24 hours.

An injection volume of 100 uL is sufficient to detect levels as low as 0.01 ppb. Injection volume can be increased up to 1000 uL if needed.

Analytical Conditions

IC System: ICS 900 or equivalent IC system (Thermo Scientific)

Analytical Column: IonPac AS22, 4x250 mm (Thermo Scientific)

Guard: IonPac AG22, 4x50 mm (Thermo Scientific)

Flow Rate: 1 mL/min

Column Temperature: 30 °C

Mobile Phase: 250 mM Ammonium Sulfate, 50 mM Ammonium Hydroxide in water

Injection Volume: 100 uL

Post-Column Conditions

Post-Column System: Onyx PCX or Pinnacle PCX for Chromium (VI) Analysis

Reactor Volume: 1.0 mL

Reactor Temperature: 30 °C

Reagent: 2 mM Diphenylcarbazide, 1 N Sulfuric Acid, 10% Methanol

Reagent Flow Rate: 0.33 mL/min

Detection: UV/VIS, 530 nm

Table 1. Recoveries Data for Tap Water Spiked With Chromium (Vi).

Spike Concentration	Recoveries	RSD N=3
0.1 ppb	104%	10.6%
0.5 ppb	96%	4.3%

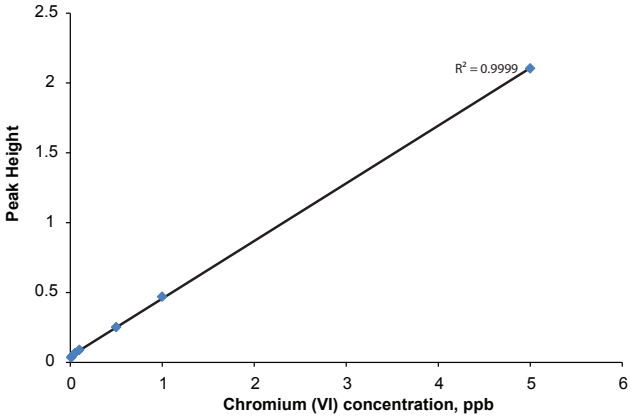


Fig 1. *Calibration curve for Chromium (VI) 0.01 – 5 ppb range*

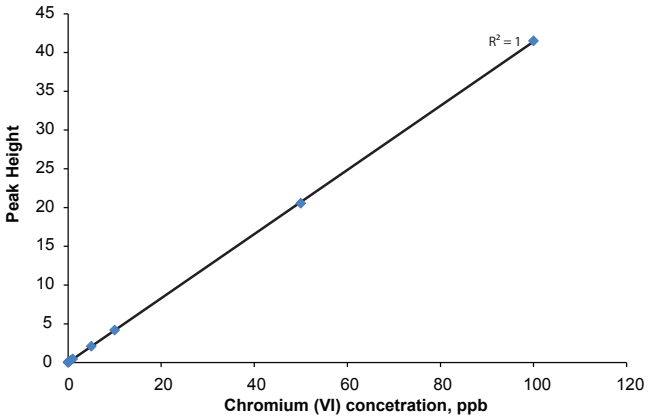


Fig 2. *Calibration curve for Chromium (VI) 0.01 – 100 ppb range*

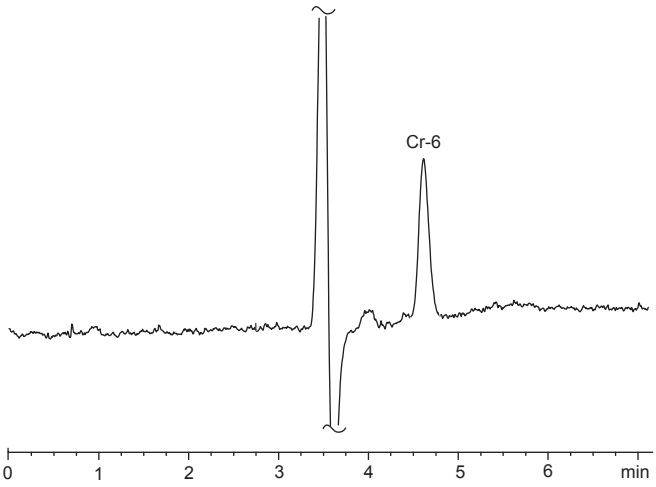


Fig 3. *Chromatogram of tap water spiked with 0.5 ppb of Chromium (VI)*

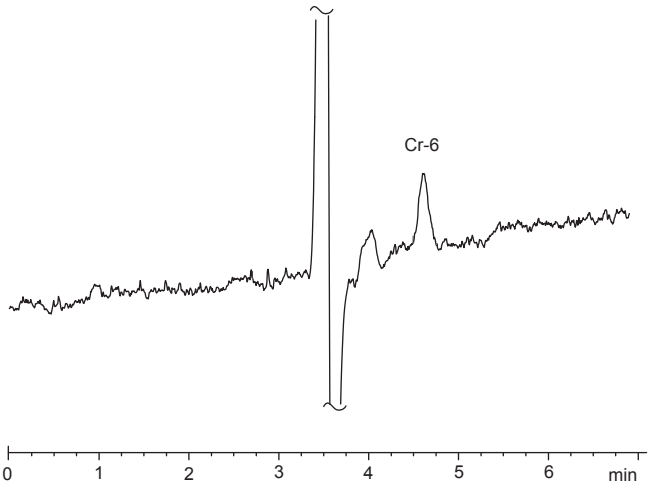


Fig 4. *Chromatogram of 0.02 ppb standard solution of Chromium (VI)*

Simultaneous Determination of Nitrite and Nitrate in Baby Food (MA-121)

Nitrite and Nitrate are added to food to preserve color and taste, and to prevent foods from becoming rancid. They are also used in food for their anti-microbial properties. Higher levels in vegetables and leafy greens are possible from the use of Nitrate fertilizers and/or livestock manure. Nitrite levels in food could also be produced by reduction of Nitrate to Nitrite during processing.

AOAC official method 993.031 for the analysis of Nitrate involves reduction using spongy Cadmium, which is toxic and carcinogenic. FDA improved on this method by using Vanadium (III) chloride and heat for the post-column reduction of Nitrate to Nitrite. Nitrite reacts with this modified Griess reagent to produce a red chromophore with maximal absorbance at 535 nm. Pickering Laboratories Inc. has further improved this method by substituting the corrosive Hydrochloric Acid with Methanesulfonic Acid.

Method

Analytical Conditions

Analytical Column: Thermo Scientific Ion Pac™ AS9-HC Column (Cat. No. 051786) or AS22 (Cat. No. 064137)

Column Temperature: 50 °C

Flow Rate: 1 mL/min

Mobile Phase: 1.8 mM Na2CO3 / 1.7 mM NaHCO3 for AS9 - HC Column
or
4.5 mM Na_2CO_3 / 1.4 mM $NaHCO_3$ for AS22 Column

Injection Volume: 10 µL

Post-Column Conditions

Post-Column System: Onyx PCX or Pinnacle PCX

Reactor Volume: 0.5 mL

Reactor Temperature: 100 °C

Reagent Flow Rate: 0.1 mL/min

Reagent: (i) 1% Vanadium (III) chloride in 20% Methanesulfonic Acid

(ii) 1% wm-Nitro aniline in 20% Methanesulfonic Acid

(iii) 1% N-(1-Naphthyl)ethylenediamine dihydrochloride in 20% Methanesulfonic Acid

Mix 50 mL of (i) and (ii), and 1.25 mL of (iii) and dilute to 250 mL using 20% Methanesulfonic Acid

Detection: UV/VIS, λ_{max} = 535 nm

Recovery Data						
Sample	**Spiked Conc. (ppm)**		**Cal. Conc. (ppm)**		**% Recovery**	
	Nitrite	Nitrate	Nitrite	Nitrate	Nitrite	Nitrate
Sweet Potato	50	50	57.3	58.0	115	116
	250	250	271.4	266.7	109	107
Pears	50	50	54.4	55.0	109	110
	250	250	280.8	271.0	112	108
Apple Sauce	50	50	56.5	54.6	113	109
	250	250	283.9	265.7	114	106

Sample Preparation

To 5 g of baby food in a 50 mL centrifuge tube, add 25 mL of 50-60 °C water (for vegetables) or 15 mM Sodium acetate (for fruits) and shake for 10 min. Add 12.5 mL of acetonitrile and make up the volume to 50 mL using water (for vegetables) or Sodium acetate (for fruits). Centrifuge the mixture for 15 mins at 5000 rpm. Filter the supernatant through a 0.45 µm nylon filter and dilute to fall within the linear range.

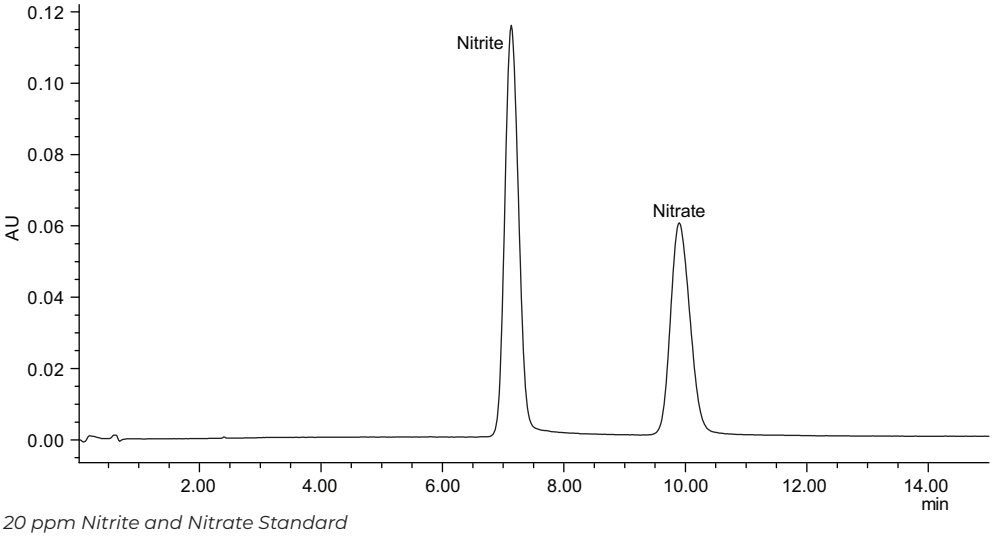
Notes

Post-column reagent solutions are stored in plastic or Teflon containers.

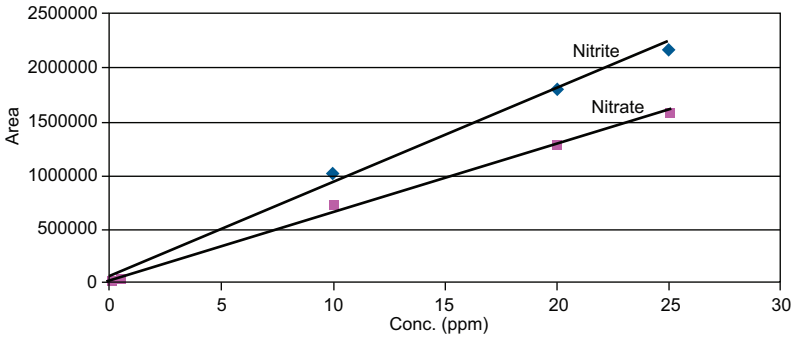
All solutions are filtered through 0.45 µm nylon filter before use.

Nitrate/Nitrite standards should be checked prior to use for oxidation.

Sample pH should be checked to determine the choice of extraction solution since acidic pH facilitates the conversion of Nitrite to Nitrate.



20 ppm Nitrite and Nitrate Standard



Calibration Curve for Nitrite and Nitrate

Acknowledgments

John A. Casanova, Food and Drug Administration, 60 8th Street, Atlanta, GA 30309

References

1. AOAC- Official Methods of Analysis of AOAC International (2000) 17th Ed., Section 50.1.11.
2. Use of Griess Reagents Containing Vanadium (III) for the Post-Column Derivatization and Simultaneous Determination of Nitrite and Nitrite in Baby Food, John A. Casanova, Lois K. Gross, Sarah E. McMullen and Frank Schenck, Food and Drug Administration, 60 8th Street, Atlanta, GA 30309.

Sensitive and Selective Analysis of Nitrite and Nitrate in Drinking Water by Ion Chromatography (IC) With Post-Column Derivatization and UV/VIS Detection (MA-129)

Nitrite and Nitrate are formed naturally in soil and water when bacteria break down waste and organic material from plants, humans and animals. Nitrate is also one of the main components of chemical fertilizers. Contamination of the drinking water supply with Nitrite and Nitrate occurs due to runoff and seepage into ground water from farms, golf courses, landfields, improperly managed animal feedlots and sewage systems.

Under certain conditions, the human body converts Nitrate to Nitrite, which can react with hemoglobin in blood and decrease its ability to carry oxygen. Nitrite can also form a variety of N-Nitroso compounds, many of which are known carcinogens. Elevated concentrations of Nitrite and Nitrate in water are especially dangerous to infants younger than 6 months old and pregnant women. To prevent harmful health effects from Nitrite and Nitrate contamination of drinking water, the USEPA has established Maximum Contamination Levels (MCLs) at 10 ppm for Nitrate and 1 ppm for Nitrite.

The USFDA method for Nitrite and Nitrate calls for post-column derivatization using a Vanadium (III) Chloride reagent containing HCl. Pickering Laboratories has improved this method by substituting the volatile and corrosive Hydrochloric Acid with Methanesulfonic Acid. When coupled with modern IC systems and columns, the Pickering post-column derivatization systems allow for fast, sensitive and selective analysis of Nitrite and Nitrate in drinking water, and additionally in food matrices*.

Method

Analytical Conditions

IC System: ICS 900 or equivalent IC system (Thermo Scientific)

Analytical Column: IonPac AS9-HC, 4 x 250 mm (Thermo Scientific)

Flow Rate: 1 mL/min

Column Temperature: 30 °C

Mobile Phase: 9.0 mM Sodium Carbonate

Injection Volume: 20 uL

Post-Column Conditions

Post-Column System: Onyx PCX, Pinnacle PCX or Vector PCX

Reactor Volume: 0.5 mL

Reactor Temperature: 100 °C

Reagent Flow Rate: 0.1 mL/min

Detection: UV/VIS, 535 nm

Reagent : Mix 50 mL of (i) and (ii) and 1.25 mL of (iii) in 250 mL volumetric flask. Bring to volume with 20/80 Methanesulfonic Acid / Water.

(i) 1% Vanadium (III) Chloride in 20/80 Methanesulfonic Acid / Water.

(ii) 1% m-Nitro Aniline in 20/80 Methanesulfonic Acid / Water.

(iii) 1% N-(1-Naphthyl)ethylenediamine Dihydrochloride in 20/80 Methanesulfonic Acid / Water.

Table 1. Recoveries Data for Nitrite and Nitrate

Spike Concentration	Nitrite %	Nitrate %
10 ppm	100.6	102.4
0.5 ppm	97.5	85.5
0.5 ppm in 100 ppm NaCl	100.1	87.1

*Inquire about Pickering Laboratories Method Abstract 123 “Simultaneous Determination of Nitrite and Nitrate in Processed Foods” and Method Abstract 121 “Nitrite and Nitrate Analysis in Baby Food”

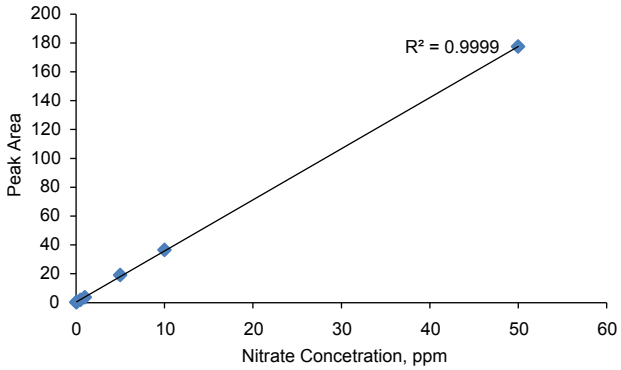


Fig 1. *Calibration Curve for Nitrate 0.05-50 ppm range*

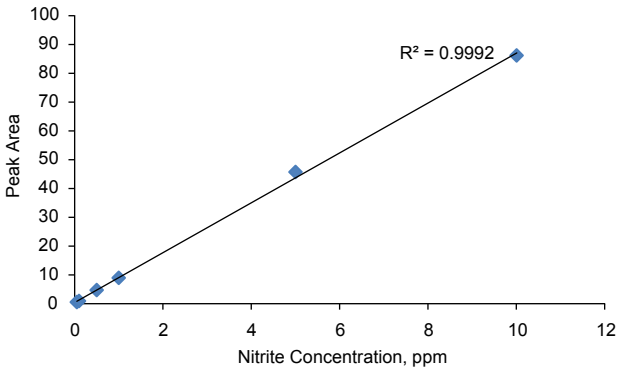


Fig 2. *Calibration Curve for Nitrite 0.05-10 ppm range*

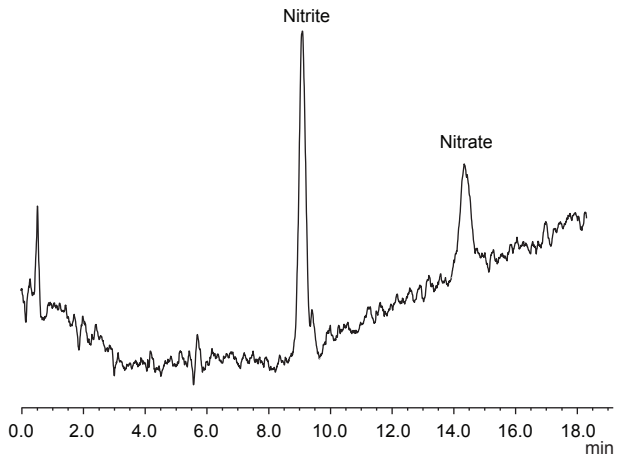


Fig 3. *Chromatogram of 0.05 ppm Nitrite and 0.05 Nitrate solution.*

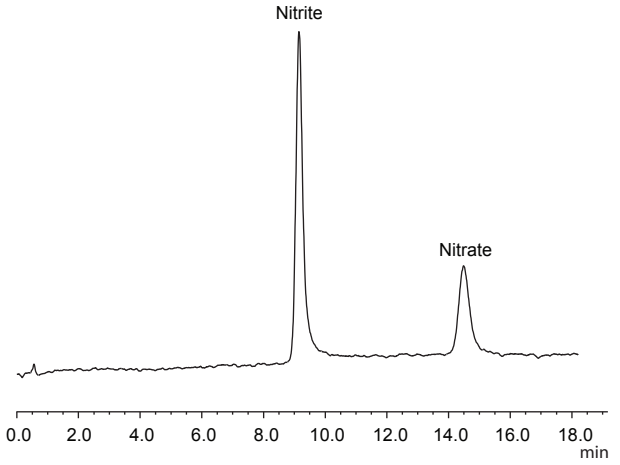


Fig 4. *Chromatogram of 0.5 ppm Nitrite and 0.5 ppm Nitrate solution in presence of 100 ppm NaCl.*

References

1. Official Methods of Analysis of AOAC International (2000) 17th Ed, Section 50.1.11
2. Use of Griess Reagents Containing Vanadium (III) for Post-Column Derivatization and Simultaneous Determination of Nitrite and Nitrate in Baby Food. John A. Casanova, Lois K. Gross, Sarah E. McMullen and Frank Schenk, Food and Drug Administartion, 60 8th Street, Atlanta, GA 30309

Simultaneous Determination of Nitrite and Nitrate in Processed Foods (MA-123)

Nitrite either alone or in combination with nitrate is added to food to preserve color and taste and to prevent foods from becoming rancid. They are also used in food for their anti-microbial properties. Higher levels in vegetables and leafy greens are possible from the use of nitrate fertilizers and/or livestock manure.

Nitrate can be reduced to nitrite at certain physiological conditions in the human body. Nitrite however can oxidize Fe (II) in hemoglobin to methemoglobin, an Fe (III) product. The oxidized product is incapable of binding molecular oxygen and high concentrations of methemoglobin can result in methemoglobinemia especially in infants. Nitrite can also react with secondary amines present in food products or in the digestive system to form nitrosamines, a class of carcinogenic compounds. Nitrite levels in food could also be produced by reduction of nitrate to nitrite during processing.

The AOAC Official Method 993.03[1] for the analysis of nitrate involves reduction using spongy Cadmium which is toxic and carcinogenic. The USFDA improved on this method by using Vanadium (III) chloride and heat for the post-column reduction of nitrate to nitrite. Nitrite reacts with this modified Griess reagent to produce a red chromophore with maximal absorbance at 535 nm. Pickering Laboratories Inc. has further improved this method by substituting the corrosive and volatile hydrochloric acid with methane sulfonic acid.

Method

Analytical Conditions

IC System: ICS 900 or equivalent IC system (Thermo Scientific)

Analytical Column: IonPac AS9-HC, 4 x 250 mm (Thermo Scientific)

Flow Rate: 1 mL/min

Column Temperature: 30 °C

Mobile Phase: 9.0 mM Sodium Carbonate

Injection Volume: 20 uL

Post-Column Conditions

Post-Column System: Onyx PCX, Pinnacle PCX or Vector PCX

Reactor Volume: 0.5 mL

ReactorTemperature: 100 °C

Reagent Flow Rate: 0.1 mL/min

Detection: UV/VIS, 535 nm

Reagent : Mix 50 mL of (i) and (ii) and 1.25 mL of (iii) in 250 mL volumetric flask. Bring to volume with 20/80 Methanesulfonic Acid / Water.

(i) 1% Vanadium (III) Chloride in 20/80 Methanesulfonic Acid / Water.

(ii) 1% m-Nitro Aniline in 20/80 Methanesulfonic Acid / Water.

(iii) 1% N-(1-Naphthyl)ethylenediamine Dihydrochloride in 20/80 Methanesulfonic Acid / Water.

Extraction Procedure

Fruits and Vegetables

To 5 g of baby food in a 50 mL centrifuge tube add 25 mL of 50-60 °C water (for vegetables) or 15 mM Sodium acetate (for fruits) and shake for 10 min. Add 12.5 mL of acetonitrile and make up the volume to 50 mL using water (for vegetables) and sodium acetate (for fruits). Centrifuge the mixture for 15 mins at 5000 rpm. Filter the supernatant through a 0.45 µ nylon filter and dilute to fall within the linear range.

Processed Meat

To 5 g of homogenized processed meat in a blender add 25 mL of 50-60 °C water and blend for 2 min. Add 25 mL of acetonitrile and blend for an additional 2 min. Transfer into a beaker and make up the volume to 100 mL using warm water. Filter the mixture using Whatman filter paper. Filter further through a 0.45 µ nylon filter and dilute to fall within the linear range.

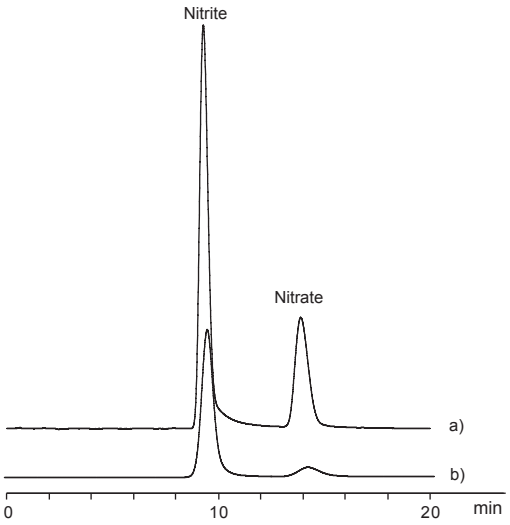
Notes

Post-column reagent solutions are stored in plastic or Teflon containers.

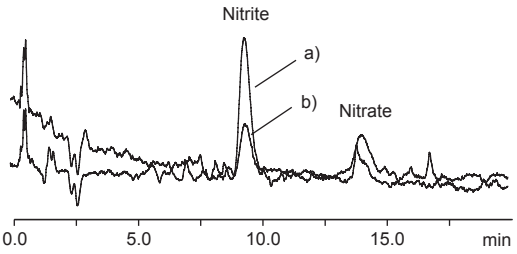
All solutions are filtered through 0.45 µ nylon filter before use.

Nitrate/Nitrite standards should be checked prior to use for oxidation.

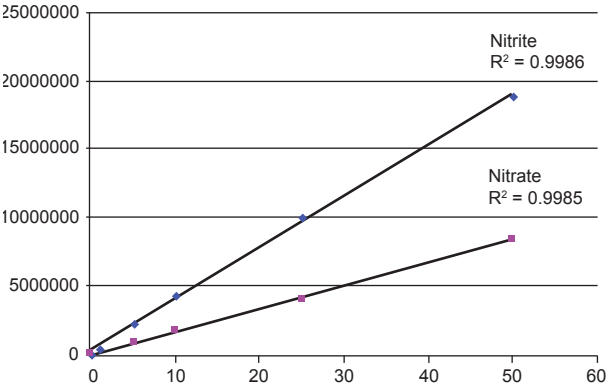
Sample pH should be checked to determine the choice of extraction solution since acidic pH facilitates the conversion of nitrite to nitrate.



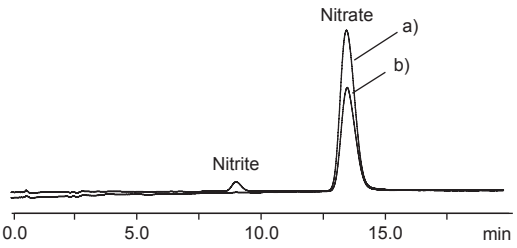
10 ppm nitrite-nitrate standard using (a) 20% methane-sulfonic acid , (b) 20% hydrochloric acid



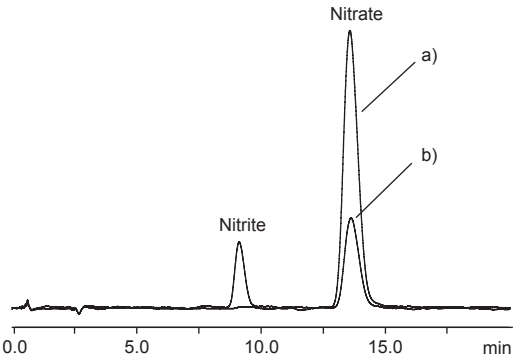
(a) 0.05 ppm, (b) 0.01 ppm nitrite-nitrate standard



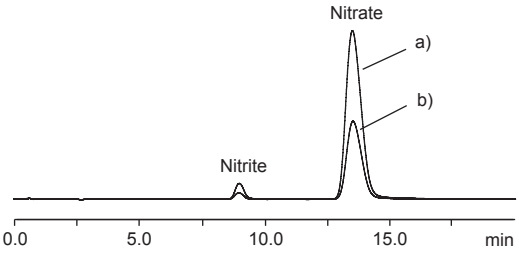
Calibration Curve for Nitrite-Nitrate (0.05 ppm – 50 ppm)



Banana (baby food): (a) spiked with 100 ppm nitrite-nitrate solution, (b) blank



Carrot (baby food): (a) spiked with 100 ppm nitrite-nitrate solution, (b) blank



Corned Beef: (a) spiked with 50 ppm nitrite and 500 ppm nitrate solution, (b) blank

Recovery Data						
	Nitrite			Nitrate		
Sample	Spiked Conc. (ppm)	Cal. Conc. (ppm)	Recovery (%)	Spiked Conc. (ppm)	Cal. Conc. (ppm)	Recovery (%)
Baby Food						
Carrot	100	92.25	92	100	79.3	79
Banana	100	94.6	95	100	102.5	103
Processed Food						
Corned Beef	50	54.4	109	500	478.9	96

Acknowledgments

John A. Casanova, Food and Drug Administration, 60 8th Street, Atlanta, GA 30309

References

1. AOAC- Official Methods of Analysis of AOAC International (2000) 17th Ed., Section 50.1.11.
2. Use of Griess Reagents Containing Vanadium (III) for the Post-Column Derivatization and Simultaneous Determination of Nitrite and Nitrite in Baby Food, John A. Casanova, Lois K. Gross, Sarah E. McMullen and Frank Schenck, Food and Drug Administration, 60 8th Street, Atlanta, GA 30309.

