PINNACLE PCX

VECTOR PCX

PINNACLE VS. VECTOR

UVE PHOTOCHEMICAL REACTOR

COLUMNS & APPS

# PCX
PRODUCT LINE

POST-COLUMN ANALYSIS PRODUCTS


PICKERING
LABORATORIES

## SIGMA SERIES POST-COLUMN DERIVATIZATION INSTRUMENT

# PINNACLE PCX

Part of the complete integrated system of instruments, chemicals, columns, methods and support from Pickering Laboratories.

The Pinnacle PCX Sigma Series is an optimized HPLC post-column derivatization system for analysis of Amino Acids, Carbamates, Mycotoxins, Antibiotics and many other applications. This instrument is the culmination of Pickering Laboratories' 30 years of experience in post-column analysis instrument manufacturing. Each component is specifically designed to optimize the sensitivity and selectivity of post-column analysis.

Only Pickering Laboratories offers the complete package of chemicals, columns, methods and post-column systems. Because each part of the method is designed to work together, Pickering Laboratories can offer the extraordinary promise that the analysis is guaranteed to work for the intended application.

The Pinnacle PCX reflects the ease of use, reliability and ruggedness you have come to expect from Pickering Laboratories.

### SYSTEM DESIGN ADVANCEMENTS RESULT IN OPTIMIZED ANALYSIS

- The electronic syringe pump provides true pulse-free flow for superior sensitivity and consistency. The pump cylinder and head is made form a single piece of inert ceramic for durability and non-reactivity.
- Electronic valves eliminate troublesome check valves and allow automated pump flushing.
- The quick-change reactor cartridge makes application switching easy and replacements quick and inexpensive.
- The Column oven utilizes circulating air for consistency of heating and quick cooling within 1°C of set point.
- Inert flow paths extend system life and reduce maintenance.
- The PCX Control software allows for precise control of the reagent delivery and conservation.
- Column oven temperature gradient programming improves separation and run times. Pinnacle PCX is the only Post-column system with this feature.
- Works with any HPLC system.

### FEATURES & BENEFITS

➔ WORKS WITH ALL HPLC SYSTEMS
Expand the usefulness of your existing HPLC

➔ ALL COMPONENTS SPECIFICALLY DESIGNED FOR POST-COLUMN DERIVATIZATION
No disadvantages of 'off the shelf ' component compromises

➔ ELECTRONIC SYRINGE PUMP
True pulse free flow for greater sensitivity

➔ AUTOMATIC PISTON WASH AND PROGRAMMABLE SYSTEM FLUSH
System protection and longer system life

➔ TEMPERATURE GRADIENT PROGRAMMABLE COLUMN OVEN
Improves separation, provides analytical flexibility, improves run-times and speeds up column cleaning

- ELECTRONIC VALVES
  No expensive check valves to service and replace
- QUICK CHANGE REACTOR CARTRIDGES
  Fast application switching and cartridge replacements
- ALL FLUIDICS ON FRONT PANEL
  Easy leak checks, easy access to finger tight fittings
- INERT FLOW PATH
  No metal contamination and long system life
- AMINO ACID ANALYSIS
  Use your existing HPLC, no need to purchase a dedicated amino acid analyzer
- LCD DISPLAY
  Continuous system monitoring
- FULL 32 BIT PC CONTROL WINDOWS SOFTWARE
  Ease of operation and reagent conservation
- PROGRAM STORAGE
  Flexible application setup
- LOG FILES
  Continuous data collection of system status and error messages can be sent to Pickering Laboratories support.

## TEMPERATURE GRADIENT FEATURES

- THE PROGRAMMABLE TEMPERATURE GRADIENT ADVANTAGE

The Pinnacle PCX provides a unique opportunity to combine eluant gradient capabilities of modern HPLC instruments with programmable column temperature gradients. As might be expected this capability helps reduce analysis time. Even more significantly is the ability to resolve coelutions: consider such metabolic markers as alloIsoleucine (MSUD) and Agrininosuccinic acid (ASA). Under standard isothermal conditions these amino acids coelute with Cystathionine and Isoleucine respectively but are resolved using a targeted temperature gradient program.

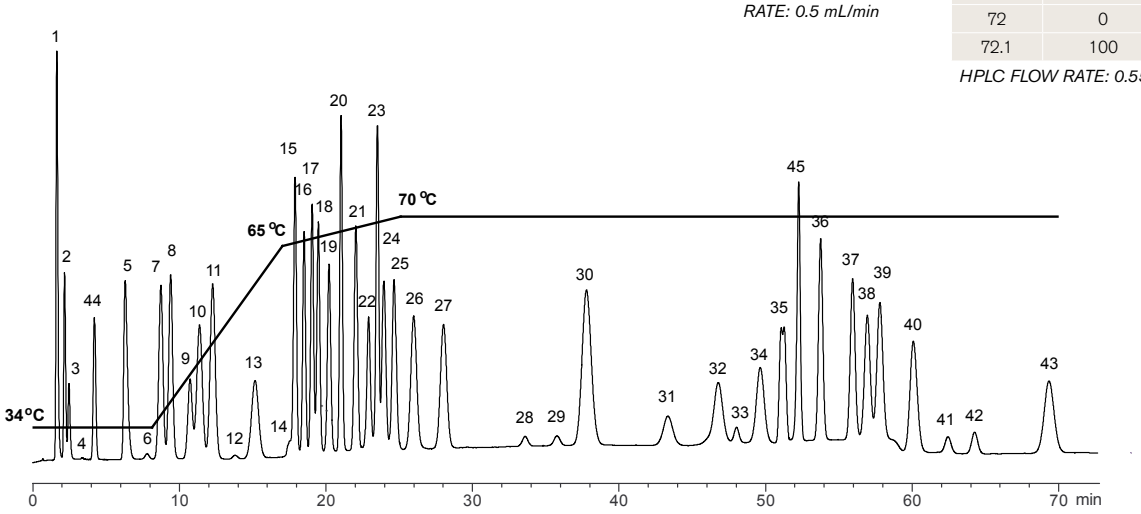
The ability to accomplish this derives from the multiple retention mechanisms of the gel-type resins employed in ion-exchange. That all the amino acids appear in the same chromatogram is testament to the dominance of ionexchange. However, the exact position is influenced by an array of mechanisms including partitioning, adsorption, charge exclusion, etc. So even though two amino acids might coelute their proximity is incidental. And since retention processes are affected differently by changes in pH, salt concentration and temperature all the parameters have significant influence on selectivity.