Analysis of Paralytic Shellfish Toxins in Bivalve Mollusks using HPLC Method with Post-Column Derivatization and Fluorescence Detection (MA-105)

The paralytic shellfish toxins are a group of 18 secondary metabolites deposited in bivalve mollusks by dinoflagelates. Dinoflagelate blooms are seasonal, occurring during warm months. Since it is unpredictable when the infestation will occur, the shellfish population should be regularly monitored for toxins. Ingestion of contaminated shellfish can lead to paralytic shellfish poisoning: a life-threatening illness.

The Mouse Bioassay method used to detect dinoflagelate-derived neurotoxins has major drawbacks, which led to the exploration of chromatographic methods of analysis. An HPLC method that utilizes post-column oxidation of the toxins under acidic conditions has been approved as a new official AOAC method – OMA 2011.02. Two different columns and gradients are utilized to separate GTX/STX toxins and C-toxins. The derivatized analytes can be detected with high sensitivity using a fluorescence detector, leading to the determination of toxin type and concentration. We describe the use of Pickering Laboratories' post-column derivatization system, reagents, and mobile phase for analysis of paralytic shellfish toxins according to AOAC Method 2011.02 in the below abstract.

Method

Sample Preparation

- Transfer 5 g of homogenized shellfish tissue into a 50 mL centrifuge tube and add 5 mL of 0.1 N HCl
- Vortex the mixture, adjust the pH to be in range of pH 2-4 as necessary
- Heat the mixture in the boiling water bath for 5 min, cool to room temperature, and recheck the pH, adjusting if necessary
- Centrifuge the mixture and transfer 500 uL of supernatant into a microcentrifuge tube
- Add 25 uL of 30% trichloroacetic acid (TCA) to deproteinate the extract, mix well and centrifuge
- Adjust pH with 1 M NaOH to optimum range of pH 2-4
- Filter through 0.2 um filter and inject

Analytical Conditions for GTXs and STXs

Column: Zorbax Bonus RP column, 3.5 um, 4.6 x 150 mm (Agilent Technologies)

Flow Rate: 0.8 mL/min

Mobile Phase A: 11 mM heptane sulfonate, 5.5 mM phosphoric acid, adjusted to pH 7.1 with ammonium hydroxide (Cat No PSP-0001)

Mobile Phase B: 11 mM heptane sulfonate, 16.5 mM phosphoric acid, 11.5% acetonitrile, adjusted to pH 7.1 with ammonium hydroxide (Cat No PSP-0002)

Table 1. HPLC Gradient Program for GTXs and STXs

Time (Min)	%A	%B
0	100	0
7.9	100	0
8	0	100
18.5	0	100
18.6	100	0
24	100	0

Sample Injection Volume: 10 uL

Analytical Conditions for C-toxins

Column: Thermo BetaBasic 8, 5 um, 4.6 x 250 mm (Thermo Fisher Scientific)

Flow Rate: 0.8 mL/min

Mobile Phase A: 2 mM tetrabutylammonium phosphate, pH 5.8 (Cat. No PSP-C003)

Mobile Phase B: 2 mM tetrabutylammonium phosphate, pH 5.8 with 4% MeCN (Cat No PSP-C004)

Post-Column Conditions

Post-Column System: Onyx PCX, Pinnacle PCX or Vector PCX

Reactor Volume: 1.0 mL

Reactor Temperature: 85 °C

Reagent 1: 100 mM phosphoric acid, 5 mM periodic acid, adjusted to pH 7.8 with 5 M sodium hydroxide (Cat No PSP-R1)

Reagent 2: 0.75 M nitric acid (Cat No PSP-R2)

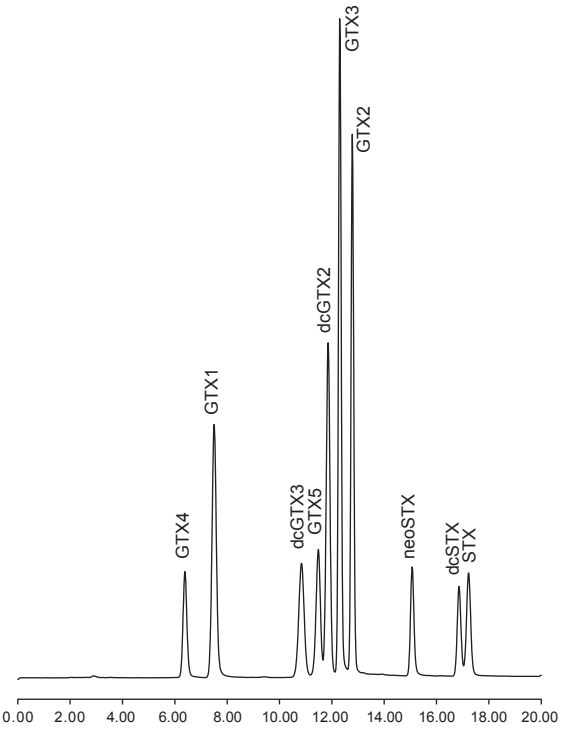
Reagent Flow Rates: 0.4 mL/min

Detection: Fluorescence detector, λ_{ex} : 330 nm, λ_{em} : 390 nm

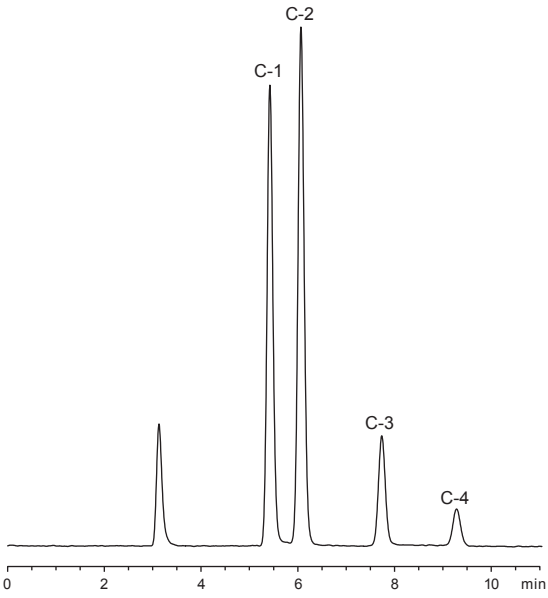
Table 2. HPLC Gradient Program for C-toxins

Time (Min)	%A	%B
0	100	0
8	100	0
15	0	100
16	0	100
19	100	0
24	100	0

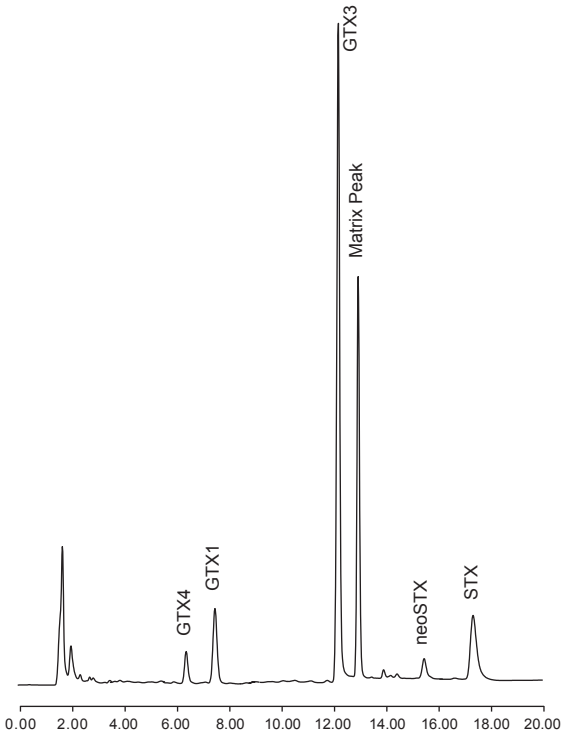
Sample Injection Volume: 5 uL



Chromatogram of GTX and STX mixed toxins standard



Chromatogram of C toxins standard



Chromatogram of mussels sample naturally contaminated with paralytic shellfish toxins

Acknowledgements:

We would like to thank Jeffrey van de Riet and Ryan Gibbs (Canadian Food Inspection Agency) for their help with this project.

References:

1. van de Riet, J.M., Gibbs, R.S., Chou, F.W., Muggah, P.M., Rourke, W.A., Burns, G., Thomas, K, Quilliam, M.A. (2009) J. AOAC Int. 92, 1690-1704.
2. AOAC Official Method 2011.02. Paralytic Shellfish Toxins in Mussels, Clams, Oysters, and Scallops. Post-Column Oxidation (PCOX) Method.

Analysis of Formaldehyde (MA-325)

Formaldehyde is widely used in manufacturing of building materials, cars, plywood, polymers, glues and adhesives. It is also commonly used in household items as a preservative and disinfectant. Due to high toxicity and suspected carcinogenicity products are tested to determine free formaldehyde content and ensure the safety of personal care products and other items. Free formaldehyde testing is also important to monitor production of resins and other polymers and to ensure the quality of final materials.

The HPLC method with post-column derivatization allows for quantifying free formaldehyde in a wide range of products and materials, from shampoos to emulsion polymers and phenolic resins. The method is simple, selective and very sensitive. Potential interferences are either separated from formaldehyde on the HPLC column or don't react with post-column reagent and so are not interfering with the detection.

Sample Preparation

Leather samples (According to ISO 177226-1)
Weigh approximately 2 g of leather pieces to the nearest 0.01 g into a 100 ml glass Erlenmeyer flask. Add 50 ml of 0.1% sodium dodecylsulfonate or sodium dodecylsulfate solution (previously preheated at 40 °C) and fit the Erlenmeyer flask with a glass stopper. Shake the contents of the flask in the water bath for 60 min at 40 °C. Immediately filter the warm extract solution by vacuum through a glass fiber filter into a flask. Cool the filtrate and analyze.

Fabric Samples (According to ISO 14184-1)
Cut specimens into small pieces, and weigh approximately 1 g of the pieces to an accuracy of 10 mg. If the formaldehyde content is low, increase the test specimen weight to 2.5 g in order to achieve a sufficient accuracy. Put the weighed pieces into a 250 ml flask with a stopper and add 100 ml of water. Cap tightly and place in a water bath at 40 °C for 60 min. Shake the flask at least every 5 min. Filter the solution into another flask through a glass filter and analyze.

Resins and Other Samples
Extract samples with water by shaking for 60 min at 40 °C. Choose sample/extraction solution ratio based on expected Formaldehyde content. Use additional dilutions as necessary.

Method

Analytical Conditions

Column: Waters Atlantis® HILIC Silica, 5 um, 4.6 x 250 mm

Column Temperature: 40 °C

Flow Rate: 0.5 mL/min

Mobile Phase: 15% 0.05 M Ammonium Acetate in water
85% Acetonitrile

Injection Volume: 10-50 uL

Post-Column Conditions

Post-Column System: Onyx PCX, Pinnacle PCX or Vector PCX

Reactor Volume: 0.5 mL

Reactor Temperature: 100 °C

Reagent: 0.81 M Ammonium Acetate
0.12 M Glacial Acetic Acid
0.05 M 2,4-Pentanedione

Reagent Flow Rate: 0.4 mL/min

Detection: FLD, λ_{EX} : 412 nm, λ_{EM} : 510 nm Or UV/Vis 410 nm

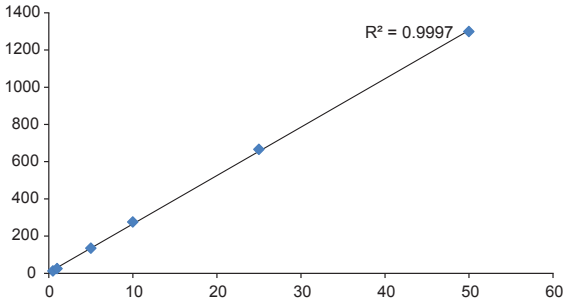


Fig 1. *Calibration curve for Formaldehyde (0.5 – 50 ppm)*

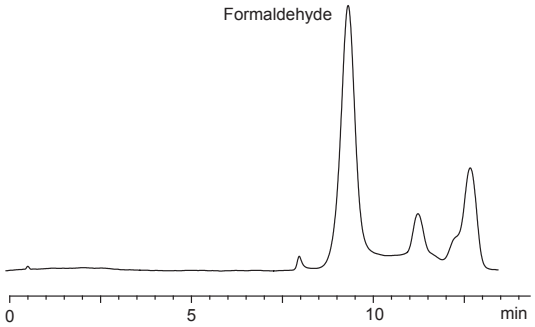


Fig 2. *Chromatogram of phenolic resin sample containing 0.02% of Formaldehyde*

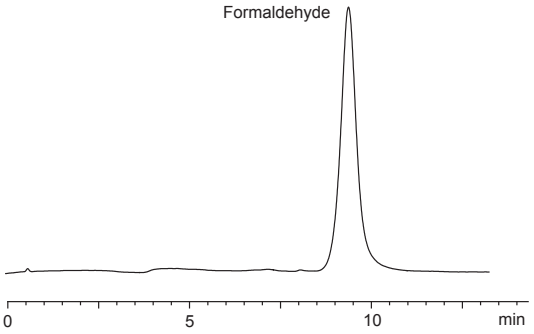


Fig 3. *Chromatogram of phenolic resin containing 0.1% of Formaldehyde*

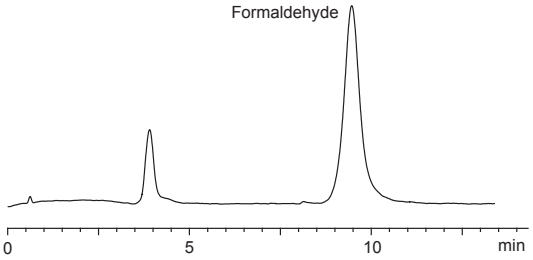


Fig 4. *Chromatogram of shampoo sample spiked with 0.01% of Formaldehyde*

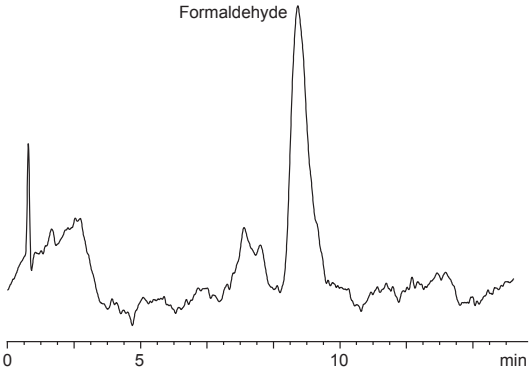


Fig 5. *Chromatogram of leather sample containing 5 ppm of Formaldehyde*

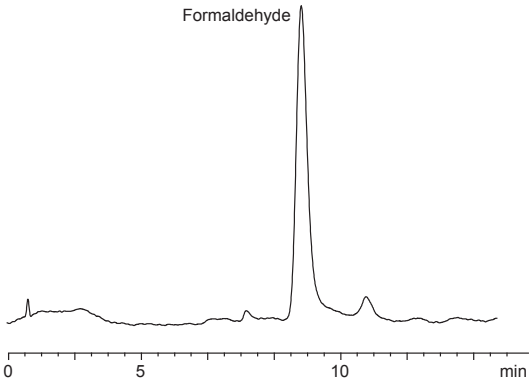


Fig 6. *Chromatogram of fabric sample containing 160 ppm of Formaldehyde*

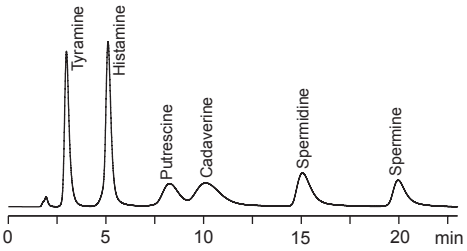
Analysis of Biogenic Amines (MA-102)

Food and Physiological Samples

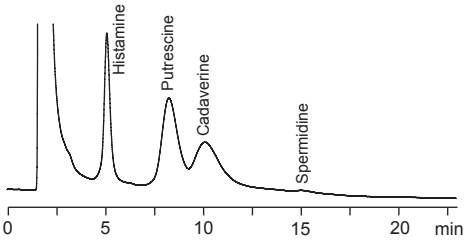
Histamine and other polyamines such as Putrescine, Cadaverine, Spermidine and Spermine are products of decomposition. The level of histamine, in particular, has been used as a regulatory guideline for degree of decomposition in fish. Also, Histamine and Tyramine levels are of interest to producers of wine and cheese.

The polyamines, Spermidine, Spermine and Putrescine are elevated in neoplastic and damaged, e.g., burned tissue. In fact, the level of circulating and urinary polyamines is an indicator of total body damage. The levels can be monitored for the effect of diet and other modalities on recovery.

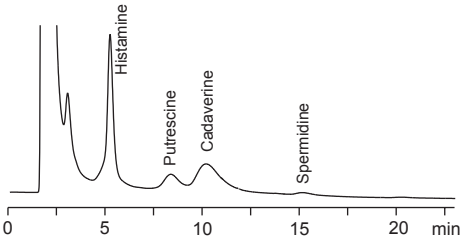
This method employs ion-exchange chromatography so, after extraction, centrifugation/filtration are the only sample preparations necessary.



Chromatogram of calibration standard, 100 µM



Chromatogram of fish sample



Chromatogram of fish sauce sample

Method

Analytical Conditions

Analytical Column: ALKION™ cation-exchange, K+ form, 4 x 150 mm, Catalog No. 9410917

Guard: ALKION™ Guard column, 3 x 20 mm, Catalog No. 9493020

Column Temperature: 45 °C

Flow Rate: 0.8 mL/min

Mobile Phase: K600, K563, K130

Extraction Procedure

Weigh 10 g of sample into a small glass blender cup (any fortifications should be added at this time). Add 50 mL of extraction solution, (80% HPLC Methanol and 20% 0.1N HCl) and homogenize for two minutes. Centrifuge for five minutes. Mix equal portions of the supernatant with the mobile phase (K600). Allow to coagulate at -4 °C for 15 minutes, then centrifuge for five minutes. The clear supernatant is filtered (0.45 µm Nylon) and placed in an autosampler vial.

Post-Column Conditions

Post-Column System: Onyx PCX, Pinnacle PCX or Vector PCX

Reactor Volume: 0.15 mL

Reactor Temperature: 45 °C

Reagent: 300 mg of OPA, 2 g of Thiofluor™, 3 mL of 30% Brij® 35 in 950 mL of OD104

Flow Rate: 0.3 mL/min

Detection: Fluorometer
λ_{ex} : 330 nm, λ_{em} : 465 nm

Conditions			
Time	**K600 %**	**K563 %**	**K130 %**
0	100	0	0
6	100	0	0
15	0	100	0
21	0	100	0
21.1	0	0	100
23	0	0	100
23.1	100	0	0
29	100	0	0

Flow Rate: 0.8 mL/min,
Column Temp.: 45 °C,
Injection Volume: 10 mL

Analysis of N-Nitroso-diethanolamine (NDELA) in Cosmetics (MA-386)

Nitrosamines are chemical contaminants that could be present in food, pharmaceuticals, tobacco, toys and personal care products. They are classified as human carcinogens and limits are established for different products. Cosmetics containing more than 50 ug/kg of nitrosamines, including NDELA, are banned in European Union.

Analysis of NDELA by HPLC with photolysis and post-column derivatization has high specificity and sensitivity and allows for accurate quantification of NDELA in cosmetics and raw materials used in cosmetics. The procedure is described in Official Method DIN EN ISO 10130:2009. Pickering Laboratories' UVE™ photochemical reactor and Onyx PCX post-column derivatization system are uniquely suited for NDELA analysis.

Method

Analytical Conditions

Analytical Column: Spherisorb ODS-2, 4.6 x 150 mm

Guard Column: Reversed-phase C_{18}, 4.6 x 20 mm

Temperature: 30 °C

Flow Rate: 0.5 mL/min

Mobile Phase: 0.02 M Ammonium Acetate in water, pH 6.8

Injection Volume: 10 – 50 µL

Post-Column Conditions

Post-Column System: Onyx PCX, Pinnacle PCX or Vector PCX

Photochemical Reactor: UVE™

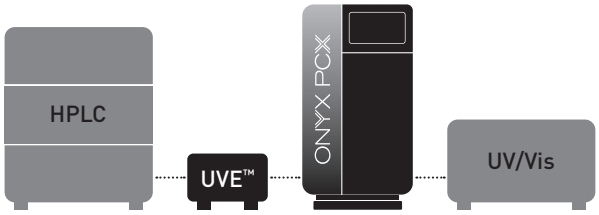
Reactor Volume: 1.0 mL

Reactor Temperature: 50 °C

Reagent: Dissolve 0.25 g of N-(1-naphthyl) ethylenediamine dihydrochloride in 250 mL of water. Dissolve 4.0 g of sulfanilamide in 250 mL of a 5% (w/v) aqueous solution of 85% orthophosphoric acid. Mix the reagents together in an amber glass bottle and keep the mixture away from light.

Reagent Flow Rate: 0.5 mL/min

Detection: UV/Vis 540 nm



Flow diagram for NDELA analysis according to DIN EN ISO 10130:2009

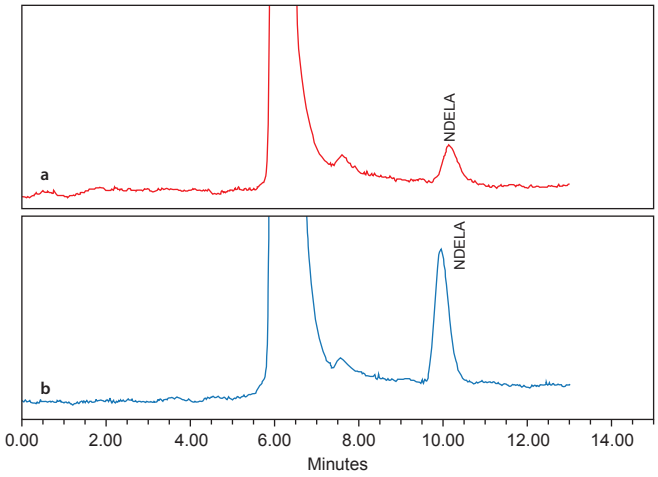


Fig 1. *Chromatograms of NDELA standard: a. 4.8 ppb; b. 16 ppb*

References

1. DIN EN ISO 10130:2009

Cosmetics - Analytical methods - Nitrosamines: Detection and determination of N-nitrosodiethanolamine (NDELA) in cosmetics by HPLC, post-column photolysis and derivatization.

2. A method for the determination of N-Nitrosodiethanolamine (NDELA) in Personal Care Products – Collaboratively evaluated by the CTPA Nitrosamines Working Group: Chris Flower, Stephen Carter, Andy Earls, Richard Fowler, Stewart Hewlins, Sam Lalljie, Mark Lefebvre, Jacqueline Mavro, David Small, and Nathalie Volpe

Analysis of Bromate in Drinking Water by HPLC and Post-column Derivatization (MA-120)

Bromate is a disinfection by-product that is formed when Ozone reacts with naturally occurring Bromide in drinking water. Bromate is a known animal carcinogen and has also been listed as a Group 2B toxin: probable human carcinogen. The U.S.EPA Method 300.1 employs conductivity as the means of detection which works well for most anions. However, the method is non-specific and coeluting interferences cannot be identified. The more recent U.S.EPA Method 317.0 utilizes a Bromate-specific reagent in a post-column reaction. This allows for a very specific and sensitive assay for Bromate in complex matrices.

Method

Equipment

- LC with a binary pump
- UV/VIS detector
- Pickering Laboratories Vector PCX or Onyx PCX
- Thermo Scientific Ion Pac™ AS9-HC Column Catalog No. 051786

Chemicals

- 9.0 mM Sodium carbonate
- Conc. Nitric acid (70 %)
- Potassium bromide
- o–Dianisidine
- Methanol

LC Conditions

LC Column Temperature: 42 ˚C

Sample Injection Volume: 250 µL

LC Flow Rate: 1.3 mL/min

Mobile Phase: 9 mM Na_2CO_3

Post-column Conditions

Post-column system: Pinnacle PCX

Reactor Volume: 0.5 mL

Reactor Temperature: 60 ˚C

Reagent: o–Dianisidine dihydrochloride (Add 40 mL of 70 % HNO_3 to 300mL deionized water in a 500mL volumetric flask. Dissolve 2.5g KBr in this solution. Dissolve 250 mg of o–Dianisidine dihydrochloride in 100 mL of Methanol and add to the Nitric acid/KBr solution and dilute to volume.)

Flow Rate: 0.7 mL/min

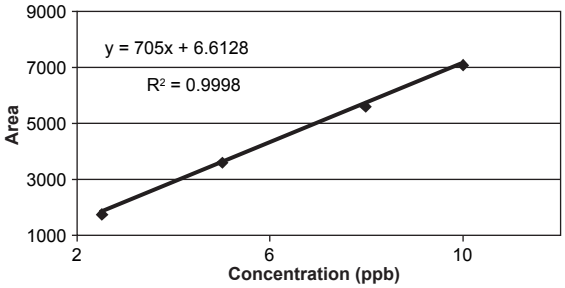
Detection: UV/VIS detector, λ max = 450 nm

References

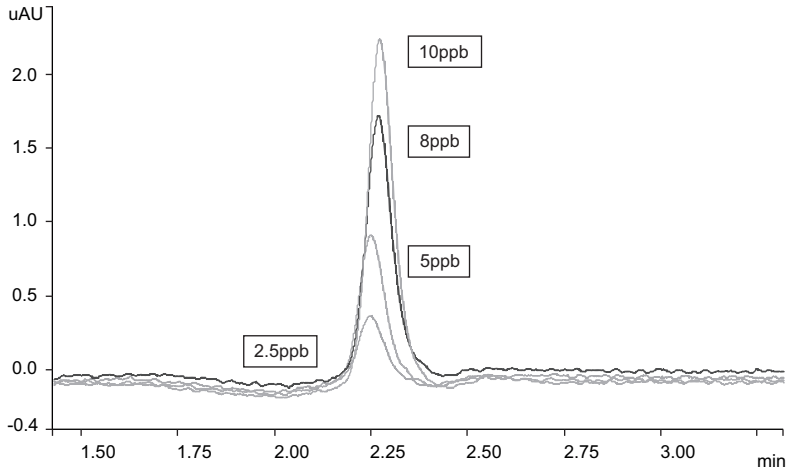
U.S.EPA Method 317.0. Determination of inorganic oxyhalide disinfection by-products in drinking water using ion chromatography with the addition of a post-column reagent for trace bromate analysis.

H.P.Wagner, B.V.Pepich, D.P.Hautman and D.J.Munch, J.Chromatography A, 882 (2000) 309 – 319.

C.R.Warner, D.H.Daniels, F.L.Joe and G.W.Diachenko, Food Additives and Contaminants, vol. 13, No.6 (1996) 633 – 638.



Calibration Curve



Overlaid chromatograms of Bromate standards

Analysis of Supplements

Analysis of Theanine in Tea by HPLC With Post-Column Derivatization (MA-378)

Theanine is a neurologically active amino acid found in tea plants. Theanine is a dominant amino acid in green tea and is responsible for its unique pleasant taste as well as known relaxation effect. Theanine also has been proven to reduce physical and mental stress, and improve cognition and mood.

Cation-exchange chromatography using post-column Ninhydrin reagent Trione and UV detection has shown unmatched reproducibility and selectivity in the analysis of free amino acids in complex matrices. The Pinnacle PCX post-column derivatization system allows shortening run times by utilizing column temperature gradients.

We introduce a simple, fast and robust method for analysis of Theanine. The same column and solutions could be used for extended analysis to determine other free amino acids in tea leaves.

Method

Sample Preparation

Homogenize 2 g of dry tea leaves with 25 mL of Li220 for 5 min. Centrifuge and filter through 0.45 um Nylon filter.

Analytical Conditions

Column: High-efficiency Lithium cation-exchange column, 4.6x75 mm, Catalog number 0354675T

Flow Rate: 0.55 mL/min

Mobile Phase: see method

Injection Volume: 10 uL

Post-Column Conditions

Post-Column System: Onyx PCX or Pinnacle PCX

Reactor Volume: 0.5 mL

Reactor Temperature: 130 °C

Reagent: Trione Ninhydrin reagent

Flow Rate: 0.3 mL/min

Detection: UV/VIS 570 nm for primary amino acids, 440 nm for secondary amino acids

HPLC Gradient			
Time	**1700-1125 %**	**Li365 %**	**RG003 %**
0	100	0	0
10	100	0	0
19	40	60	0
19.1	0	0	100
24	0	0	100
24.1	100	0	0
36	100	0	0

Column Oven Program	
Time	**Temperature °C**
0	34
6	34
17	65
19	34

Run Time: 24 Min
Equilibration Time: 12 Min

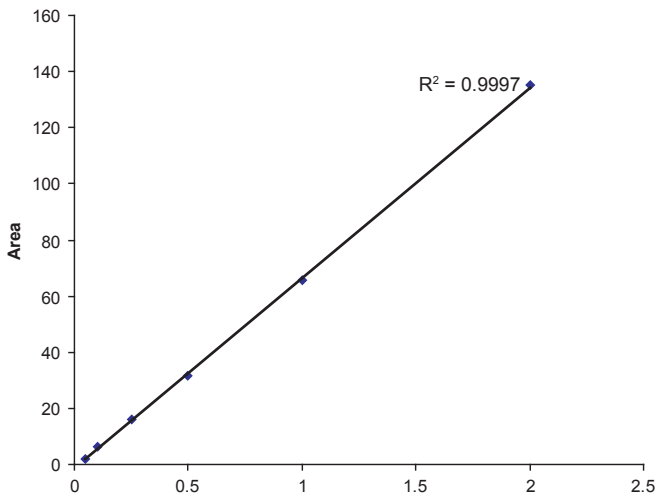


Fig 1. *Calibration curve for Theanine. Concentration range 0.05-2 umole/mL*

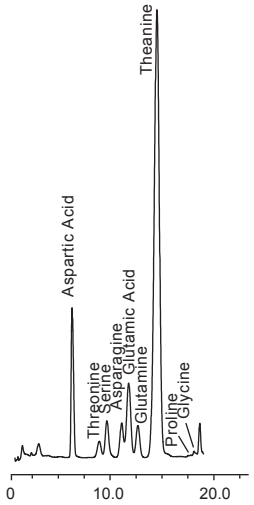


Fig 2. *Chromatogram of green tea sample*

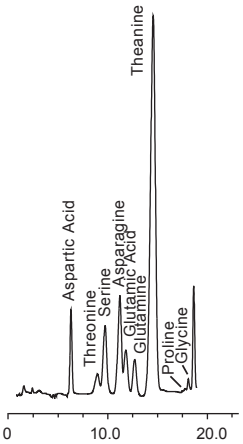


Fig 3. *Chromatogram of white tea sample*

Simultaneous Analysis of Vitamins B1, B2, B3 and B6 in Protein Powders and Supplements (MA-239)

B vitamins are a group of water soluble vitamins that play an important role in cell metabolism. This group consists of a number of compounds including Thiamine (Vitamin B1), Riboflavin (Vitamin B2), Niacin and Nicotinamide (Vitamin B3) and Pyridoxine and Pyridoxal (Vitamin B6). B vitamins are found in plant and animal food sources, such as legumes, nuts, green leafy vegetables, red meat and poultry. Many commercial food products are fortified with vitamin B complex and supplementation with multi-vitamins is common to fight deficiencies.

Pickering Laboratories offers a method for simultaneous determination of Vitamins B1, B2, B3 and B6 in supplements and protein powders. The method uses chemical and photochemical post-column derivatization with Fluorescence detection that increases sensitivity and selectivity of analysis. Photochemical derivatization is required for Niacin and Nicotinamide and chemical derivatization is needed for Thiamine. Vitamins B2 and B6 have natural fluorescence.

Method

Calibration

Calibration Ranges
Thiamine: 0.03 – 10 ug/mL; Niacin: 0.125-10 ug/mL; Nicotinamide: 0.3 – 100 ug/mL; Riboflavin: 0.03-10 ug/mL; Pyridoxal: 0.125-10 ug/mL, Pyridoxine: 0.125-10 ug/mL.

To Make Riboflavin Stock Solution
Dissolve 20 mg of Riboflavin in 5 mL of 0.1 M NaOH and immediately add 50 mL of 0.1 M HCl and make up the solution with DI water to 500 mL. Make working standards by diluting the stock solution with 0.01 M HCl. Store protected from light.

Stock and working standards for Thiamine, Niacin, Nicotinamide, Ryridoxal and Pyridoxine are made in 0.01 M HCl and stored protected from light.

Instrument Set Up

Connect the Instruments in the Following Order
Onyx PCX post-column derivatization instrument

- UVE™ photochemical reactor – Fluorescence detector.

Sample Preparation

For Protein Powders
To 0.5 g of samples add 50 mL of extraction buffer (0.1 N NaOH adjusted to pH 2 with Phosphoric acid). Homogenize using hand held homogenizer for 30 sec and heat on a water bath at 100 °C for 30 min. Cool the solution down, filter through 0.45 um nylon filter and inject. Protect from light.

For Multi-Vitamins Supplements Tablets
Blend at least 10 tablets to a fine powder and mix the entire sample thoroughly. Weight 250 mg of sample and add 90 mL of DI water acidified to pH 2.6 with 0.1 N HCl. Stir using magnetic stirring plate for 2 hours, protecting from light. Make the volume up to 100 mL with acidified water. Filter the sample through 0.45 um nylon filter and inject.

Analytical Conditions

Analytical Column: Thermo Hypersil, Aquasil C_{18} (4.6x150 mm)

Column Temperature: 40 °C

Flow Rate: 1 mL/min

Mobile Phase:
Solvent A: Dissolve 4.77 g of Potassium Phosphate Monobasic in 900 mL of DI water, adjust pH to 5.9 with KOH. Bring volume to 1 L with DI water.

Solvent B: Acetonitrile. See Table 1 for gradient conditions.

Injection Volume: 20 uL

Post-Column Conditions

Post-Column Derivatization System: Onyx PCX, Pinnacle PCX

Reactor Volume: 0.5 mL

Reactor Temperature: 30 °C

Reagent: Dissolve 10 g of Sodium Hydroxide in 500 mL of DI water, add 2 g of Sodium Sulfite, mix till fully dissolved

Reagent Flow Rate: Initial flow rate 0 mL/min. See Table 2 for pump program

Detection: FLD, see Table 3 for detector program
UVE™ Photochemical Reactor

Table 1. HPLC Gradient		
Time	Solvent A %	Solvent B %
0	100	0
8	100	0
15	90	10
22	90	10
30	40	60
35	40	60
35.1	100	0
45	100	0

Table 3. FLD Program		
Time	Excitation (nm)	Emission (nm)
0	322	400
17.9	322	400
18	370	440
25	370	440
25.1	350	470
35	350	470

Table 2. Post-Column Pump Program	
Time	Flow Rate (mL/min)
0	0
16.9	0
17	0.5
23	0.5
23.1	0
35	0

Run Time: 35 Min
Equilibration Time: 10 Min

Table 4. Analysis of NIST Samples						
Compounds	Found in Protein Powder	RSD n=3	NIST Value for Protein Powder	Found in Vitamins Tablets	RSD n=3	NIST Value for Vitamins Tablets
Nicotinamide	282 ug/g	4.5%	258 ug/g	15288 ug/g	0.8%	13907 ug/g
Thiamine	12.6 ug/g	9.0%	15.8 ug/g	1145 ug/g	0.6%	1045 ug/g
Riboflavin	25.9 ug/g	11.2%	26.9 ug/g	1204 ug/g	1.8%	1302 ug/g

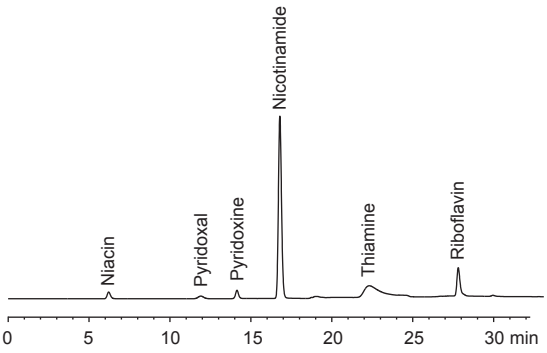


Fig 1. *Chromatogram of mixed B Vitamins standard solution. Niacin, Riboflavin, Pyridoxine, Pyridoxal, Thiamine – 1 ug/mL; Nicotinamide – 10 ug/mL*

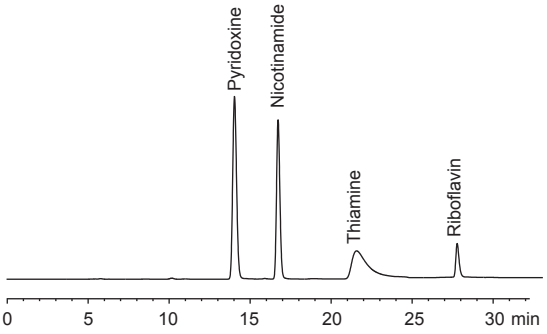


Fig 3. *Chromatogram of soy protein shake powder sample*

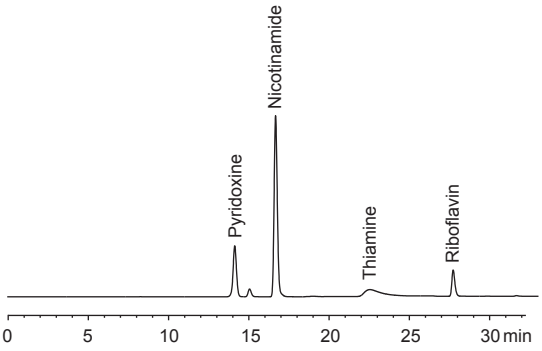


Fig 2. *Chromatogram of NIST Multivitamin tablets sample*

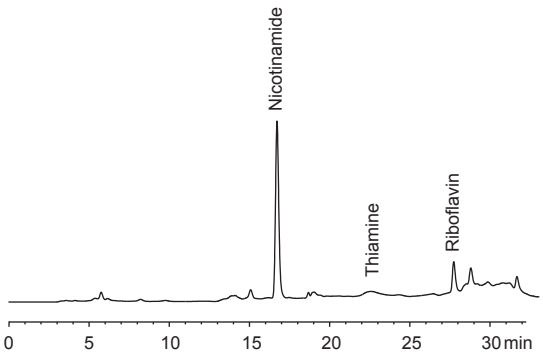


Fig 4. *Chromatogram of NIST chocolate protein drink mix sample*

Analysis of Antioxidants in Foods and Dietary Supplements Using HPLC With Post-Column Derivatization (MA-231)

Antioxidants protect cells from the damaging effects of free radicals and offer numerous benefits for human health. Many phenolic compounds found in plants, as well as some vitamins, exhibit antioxidant activity. Several colorimetric assays exist to measure the total antioxidant capacity, typically expressed as Trolox or Gallic acid equivalents.

An increased interest in antioxidants has created a demand for methods that are not only capable of determining the total antioxidant activity of the sample, but also are able to identify and quantify individual compounds known for their biological benefits. Our method abstract demonstrates that the well-known colorimetric reagents, such as Folin-Ciocalteu and 2,2'-azinobis(3-ethylbenzothiazoline)-6-sulfonate (ABTS), can be successfully used for analysis of antioxidants in foods and dietary supplements by HPLC with post-column derivatization.

Reagents Overview

Folin-Ciocalteu Reagent (FCR) reacts with reducing compounds to form a chromophore that can be detected using UV/Vis detection. Phenolic and non-phenolic antioxidants, including Vitamin C, Vitamin B1 and Folic acid, can be analyzed using this reagent with high sensitivity. Many antioxidants can be easily detected in solution at levels as low as 1 ug/mL.

The ABTS reagent is used in the Trolox-equivalent antioxidant capacity (TEAC) assay. The reaction is based on suppression of the absorbance of radical cations by antioxidants, and can be detected by UV/Vis detection as negative peaks on the chromatograms. This indirect detection method allows for analyzing common antioxidants at levels between 40 and 200 ug/mL.

Using both reagents, it is possible both to identify and quantify specific target compounds, as well as to calculate the total antioxidant capacity of the sample by comparing the total area of all the peaks on the chromatogram with Trolox or Gallic Acid calibration curves.

Method

Sample Preparation

- *Fresh Fruit:* Homogenize 10 g of fresh fruit with 20 mL of water/methanol (50:50) extraction solution for 5 min. Centrifuge and filter through 0.45 um Nylon filter
- *Dried Fruit:* Mix 4 g of finely ground dried fruit with 20 mL of 100% Methanol. Shake for 1 h on a mechanical shaker. Centrifuge and filter through 0.45 um Nylon filter.
- *Dietary Supplements:* Mix 100 mg of finely ground sample with 10 mL of 100% Methanol. Shake for 30 min on a mechanical shaker. Centrifuge and filter through 0.45 um Nylon filter.
- *Dry Tea Leaves:* Homogenize 1 g of dry tea leaves with 25 mL of water/methanol (50:50) extraction solution for 5 min. Centrifuge and filter through 0.45 um Nylon filter. Alternatively, mix 1 g of dry tea leaves with 25 mL of hot water, stir on a hot plate for 5 min. Cool down, centrifuge and filter through 0.45 um Nylon filter.
- *All extracts can be diluted if needed with water/methanol (50:*50).

Analytical Conditions

Column: Reversed-phase C_{18} column, 4.6x150 mm

Temperature: 40 °C

Flow Rate: 1 mL/min

Mobile Phase: 4.8% Acetic acid in water, Methanol

Injection Volume: 10-50 uL

Post-Column Conditions

Post-Column System: Onyx PCX, Pinnacle PCX or Vector PCX

Reactor Volume: 1.4 mL

Reactor Temperature: 130 °C

Reagents:

Option 1: 40% of Folin-Ciocalteu reagent in water

Option 2: 15% of ABTS stock in Methanol. ABTS stock solution: 7 mmole/L of 2,2'- azinobis(3-ethylbenzothiazoline)-6-sulfonate is dissolved in a 2.45 mmole/L solution of Sodium Persulphate in water. Stock solution should be stored in the dark for at least 12 h before making a working reagent.

Flow Rate: 0.1 mL/min

Detection: UV/Vis 635 nm

HPLC Gradient		
Time	**Acetic Acid / water %**	**Methanol %**
0	95	5
3	95	5
25	40	60
30	40	60
30.1	95	5
40	95	5

Calculated Total Antioxidant Capacity of Selected Samples		
Matrix	**Trolox Equivalent, ABTS Reagent**	**Gallic Acid Equivalent, Folin-Ciocalteu Reagent**
Fresh Raspberries	1.03 mg/g	0.63 mg/g
Fresh Blueberries	0.66 mg/g	0.35 mg/g
Dry Goji berries	2.39 mg/g	3.30 mg/g
Green tea	-	50.0 mg/g
Antioxidant Dietary Supplement	477.24 mg/g	120.0 mg/g

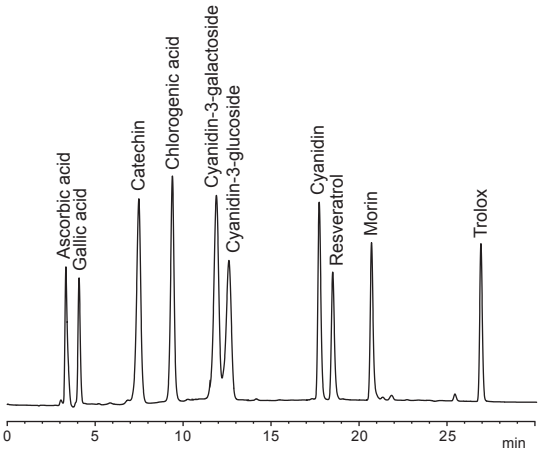


Fig 1. *Mixture of 10 common antioxidants, analyzed using Folin-Ciocalteu reagent*

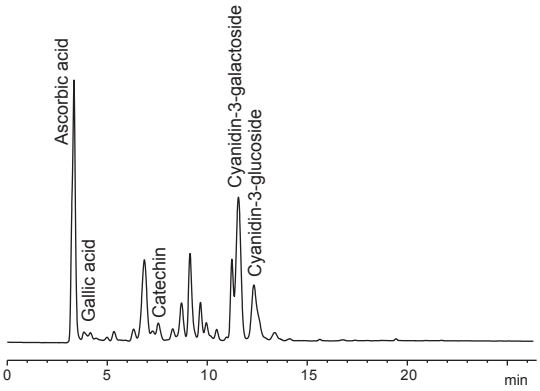


Fig 2. *Fresh raspberries extract, analyzed using Folin-Ciocalteu reagent*

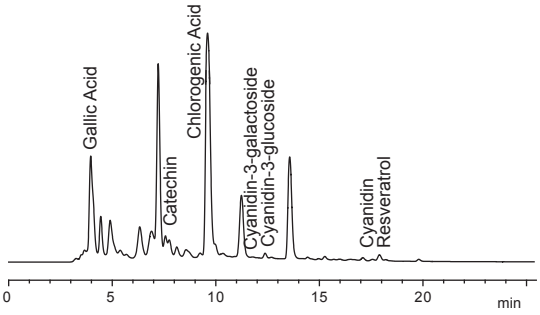


Fig 3. *Hot water extract of green tea, analyzed using Folin-Ciocalteu reagent*

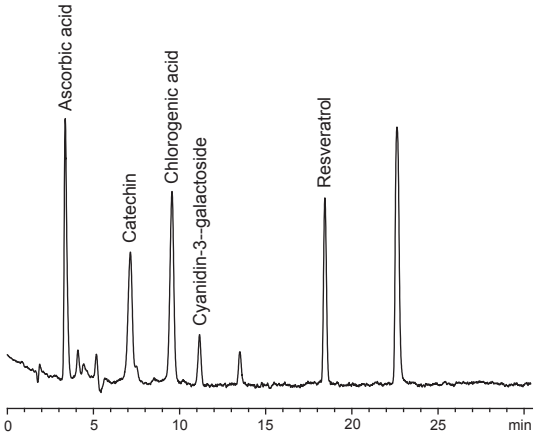


Fig 4. *Extract of dietary supplement, analyzed using ABTS reagent*

References:

Barbara Kusznierewicz, Anita Piasek, Agnieszka Bartoszek, Jacek Namiesnik, "The Optimization of Analytical Parameters for Routine Profiling of Antioxidants in Complex Mixtures by HPLC Couples with Post-column Derivatization," Phytochem. Anal. 2011, 22, 392-402

Analysis of Bioavailable Niacin (Vitamin B3) by HPLC With Post-Column Photochemical Derivatization in Foods and Supplements (MA-237)

Niacin (Nicotinic acid) is an essential nutrient important to human health. Free Nicotinic Acid and Nicotinamide have similar biological activity and both are used as dietary supplements to prevent Vitamin B3 deficiency. In supplements, the use of Nicotinamide is often preferred due to absence of common side effects of Nicotinic Acid, such as skin flushing.

The European Committee for Standardization has approved the HPLC method with post-column photochemical derivatization to measure Vitamin B3 in foodstuff. UV irradiation converts Nicotinic Acid and Nicotinamide into highly fluorescent derivatives. The addition of Pickering Laboratories' UVE™ Photochemical reactor to any HPLC system allows for highly sensitive and selective analysis of both Nicotinic Acid and Nicotinamide in a variety of different matrices.

Method

Calibration

- *Calibration Range:* Nicotinic Acid: 0.1 – 50 ug/mL, R2=0.999; Nicotinamide: 0.1 – 50 ug/mL, R2=0.998

Sample Preparation

The sample preparation described below is designed to measure bioavailable (or free) Nicotinic Acid and Nicotinamide in foods. Different procedures can be employed to prepare samples for the analysis of total Niacin.

- *Food Samples:* To 5 g of sample, add 30 mL of 0.1 N HCl. Blend at high speed for 2-3 min and heat the mixture at 100 °C for 1 h. Cool the mixture to room temperature, transfer into a graduated cylinder and adjust the volume to 50 mL with DI water. Dilute further with water to fit the calibration curve as needed. Filter the solution through a 0.45 um filter.
- *For High Protein / High Fat Matrices:* Proceed as directed above. After filtering, pipette 4 mL of the solution into a centrifuge tube. Add 1 mL of 50% (w/v) solution of Trichloroacetic Acid in water to precipitate proteins. Cool the mixture in an ice water bath for 5 min. Centrifuge and filter through a 0.45 um syringe filter.
- *Dietary Supplement:* Thoroughly mix the content of at least 10 finely ground capsules / tablets. Dissolve 100 mg portion in 100 mL of DI water. Dilute further with water to fit the calibration curve as needed. Filter through a 0.45 um filter.

Analytical Conditions

Analytical Column: ThermoHypersil, Aquasil C_{18} (150 x 4.6 mm)

Temperature: 40 °C

Flow Rate: 1 mL/min

Mobile Phase: Methanol / Phosphate buffer (0.035 mol/L of Potassium Phosphate Monobasic adjusted to pH 4.45). See Table 1 for gradient conditions.

Injection Volume: 20 uL

Post-Column Conditions

UVE™ Photochemical Reactor

Detection: FLD, λ_{ex} : 322 nm, λ_{em} : 370 nm

Table 1. HPLC Gradient

Time	Phosphate Buffer %	Methanol %
0	100	0
25	100	0
25.1	0	100
29	0	100
29.1	100	0
35	100	0

Table 1. Niacin Analysis in Foods

Sample	Fresh Peas		Fresh Tomatoes		Raw Ground Pork		Oat Cereal	
	Nicotinic Acid	Nicotinamide	Nicotinic Acid	Nicotinamide	Nicotinic Acid	Nicotinamide	Nicotinic Acid	Nicotinamide
Found in the sample	2.77 ug/g	5.35 ug/g	1.30 ug/g	3.33 ug/g	2.48 ug/g	60.95 ug/g	7.91 ug/g	243.30 ug/g
RSD, N=4	1.8%	1.5%	1.6%	2.0%	2.3%	0.7%	3.8%	0.6%
Spike	10 ug/g	10 ug/g	10 ug/g	10 ug/g	10 ug/g	50 ug/g	20 ug/g	300 ug/g
Recoveries	93%	94%	98%	92%	70%	102%	109%	96%
RSD, N=3	3.8%	2.0%	1.1%	1.4%	0.8%	1.5%	3.2%	2.1%

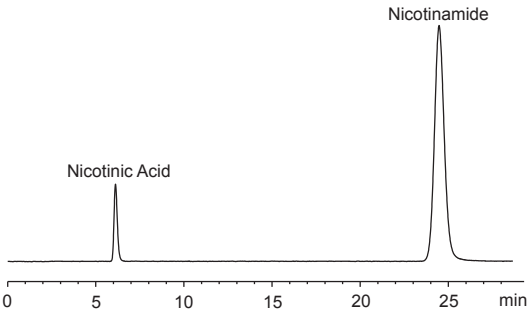


Fig 1. *Chromatogram of 1 ug/mL calibration standard of Nicotinic Acid and Nicotinamide*

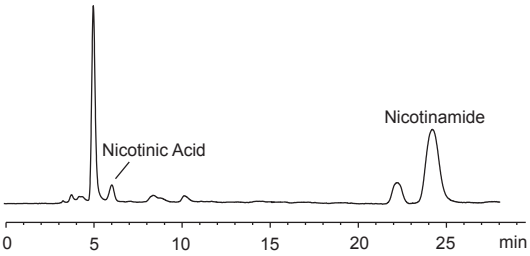


Fig 2. *Chromatogram of fresh peas sample*

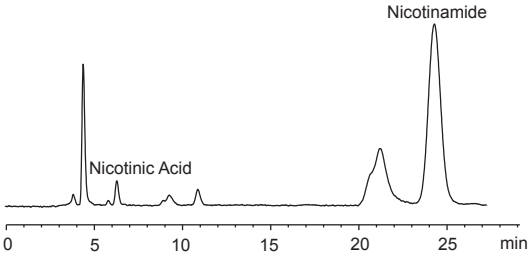


Fig 3. *Chromatogram of fresh tomatoes sample*

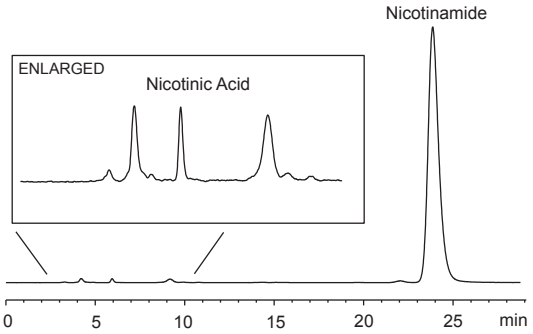


Fig 4. *Chromatogram of raw pork sample*

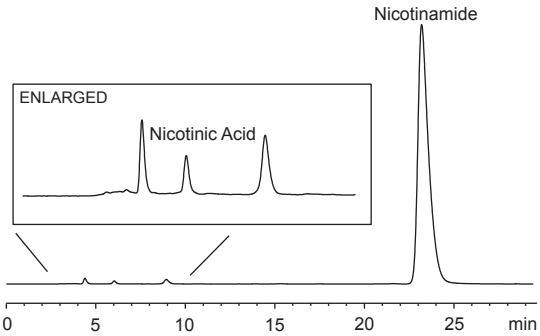


Fig 5. *Chromatogram of oat cereal sample*

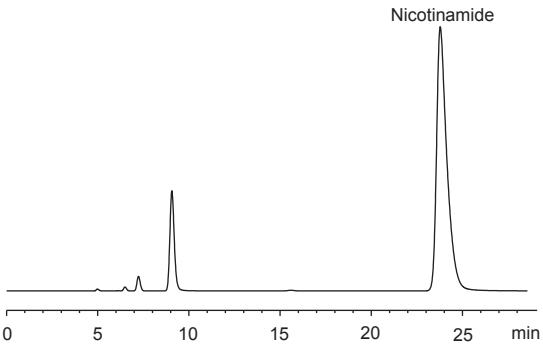


Fig 6. *Chromatogram of Vitamin B Complex dietary supplement*

Analysis of Vitamin B1 in Foods and Dietary Supplements by HPLC With Post-Column Derivatization and Fluorescence Detection (MA-233)

Thiamine (vitamin B1) plays important role in many cellular processes and its deficiency can quickly lead to serious health problems. Since humans and animals can't synthesize vitamin B1, they must obtain a sufficient amount through their diet. The requirements of nutritional labeling have led to increased demand for methods to analyze vitamin B1 in different matrices.