| COLUMN OVEN PROGRAM | |
|---|---|
| TIME | TEMP °C |
| 0 | 34 |
| 6 | 34 |
| 17 | 65 |
| 25 | 70 |
| 70 | 70 |
| 71 | 34 |

*REAGENT FLOW RATE: 0.5 mL/min*

| 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 |

*HPLC FLOW RATE: 0.55 mL/min, INITIAL TEMP.: 34 °C*




| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **1** | Phosphoserine | **10** | Glutamic acid | **19** | Valine | **28** | b-Alanine | **37** | Lysine |
| **2** | Taurine | **11** | Glutamine | **20** | Cystine | **29** | b-Amino-i-butyric acid | **38** | 1-Methylhistidine |
| **3** | Phosphoethanolamine | **12** | Sarcosine | **21** | Methionine | **30** | Homocystine | **39** | Histidine |
| **4** | Urea | **13** | a-Aminoadipic acid | **22** | Allo-Isoleucine | **31** | g-Aminobutyric acid | **40** | 3-Methylhistidine |
| **5** | Aspartic acid | **14** | Proline | **23** | Cystathionine | **32** | Tryptophan | **41** | Anserine |
| **6** | Hydroxyproline | **15** | Glycine | **24** | Isoleucine | **33** | Ethanolamine | **42** | Carnosine |
| **7** | Threonine | **16** | Alanine | **25** | Leucine | **34** | Ammonia | **43** | Arginine |
| **8** | Serine | **17** | Citrulline | **26** | Tyrosine | **35** | Hydroxylysines | **44** | Glucosaminic Acid* |
| **9** | Asparagine | **18** | a-Amino-n-butyric acid | **27** | Phenylalanine | **36** | Ornitine | **45** | 2-Aminoethyl-cysteine (AEC)* |

**Internal Standard*

*NOTE: This method utilizes column temperature gradient. Use Pinnacle PCX column oven or HPLC column oven with temperature gradient capabilities.*

***High-efficiency Lithium for Physiological samples using temperature gradient***

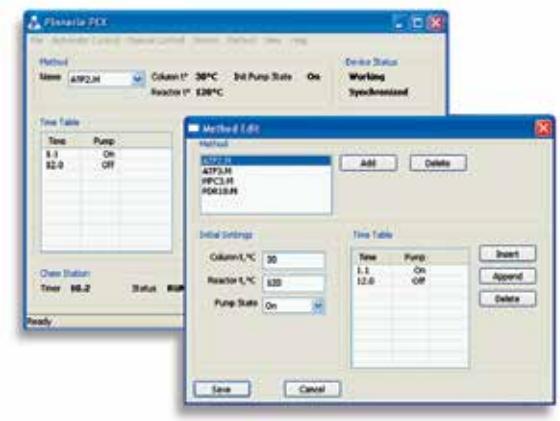
## PCX CONTROL SOFTWARE

The Pinnacle PCX Sigma Series is controlled by the PCX Control Software running on Microsoft Windows. Compatible with Windows XP, Vista or Windows 7. Using the same computer as the HPLC the PCX Control Software interfaces easily with Agilent Chemstation or with any other modern HPLC using a relay connection. The computer can physically connect to the Pinnacle PCX unit through USB cable or Ethernet network protocol.

After an easy installation and configuration the software runs in a window or as an icon on the system desktop. The main screen displays an emulation of the digital LCD display of the system where all PCX functions of temperature, flow rate and system status are displayed in real time. This allows for monitoring and control of the PCX and HPLC from one computer.

Methods are managed in the Method window. Here methods are created, edited and saved to create a library for all application parameters. A sequence table is used to schedule multiple runs of the same or different methods in a series. At the end of the sequence a full system flush can be programmed.

System & pump performance can be evaluated in the maintenance menu where you'll find a pump pressure test and system pressure test. A system log file continually records system status and error messages for later reference and can be sent to Pickering Support for remote diagnostics.




*Realtime synchronization with Pinnacle PCX.*

## COMPONENTS

➔ ELECTRONIC SYRINGE PUMP AND VALVES

The syringe pump cylinder and head is made from a single piece of 99.9 % Alumina for ruggedness and nonreactivity. The piston surface is made from peek with an inert o-ring seal. The piston seal is protected by an automatic piston wash system that provides long seal life. The programmable flow rates range from 50 µl to 1500 µl/minute with less than a 60 second refill cycle. The electronic valves utilize peek and teflon in an interference fit interface with a port layout that eliminates cross contamination.

➔ REACTOR

The reactor is designed for quick heating and ease of application switching. The heating and control electronics are in a base unit of the reactor while the coil volumes are inserted with a 'quick-change' cartridge in the front of the fluidics panel. The temperature range holds within 1 °C resolution from 5 °C above ambient to 130 °C.

➔ COLUMN HEATER

The column heater utilizes recirculating air flow technology to provide quick, uniform column heating. Fast column cooling is assisted by the introduction of a fresh air flow into the chamber. The temperature range holds within 1 °C resolution from 5 °C above ambient to 75 °C. The temperatures can be programmed for a gradient with as many steps as required for fine-tuning an analysis.