This application note describes a sensitive and accurate HPLC method capable of measuring Thiamine and its biologically active phosphorylated derivatives in foods and dietary supplements. Thiamine and its derivatives are separated on a reversed-phase column and converted using post-column derivatization into highly fluorescent compounds. To determine total vitamin B1 content in foods, an enzymatic reaction with Taka-diastase was employed to convert all Thiamine esters to free Thiamine.

Method

Sample Preparation

- *Food Samples:* To 5 g of sample add 60 mL of 0.1 N HCl, blend at high speed for 2-3 min and heat the mixture at 100 °C for 1 h. Cool the mixture to room temperature and adjust pH to 4.0 – 4.5 using 2.5 M Sodium Acetate solution. Add 200 mg of Taka-diastase, shake well and incubate for 18 h at 45 °C. After enzymatic hydrolysis is complete add 2 mL of 50% Trichloroacetic acid solution in water and heat at 100 °C for 15 min to precipitate proteins. Adjust pH to 2.6 – 2.8 with Sodium Acetate and bring the volume to 100 mL with DI water. Filter through 0.45 um filter.
- *Dietary Supplements:* Mix the contents of at least 10 capsules. Take 250 mg portion and dissolve in 100 mL of DI water acidified to pH 2.6-2.8 with 0.1 N HCl. Dilute the solution further with acidified water to fit the calibration curve as needed. Filter through 0.45 um filter.

Standards Preparation

- *Standard solutions:* Prepare standard solutions of Thiamine and its derivatives in water acidified to pH 2.6 – 2.8 with HCl. Make fresh daily.
- *Calibration Range:* Thiamine: 0.1 – 25 ug/mL, R^2=0.999; Thiamine Pyrophosaphate: 0.02 – 5 ug/mL, R^2=0.999; Thiamine Monophosphate: 0.02 – 5 ug/mL, R^2=0.999.

Analytical Conditions

Analytical Column: ThermoHypersil, Aquasil C_{18}

Flow Rate: 1 mL/min

Column Temperature: 40 °C

Mobile Phase: 10% Acetonitrile - 90% Phosphate Buffer (6 g/L of Phosphoric Acid adjusted to pH 5.9 with NaOH)

Injection Volume: 10 uL

Post-Column Conditions

Post-Column System: Onyx PCX, Pinnacle PCX or Vector PCX

Reactor Volume: 0.5 mL

Reactor Temperature: 55 °C

Reagent: 40 g/L NaOH, 600 mg/L Potassium Ferricyanide in water

Reagent Flow Rate: 0.5 mL/min

Detection: FLD, λ_{ex} : 375 nm, λ_{em} : 430 nm

Table 1. Thiamine Analysis in Foods

Sample	Thiamine Found in the Sample	RSD, N=4	Thiamine Spike Concentration	Recoveries	RSD, N=4
Whole Yellow Peas	13 ug/g	1.5%	50 ug/g	92%	7.8%
Cereal	21.5 ug/g	3.6%	50 ug/g	102%	2.8%
Pork Sausages	5.0 ug/g	2.2%	50 ug/g	108%	1.6%

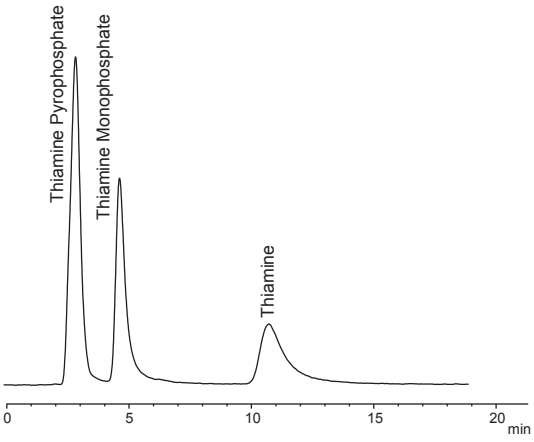


Fig 1. *Chromatogram of 1 ug/mL calibration standard of Thiamine and its phosphorylated derivatives*

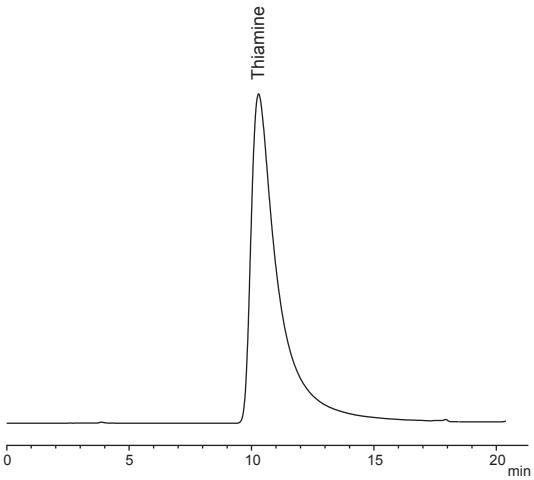


Fig 2. *Chromatogram of Vitamin B Complex supplement*

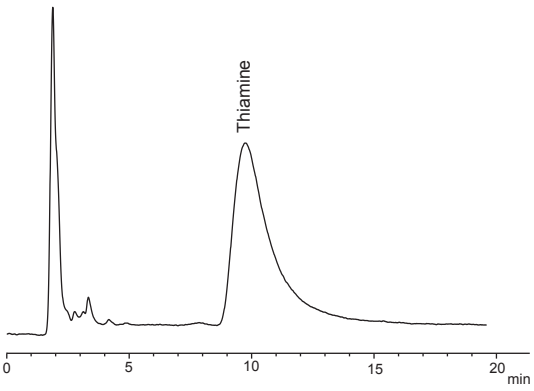


Fig 3. *Chromatogram of cereal sample*

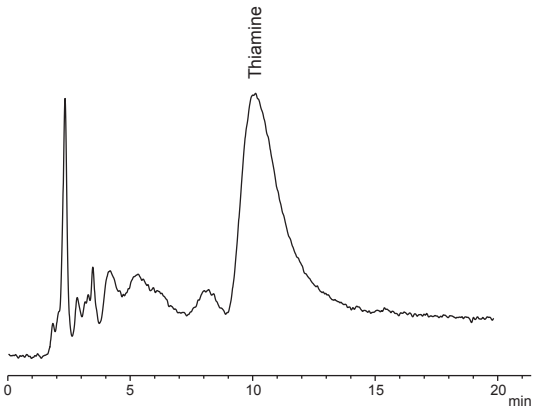


Fig 4. *Chromatogram of pork sausage sample*

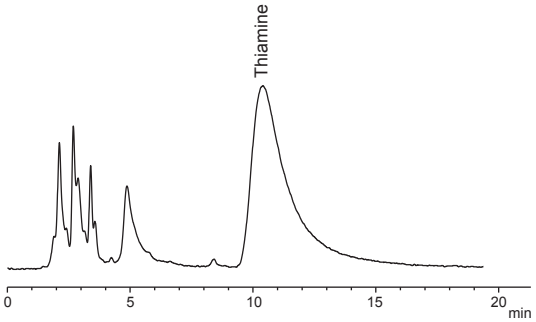
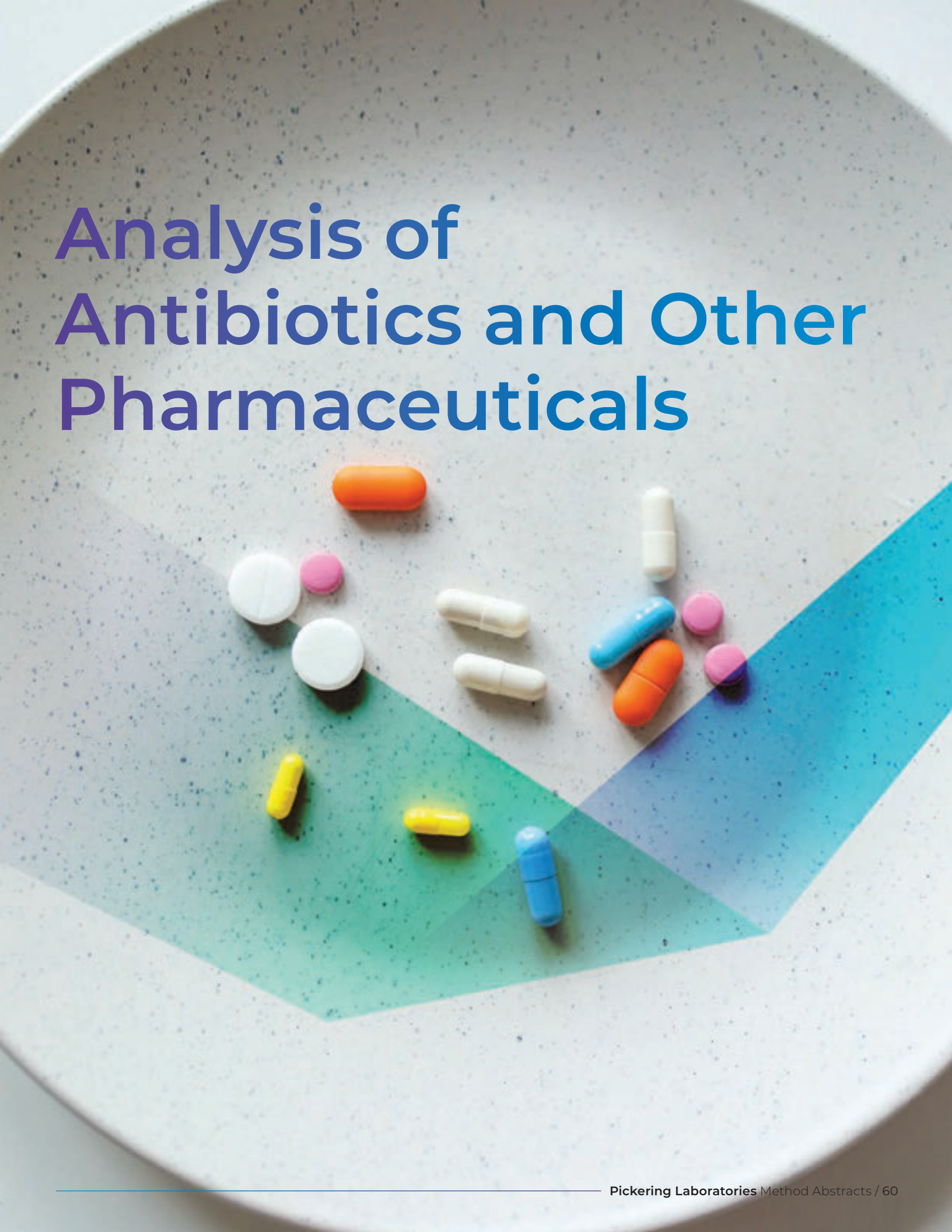


Fig 5. *Chromatogram of whole yellow peas sample*

Analysis of Antibiotics and Other Pharmaceuticals



Analysis of Alprostadil by HPLC With Post-Column Derivatization (MA-334)

Alprostadil (Prostaglandin E1) is a drug that has vasodilation properties and is used to treat erectile dysfunction (ED) and other medical conditions. This medication is available in injectable form and in suppository form.

Since Prostaglandin E1 has low UV absorbance, the analysis of the formulations could be difficult. The addition of post-column derivatization increases the sensitivity and specificity of the analysis.

Method

Analytical Conditions

Analytical Column: Reversed-phase C_{18} column (150 x 4.6 mm)

Temperature: 37 °C

Flow Rate: 1 mL/min

Mobile Phase: 30% Acetonitrile – 70% of 0.02 M Potassium Phosphate monobasic (adjusted to pH 3)

Or alternative conditions:

Mobile Phase: 25% Acetonitrile – 75% of 0.0067 M Potassium Phosphate buffer pH 6.3

Injection Volume: 20 uL

Post-Column Conditions

Post-Column System: Onyx PCX or Pinnacle PCX

Reactor Volume: 2 mL

Reactor Temperature: 60 °C

Reagent: 1 mol/L Potassium Hydroxide

Reagent Flow Rate: 1 mL/min

Detection: UV 278 nm

Calibration

Alprostadil: 0.1 ug/mL – 10 ug/mL

β-Naphthol (Internal Standard): 0.25 ug/mL – 10 ug/mL

Make stock solutions of Alprostadil and β-Naphthol in anhydrous Ethanol. Prepare working standards by appropriate dilution of stock solution with mobile phase.

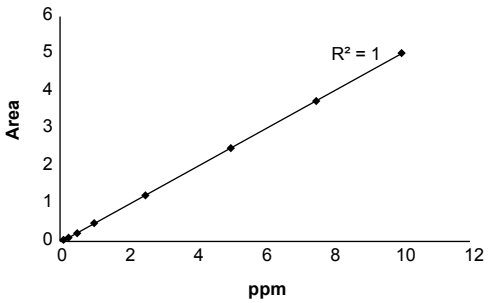


Fig 1. *Calibration curve for Alprostadil*

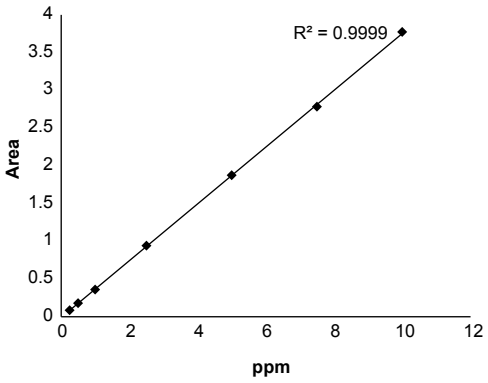


Fig 2. *Calibration curve for β-Naphthol*

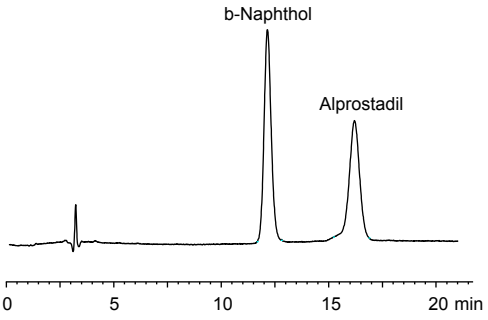


Fig 3. *Chromatogram of Alprostadil and β-Naphthol (Internal Standard)*

Aminoglycoside Antibiotics in Feeds (MA-205)

Apramycin, Gentamicin, Neomycin

Aminoglycoside antibiotics are among those commonly used in animal feeds to manage intestinal microorganisms. The beneficial effects include improved growth and generally healthier animal populations. Use of antibiotics creates a demand for analytical procedures to verify concentrations in pre-mixes and feeds and in some instances for residue analysis in animal products.

This note describes a simple, robust analytical method for the family of Aminoglycoside antibiotics in feeds and animal products. The sample is homogenized with a generic extraction solution and the crude soluble portion is directly injected into an HPLC ion-exchange column. The column effluent is then mixed with an OPA/Thiofluor™ reagent which forms highly fluorescent derivatives with the primary amine moieties of the antibiotics.

Method

Extraction Procedure

Take one part feed: 10 parts Extraction solution (30 g/L of Sulfosalicylic Acid) and homogenize for five minutes. Centrifuge for 10 minutes. Three layers will form: the pellet, a supernatant emulsion and a soft layer of floating fat. Carefully lift the floating fatty layer with a spatula and discard. Transfer the emulsion to a sealable vial. Coagulate the emulsion by placing the vial in a boiling water bath for 15 minutes. Centrifuge for 10 minutes. The clear supernate is filtered (0.45 um Nylon) and placed in an autosampler vial.

Analytical Conditions

Analytical Column: ALKION™ cation-exchange, 4 x 150 mm, Catalog No. 9410917

Guard Column: ALKION™, 3 x 20 mm, Catalog No. 9493020

Column Temperature: 40 °C

Flow Rate: 0.8 mL/min

Mobile Phase: 1700-1101, Potassium buffer, K01
1700-1102, Potassium titrant, K02
1700-1103, Potassium ionic strength adjuster, K03

Post-Column Conditions

Post-Column System: Onyx PCX, Pinnacle PCX or Vector PCX

Reactor Volume: 0.15 mL

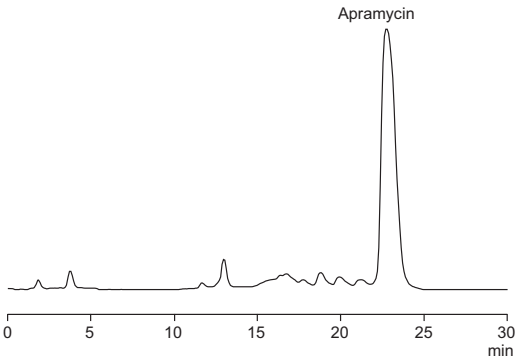
Reactor Temperature: 45 °C

Reagent: 300 mg of OPA, 2 g Thiofluor™, 3 mL of 30% Brij® 35 in 950 mL of OD104

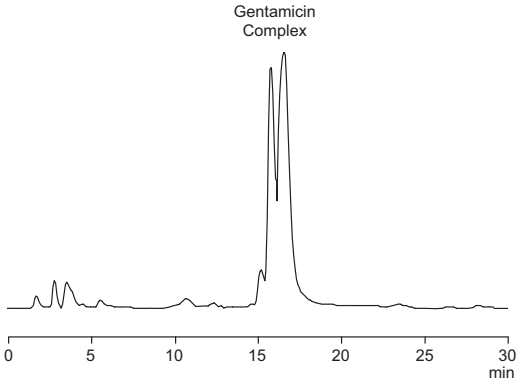
Flow Rate: 0.3 mL/min

Detection: FLD, λ_{ex} : 330 nm, λ_{em} : 465 nm

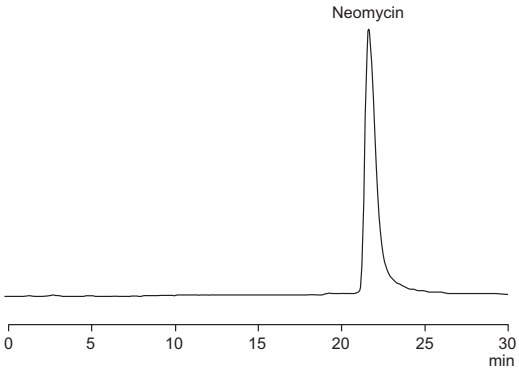
Conditions for Apramycin Analysis			
Time	1700-1101 %	1700-1102 %	1700-1103 %
0	67	33	0
5	67	33	0
15	14.7	7.3	78
20	14.7	7.3	78
20.1	0	22	78
21	0	22	78
21.1	67	33	0
28	67	33	0



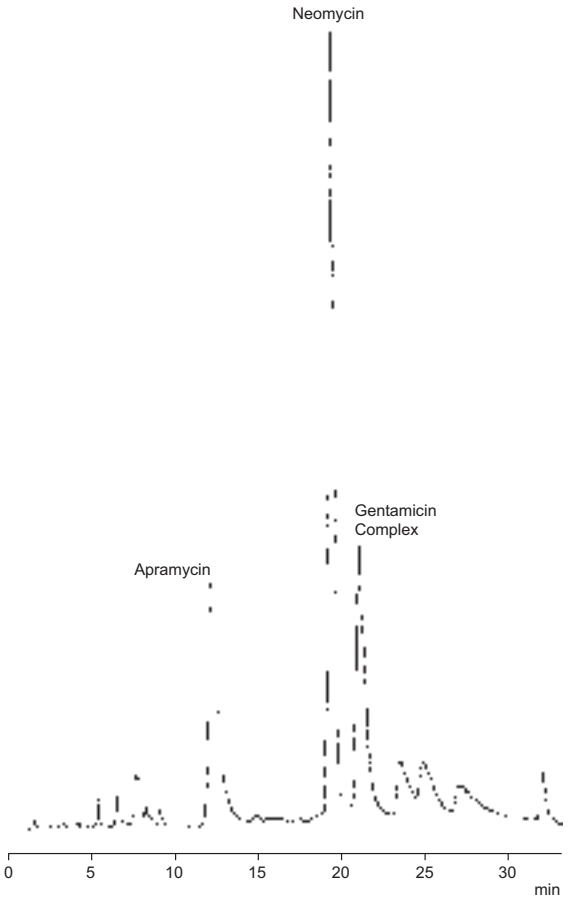
Conditions for Gentamicin Analysis			
Time	**1700-1101 %**	**1700-1102 %**	**1700-1103 %**
0	43	31	26
20	9	13	78
30	9	13	78
30.1	0	22	78
31	0	22	78
31.1	43	31	26
38	43	31	26



Conditions for Neomycin Analysis			
Time	**1700-1101 %**	**1700-1102 %**	**1700-1103 %**
0	60	40	0
15	13.2	8.8	78
25	11	11	78
25.1	0	22	78
26	0	22	78
26.1	60	40	0
32	60	40	0



Conditions for Separation of Apramycin Gentamicin and Neomycin			
Time	**1700-1101 %**	**1700-1102 %**	**1700-1103 %**
0	60	40	0
15	13.2	8.8	78
15.1	12	10	78
30	12	10	78
30.1	0	22	78
31	0	22	78
31.1	60	40	0
37	60	40	0



Analysis of Polyether Antibiotics in Animal Feeds by HPLC With Post-Column Derivatization (MA-264)

Polyether Antibiotics are commonly used for preventing coccidiosis and other infections in poultry and for improving feed efficiency for beef cattle and swine. The use of Polyether Antibiotics is strictly regulated, with only specific ionophores approved for use in feeds intended for different animals.

Analysis of Polyether Antibiotics by HPLC with post-column derivatization and UV/Vis detection has been proven to successfully identify and quantify Monensin, Narasin and Salinomycin in medicated feeds, supplements and premixes as well as to determine trace contamination levels in non-medicated feeds [1, 2].

Post-column derivatization of Polyether Antibiotics is done using highly acidic Vanillin or DMAB reagents. The Pinnacle PCX derivatization system (Pickering Laboratories, Inc.) has an inert flow path and automated system wash capabilities that make it uniquely suitable for handling corrosive reagents. The two-pump system is recommended to extend reagent stability, but the single-pump system for this application is also available.

Adding a Fluorescence detector to the instrumentation allows for using the same extraction procedure and HPLC conditions to also determine Lasalocid, which doesn't require post-column derivatization.

Method

Sample Preparation

To 25 g of finely ground feed sample, add 100 mL of extraction solution (90% Methanol - 10% water). Shake for 1 hour at high speed using mechanical shaker. Let the solids settle and filter an aliquot of the extract for injection. Dilute with extraction solution if needed to fit the calibration curve. Use 2.5 g portion when testing premixes.

Analytical Conditions

Analytical Column: Polyether Column, C_{18}, 4.6 x 250 mm, Catalog No 2381750

Guard Column: Reversed-phase guard cartridge, Catalog No 18ECG001

Temperature: 40 °C

Flow Rate: 0.7 mL/min

Mobile Phase: 90% Methanol, 10% of 5% Acetic Acid solution in water, isocratic

Injection volume: 20 µL

Post-Column Conditions

Post-Column System: Onyx PCX, Pinnacle PCX or Vector PCX

Reactor Volume: 1.4 mL

Reactor Temperature: 90 °C

Reagent 1: Concentrated Sulfuric acid / Methanol (4:96 v/v)

Reagent 2: 60 g of Vanillin in 950 mL of Methanol

Reagents Flow Rate: 0.3 mL/min

Detection: UV/VIS 570 nm (for Lasalocid – FLD, λ_{ex} : 322 nm, λ_{em} : 370 nm)

Calibration

Monensin A: 0.1 ppm – 50 ppm, R^2 = 0.999

Monensin B: 0.0035 ppm – 0.7 ppm, R^2 = 0.999

Lasalocid Acid: 0.25 ppm – 50 ppm, R^2 = 0.999

Table 1. Polyether Antibiotics in Certified Medicated Feeds

Antibiotic	Feed Type	Certified Amount	Found In Sample	Recoveries	RSD, N=4
Monensin	Beef feed	267 g/ton	275 g/ton	103%	0.7%
Lasalocid	Milk Replacer	72 g/ton	69 g/ton	96%	3.3%

Table 2. Spike Recoveries for Monensin

	Non-Medicated Bird Feed		Non-Medicated Rabbit Feed	
Antibiotic	Monensin A	Monensin B	Monensin A	Monensin B
Spike Level	172 g/ton	8 g/ton	86 g/ton	4 g/ton
Recoveries	100%	100%	101%	102%
RSD, N=3	1.9%	2.1%	1.1%	0.6%
Spike Level	3.44 g/ton	0.16 g/ton	3.44 g/ton	0.16 g/ton
Recoveries	96%	95%	94%	88%
RSD, N=3	0.7%	3.1%	0.9%	1.6%

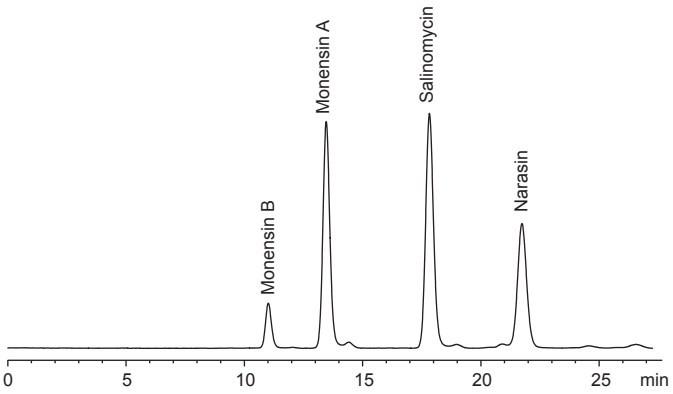


Fig 1. *Standard mixture of Monensin, Salinomycin and Narasin*

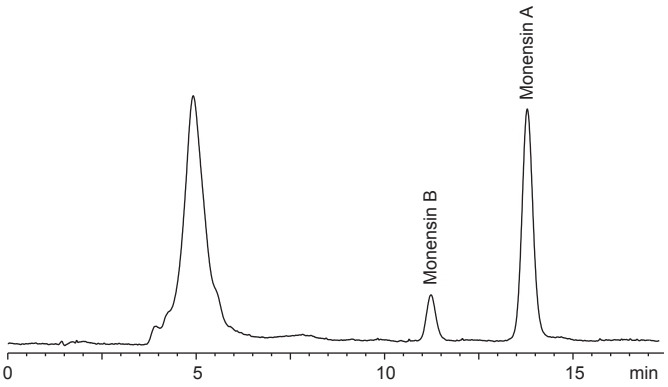


Fig 3. *Non-medicated bird feed sample spiked with Monensin A (3.44 ug/g) and Monensin B (0.16 ug/g)*

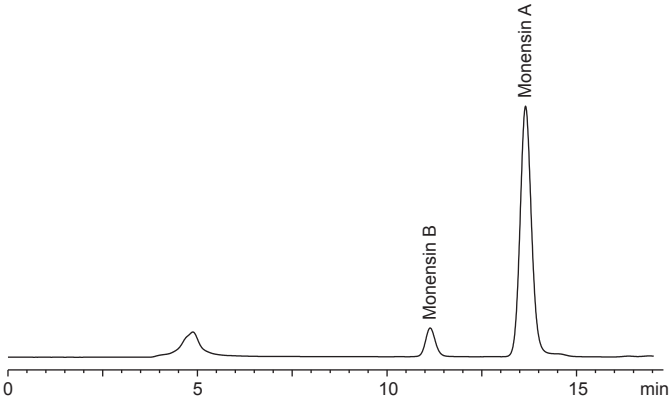


Fig 2. *Certified medicated beef feed sample containing 267 g/ton of Monensin*

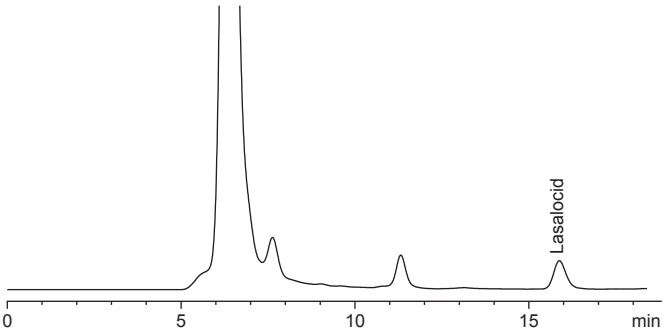


Fig 4. *Certified medicated milk replacer containing 72 g/ton of Lasalocid*

Conclusion

Analysis of Polyether Antibiotics by HPLC with post-column derivatization is a robust and sensitive method that utilizes standard equipment and could easily be adopted by testing laboratories. It allows for testing of different ionophores at wide range of concentrations, including at trace levels. Using Pickering Laboratories' post-column derivatization system, factory configured for the analysis, guarantees stable and reproducible results.

References

1. Campbell, H., Nayeri, G. (2006) J. AOAC Int. 89, 1229-1242, Determination of Monensin, Narasin, and Salinomycin in Mineral Premixes, Supplements, and Animal Feeds by Liquid Chromatography and Post-Column Derivatization: Collaborative Study
2. AOAC Official Method 997.04. Monensin in Premix and Animal Feeds

Analysis of Voglibose in Pharmaceutical Formulations (MA-327)

By HPLC With Post-Column Derivatization

Voglibose is an Alpha-Glucosidase inhibitor widely used for the treatment of diabetes. Alpha-glucosidase inhibitors are agents that delay the glucose absorption at the intestinal level and thereby prevent a sudden surge of glucose after a meal. Vogilbose is the safest and most effective drug of its class.

Since Vogibose has no UV chromophore, post-column derivatization is employed to produce a fluorescent derivative.

This abstract describes a very sensitive and robust analytical method for the analysis of Voglibose in pharmaceutical tablets. Simple sample preparation and fast analysis time allow for using this method in high throughput environments.

Method

Analytical Conditions

Column: Restek Pinnacle II Amino, 5 um, (250 x 4.6) mm, Catalog # 9217575

Temperature: 35 °C

Flow Rate: 0.6 mL/min

Mobile Phase: Sodium phosphate buffer, 20 mM pH 6.5 / Acetonitrile (37 : 63)

Injection Volume: 50 µL

Note: We strongly recommend that the column be flushed with acetonitrile water (80:20) for twenty minutes before making any injections.

Sample Preparation

Crush 5 tablets and mix with 25 mL of mobile phase. Sonicate for 10 min and filter liquid portion through 0.45 µm filter. Put in HPLC autosampler vial and inject 50 µL.

Repeatability Studies for Different Concentration Levels		
	0.5 ppm	**100 ppm**
Average RT, min	21.25	21.26
RSD, %, N = 6	0.36	0.08
Average Peak Area	9.22	1,562.69
RSD, %, N = 6	1.48	0.79

Post-Column Conditions

Post-Column System: Onyx PCX, Pinnacle PCX or Vector PCX

Heated Reactor Volume: 3.5 mL

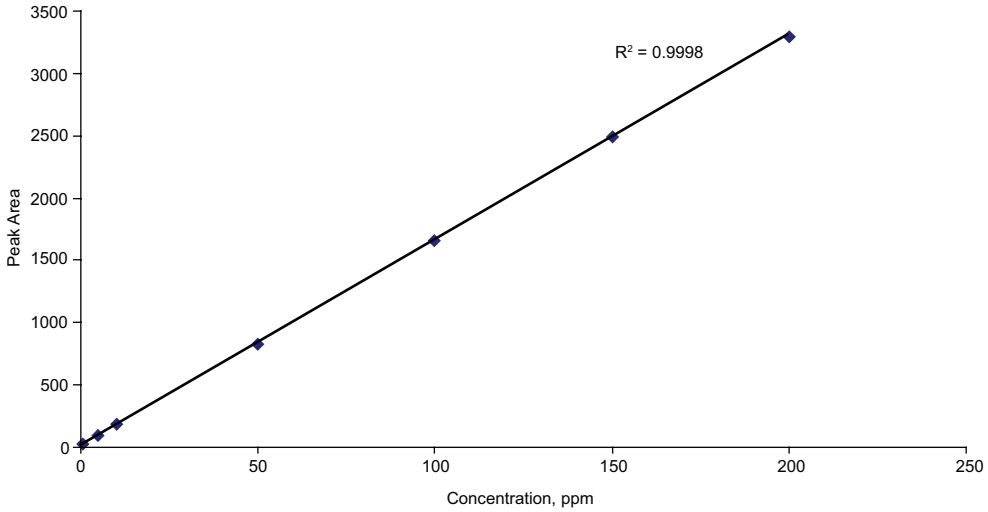
Temperature: 100 °C

Cooling Coil: 0.15 mL (at room temperature)

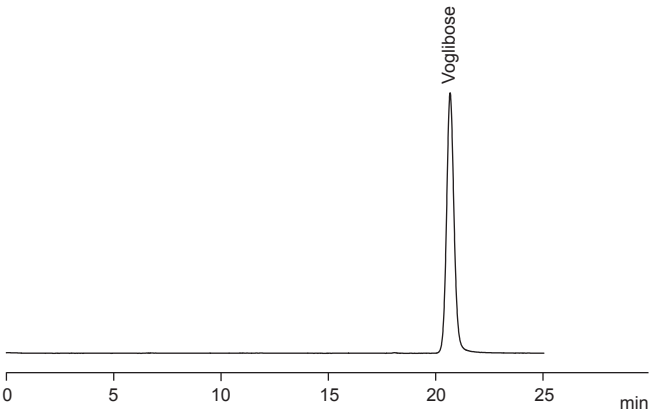
Reagent: Taurine (6.25 g), Sodium Periodate (2.56 g) in 1000 mL of water

Flow Rate: 0.6 mL/min

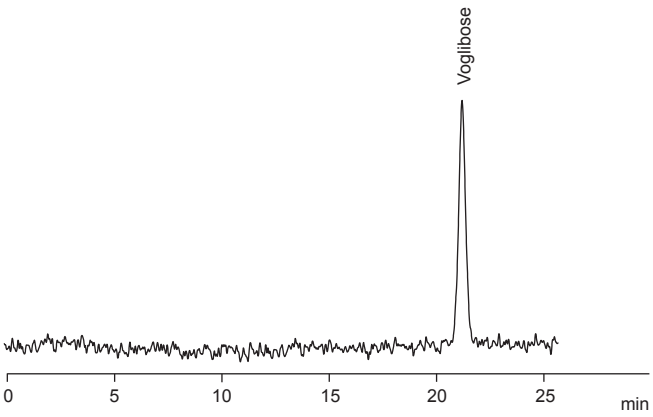
Detection: FLD
λ_{ex}: 350 nm, λ_{em}: 430 nm



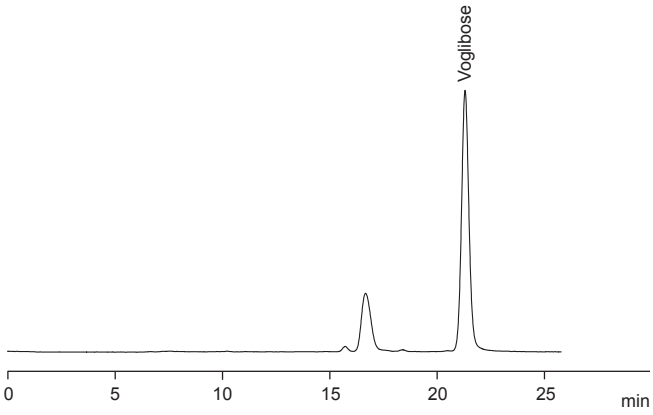
Calibration Curve for Analytical Range 0.5-200 ppm



Chromatogram of Voglibose Standard, 50 ppm, 50 µL Injection



Chromatogram of Voglibose Standard, 0.5 ppm, 50 uL Injection



Chromatogram of Voglibose Tablets (VolixTM, 0.2 mg), 50 µL Injection

Analysis of Apramycin Sulfate According to British Veterinary Pharmacopeia (MA-267)

Apramycin Sulfate is an aminoglycoside antibiotic that is used to treat colibacillosis and salmonellosis infections in calves, pigs, lambs and poultry. It is used as oral dosages as well as feed premixes and injectable formulations.

British Veterinary Pharmacopeia specifies tests to determine purity and quality of Apramycin Sulfate. Cation-exchange chromatography with post-column derivatization using Ninhydrin reagent is the recommended method for analysis of 3-hydroxyapramycin, lividamine, caerulomycin, 2-deoxystreptamine and other related substances commonly found as impurities in Apramycin Sulfate.

Pickering Laboratories' post-column derivatization systems and patented Trione Ninhydrin reagent can be successfully used to analyze the purity of Apramycin Sulfate according to Veterinary Pharmacopeia.

Method

Analytical Conditions

Column: Dionex IonPac Fast Cation I column, 4 x 250 mm (Product # SP5391)

Flow Rate: 0.8 mL/min

Column Temperature: 30 °C

Mobile Phase A: Aqueous solution of 1.961% w/v of sodium citrate, 0.08% w/v of phenol and 0.5% v/v of thiodiglycol, adjusted to pH 4.25

Mobile Phase B: Aqueous solution of 4.09% w/v of sodium chloride, 3.922% w/v of sodium citrate and 0.08% w/v of phenol, adjusted to pH 7.4

Injection Volume: 20 uL

Post-Column Conditions

Post-Column System: Onyx PCX, Pinnacle PCX or Vector PCX

Reagent 1: Trione® Ninhydrin reagent

Reactor Volume: 1.0 mL

Reactor Temperature: 130 °C

Flow Rate: 0.5-1.0 mL/min

Detection: DAD 568 nm

HPLC Program		
Time	**Mobile Phase A %**	**Mobile Phase B %**
0	75	25
3	75	25
9	0	100
30	0	100
30.1	75	25
40	75	25

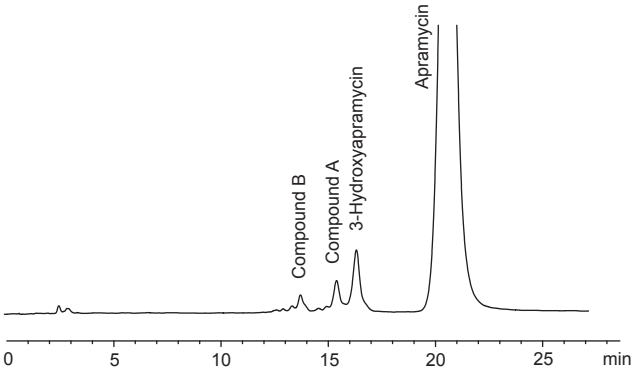


Fig 1. *Chromatogram of British Pharmacopeia Chemical Reference Substance (BPCRS)*

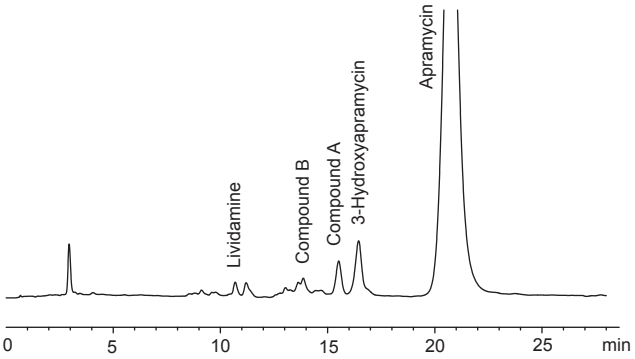
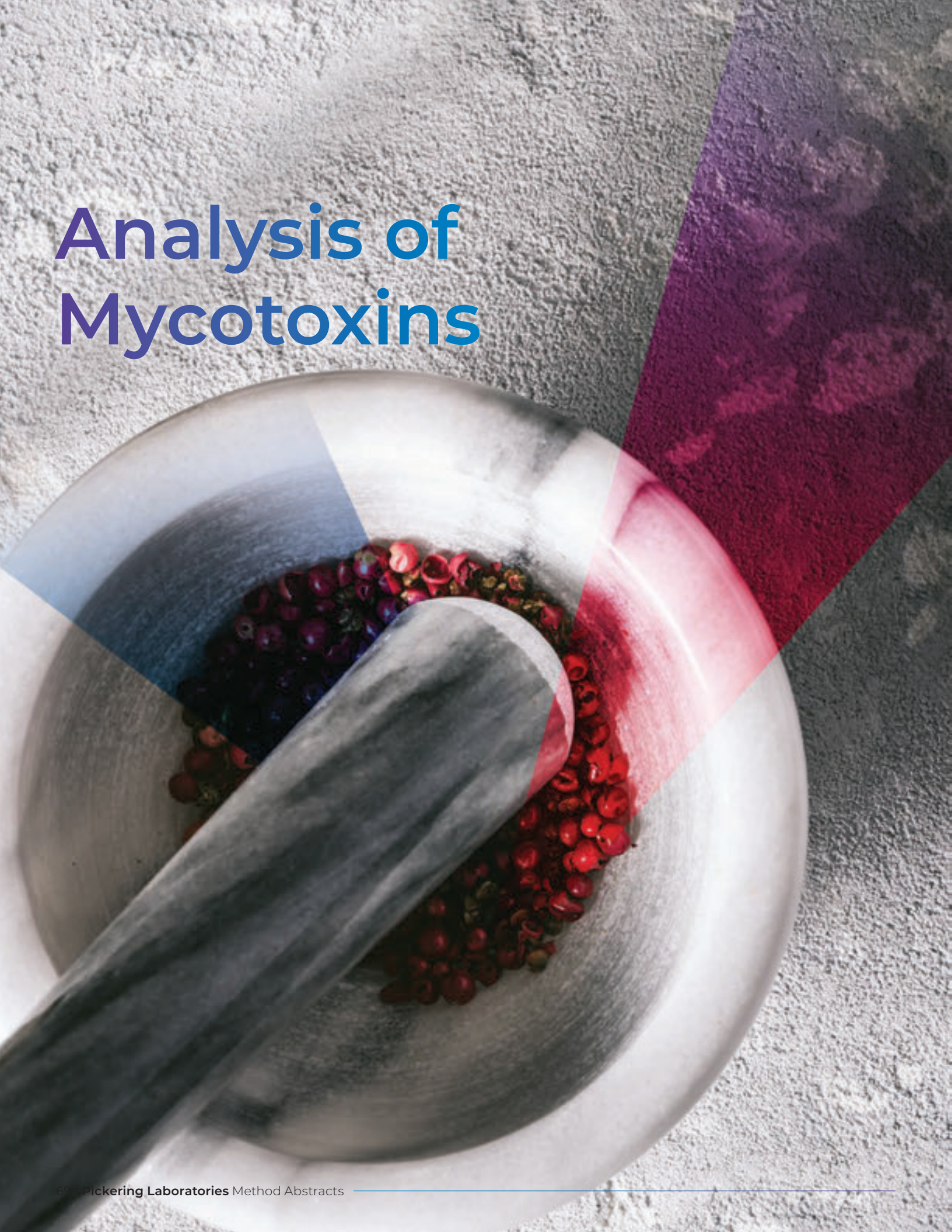


Fig 2. *Chromatogram of commercially available Apramycin Sulfate*

Analysis of Mycotoxins



Clean-Up and Analysis of Aflatoxins and Ochratoxin A in Herbs and Spices (MA-218)

The production of herbal supplements and spices is a fast growing industry. Unfortunately, the raw materials are often imported from countries that lack adequate quality control and whose weather conditions during the growing season, along with improper harvesting and storage practices, can cause toxic mold contamination. There are numerous reports on the presence of Mycotoxins in commercially available herbs and spices such as chamomile, black and white tea leaves, ginkgo leaves, paprika and cumin.

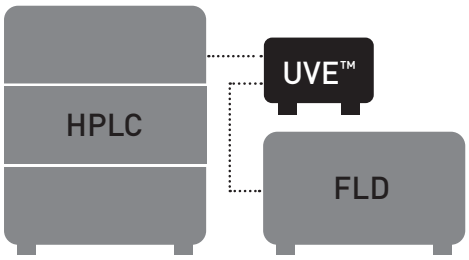
We developed a simple, sensitive and robust HPLC method for analyzing Aflatoxins B1, B2, G1, G2 and Ochratoxin A in herbs and spices. AflaOTAClean™ Immunoaffinity columns contain antibodies specific for both classes of Mycotoxins and allow for fast and efficient sample clean-up. We used the AcceClean™ automated workstation, which processes three samples simultaneously.

Post-column photochemical derivatization was used to increase the sensitivity of detection of Aflatoxins B1 and G1. The UVE™ (LCTech, Germany) photochemical reactor requires no additional reagents and is easy to install between the HPLC column and fluorescence detector. Ochratoxin A is a naturally fluorescent compound that does not require derivatization and can be analyzed together with all four Aflatoxins.

HPLC Gradient:

Time	1700-1108 %	Methanol %	Acetonitrile %
0	57	28	15
13	57	28	15
13.1	40	60	0
23	40	60	0
23.1	0	100	0
28	0	100	0

Equilibration: 10 min



Flow Diagram for UVE™ Photochemical Reactor

Method

Isolation of Aflatoxins B1, B2, G1, G2 and Ochratoxin A

Mix 5 g of finely ground sample with extraction solution (25 mL of Methanol:water 80:20, 12.5 mL of Hexane, 0.5 g of NaCl) and shake on a mechanical shaker for 1 hour. Filter the extract through fluted paper. Dilute 14 mL of the aqueous layer with 86 mL of PBS buffer (pH 7.2), filter and apply 11 mL of solution to AflaOTAClean™ Immunoaffinity column at a flow rate of 2 mL/min. Wash the column with 10 mL of water at a flow rate of 2 mL/min. Elute the toxins with two 1 mL portions of Methanol at a flow rate of 0.3 mL/min. Allow 5 min before applying the second portion of the Methanol to ensure complete breaking of the antibody-toxin bond.

Analytical Conditions

Analytical Column: MYCOTOX™ Reversed-phase Column, 4.6 x 250 mm, P/N 1612124

Guard Column: Reversed-phase guard cartridge, P/N 18ECG001

HPLC Eluent: Sodium Phosphate buffer (Cat #1700-1108), Methanol, Acetonitrile

Flow Rate: 1 mL/min

Post-Column Photochemical Reactor: UVE™ (Cat # 10519 (240V), Cat # 10742 (120 V)

Injection Volume: 30 µL

FLD: λ_{ex} : 365 nm, λ_{em} : 430 nm for Aflatoxins
λ_{ex} : 335 nm, λ_{em} : 455 nm for Ochratoxin A

Results & Discussion

The 6-point calibration curves were built in the range of 0.12-11.49 ppb for B1, 0.04-3.29 ppb for B2 and G2, 0.1-9.85 ppb for G1, 0.263-25.23 ppb for Ochratoxin A with R2 exceeding 0.999.

The samples of echinacea, ginger and ginseng were purchased from a local herbal store. These samples were not naturally contaminated with Mycotoxins.

There were no matrix interferences present after Immunoaffinity clean-up. The samples were spiked with five Mycotoxins at two levels and processed, along with sample blanks, as described above. The recovery data for Aflatoxins B1, B2, G1, G2 and Ochratoxin A are presented in Tables 1-3.

Table 1. Recovery Results for Echinacea Sample

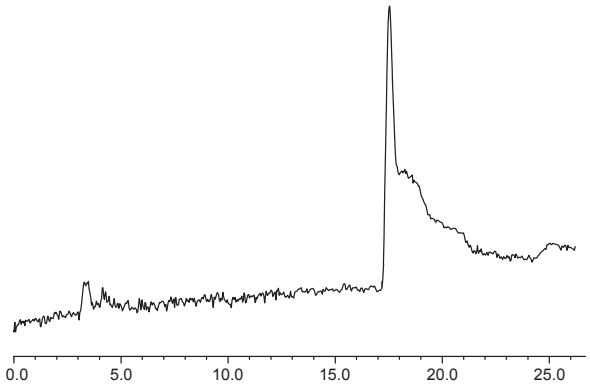
Mycotoxins	Aflatoxin B1	Aflatoxin B2	Aflatoxin G1	Aflatoxin G2	Ochratoxin A
Spike level, ng/g	5.06	1.45	4.33	1.45	10.1
Recoveries, %	96	97	84	62	72
Spike level, ng/g	2.53	0.72	2.16	0.72	5.05
Recoveries, %	77	80	78	63	80

Table 2. Recovery Results for Ginger Sample

Mycotoxins	Aflatoxin B1	Aflatoxin B2	Aflatoxin G1	Aflatoxin G2	Ochratoxin A
Spike level, ng/g	5.06	1.45	4.33	1.45	10.1
Recoveries, %	72	78	86	75	62
Spike level, ng/g	2.53	0.72	2.16	0.72	5.05
Recoveries, %	66	89	74	59	60

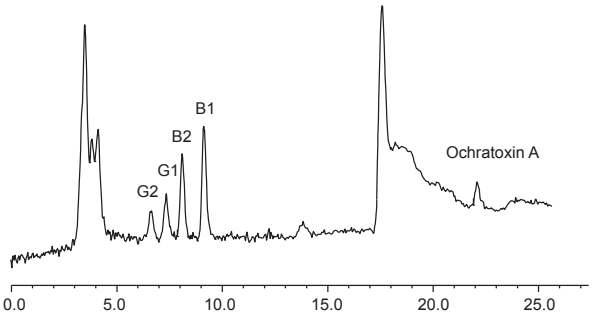
Table 3. Recovery Results for Ginseng Sample

Mycotoxins	Aflatoxin B1	Aflatoxin B2	Aflatoxin G1	Aflatoxin G2	Ochratoxin A
Spike level, ng/g	5.06	1.45	4.33	1.45	10.1
Recoveries, %	87	89	86	97	68
Spike level, ng/g	2.53	0.72	2.16	0.72	5.05
Recoveries, %	75	69	64	58	68

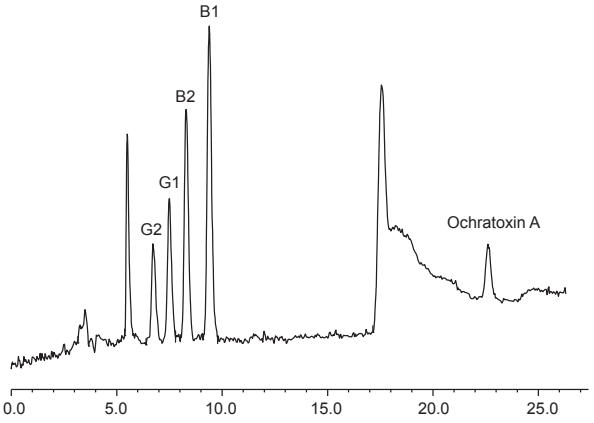


Pic 1. *Ginseng sample blank.*

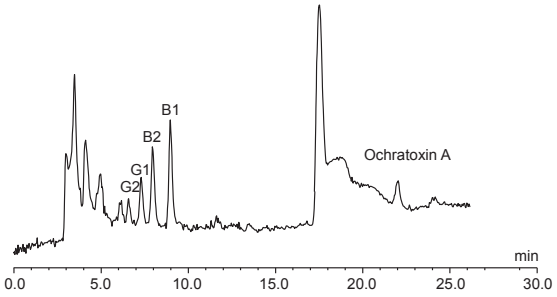
NOTE: The shift on the baseline at 17 min is due to changes in gradient conditions and detector settings.



Pic 2. *Ginseng sample spiked with Mycotoxins. Aflatoxin B1 - 2.53 ng/g, Aflatoxin B2 - 0.72 ng/g, Aflatoxin G1 - 2.16 ng/g, Aflatoxin G2 - 0.72 ng/g, Ochratoxin A – 5.05 ng/g.*



Pic 3. *Ginger sample spiked with Mycotoxins. Aflatoxin B1 – 5.06 ng/g, Aflatoxin B2 – 1.45 ng/g, Aflatoxin G1 – 4.33 ng/g, Aflatoxin G2 – 1.45 ng/g, Ochratoxin A – 10.1 ng/g.*



Pic 4. *Echinacea sample spiked with Mycotoxins. Aflatoxin B1 - 2.53 ng/g, Aflatoxin B2 - 0.72 ng/g, Aflatoxin G1 - 2.16 ng/g, Aflatoxin G2 - 0.72 ng/g, Ochratoxin A – 5.05 ng/g.*

Clean-Up and Determination of Aflatoxins in Peanuts and Peanut Butter (MA-215)

Aflatoxins occur naturally in peanuts, cottonseed, corn, and dried chili pepper as well as many mixed or processed foods and feeds. Of significant assistance is the cleanup of extracts by an Immunoaffinity column containing antibodies specific to the Mycotoxin of interest. We used a simple, sensitive and robust HPLC method with post-column photochemical derivatization and fluorescence detection to analyze Aflatoxins B1, B2, G1, G2 in peanut butter and ground peanuts. The UVE™ (LCTech, Germany) photochemical reactor requires no additional reagents and is easy to install between the HPLC column and FLD detector. This method and instrumentation allows for quick and interferencefree detection of Aflatoxins at the low ppb level.

Project Overview

As participants in an NIST study, we analyzed samples of peanuts and peanut butter (table 1, 2). Four other laboratories that use other HPLC methods for analysis of Aflatoxins participated in this study. Community results for peanuts are presented in table 2. The extracts were cleaned up using the AflaCLEAN™ (LCTech, Germany) Immunoaffinity columns for Aflatoxin B1, B2, G1, G2. The prepared samples were analyzed by HPLC with post-column photochemical derivatization using the UVE™ Photochemical Reactor.

Method

Isolation of Aflatoxins B1, G1, B2, G2

To 20 g of sample add 2 g of NaCl, 100 mL of extraction solution (80/20 Methanol/water) and 50 mL of Hexane. Blend at high speed and filter through fluted paper. To 14 mL of aqueous layer add 86 mL of PBS buffer pH 7, mix well and filter. Open the AflaClean Immunoaffinity column and drain the storage buffer. Load 11 mL of extract/PBS mixture and drain to the top of the sorbent bed. Wash the column with 10 mL of water. Elute Aflatoxins with 2 portions of 1 mL Methanol. Leave the first portion of Methanol in the column for 5 min before adding the second portion to ensure the breaking of the Aflatoxins bond with the antibodies. Analyze Aflatoxins as described below.