➔ USABILITY DESIGN

The design of the Pinnacle PCX Delta Series is focused on ease of use, quick monitoring, and rapid service. Lab bench space is conserved by the narrow and tall configuration. All the fluidics are on the front panel for easy monitoring and configuration. A convenient drip tray is locked below to catch fluids during tubing changes. The side panels quickly slide off to reveal all the mechanical components on one side and all the electronics on the other side. A removable reagent tray is integrated into the top of the unit for easy inspection and access. The gas manifold is integrated into the system for ease of set-up and especially for reagent preparation and switching. The temperature gradient column oven is oriented for easy column attachment and switching. The LCD provides real time monitoring of the system status and programs.


| | | | |
|---|---|---|---|
| 1 | REAGENTS | 7 | REACTOR COIL |
| 2 | DISPLAY | 8 | TRANSDUCER |
| 3 | PUMP TWO | 9 | VALVE ONE |
| 4 | VALVE TWO | 10 | PUMP ONE |
| 5 | COLUMN OVEN | 11 | DRIP TRAY |
| 6 | AMBIENT REACTOR COIL | | |

## SPECIFICATIONS

- DIMENSIONS
  - 21.25 H x 10.5 W x 18.25 D
- WEIGHT
  - 48 lbs
- ELECTRICAL
  - 100-120 V, 50/50 Hz, 1.7 A, 200 W or 200-240 V, 50/60 Hz, 0.8 A, 200 W
  - Mains voltage ± 10 % of nominal
  - Installation (over voltage) category II, pollution degree 2
- ENVIRONMENTAL
  - Indoor use only
  - Altitude up to 6500 ft
  - Ambient Temperature 15 ° - 25 °C
  - Relative humidity up to 80 % at 31 °C
- REAGENT PUMPS
  - True pulse-free syringe pump
  - Single piece ceramic barrel
  - Completely inert flow path
  - Maximum operating pressure 500 psi
  - Programmable flow-rate
  - Flow range; 50 µL to 1500 µL/minute
  - Refill cycle of 60 seconds
  - Automatic piston wash
  - Automatic reagent flush cycle
  - No check valves
- REACTOR
  - Heated reactor for temperature from 5 °C above ambient to 130 °C
  - Easy replacement coil cartridges
  - Range of reactor dwell volumes; 0.1 mL to 3 mL
  - Reaction coil withstands up to 42 bar (600 psi) inlet pressure at 130 °C
  - Thermal safety switch limits temperature to 150 °C to prevent damage
  - Fast response
- SAFEGUARDS
  - In-line check valve: Prevents reagent back flow into the column when HPLC pressure drops
  - Replaceable reagent filters: Prevent reactor fouling
  - Post-column system over pressure: A pre-calibrated relief valve opens at 35 bar (500 psi) to prevent rupture of the post-column reactor tubing in the event of down-stream blockage
  - Back-pressure regulator: Applies 7 bar (100 psi) to the detector flow cell outlet (waste) to prevent detector noise and precipitation due to out-gassing or boiling
- COLUMN HEATER AND REACTOR CONTROLLER
  - Heater accepts 6 or 8 mm OD (0.25 or 0.31 inch) x 50-250 mm in length Column and guard
  - Programmable Temperature gradient
  - Temperature holds within ± 1 °C resolution from 5 °C above ambient to 75 °C
  - Easy column access
- INSTRUMENT PACKAGE AND FLOW PATH
  - Advanced fluidics valve management system
  - Easy access to internal components
  - Standard fittings
  - Post-column pressure relief valves
  - Side panels easily remove for service
  - Integrated Reagent reservoir tray
  - Corrosion proof pan and panels
- DISPLAY
  - Back light LCD
  - Real time temperature and pressure display
  - System Status icons
  - Simple system control interface
- GAS PRESSURE MANIFOLD AND REGULATOR
  - Panel mount manifold
  - Regulator maintains 0.3 bar (3-5 psi) on reagent reservoirs with 3-5 bar (45-75 psi) source pressure
  - Safety Pressure-relief valve opens at 6 bar (8 psi)
  - Manifold with anti-siphon valves has two 1/4-28 fittings

## APPLICATIONS

- AMINO ACIDS
- CARBAMATE PESTICIDES
- GLYPHOSATE HERBICIDE
- MULTI RESIDUE MYCOTOXIN IN FEEDS
- AMINOGLYCOSIDE ANTIBIOTICS
- BIOGENIC AMINES
- POLYETHER ANTIBIOTICS
- BROMATE
- FORMALDEHYDE
- CHROMIUM VI
- GUANIDINOS
- HEXOSAMINES
- PARALYTIC SHELLFISH TOXINS
- PKU / MSUD
- PARAQUAT & DIQUAT
- POLYPHOSPHATES/PHOSPHONATES
- SULFA DRUGS
- TRANSITION/RARE EARTH METALS
- VITAMINS B1, B6
- CUSTOM APPLICATIONS

## INTEGRATED SYSTEM

- COMPLETELY INTEGRATED ANALYSIS SYSTEM

Only Pickering Laboratories offers the complete package of reagents, columns, methods and post-column systems. Because each part of the method is designed to work together, Pickering Laboratories can offer the extraordinary promise that the analysis is guaranteed to work for the intended application.

- COLUMNS

Pickering Laboratories manufactures optimized cation-exchange and reverse-phase columns for specific analytical applications. Each column is guaranteed to separate the analytes of interest when used according to the specific protocol. Each column is manufactured to GMP quality standards and each column packaged with the specific quality assurance application chromatogram.

Cation-exchange columns are packed with fully sulfonated polystyrene divinylbenzene resin. These resins are very durable under high pressures with wide pH stability and high selectivity and reproducibility. These columns offer long lifetimes and reproducibility over hundreds of injections.

- CHEMISTRY

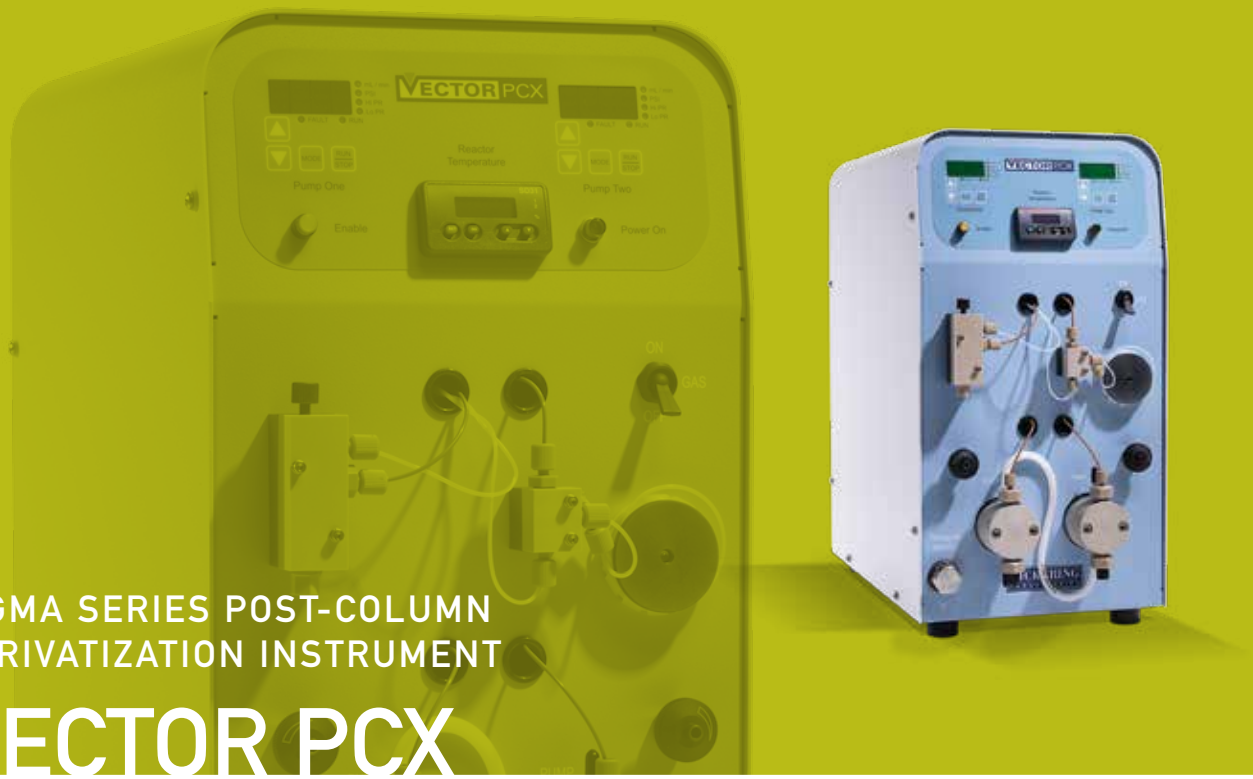
All Pickering Laboratories chemistries are Chromatographic Grade™ and optimized for analytical use. Through exhaustive validation and confirmation by analytical laboratories the Pickering Laboratories Reagents and Chemicals have a reputation worldwide for quality and reliability in all analytical systems and methods.

- SUPPORT

All applications, systems and products are supported by a team of application chemists available for phone and email consultation. We believe the quality of our products includes the quality of our support for your analysis.

- FACTORY AUTHORIZED SERVICE CONTRACTS

Now Pickering Laboratories offers Services Contracts that include on site visits and Preventative Maintenance visits. Visit our web site for more information.




## SIGMA SERIES POST-COLUMN DERIVATIZATION INSTRUMENT

# VECTOR PCX

The Vector PCX instrument is the newest addition to the Pickering family of post-column derivatization systems. Introduced a year after the Pinnacle PCX launch, the new Vector PCX serves as another post-column choice ideal for application-specific methods.

Vector PCX provides the selectivity and sensitivity required for most standard post-column applications while being reliable and easy to use. Since Vector PCX does not have a column oven it is important to use the HPLC column oven to ensure stable column temperature and prevent retention time drifts and separation problems.

Designed as a step-up from our previous model PCX5200, the Vector PCX has several improvements and innovations.

### FEATURE HIGHLIGHTS

- WORKS WITH ANY HPLC
- RUGGED AND DEPENDABLE
- AUTOMATIC PISTON-WASH
- LOW-PULSATION FLOW

### APPLICATIONS

- AMINO ACIDS
- CARBAMATE PESTICIDES
- GLYPHOSATE HERBICIDE
- MULTI RESIDUE MYCOTOXIN IN FEEDS
- AMINOGLYCOSIDE ANTIBIOTICS
- BIOGENIC AMINES
- POLYETHER ANTIBIOTICS
- BROMATE
- FORMALDEHYDE
- CHROMIUM VI
- GUANIDINOS
- HEXOSAMINES
- PARALYTIC SHELLFISH TOXINS
- PKU / MSUD
- PARAQUAT & DIQUAT
- POLYPHOSPHATES/PHOSPHONATES
- SULFA DRUGS
- TRANSITION/RARE EARTH METALS
- VITAMINS B1, B6
- CUSTOM APPLICATIONS