Analytical Conditions

Analytical Column: MYCOTOX™ Reversed-phase Column, 4.6 x 250 mm, P/N 1612124

Guard Column: Reversed-phase guard cartridge, P/N 18ECG001

HPLC Eluent: Sodium Phosphate buffer (Cat #1700-1108)/Methanol/Acetonitrile (57/28/15)

Flow Rate: 1 mL/min

Injection Volume: 30 µL

FLD: λ_{ex} : 365 nm, λ_{em} : 430 nm

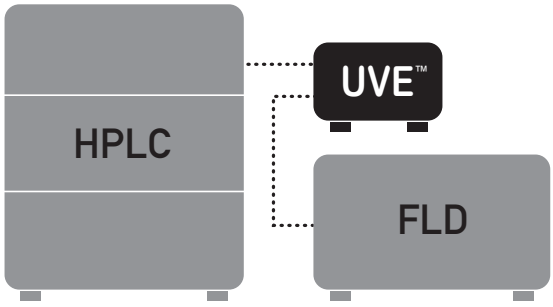
Results & Discussion

Isolation of Aflatoxins B1, G1, B2, G2

The 6-point calibration curves were built in a range of 11.49 – 0.24 ppb for B1, 3.29 – 0.07 ppb for B2 and G2, 9.85 – 0.21 ppb for G1 with R2 exceeding 0.999. There were no matrix interferences present after the sampleclean-up using the Immunoaffinity columns.

The results for all Aflatoxins are in good agreement with certified NIST values and with the results obtained by other methods.

Using the IAC columns, and the UVE reactor to derivatize, we were able to detect low levels of Aflatoxin quickly and efficiently.



Flow diagram for UVE™ Photochemical Reactor

Table 1. *Peanut butter (NIST SRM2387) – control sample*

	Aflatoxin B1	Aflatoxin B2	Total Aflatoxins
Target value, ng/g	4.2 ± 0.9	0.7 ± 0.3	5.0 ± 0.5
Packet A, ng/g	4.47	0.73	5.2
Packet B, ng/g	4.76	0.96	5.72
Packet C, ng/g	4.74	0.8	5.54

Table 2. *Ground peanut sample*

	Aflatoxin B1	Aflatoxin B2	Aflatoxin G1	Aflatoxin G2	Total Aflatoxins
Packet A, ng/g	6.21	1.82	1.74	1.24	11.01
Packet B, ng/g	6.45	1.65	2.02	1.3	11.42
Packet C, ng/g	5.73	1.78	2.07	1.52	11.1
Mean, ng/g	6.1	1.8	1.9	1.4	11.2
RSDr %	6.5	5.6	10.5	7.1	1.8
Community results*	4.02-6.48	1.38-1.75	1.54-2.22	1.34-1.45	8.4-11.6
NIST assessed value**, ng/g	7.47 ± 3.28	1.82 ± 0.79	2.57 ± 1.13	1.64 ± 0.72	13.5 ± 5.9

* Results from 5 participating laboratories are presented as (minimum reported value – maximum reported value), ng/g

** ± 95% confidence interval about the NIST assessed value

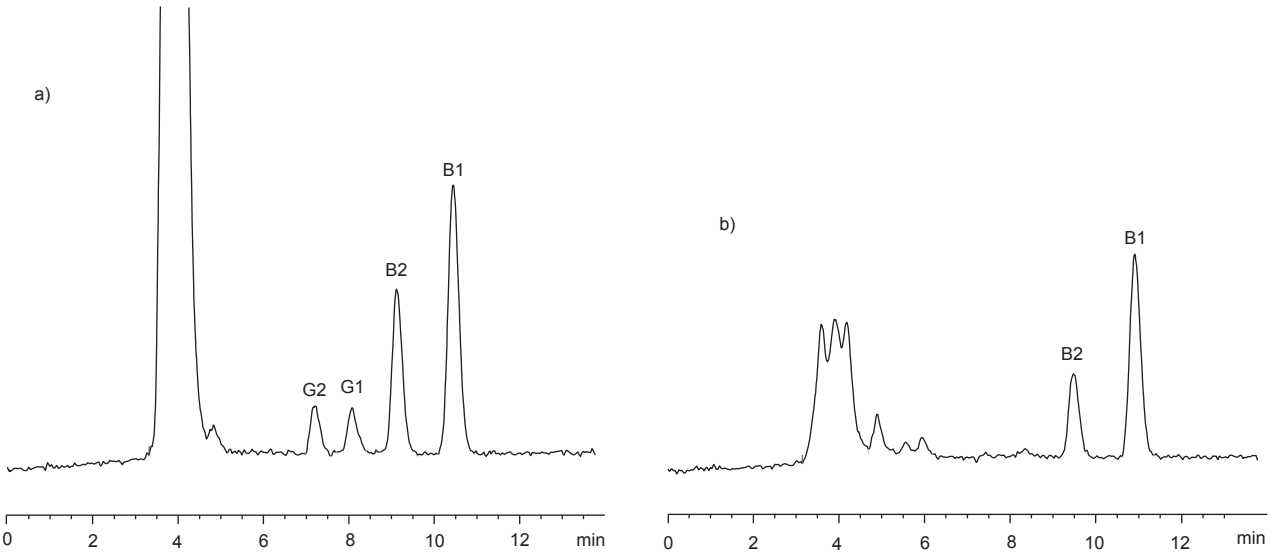


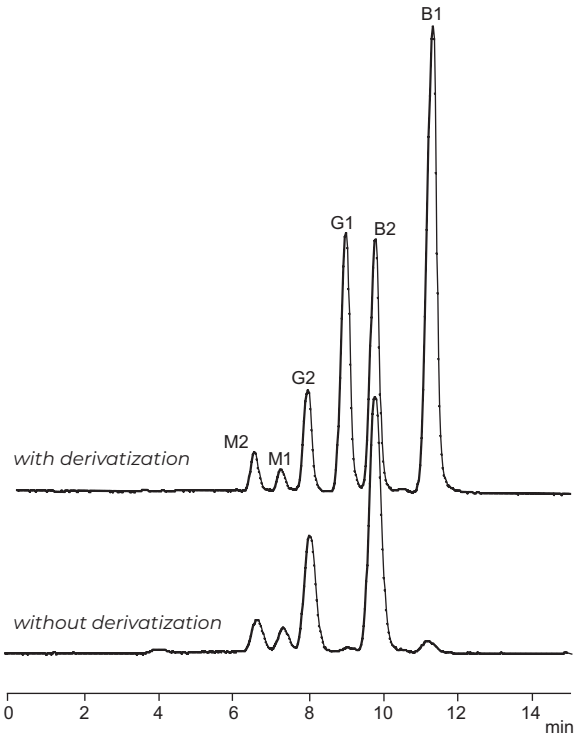
Fig 1. *Chromatograms of a) Ground peanuts; b) NIST SRM2387 peanut butter sample. All samples are part of NIST Exercise E (April 2010).*

Aflatoxins (MA-110)

Toxins in Peanuts, Vegetable Matter, and Milk

Aflatoxins occur naturally in peanuts, peanut meal, cottonseed meal, corn, dried chili pepper, etc. However, the growth of mold does not always indicate the presence of toxin since the yield of Aflatoxins is dependent on growth conditions such as moisture, temperature, and aeration. The Aflatoxins are characterized as B for blue fluorescence and G for green fluorescence. The numerical subscripts indicate relative chromatographic mobility. Besides the toxins commonly found in vegetable matter (B1, B2, G1, and G2), Aflatoxins M (for milk) are found in milk of cows fed toxic meals. The highly toxic M metabolites are 4-hydroxylated Bs.

The most important feature of the post-column method described here is that all four Aflatoxins are detectable at the same fluorescence emission wavelength in a single run.



Method

Analytical Conditions

Column: MYCOTOX™ Reversed-phase Column, 4.6 x 250 mm, P/N 1612124

Guard Column: Reversed-phase guard cartridge, P/N 18ECG001

Column Temperature: 42 °C

Flow Rate: 1.0 mL/min

Mobile Phase: MeOH, CH_3CN, H_2O; 22:22:56, Isocratic

Injection Volume: 10-50 uL

Post-Column Conditions

Post-Column System: Onyx PCX, Pinnacle PCX or Vector PCX

Reactor Volume: 1.4 mL

Reactor Temperature: 95 °C

Reagent: Iodine 100 mg/L in water

Flow Rate: 0.3 mL/min

Detection: FLD Detector, λ_{ex} : 365 nm, λ_{em} : 430 nm

References

1. R. Buchi in “Aflatoxins,” L. Goldblat, Ed., Academic Press, New York, NY (1969)
2. C.W. Thorp, G.M. Ware, and A.E. Pohland,“Proceedings of the 5th International IUPAC Symposium on Mycotoxins and Phycotoxins,” W. Pfannhauser and P.B. Czedic-Eysenberg (Eds.), Technical University, Vienna (1982) 52–55
3. J.W. Dorner & R.J. Cole, J.A.O.A.C., 71 (1988) 43–47
4. M.J. Shepherd and J. Gilbert, Food Additives Contaminants, 1 (1984) 325–335

Analysis of Fumonisins FB1, FB2 and FB3 Using HPLC With Post-Column Derivatization (MA-249)

Fumonisins are a group of naturally occurring Mycotoxins produced by *Fusarium moniliforme* fungi species that grow on corn and other commodities. Fumonisins are suspected human carcinogens and are toxic to pigs, poultry and horses. Environmental factors, such as temperature and humidity, affect the occurrence of Mycotoxins and contamination can happen in the field as well as during storage. Many countries set limits on the presence of Fumonisins in foods and feeds and testing of raw crops as well as finished products is done on a regular basis.

A simple and sensitive method to detect Fumonisins involves using an HPLC to separate the toxins and then converting them using post-column derivatization with OPA into highly fluorescent derivatives.

Method

Analytical Conditions

Analytical Column: MYCOTOX™ Reversed-phase Column, 4.6 x 250 mm, P/N 1612124

Guard Column: Reversed-phase guard cartridge, P/N 18ECG001

Temperature: 40 °C

Flow Rate: 0.8 mL/min

Mobile Phase: Eluant A: Dilute 1 mL of formic acid to 1 L with D.I. water

Eluant B: MeOH

Injection Volume: 10–50 µL

Post-Column Conditions

Post-Column System: Onyx PCX, Pinnacle PCX or Vector PCX

Reactor Volume: 1.4 mL

Reactor Temperature: 65 °C

Flow Rate: 0.4 mL/min

Reagent: 300 mg *o*-Phthalaldehyde, 2g Thiofluor and 3 mL of 30% Brij 35 solution in 950 mL OD104 Diluent

Detection: FLD detector, λ_{ex} : 335 nm, λ_{em} : 440 nm

HPLC Gradient		
TIME	**Eluent A %**	**Eluent B %**
0	45	55
2	45	55
9	30	70
14	10	90
16	10	90
16.1	45	55
22	45	55

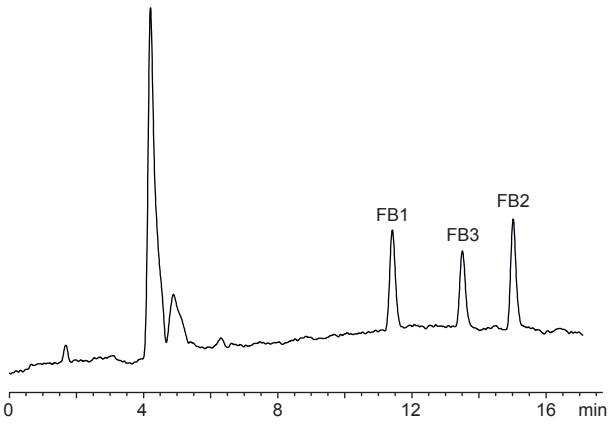


Fig 1. *Chromatogram of Fumonisins standard (25 ppb)*

Analysis of Fumonisins in Grains and Feed (MA-252)

Fumonisins are naturally occurring Mycotoxins produced by Fusarium mold species. Fumonisins contamination happens worldwide in many agricultural commodities, especially in corn, and is usually associated with dry and hot weather followed by periods of high humidity. Out of more than ten types of Fumonisins, Fumonisin FB1 is the most prevalent and toxic, followed by Fumonisins FB2 and FB3. Fumonisins are classified as possibly carcinogenic for humans and also cause health problems in animals, especially in equids and swine. FDA sets total Fumonisins limits in human foods between 2-4 ug/g and in animal feed between 5-100 ug/g.

Since Fumonisins don't have a chromophore and don't fluoresce derivatization is needed to achieve the required sensitivity of detection. We developed a fast and sensitive HPLC method with post-column derivatization that is capable of analyzing Fumonisins in grains and animal feed at levels as low as 0.01 ug/g.

Method

Analytical Conditions

Analytical Column: MYCOTOX™ Reversed-phase Column, 4.6 x 250 mm, P/N 1612124

Guard Column: Reversed-phase guard cartridge, P/N 18ECG001

Temperature: 40 °C

Flow Rate: 0.8 mL/min

Mobile Phase: Eluant A: Dilute 1 mL of formic acid to 1 L with D.I. water

Eluant B: MeOH

Injection Volume: 10–50 µL

Post-Column Conditions

Post-Column System: Onyx PCX, Pinnacle PCX or Vector PCX

Heated Reactor Volume: 1.4 mL

Temperature: 65 °C

Reagent: 950 mL GA 104, 300 mgs OPA, 2 g Thiofluor, 3 mL of 30% Brij 35 solution

Detection: FLD, λ_{ex} : 335 nm, λ_{em} : 440 nm

HPLC Gradient		
TIME	**Eluent A %**	**Eluent B %**
0	45	55
2	45	55
9	30	70
14	10	90
16	10	90
16.1	45	55
22	45	55

Sample Extraction and Clean-Up

Immunoaffinity Clean-Up Columns: Fumonitest™ WB (Vicam)

Extraction Solution: Water/Methanol (20/80)

PBS Solution: Dilute 100 mL of 10X PBS (Vicam, P/N G1113 to 1 L with DI water

To 25 g of finely ground sample add 2.5 g of NaCl and 50 mL of extraction solution. Blend at high speed for 5 min, filter through fluted filter. Take 10 mL of extract and add 40 mL of PBS solution, mix well, filter through microfiber filter. Load 10 mL of diluted extract to Immunoaffinity column, let the solution pass through at the flow rate about 1-2 drops/sec. Wash the column with 10 mL of PBS solution, elute with 1 mL of Methanol followed by 1 mL of DI water. Evaporate the solution to dryness under the stream of Nitrogen, reconstitute in 1 mL of Methanol/water (50/50). Inject 10–50 uL.

Analysis of Fumonisins						
Sample	**FB1 Found in Sample**	**FB2 Found in Sample**	**FB1 Spike Concentration**	**FB2 Spike Concentration**	**FB1 Recoveries**	**FB2 Recoveries**
Barley	0 ug/g	0 ug/g	0.2 ug/g	0.07 ug/g	92.3%	87.5%
Milo	0.04 ug/g	0.01 ug/g	0.2 ug/g	0.07 ug/g	85.4%	81.8%
Saf-flower seeds	0 ug/g	0 ug/g	0.2 ug/g	0.07 ug/g	92.0%	80.8%
Corn	0.17 ug/g	0.03 ug/g	0.4 ug/g	0.13 ug/g	91.7%	88.7%
Oats	0 ug/g	0 ug/g	0.2 ug/g	0.07 ug/g	91.5%	83.3%
Mixed feed	0.08 ug/g	0.02 ug/g	0.3 ug/g	0.1 ug/g	88.0%	80.6%

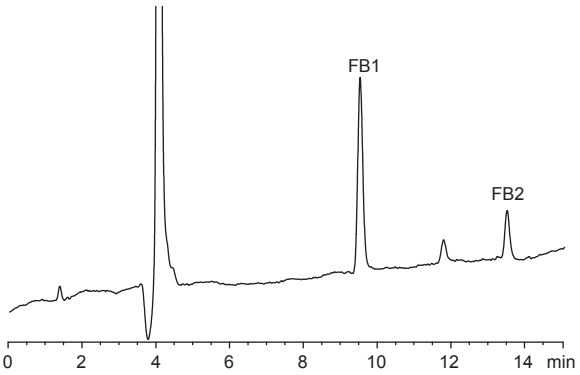


Fig 1. *Chromatogram of corn sample contaminated with 0.17 ug/g of FB1 and 0.03 ug/g of FB2*

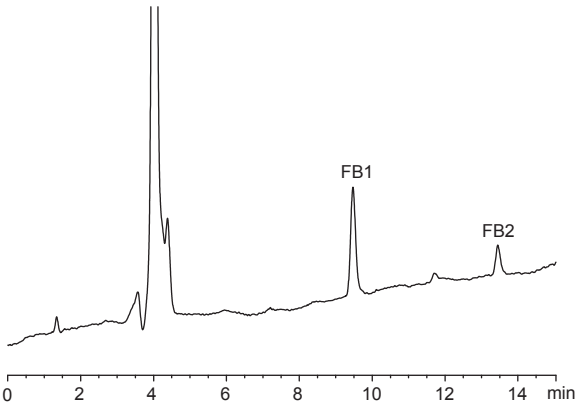


Fig 2. *Chromatogram of mixed feed sample contaminated with 0.08 ug/g of FB1 and 0.02 ug/g of FB2*

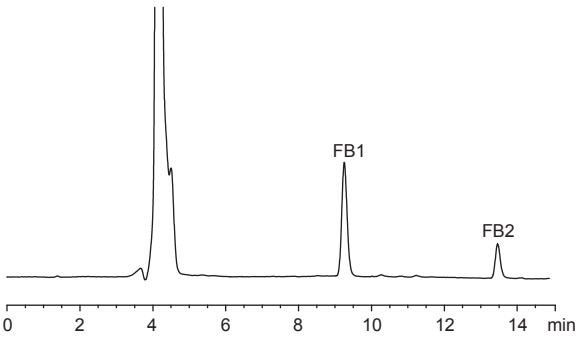


Fig 3. *Chromatogram of barley sample spiked with 0.2 ug/g of FB1 and 0.07 ug/g of FB2*

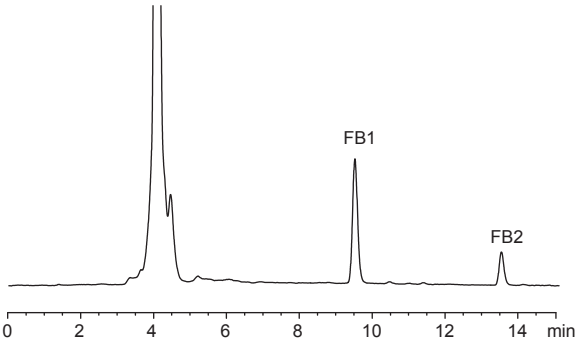


Fig 4. *Chromatogram of safflower seeds sample spiked with 0.2 ug/g of FB1 and 0.07 ug/g of FB2*

Multi-Residue Mycotoxin Analysis—Single Run Analysis of Deoxynivalenol, Aflatoxins, Ochratoxin A, Zearalenone and Fumonisin by HPLC and Post-Column Derivatization (MA-203)

Although Aspergillus (Aflatoxins, Ochratoxin A) are generally associated with peanuts and Fusarium (Deoxynivalenol, Zearalenone) with wheat, these fungi and those that produce other toxins are not host selective and so can cross plant species. This situation is complicated by the fact that the microscopic mold may not be visible to the naked eye. Also, when infected grains are processed, any visible mold is lost but the toxic metabolites carry over into the finished products. Thus, multi-residue analytical screens for toxins in grain and finished goods are a wiser choice than single-family protocols. We present a single screen to cover five families of toxins. This method is suitable for analyzing beverages, grains and feeds.

Sample Extraction and Clean Up

25 g of finely grounded sample is extracted with 150 mL of water/Methanol mixture (30/70). 20 mL of filtered extract is diluted with 70 mL of Phosphate Buffered Saline (PBS). Aflatoxins, Zearealenone and Ochratoxin A are isolated using AOZ Immunoaffinity column (Vicam, USA) according to the procedure from the column manufacture. Toxins are eluted with 2 x 2 mL of Methanol. Fumonisins are isolated using FumoniTest Immunoaffinity column (Vicam, USA) according to the procedure from the column manufacture. Toxins are eluted with 2 x 1.5 mL of Methanol.

To isolate DON 3 mL of filtered extract is mixed with 6 mL of Acetonitrile and cleaned with MycoSep 277 column (Romer Labs, USA) according to the manufacture's instructions. The cleaned solution is filtered and 5.5 mL of it is combined with eluants from AOZ and FumoniTest columns. The solution is evaporated to 0.5 mL and final volume is adjusted to 1 mL with Methanol.

Method

Analytical Conditions

Analytical Column: MYCOTOX™ Reversed-phase Column, 4.6 x 250 mm, P/N 1612124

Guard Column: Reversed-phase guard cartridge, P/N 18ECG001

Temperature: 40 °C

Flow Rate: 1 mL/min

Mobile Phase: Sodium Phosphate buffer, pH 3.5 Catalog No 1700-1108/MeOH/ACN

HPLC Program			
Time	**1700-1108 %**	**Methanol %**	**Acetonitrile %**
0.0	85	0	15
5.0	85	0	15
5.1	57	28	15
20.0	57	28	15
23.0	40	60	0
40.0	40	60	0
50.0	20	0	80
60.0	20	0	80

Post-Column Conditions

Post-Column System: Onyx PCX and Pinnacle PCX

Reactor Volume: 1.4 mL

Temperature: 60 °C

Reagent: OPA, Thiofluor, Brij 35® in GA104

Photochemical Reactor: UVE™

Detection:
Fluorescence
Aflatoxins (photochemical derivatization)
λ_{ex} = 365 nm; λ_{em} = 455 nm

Fumonisins (post-column derivatization with OPA)
λ_{ex} = 330 nm; λ_{em} = 465 nm

Ochratoxin A
λ_{ex} = 335 nm; λ_{em} = 455 nm

Zearalenone
λ_{ex} = 275 nm; λ_{em} = 455 nm

UV/Vis
Deoxynivalenol
λ=218 nm

References:

Ofitserova, M., Nerkar, S., Pickering, M., Torma, L., Thiex, N., Multiresidue Mycotoxin Analysis in Corn Grain by Column High-performance Liquid Chromatography with Post-column Photochemical and Chemical Derivatization: Single-Laboratory Validation., (2009), J AOAC Int., **92**, 15-25

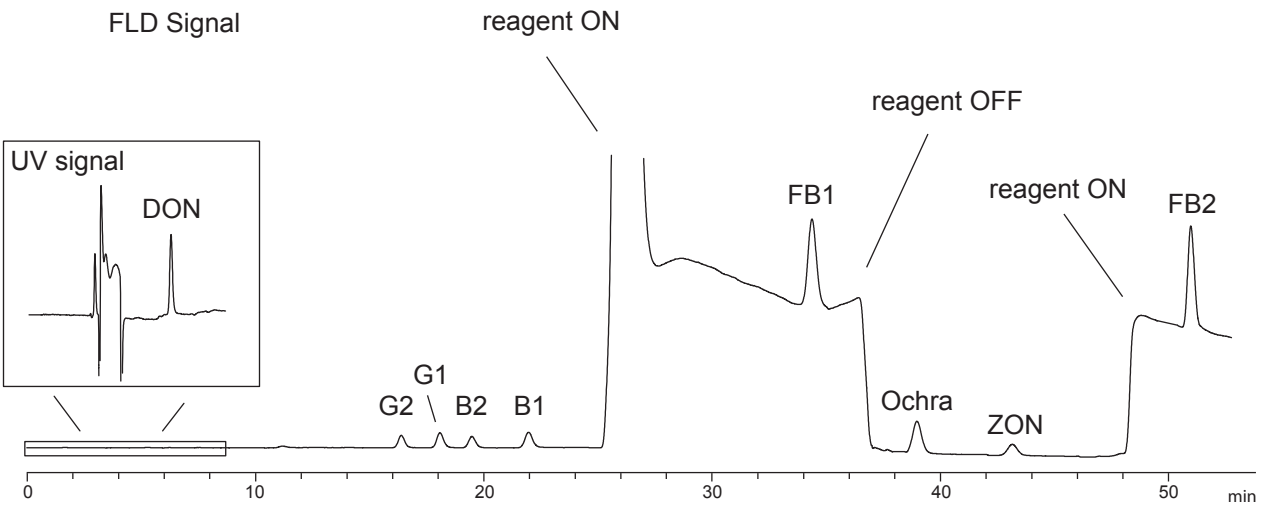


Fig 1. *Chromatogram of a standard solution of mycotoxins. Concentrations of toxins (ng/mL): deoxynivalenol (DON) - 930, aflatoxin B1 - 4.5, aflatoxin B2 - 1.6, aflatoxin G1 - 4.7, aflatoxin G2 - 2, ochratoxin A - 92, zearalenone - 481, fumonisin B1 - 474, and fumonisin B2 - 627.*

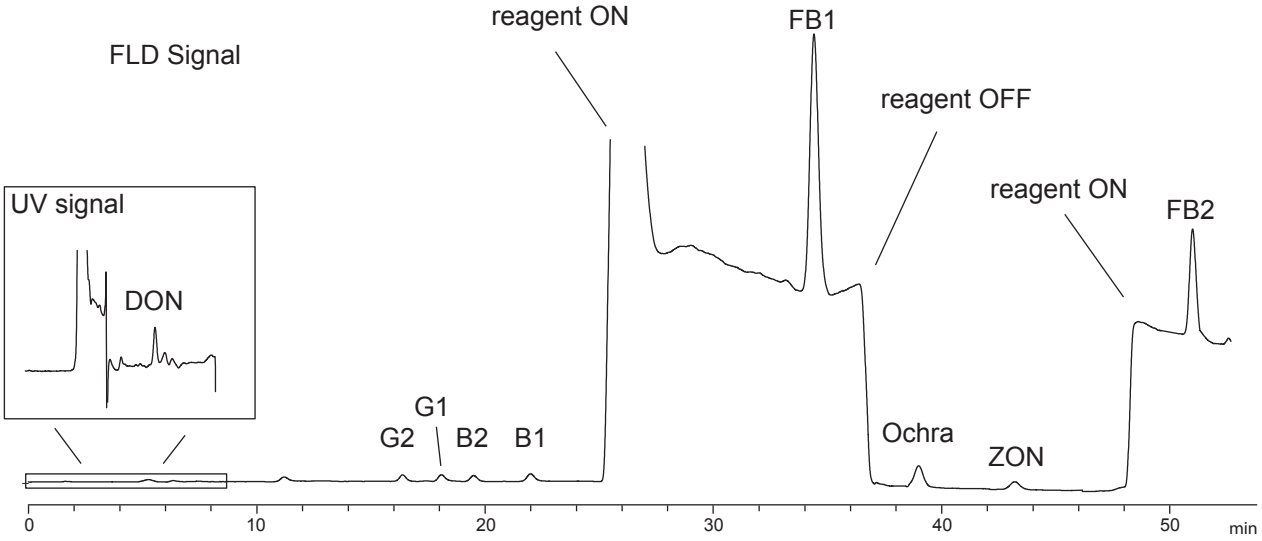
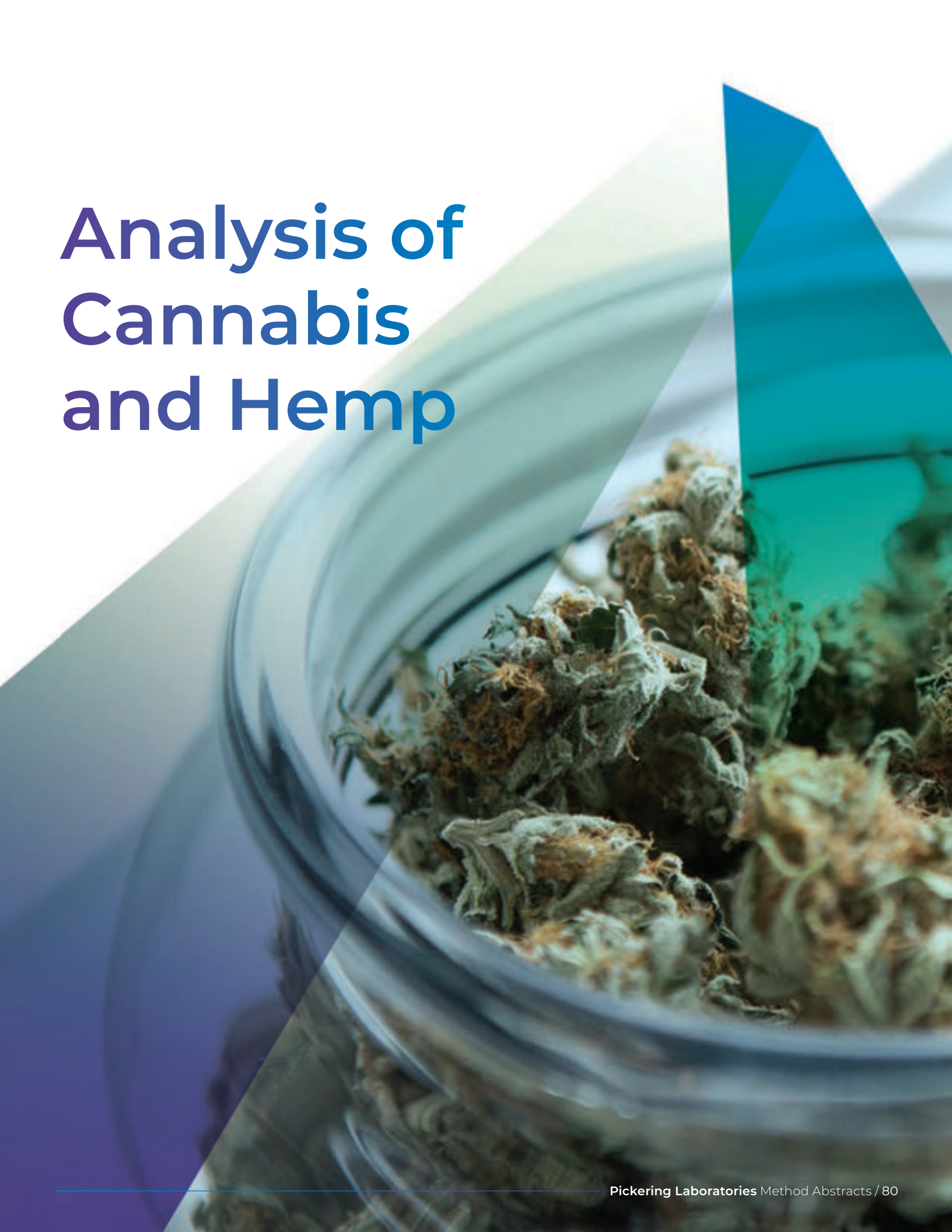


Fig 2. *Chromatogram of a corn grain sample naturally contaminated with fumonisins FB1 and FB2 and spiked with DON; aflatoxins B1, B2, G1, and G2; ochratoxin A; and zearalenone. Concentrations of toxins in the sample (ng/g): deoxynivalenol - 930, aflatoxin B1 - 5.0, aflatoxin B2 - 1.7, aflatoxin G1 - 5.1, aflatoxin G2 - 2.2, ochratoxin A - 102, zearalenone - 529, fumonisin B1 - 1838, and fumonisin B2 - 1107.*

Analysis of Cannabis and Hemp



Analysis of Cannabinoids in Hemp and Hemp-Containing Products Using HPLC With Post-Column Derivatization (MA-242)

The legalization of hemp under the 2018 Farm Bill brought new opportunities as well as new challenges to both growers of industrial hemp and manufacturers of hemp-containing products. The Farm Bill classifies hemp as the plant *Cannabis sativa L.* and all its derivatives with delta-9 tetrahydrocannabinol (THC) of not more than 0.3% concentration. To comply with Federal laws, all producers need to test their products to determine THC content as well as the concentration of other cannabinoids, particularly CBD, that are associated with pharmacological activity of *Cannabis sativa L.* plant.