## SPECIFICATIONS

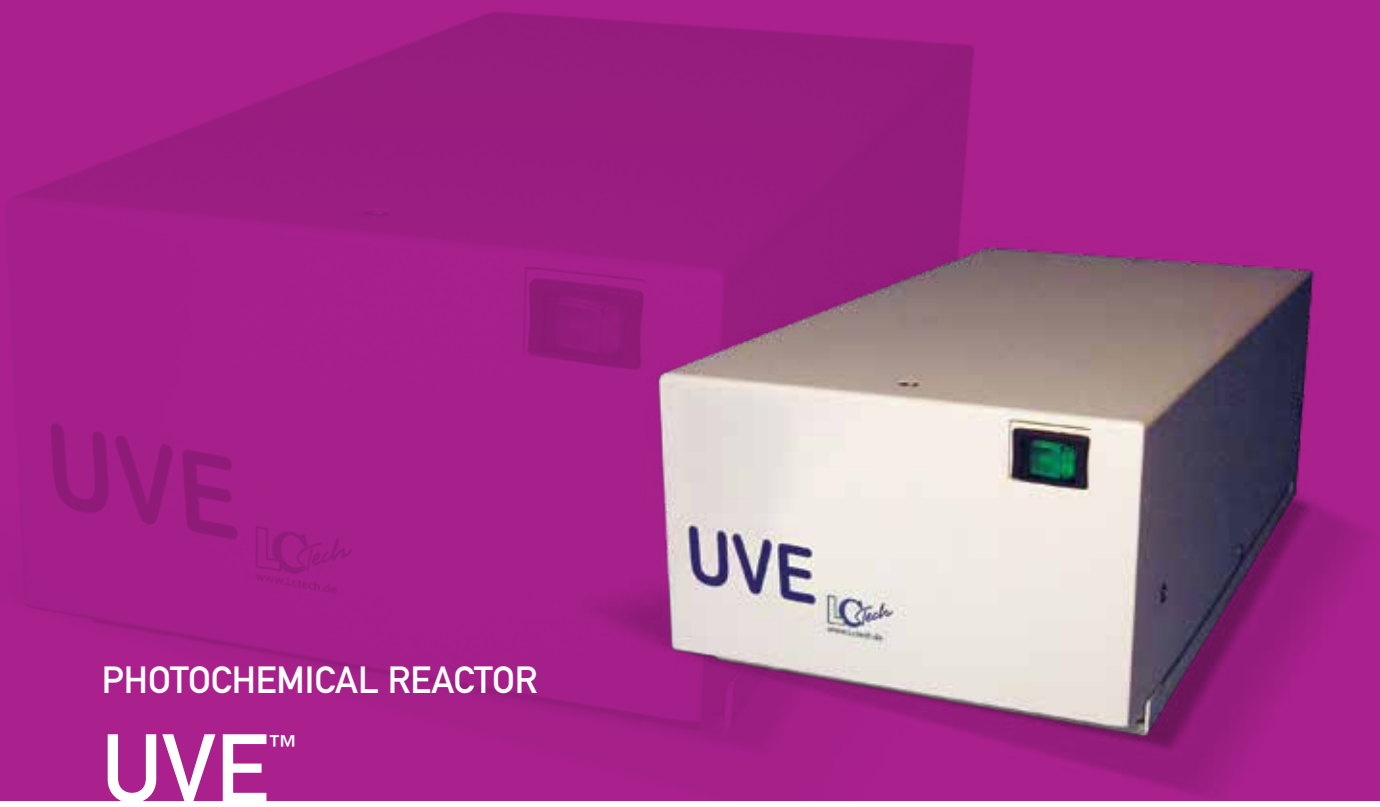
- DIMENSIONS
  - (H x W x D): 43 x 21.6 x 41.2 cm (17 x 8.75 x 16 inches)
- WEIGHT
  - 11.5 kg (25.3 lb.)
- ELECTRICAL
  - 100 - 120 V, 50/60 Hz 1.7 A, 200 W or
    200-240 V, 50/60 Hz, 0.8 A, 200 W
    Mains voltage ± 10 % of nominal
    Installation (overvoltage) category II, pollution degree 2
- REAGENT PUMPS
  - Independently adjustable, low-pulsation
  - Adjustable from 0.05 to 2.00 mL/minute against back-pressures of up to 2000 psi
  - Flow Accuracy 3 % for flowrates of 0.33 ml/min and above, 0.01 ml/min for flowrates below 0.33 ml/min
  - Flow Precision 0.5 % RSD
  - Sapphire pistons
  - Liquid ends; including check valve housing PEEK
  - PEEK Bypass/purge valves for each pump located on front of instrument panel
  - Automatic Piston wash
- FLOW PATH
  - Independent pressure transducer for each pump 210 bar (0-3000 psi)
  - Diamond-packed restrictors, match to flow rate and viscosity of reagents
  - PEEK Bypass/purge valves
  - Replaceable reagent filter
  - PEEK mixing manifold
- REACTOR
  - Heater reactor controls at ± 0.4 °C for temperatures from 10 °C above ambient to 130 °C. Range of reactor dwell volumes, depending upon application
  - Reaction coil withstands up to 42 bar (600 psi) inlet pressure at 130 °C
  - LCD display of actual temperature or set point
  - Thermal safety switch limits temperature to 150 °C
- SAFEGUARDS
  - Post-column Reagent Backflow
    A pressure switch installed between LC (eluant) pump and sample injector turns off power to reagent pumps and reactor when the eluant pump pressure drops to 35 bar (500 psi), ensuring that reagent will not flow upstream and damage the analytical column. Low eluant pressure can result from power failure, eluant pump malfunction, automatic or intentional shut-down, or an empty reservoir. The Vector PCX will not restart automatically.
  - Post-column System Over-pressure
    A relief valve pre-calibrated to open at 35 bar (500 psi) prevents rupture of the post-column reactor tubing in the event of downstream blockage, and reduces the possibility that all or part of the reagent flow will be diverted to the column
  - Detector Noise, Precipitation
    Back-pressure regulator applies 7 bar (100 psi) to the detector noise and precipitation due to solvent outgassing or boiling adjustable (2-10 bar)
- GAS PRESSURE MANIFOLD AND REGULATOR
  - Regulator maintains 0.3 bar (3-5 psi) on reagent reservoirs with 3-5 bar (45-75 psi) source pressure
  - Pressure-relief valve opens at 0.7 bar (10 psi)
  - Manifold has two 1/4-28 tubing connections
- PRESSURIZED REAGENT RESERVOIR
  - One liter capacity (2 and 5 L reservoirs available)
  - Maintained under inert gas pressure to inhibit oxidation of OPA or other oxygen-sensitive reagents
  - Valve built into reservoir cap permits sparging during reagent preparation
  - Reagent reservoirs fitted with 3.1 mm OD oxygen-impermeable Saran tubing for oxygen-sensitive reagents and/or with 3.1 mm OD FEP tubing.
  - Check-values on the gas lines prevents back-flow of the reagent into the manifold in case of pressure drop.


# PINNACLE PCX VS. VECTOR PCX

The Pinnacle PCX and Vector PCX are Post-Column Derivatization instruments designed with features and capabilities that satisfy diverse laboratory requirements.

This reference chart lists comparisons of specific features and applications for the Pinnacle PCX and the Vector PCX.

| FEATURES COMPARISON OF PINNACLE PCX AND VECTOR PCX | | |
|---|---|---|
| **FEATURES** | **PINNACLE PCX** | **VECTOR PCX** |
| Works with All HPLC Systems | Yes | Yes |
| All Components specifically designed for Post-Column Derivatization | **Yes** | No |
| Pump Technology | Syringe | Reciprocating |
| Piston Wash | Automatic | Automatic |
| Programmable System Flush | **Yes** | No |
| Column Oven | **Yes** | No |
| Temperature Gradient Column Oven | **Yes** | No |
| Automatic Column Oven Cool Down (10 min) | **Yes** | No |
| Electronic Valves | **Yes** | No |
| Check Valves | No | **Yes** |
| Flow Restrictors | No | **Yes** |
| Quick Change Reactor Cartridges | **Yes** | No |
| All Fluidics on Front Panel | **Yes** | No |
| Inert Flow Path | Standard | Custom |
| LCD Display | Graphical | Text |
| Full 32 bit PC Control Windows Software | Included | No |
| Program Storage | Unlimited | No |
| Network Enabled | **Yes** | No |
| Remote Diagnostics | Yes | Yes |
| Reduced Bench Space | **Yes** | No |
| Integrated Reagent Tray | **Yes** | No |
| Integrated Spill Tray | **Yes** | No |
| Integrated Lab Gas Manifold | Yes | Yes |
| Reduce Maintenance Costs | **Yes** | No |
| Reduce Reagent Usage/Costs | **Yes** | No |




## PHOTOCHEMICAL REACTOR

# UVE™

## Used for Detection Enhancement for Aflatoxins, Phenylurea Pesticides, Barbiturates and Other Compounds.

Photochemical derivatization is a simple, inexpensive and flexible technique that improves sensitivity and selectivity of detection for a broad range of analytes. Among the applications for the photochemical reactor are analysis of Aflatoxins in foods, Phenylurea Pesticides in water and Barbiturates in biological samples. Photochemical derivatization also allows identification of closely related compounds such as polyphenols.

Pickering Laboratories Multi-residue Mycotoxins method for DON, Aflatoxins, Fumonisins, Ochratoxin A and Zearalenone employs photochemical derivatization for Aflatoxins, allowing detection at sub-ppb levels.

The photochemical reactor has a 254 nm lamp and a knitted reactor coil.

### FEATURE HIGHLIGHTS

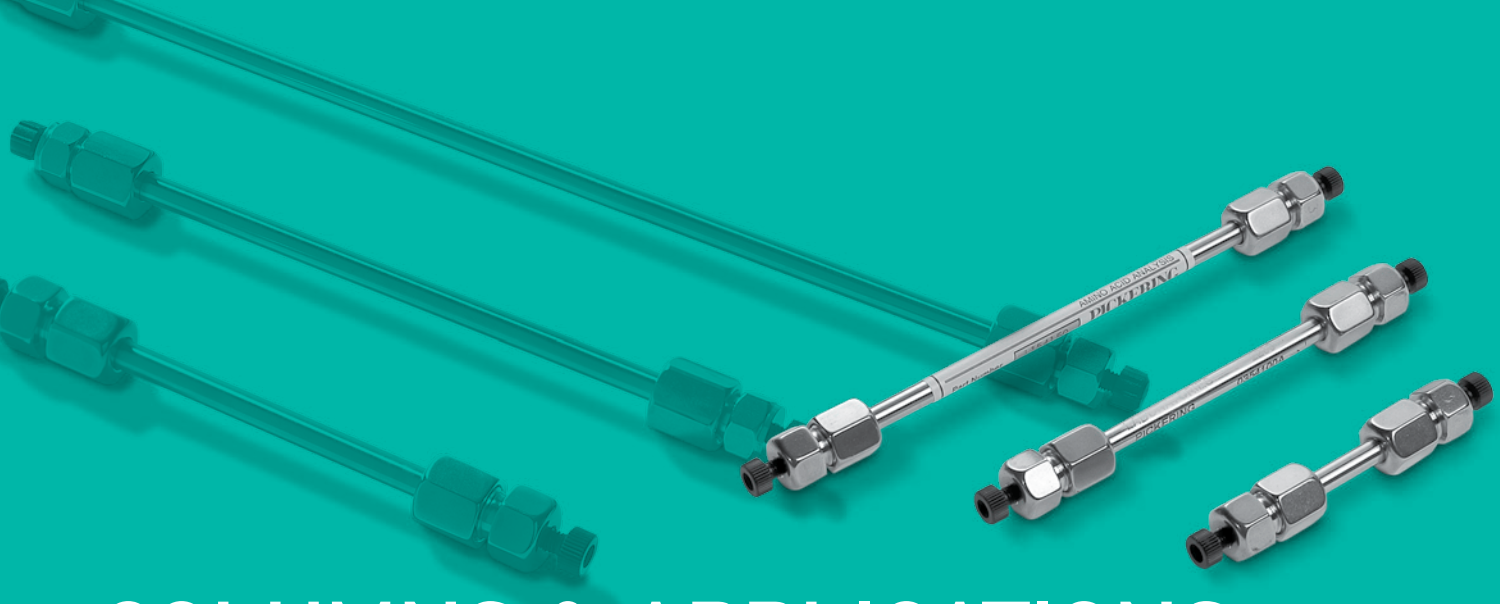
- 254 nm UV Low Pressure Lamp With Cooled Reflector Tube
- Long Term Stability Of Lamp And Coil
- High Light Transmission
- Safety Shut-off Turns Off Lamp If Cover Is Removed
- Robust Steel Housing To Meet Laboratory Requirements
- Special Designed Fluorocarbon Coil
- Comparable To Electrochemical Derivatization With Cobra Cell (DG Joint Research Center Of The European Commission In The Institute For Health And Consumer Protection)
- AOAC Accepted Methodology
- Standard Reactor Volume Is 1.0 mL