A new HPLC method with post-column derivatization was developed to analyze cannabinoids in hemp and hemp-containing edible products. This post-column method is based on reaction with Fast Blue Salt reagent under basic conditions. Detection at 475 nm is performed using a UV/Vis detector.

The method utilizes a simple extraction procedure with no additional sample clean-up and is suitable for analysis of the major neutral cannabinoids as well as cannabinoid acids with high sensitivity and selectivity of detection.

Method

Analytical Conditions

Analytical Column: C_{18} Reversed-phase Column, 4.6 x 150 mm

Guard Column: Reversed-phase guard cartridge, P/N 18ECG001

Column Temperature: 45 °C

Flow Rate: 1 mL/min

Mobile Phase: 70% acetonitrile – 30% sodium phosphate buffer (6 mM) at pH 3.5

Injection Volume: 20 uL

Post-Column Conditions

Post-Column System: Onyx PCX, Pinnacle PCX or Vector PCX

Heated Reactor Volume: 1.4 mL

Temperature: 45 °C

Reagent 1: Dissolve 0.1 g of Fast Blue Salt in 240 mL of DI water.Add 40 mL of 1 N HCl and 720 mL of Acetonitrile.
Protect the reagent from light and use within 3 days.

Reagent 2: Dissolve 8 g of NaOH in 1 L of DI water

Detection: UV/VIS at 475 nm

To avoid precipitation of the reagent as it ages, flush the postcolumn system regularly with water/methanol/0.1N HCl (49:49:2)

Sample Extraction

Dried Plant Material and Edible Products[1]

Weigh 0.5 g of a thoroughly homogenized sample into a 50-mL centrifuge tube. Add 20 mL of ethanol, then shake for 30 minutes using a horizontal shaker at 250 rpm. Centrifuge the tube for 5 minutes and filter the supernatant through filter paper into a 50-mL volumetric flask. Repeat extraction of the sample following the steps above. Combine both extracts in 50-mL volumetric flask and bring to volume with ethanol. Filter the extract through a 0.22 um PFTE syringe filter into an injection vial.

Resins, Tinctures and Oils[1]

Reduce the sample size to 0.05 g – 0.1 g. Extraction steps above are used except no re-extraction is needed. Dilute the extracts if needed.

Calibration

The following cannabinoids were analyzed: delta-9 tetrahydrocannabinol (9-THC), delta-8 tetrahydrocannabinol (8-THC), cannabinol (CBN), cannabidiol (CBD), cannabigerol (CBG), tetrahydrocannabivarin (THCV), delta-9 tetrahydrocannabinolic acid A (THCA-A), cannabidiolic acid (CBDA), cannabigerolic acid (CBGA).

The calibrators were prepared by diluting commercially available cannabinoids standards with methanol. The calibration range from 5 ppm to 75 ppm was used. Correlation coefficient R^2 exceeded 0.999 for all calibration curves.

Analysis of Cannabinoids in Hemp and Hemp-Containing Products									
Sample	**CBD**	**9-THC**	**8-THC**	**CBG**	**CBN**	**THCV**	**CBDA**	**THCA**	**CBGA**
Hemp Pre-Rolls	9.12 mg/g	1.53 mg/g	ND	6.63 mg/g	0.96 mg/g	ND	114.69 mg/g	3.62 mg/g	ND
CBD-Containing Tincture	14.82 mg/g	0.15 mg/g	ND	ND	0.42 mg/g	ND	ND	ND	ND
CBD-Containing Chocolate	14.89	ND	ND	ND	ND	ND	ND	ND	ND
CBD Oil	6.78 mg/g	0.32 mg/g	ND	ND	ND	ND	ND	ND	ND
CBD-Containing Chews	4.75 mg/g	ND	ND	ND	ND	ND	0.77 mg/g	ND	ND

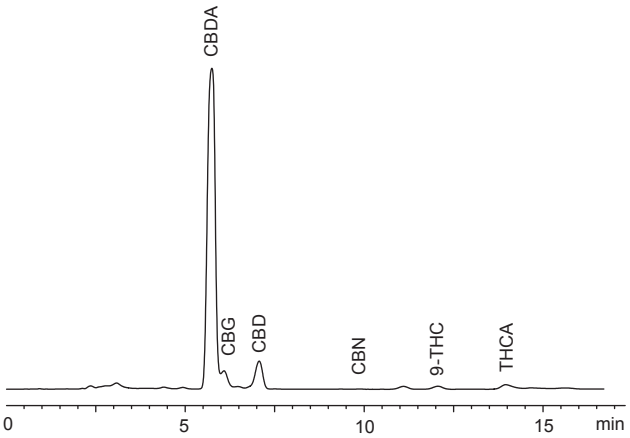


Fig 1. *Chromatogram of hemp pre-roll*

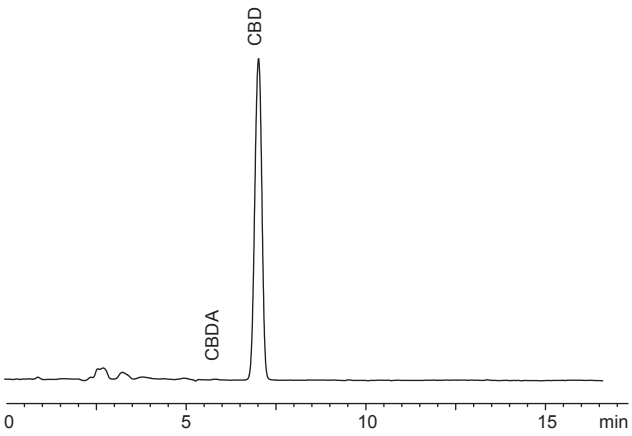


Fig 2. *Chromatogram of CBD-containing chocolate*

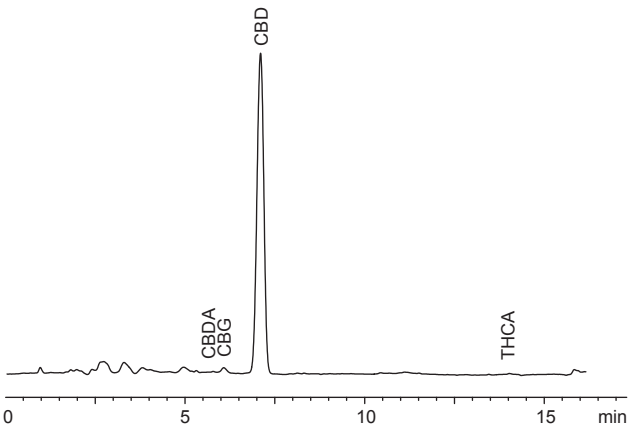


Fig 3. *Chromatogram of CBD oil supplements*

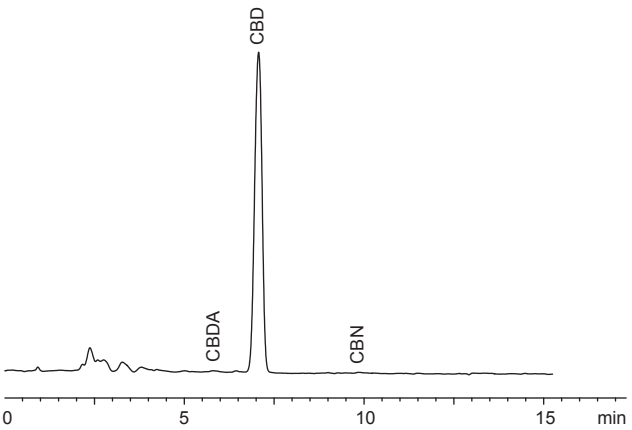


Fig 4. *Chromatogram of CBD-containing chews*

References
1. AOAC Official Method 2018.11

Analysis of Cannabinoids Using HPLC With Post-Column Derivatization (MA-243)

Cannabinoids are a class of terpenophenolic compounds that are associated with the pharmacological activity of cannabis. Broader acceptance of medical cannabis use increases the need for analytical methods capable of determining the active compounds of cannabis. Cannabinoids exist in the plant mainly as carboxylic acids and are converted to neutral analogs by light and heat while in storage or during the preparation of edible products. Acids are also converted to neutral analogs during GC analysis, which often causes differences in results when comparing with HPLC methods.

A new HPLC method with post-column derivatization was developed to analyze cannabinoids in cannabis plants as well as in cannabis containing edible products. This post-column method is based on reaction with Fast Blue Salt reagent under basic conditions, a well-known colorforming reaction that is used in drug tests to detect cannabinoids via test-tube methods and thin-layer chromatography. Detection at 475 nm is performed using a UV/Vis detector.

Our method implements a simple extraction with acidified water/acetonitrile followed by QuEChERS sample clean-up. The same procedure is applicable to both plant materials and edible products containing cannabis. The method is suitable for analysis of the major neutral cannabinoids such as THC, CBD, CBN and CBG as well cannabinoid acids THCA-A and CBDA with high sensitivity and selectivity of detection.

Method

Analytical Conditions

Analytical Column: C_{18} reversed-phase column, 4.6 x 150 mm

Guard Column: Reversed-phase guard cartridge, P/N 18ECG001

Column Temperature: 45 °C

Flow Rate: 1 mL/min

Mobile Phase: 70% acetonitrile – 30% sodium phosphate buffer (6 mM) pH 3.5

Injection Volume: 20 uL

Post-Column Conditions

Post-Column System: Onyx PCX, Pinnacle PCX or Vector PCX

Heated Reactor Volume: 1.4 mL

Temperature: 30 °C

Ambient Reactor: 0.1 mL

Reagent 1: Dissolve 0.1 g of Fast Blue Salt in 240 mL of DI water. Add 40 mL of 1 N HCl and 720 mL of Acetonitrile.
Protect the reagent from light. Use within 3 days.

Reagent 2: Dissolve 8 g of NaOH in 1L of DI water

Reagents Flow Rate: 0.25 mL/min

Detection: UV/VIS 475 nm

To avoid precipitation of aging reagents flush post-column system regularly with 49 : 49 : 2 – water : Methanol : 0.1N HCl

Supplies for Sample Preparation

Extraction Solution: Acetonitrile containing 1% of Acetic Acid / Water (50:50)

Q-SEP QuEChERS extraction salts: Restek, Cat # 26238 (AOAC 2007.01 Method)

Sample Extraction and Cleanup

Use 0.1 – 0.2 g sample size to analyze plant material and 0.2 – 0.5g sample size to analyze edible products. Place homogenized sample into 50 mL centrifuge tube and add 30 mL of Extraction Solution. Blend using a hand held blender for 1 min. For candies, chews and other products hard or viscous products let the solution sit for 30 min before blending. Shake blended extracts for 30 min using a mechanical shaker.

Centrifuge the samples for 10 min at 4,000 rpm, decant and save the extract. Repeat the extraction with fresh 30 mL of extraction solution. Centrifuge, decant the extract and combine it with the first portion.

Place 30 mL of combined extract in 50 mL centrifuge tube. Add Q-Sep QuEChERS extraction salts to each centrifuge tube according to the manufacture instructions. Vigorously shake for 1 min. Centrifuge the sample for 10 min at 4,000 rpm. Use upper layer for analysis. Filter through 0.45 Nylon filter before injecting. If needed, dilute the sample with Methanol to fit the calibration curve.

Extraction Efficiency Study

Due to availability and price constraints of cannabinoids standards, spike-recoveries studies are not practical to evaluate extraction efficiency. We performed three re-extractions of each sample to validate the extraction procedure. It was found that extraction with two 30-mL portions of extraction solution is sufficient to extract more that 97.5% of cannabinoids. Due to relatively high levels of cannabinoids in the samples and high sensitivity of the method, additional dilution of the extract due to repeated extraction didn't negatively affect the analysis.

Calibration

The following cannabinoids were analyzed: delta-9 tetrahydrocannabinol (THC), cannabinol (CBN), cannabidiol (CBD), cannabigerol (CBG), cannabichromene (CBC), delta-9 tetrahydrocannabinolic acid A (THCA-A) and cannabidiolic acid (CBDA).

The calibrators were prepared by diluting commercially available cannabinoids standards with methanol. The following calibration ranges were used: 1 ppm to 75 ppm for CBG, CBD, CBN, THC and CBDA; 5 ppm to 75 ppm for THCA-A and CBC. Correlation coefficient R2 for all calibration curves exceeded 0.999 value.

Analysis of Cannabinoids in Cannabis and Cannabis-Containing Edible Products

To demonstrate method capabilities, the method was applied for analysis of cannabinoids in medical cannabis products: dry cannabis inflorescence, commercially available pre-rolls, medical cannabis chocolate chip cookies and medical cannabis sugar-free chews.

As expected, cannabis inflorescences and pre-rolls made with dry plant material contained high levels of cannabinoid acid (THCA-A). Dry plant material may also contain cannabidiolic acid (CBDA) but this compound has not been detected in any of the samples we analyzed.

Table 1. Analysis of cannabinoids in plant material and edible products

Sample	CBG	CBD	CBN	THC	THCA-A	CBC	CBDA
Dry Cannabis Inflorescence	3.76 mg/g	ND	3.15 mg/g	3.11 mg/g	142.52 mg/g	ND	ND
Pre-rolls	3.65 mg/g	ND	3.16 mg/g	28.72 mg/g	60.33 mg/g	ND	ND
Chocolate Chip Cookie	0.45 mg/g	0.51 mg/g	0.32 mg/g	3.48 mg/g	ND	0.39 mg/g	ND
Chews	0.91 mg/g	2.73 mg/g	0.74 mg/g	29.43 mg/g	1.98 mg/g	0.62 mg/g	ND

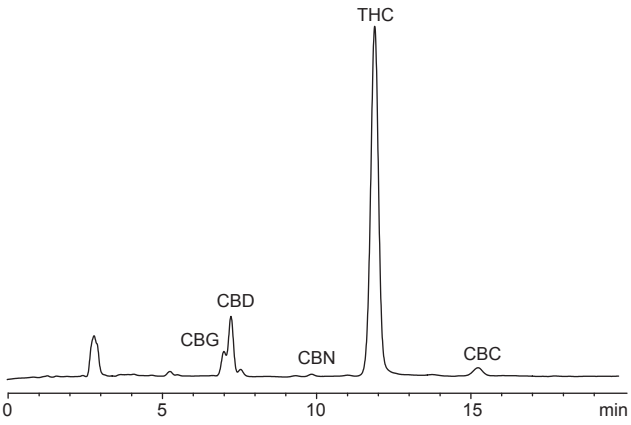


Fig 1. *Chromatogram of cannabis-containing chocolate chip cookie sample*

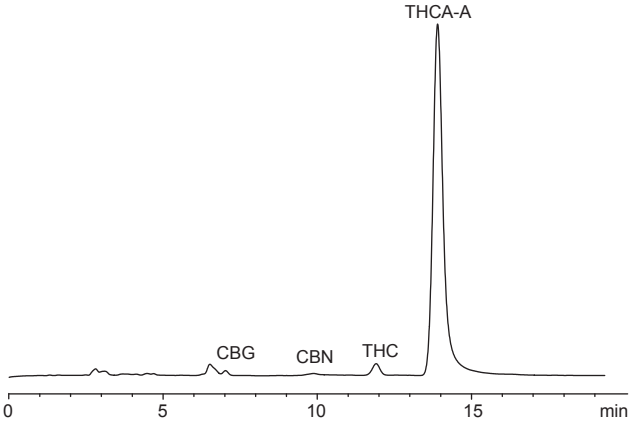


Fig 2. *Chromatogram of cannabis inflorescence*

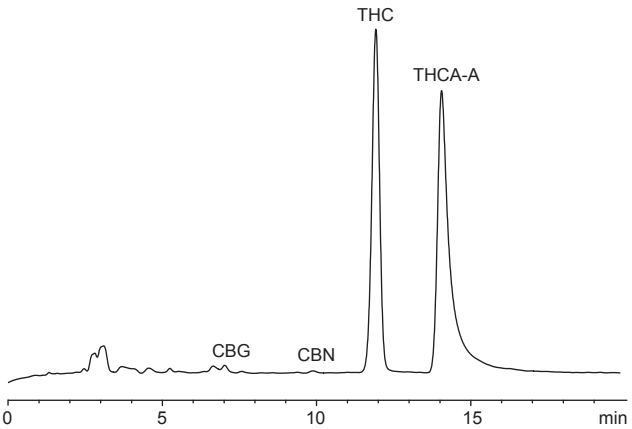


Fig 3. *Chromatogram of commercially available pre-roll sample*

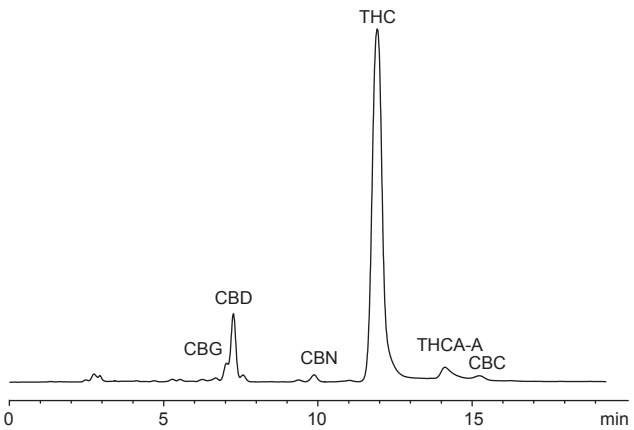


Fig 4. *Chromatogram of cannabis-containing chews*

Analysis of Mycotoxins in Cannabis Plant and Cannabis-Containing Products (MA-241)

As medical and recreational Cannabis use gains broader acceptance, regulations are being put in place to mandate the testing of consumer products containing Cannabis. Legally available Cannabis plant and cannabis-containing edible products are tested for presence of pesticides, heavy metals, residual solvents and other harmful substances. Mycotoxins is another group of contaminates that state regulations have established maximum allowed levels for. In cannabis products sold to consumers the maximum allowed levels for total Aflatoxins G1, G2, B1 and B2 are set at < 20 ppb and for Ochratoxin A at < 20 ppb.

Pickering developed an easy and sensitive method to analyze Aflatoxins B1, B2, G1, G2 and Ochratoxin A in cannabis plant and edible products. Mycotoxins are isolated using immunoaffinity clean-up columns and analyzed with fluorescence detection. To increase sensitivity of Aflatoxins B1 and G1, an in-line photochemical reactor is installed before the detector. This method utilizes standard HPLC equipment and allows laboratories to easily determine Mycotoxins at levels below the limits established by state regulations.

Method

Isolation of Aflatoxins B1, B2, G1, G2 and Ochratoxin A

Blend 1 g of finely ground sample with extraction solution (10 mL of Methanol/water 80:20, 5 mL of Hexane, 0.1 g of NaCl) using a handheld homogenizer. Centrifuge for 10 min. Mix 2 mL of the aqueous layer with 12 mL of PBS buffer (pH 7.2) containing 4% of Tween 20. Apply the solution to AflaOTAClean™ Immunoaffinity column at a flow rate of 1-2 drops/sec.

Wash the column with 10 mL of water at a flow rate of 1-2 drops/sec. Elute the toxins with two 1 mL portions of Methanol at a flow rate of 1 drop/sec. Allow 5 min before applying the second portion of the Methanol to ensure complete breaking of the antibody-toxin bond.

Evaporate to dryness at 55 °C. Reconstitute in 1 mL of Methanol/water 50:50.

Other immunoaffinity columns, such as Vicam's AflaOchra HPLC, could be used for sample clean up as well.

Analytical Conditions

Analytical Column: MYCOTOX™ Reversed-phase Column, 4.6 x 250 mm, P/N 1612124

Guard Column: Reversed-phase guard cartridge, P/N 18ECG001

HPLC Eluent: Sodium Phosphate buffer (Cat #1700-1108), Methanol, Acetonitrile

Flow Rate: 1 mL/min

Injection Volume: 100 µL

FLD: λ_{ex} : 365 nm, λ_{em} : 430 nm for Aflatoxins
λ_{ex} : 333 nm, λ_{em} : 477 nm for Ochratoxin A

HPLC Gradient			
TIME	1700-1108 %	Methanol %	Acetonitrile %
0	57	28	15
13	57	28	15
16	30	45	25
25	30	45	25

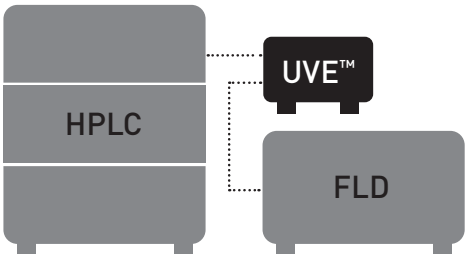
Equilibration time: 10 min

Calibration

The 5-point calibration curves were built in the ranges of 0.25-5 ppb for B1, 0.075-1.5 ppb for B2, 0.248-4.95 ppb for G1, 0.075-1.5 ppb for G2 and 1-10 ppb for Ochratoxin A. Correlation coefficient R2 >0.999 for all toxins. All calibration standards were prepared in Methanol/Water 50:50

Recoveries

For all tested samples the recoveries for toxins exceeded 77% for G2, 88% for G1, 73% for B2, 78% for B1 and 80% for Ochratoxin A



Flow Diagram for UVE™ Photochemical Reactor

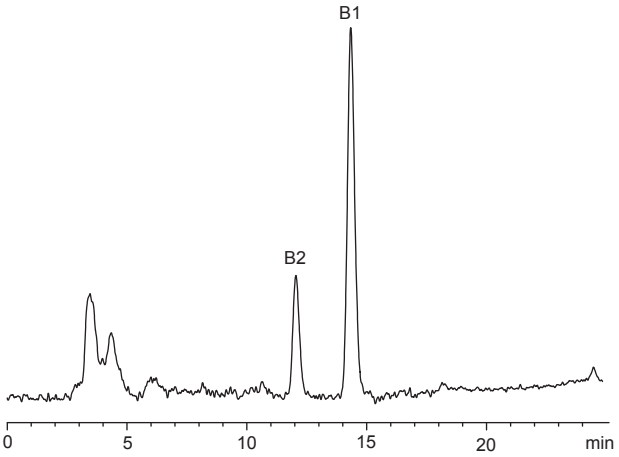


Fig 1. *Chromatogram of cannabis-containing peanut butter cookie sample naturally contaminated with 1.58 ng/g of Aflatoxins B1 and 0.26 ng/g of B2*

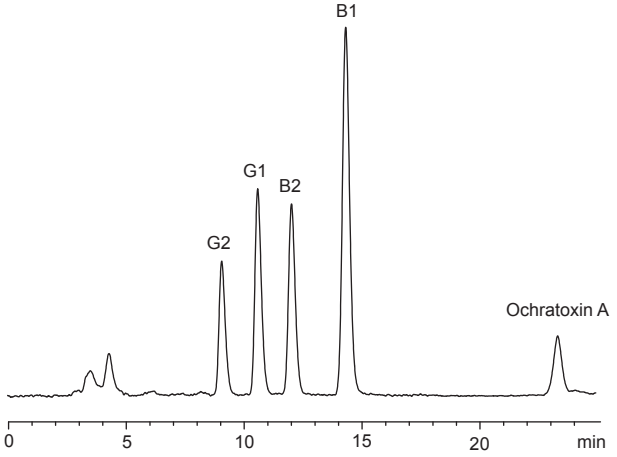


Fig 2. *Chromatogram of cannabis-containing peanut butter cookie sample spiked with additional 6 ng/g of Aflatoxin B1; 1.8 ng/g of Aflatoxin B2; 5.94 ng/g of Aflatoxin G1; 1.8 ng/g of Aflatoxins G2 and 20 ng/g of Ochratoxin A*