### APPLICATIONS

- AFLATOXIN
- PHENYLUREA PESTICIDES
- BARBITURATES
- TAMOXIFEN
- OTHER INDOLE-CONTAINING MYCOTOXINS

| UVE™ SPECIFICATIONS | |
|---|---|
| CE CERTIFIED | Yes |
| UV LAMP | 254 nm |
| REACTOR COIL | Special |
| DIMENSIONS | 14.5 x 8.5 x 27 cm |
| POWER INPUT | 50 W |
| WEIGHT | 3 kg |

| UVE™ CATALOG INFORMATION | |
|---|---|
| CATALOG NO. | DESCRIPTION |
| 1100-3347 | Photochemical Reactor 1.0 mL, 120 V |
| 1100-3348 | Photochemical Reactor 1.0 mL, 240 V |
| 1552-0024 | Lamp, 254 UV, Photochemical |
| 1100-3505 | Reactor Coil, Photochemical 1.0 mL |



# COLUMNS & APPLICATIONS

Pickering Laboratories columns and guards are intended for specific applications that require post-column derivatization. This technology guarantees detection of certain classes of compounds at very low concentrations—amino acids, carbamate pesticides and polyamines, for example.

Each column is packed and tested to separate the target compounds according to Pickering's chromatographic quality control standards and published analytical method. The following acceptance criteria apply to all of Pickering's columns:

- With guard column installed, produce a specified chromatogram of the compounds in a standard test mixture.
- Separate the compounds in the established order, with specified resolution of critical pairs.
- Operate within the specified range of back pressure.
- Be free of contaminating material which can cause baseline artifacts.

Only after all criteria have been met can the column's serial number and label be applied.

Quite simply, the column is guaranteed to produce a chromatogram for its intended application if it is operated according to the conditions and methods prescribed by Pickering Laboratories.

## ABOUT GUARD COLUMNS

While it is true that any of our columns may be run without a guard, the practical consequence is a shorter column lifetime. Guard columns provide protection against contamination without affecting column efficiency. It is far less expensive to replace or repack a guard than an analytical column.

## APPLICATIONS

- AMINO ACIDS
- CARBAMATE PESTICIDES
- GLYPHOSATE HERBICIDE
- MULTI RESIDUE MYCOTOXIN IN FEEDS
- AMINOGLYCOSIDE ANTIBIOTICS
- BIOGENIC AMINES
- POLYETHER ANTIBIOTICS
- BROMATE
- FORMALDEHYDE
- CHROMIUM VI
- GUANIDINOS
- HEXOSAMINES
- PARALYTIC SHELLFISH TOXINS
- PKU / MSUD
- PARAQUAT & DIQUAT
- POLYPHOSPHATES/PHOSPHONATES
- SULFA DRUGS
- TRANSITION/RARE EARTH METALS
- VITAMINS B1, B6
- CUSTOM APPLICATIONS

# 30-MIN HIGH-EFFICIENCY SODIUM CATION-EXCHANGE COLUMN (4.6 X 110 MM)

CATALOG NUMBER 1154110T
USE WITH CATION-EXCHANGE GARD™ COLUMN PROTECTION SYSTEM 1700-3102
USE FOR PROTEIN AND OXIDIZED FEEDS HYDROLYSATE
TEMPERATURE GRADIENT

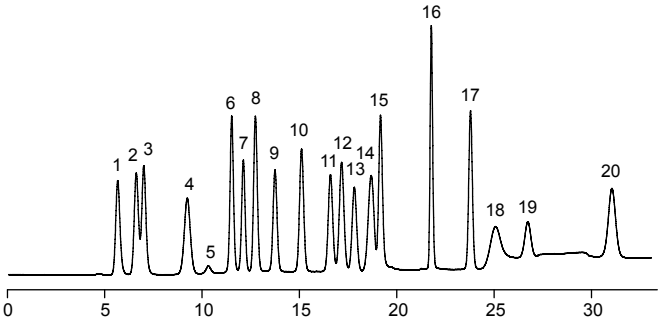



*Fig 1. Chromatogram of protein hydrolysate standard*

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

| 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 |

HPLC FLOW RATE: 0.6 mL/min
INITIAL TEMP: 46 °C
INJECTION VOLUME: 10 mL OF 0.25 mmole/mL STD.