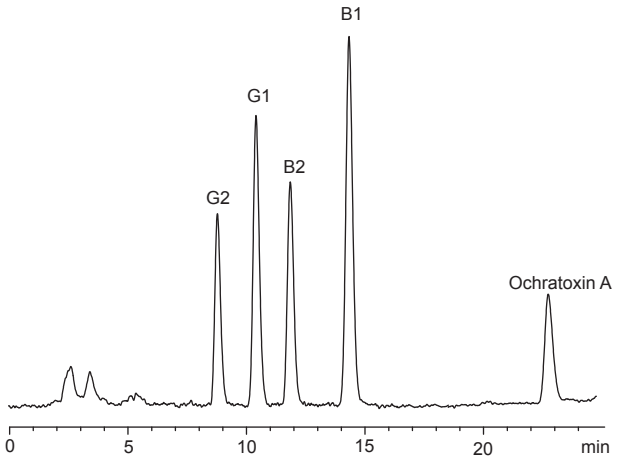


Fig 3. *Chromatogram of cannabis pre-roll sample spiked with 6 ng/g of Aflatoxin B1; 1.8 ng/g of Aflatoxin B2; 5.94 ng/g of Aflatoxin G1; 1.8 ng/g of Aflatoxins G2 and 20 ng/g of Ochratoxin A*

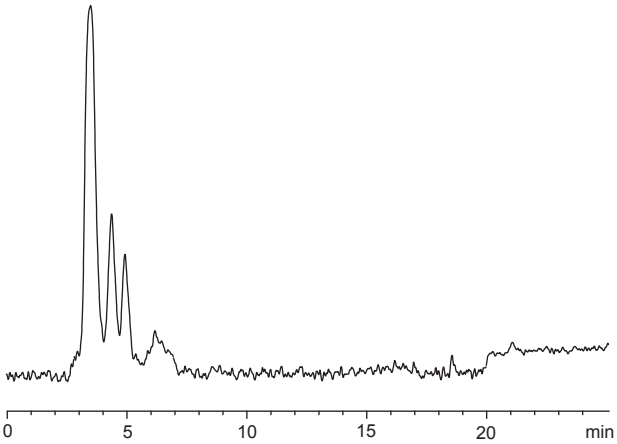


Fig 4. *Cannabis inflorescence sample blank*

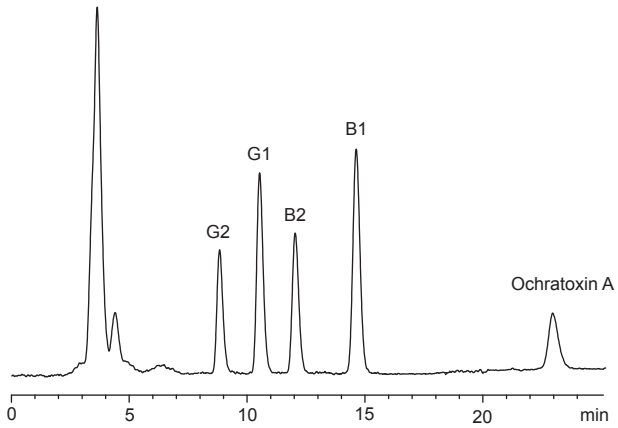


Fig 5. *Chromatogram of cannabis inflorescence sample spiked with 6 ng/g of Aflatoxin B1; 1.8 ng/g of Aflatoxin B2; 5.94 ng/g of Aflatoxin G1; 1.8 ng/g of Aflatoxins G2 and 20 ng/g of Ochratoxin A*

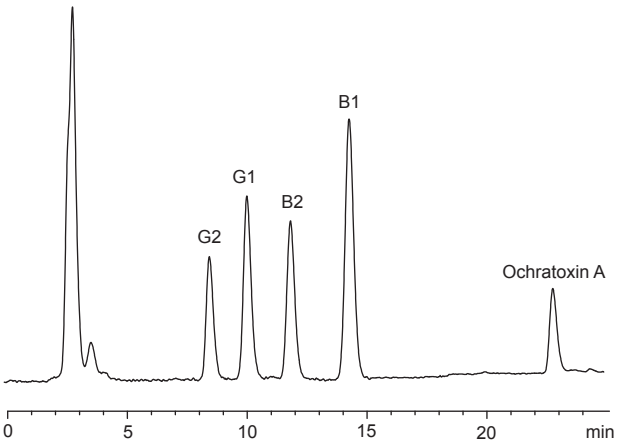


Fig 6. *Chromatogram of cannabis-containing chocolate chip cookie sample spiked with 6 ng/g of Aflatoxin B1; 1.8 ng/g of Aflatoxin B2; 5.94 ng/g of Aflatoxin G1; 1.8 ng/g of Aflatoxins G2 and 20 ng/g of Ochratoxin A*

Analysis of Mycotoxins in Hemp and Hemp-Containing Edible Products (MA-244)

The Agriculture Improvement Act (2018 Farm Bill) makes hemp production and distribution legal under federal law. Hemp has wide range of possible applications, including production of fibers, paper, certain foods, supplements, cosmetics and even the recently FDA-approved drug Epidiolex. Legally available hemp and hemp products need to be tested for presence of pesticides, heavy metals, residual solvents and other harmful substances. Similarly classified crops are routinely tested for Mycotoxins, including Aflatoxins and Ochratoxin A.

Pickering Laboratories developed an easy and sensitive method to analyze Aflatoxins B1, B2, G1, G2 and Ochratoxin A in hemp and hemp-containing edible products. Mycotoxins are isolated using immunoaffinity clean-up columns and analyzed with fluorescence detection. To increase sensitivity of Aflatoxins B1 and G1, an in-line photochemical reactor (UVE™) is installed before the detector. This method utilizes standard HPLC equipment and allows laboratories to easily determine these Mycotoxins at low ppb levels.

Method

Isolation of Aflatoxins B1, B2, G1, G2 and Ochratoxin A

Blend 1 g of finely ground sample with extraction solution (10 mL of methanol/water 80:20) using a handheld homogenizer. Centrifuge for 10 min. Mix 2 mL of the extract with 10 mL of PBS buffer (containing 2% Tween 20).

Load 10 mL of diluted extract on AflaOchra HPLC immunoaffinity column at a flow rate of 1-2 drops/sec. Wash the column with 10 mL of PBS buffer (containing 2% Tween 20) followed by 10 mL of DI water at a flow rate of 1-2 drops/sec. Elute the toxins with two 1 mL portions of methanol at a flow rate of 1 drop/sec.

Evaporate the solution to about 0.3 mL at 55 °C and bring the volume to 1.5 mL with water/methanol solution (50:50). Filter into an injection vial.

Analytical Conditions

Analytical Column: MYCOTOX™ Reversed-phase Column, 4.6 x 250 mm, P/N 1612124

Guard Column: Reversed-phase guard cartridge, P/N 18ECG001

HPLC Eluant: Sodium Phosphate Buffer (Cat #1700-1108), Methanol, Acetonitrile

Flow Rate: 1 mL/min

Injection Volume: 100 uL

Post-Column Photochemical Reactor: UVE™ (Cat # 10519 (240V), Cat # 10742 (120 V))

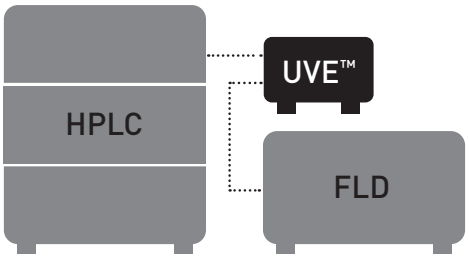
FLD: λ_{ex} : 365 nm, λ_{em} : 430 nm for Aflatoxins
λ_{ex} : 333 nm, λ_{em} : 477 nm for Ochratoxin A

HPLC Gradient			
Time	**1700-1108 %**	**Methanol %**	**Acetonitrile %**
0	57	28	15
13	57	28	15
16	30	45	25
25	30	45	25

Equilibration time: 10 min

Calibration

The 5-point calibration curves were built in the ranges of 0.325-3.25 ppb for B1, 0.088- 0.882 ppb for B2, 0.310-3.099 ppb for G1, 0.099-0.996 ppb for G2 and 1-10 ppb for Ochratoxin A. Correlation coefficient was R2 >0.999 for all toxins. All calibration standards were prepared in methanol/water (50:50).



Flow Diagram for UVE™ Photochemical Reactor

Recoveries

Toxins	Spike Levels, ng/g	CBD-Containing Tincture		CBD-Containing Chocolate		CBD Oil		Hemp Pre-Rolls		Full-Spectrum CBD Chews	
		Recoveries %	RSD %	Recoveries %	RSD %	Recoveries %	RSD %	Recoveries %	RSD %	Recoveries %	RSD %
Aflatoxin G2	1.932	91.85	2.54	78.89	1.42	88.67	7.32	84.66	10.82	93.90	6.85
Aflatoxin G1	6.198	92.50	7.86	85.89	1.21	96.35	1.24	93.56	7.05	97.93	2.42
Aflatoxin B2	1.764	92.99	5.30	88.73	2.88	99.31	1.84	94.28	6.77	101.63	1.72
Aflatoxin B1	6.498	84.73	3.51	87.88	0.56	91.62	3.08	85.78	7.50	91.97	1.99
Ochratoxin A	20.16	92.31	4.50	91.86	4.17	89.75	2.26	85.68	5.08	86.97	5.27

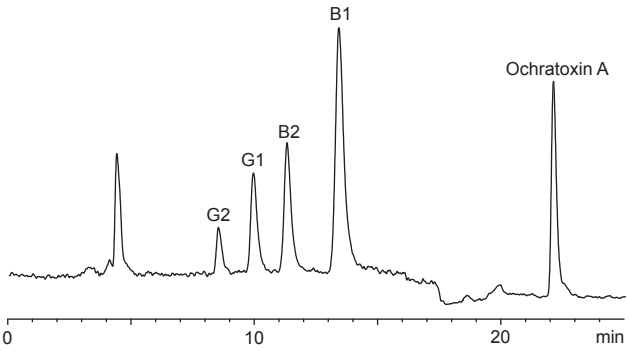


Fig 1. *Chromatogram of hemp pre-roll spiked with 6.5 ng/g of Aflatoxin B1; 1.8 ng/g of Aflatoxin B2; 6.1 ng/g of Aflatoxin G1; 1.9 ng/g of Aflatoxins G2 and 20.1 ng/g of Ochratoxin A*

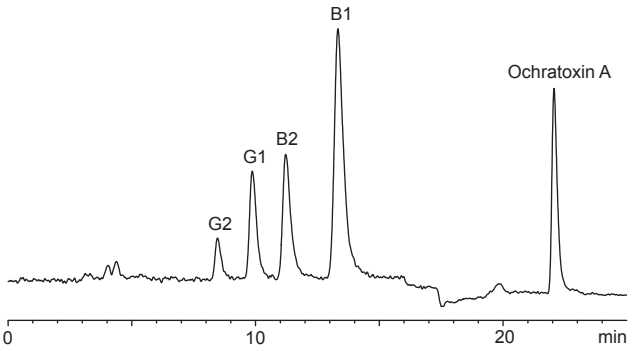


Fig 2. *Chromatogram of hemp-containing chews spiked with 6.5 ng/g of Aflatoxin B1; 1.8 ng/g of Aflatoxin B2; 6.1 ng/g of Aflatoxin G1; 1.9 ng/g of Aflatoxins G2 and 20.1 ng/g of Ochratoxin A*

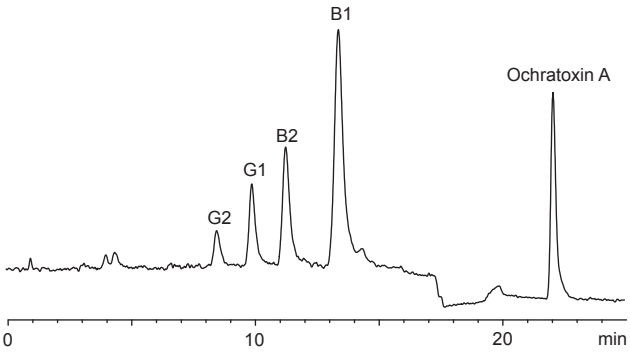


Fig 3. *Chromatogram of full spectrum hemp tincture spiked with 6.5 ng/g of Aflatoxin B1; 1.8 ng/g of Aflatoxin B2; 6.1 ng/g of Aflatoxin G1; 1.9 ng/g of Aflatoxins G2 and 20.1 ng/g of Ochratoxin A*

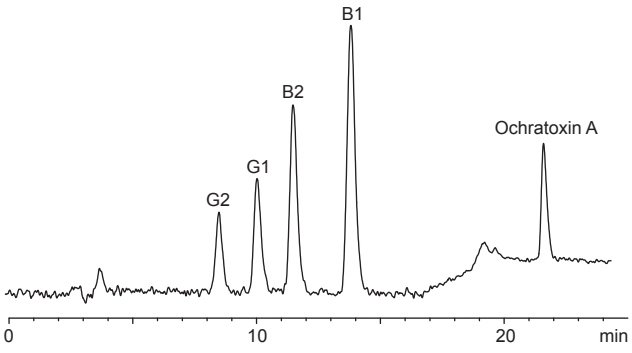
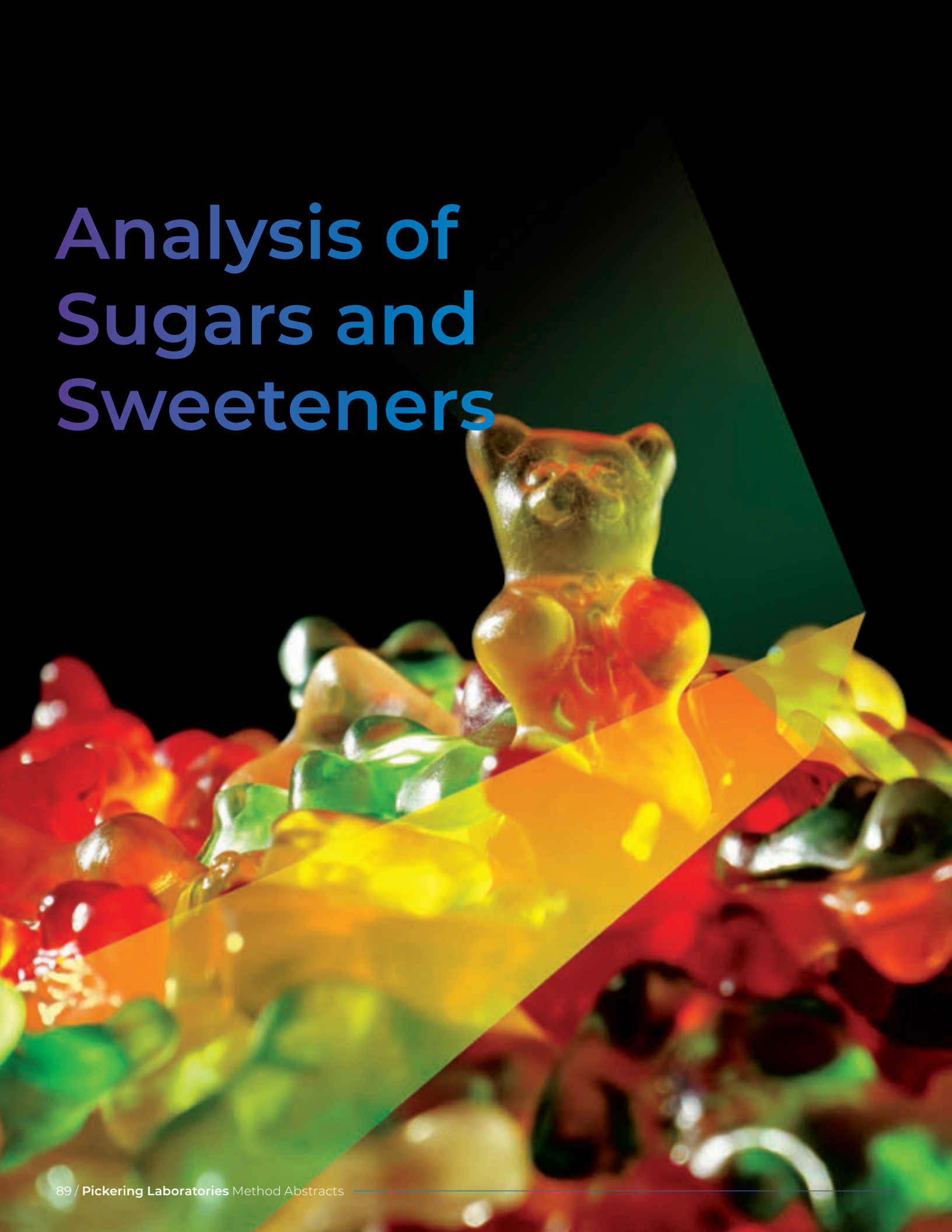


Fig 4. *Low-level Mycotoxins standard containing 0.81 ppb of Aflatoxin B1; 0.22 ppb of Aflatoxin B2; 0.31 ppb of Aflatoxin G1; 0.24 ppb of Aflatoxins G2 and 2.52 ppb of Ochratoxin A*

Analysis of Sugars and Sweeteners



Analysis of Sugars in Feeds (MA-223)

By HPLC With Post-Column Derivatization and Fluorescence Detection

The types and amounts of sugar in animal feeds are as important as the amount of protein, minerals and fats in the determination of nutritive value. We developed a simple and sensitive HPLC method for analyzing six sugars in animal feeds - Sucrose, Fructose, Glucose, Galactose, Maltose and Lactose. Post-column derivatization reagents convert reducing and non-reducing sugars into fluorescent derivatives, which greatly improves the sensitivity and selectivity of the detection.

The blends of feed examined varied from grains/vegetable products (live stock feeds) to meat/vegetable products (pet food).

Method

Sample Preparation

Mix 2.5 g of feed sample with 50 mL of water. Heat using a water bath while constantly mixing for 1 hour at 65 °C. Centrifuge and filter through 0.45 um filter.

Analytical Conditions

Column: Carbohydrate column, 4.6x150 mm

Temperature: 30 °C

Flow Rate: 1 mL/min

Mobile Phase: Acetonitrile/Water

Injection Volume: 10 uL – 50 uL

Post-Column Conditions

Post-Column System: Onyx PCX, Pinnacle PCX or Vector PCX

Reactor Volume: 1.4 mL

Temperature: 130 °C

Reagent 1: Guanidine hydrochloride 60 mM in 200 mM Boric acid adjusted to pH 11.5 with KOH

Reagent 2: 1.5 mM periodic acid adjusted to pH 11.5 with KOH

Flow Rate: 0.15 mL/min each reagent

Detection: FLD; λ_{ex} : 325 nm, λ_{em} : 465 nm

HPLC Gradient		
Time	Water %	ACN %
0.0	20	80
20.0	20	80
20.1	50	50
30.0	50	50
30.1	20	80

Calibration

A quadratic calibration curve with correlation > 0.999 is observed for monosaccharides such as Fructose, Glucose and Galactose. A linear calibration curve with correlation > 0.999 is observed for disaccharides such as Maltose, Lactose and Sucrose. Examples of calibration curves presented in Fig. 1 and Fig. 2.

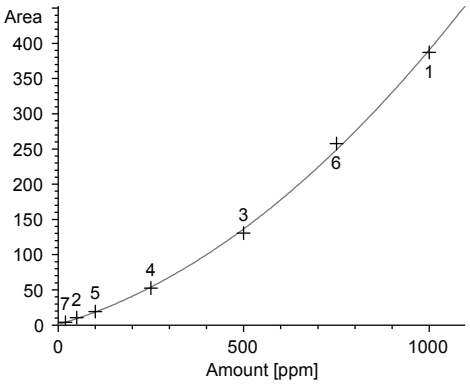


Fig 1. *Calibration curve for Frucrose.*

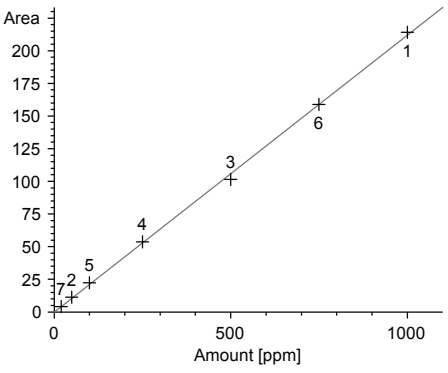


Fig 2. *Calibration curve for Maltose.*

	Fructose	Glucose	Galactose	Sucrose	Maltose	Lactose
Feed Matrix 1						
Content in feed, %	0.54	0.52	0.09	4.02	1.12	ND*
Spike Concentration, %	0.60	0.58	0.60	2.02	0.58	0.59
Recoveries, n=3, %	105	107	110	91	103	114
Spike Concentration, %	1.21	1.20	1.19	4.05	1.22	1.21
Recoveries, n=3, %	108	106	110	85	85	103
Feed Matrix 2						
Content in feed, %	0.23	0.46	ND*	3.21	0.59	ND*
Spike Concentration, %	0.42	0.57	0.56	2.50	0.57	0.58
Recoveries, n=3, %	98	101	104	106	106	107
Spike Concentration, %	0.81	1.14	1.12	4.80	1.11	1.15
Recoveries, n=3, %	103	101	101	102	102	106
Feed Matrix 3						
Content in feed, %	0.14	0.11	ND*	0.51	0.02	ND*
Spike Concentration, %	0.38	0.56	0.57	2.40	0.55	0.56
Recoveries, n=3, %	95	101	116	102	95	101
Feed Matrix 4						
Content in feed, %	0.17	0.13	ND*	1.35	0.21	ND*
Spike Concentration, %	0.41	0.57	0.58	2.43	0.56	0.56
Recoveries, n=3, %	92	97	116	101	95	102

*Not Detected

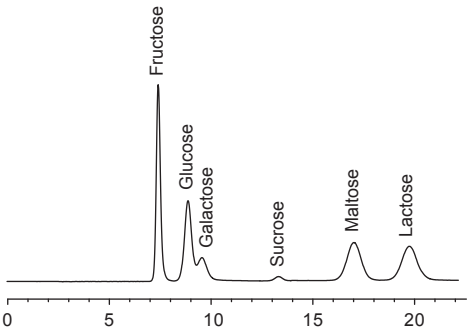


Fig 3. *Chromatogram of standard solution of sugars. Fructose 500 ppm, Glucose 500 ppm, Galactose 500 ppm, Sucrose 3000 ppm, Maltose 500 ppm, Lactose 500 ppm.*

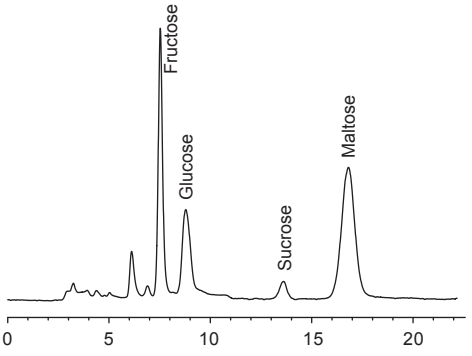


Fig 4. *Chromatogram of Feed Matrix 1. Levels of sugars present in the sample: Fructose 0.54%, Glucose 0.52%, Galactose 0.09%, Sucrose 4.02%, Maltose 1.12%.*

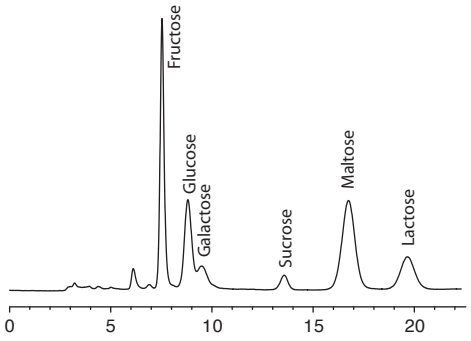


Fig 5. *Chromatogram of Feed Matrix 1 spiked with sugars. Total levels for sugars: Fructose 1.14%, Glucose 1.52%, Galactose 0.69%, Sucrose 6.02%, Maltose 1.72%, Lactose 0.6%.*

Sulfonamide Artificial Sweeteners in Food Products (MA-124)

Analysis of Acesulfame, Saccharin and Cyclamate by HPLC With Post-Column Derivatization

Non-nutritive sweeteners are widely used in foods and beverages. Since some studies raised questions about long-term safety of these compounds, their concentration in food products is regulated. Among the artificial sweeteners used worldwide Saccharin and Cyclamate cause most controversy and their use is restricted in many countries, including the US. Liquid chromatography is a method of choice for artificial sweeteners but Cyclamate analysis is complicated by the fact that this compound does not exhibit noticeable UV absorbance.

This method allows for simultaneous analysis of Acesulfame, Saccharin and Cyclamate by LC with post-column derivatization followed by fluorescence detection. Since the sweeteners are commonly used in combination, a single method of analysis is preferred.

Method

Analytical Conditions

Analytical Column: Reversed-phase column, C_{18}, 2x100 mm

Temperature: 50 ˚C

Flow Rate: 0.2 mL/min

Mobile Phase:
0.02 M Potassium Phosphate Monobasic in water. Isocratic for 8 min, followed by washing the column with 70% ACN – 30% 0.02 M KH_2PO4

Injection Volume: 10-50 uL

Post-Column Conditions

Post-Column System: Onyx PCX, Pinnacle PCX or Vector PCX

Reagent: Hexadecyltrimethyl ammonium bromide (10^{-3} M), 1,6 Diphenyl-1,3,5-hexatriene ($4x10^{-6}$ M)

Reagent Preparation:
To 250 mL of DI water add 0.09 g of Hexadecyltrimethylammonium bromide and sonicate until fully dissolved. Add 300 uL of 1,6-diphenyl-1,3,5-hexatriene (DPH) stock solution (i), mix well. Protect the reagent from light.

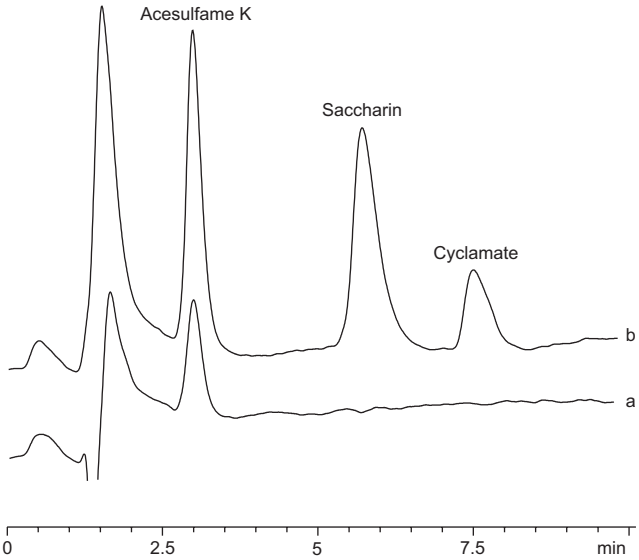
(i) Dissolve 0.007 g of 1,6-diphenyl-1,3,5-hexatriene (DPH) in 10 mL of dry THF. Store the stock solution in refrigerator, protected from light.

Reactor Volumn: 0.15 mL

Reactor Temperature: 40 ˚C

Flow Rate: 0.3 mL/min

Detection: FLD Detector,
λ_{ex} : 365 nm, λ_{em} : 460 nm



Chromatograms of a) Diet cola containing Acesulfame K; b) Soft drink spiked with 60 ppm of artificial sweeteners.

References

James F. Lawrence, Analyst, vol. 112, No 6 (1987), 879-881

James F. Lawrence, Claudette F. Charbonneau, J. Assoc. Anal. Chem., vol. 71, No 5 (1988), 934-937

Notes

Notes

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