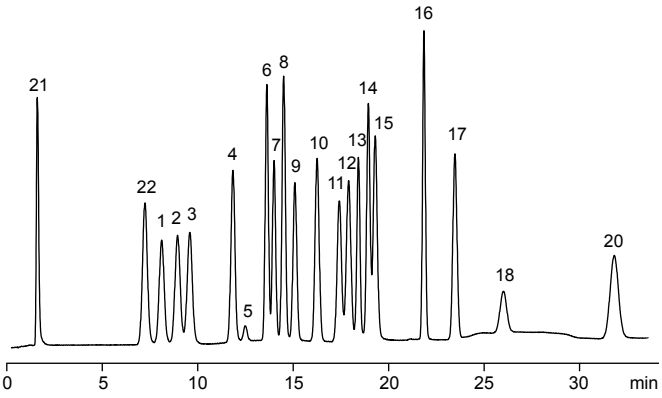



*Fig 2. Chromatogram of oxidized feeds hydrolysate standard*

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

| 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 | 100 | 0 | 0 | 0 |

| COLUMN OVEN PROGRAM | |
|---|---|
| TIME | TEMP °C |
| 0 | 55 |
| 12 | 55 |
| 17 | 70 |
| 32 | 70 |
| 33 | 55 |

HPLC FLOW RATE: 0.6 mL/min
INITIAL TEMP: 55 °C
INJECTION VOLUME: 10 mL OF 0.25 mmole/mL STD.

# 70-MIN HIGH-EFFICIENCY LITHIUM CATION-EXCHANGE COLUMN (4.6 X 75 MM)

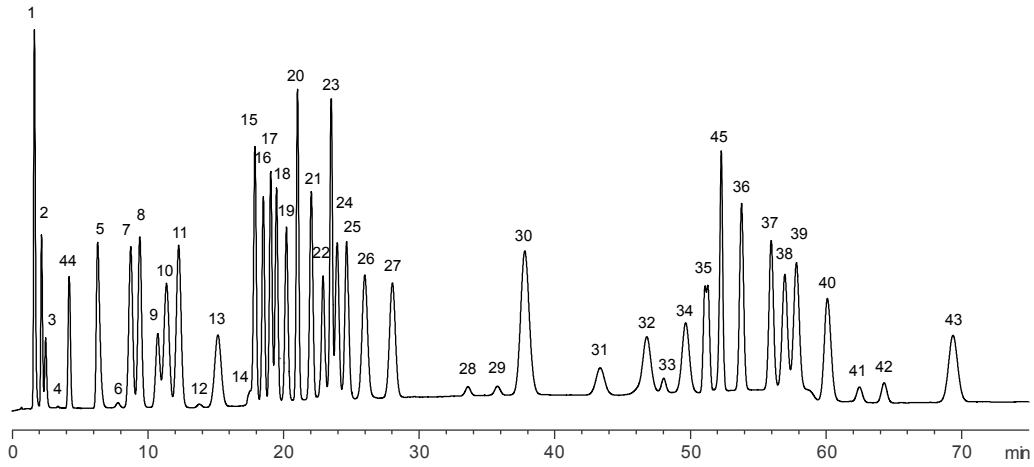
**CATALOG NUMBER 0354675T**
**USE WITH CATION-EXCHANGE GARD™ COLUMN PROTECTION SYSTEM**
**USE FOR PHYSIOLOGICAL SAMPLES**
**TEMPERATURE GRADIENT**

| 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 |

*HPLC FLOW RATE: 0.55 mL/min, INITIAL TEMP.: 34 °C*

| COLUMN OVEN PROGRAM | |
|---|---|
| TIME | TEMP °C |
| 0 | 34 |
| 6 | 34 |
| 17 | 65 |
| 25 | 70 |
| 70 | 70 |
| 71 | 34 |

*REAGENT FLOW RATE: 0.5 mL/min*




| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **1** | Phosphoserine | **13** | a-Aminoadipic acid | **25** | Leucine | **37** | Lysine |
| **2** | Taurine | **14** | Proline | **26** | Tyrosine | **38** | 1-Methylhistidine |
| **3** | Phosphoethanolamine | **15** | Glycine | **27** | Phenylalanine | **39** | Histidine |
| **4** | Urea | **16** | Alanine | **28** | b-Alanine | **40** | 3-Methylhistidine |
| **5** | Aspartic acid | **17** | Citrulline | **29** | b-Amino-i-butyric acid | **41** | Anserine |
| **6** | Hydroxyproline | **18** | a-Amino-n-butyric acid | **30** | Homocystine | **42** | Carnosine |
| **7** | Threonine | **19** | Valine | **31** | g-Aminobutyric acid | **43** | Arginine |
| **8** | Serine | **20** | Cystine | **32** | Tryptophan | **44** | Glucosaminic Acid* |
| **9** | Asparagine | **21** | Methionine | **33** | Ethanolamine | **45** | 2-Aminoethyl-cysteine (AEC)* |
| **10** | Glutamic acid | **22** | Allo-Isoleucine | **34** | Ammonia | | |
| **11** | Glutamine | **23** | Cystathionine | **35** | Hydroxylysines | | |
| **12** | Sarcosine | **24** | Isoleucine | **36** | Ornitine | | |

**Internal Standard*

*NOTE: This method utilizes column temperature gradient. Use Pinnacle PCX column oven or HPLC column oven with temperature gradient capabilities.*

# CARBAMATE PESTICIDE ANALYSIS COLUMNS

Pickering Laboratories carbamate columns are guaranteed to produce the separation of carbamate residues, specified by EPA and AOAC methods. For $C_{18}$ columns, two water/methanol gradient conditions are available for analysis of methanolic and water samples. Expanded resolution $C_8$ column is capable of separating as many as 23 carbamates with either water/methanol or water/acetonitrile gradients. The difference in selectivity between Methanol and Acetonitrile protocols could be used for confirming peak identification.

***Post-column conditions for pesticide analysis:***

*Reagent 1:* Hydrolysis reagent CB130 or CB130.2

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

*Reactor 1:* 100 °C, 0.5 mL

*Reactor 2:* ambient. 0.1 mL

*Reagents flow rate:* 0.3 mL/min

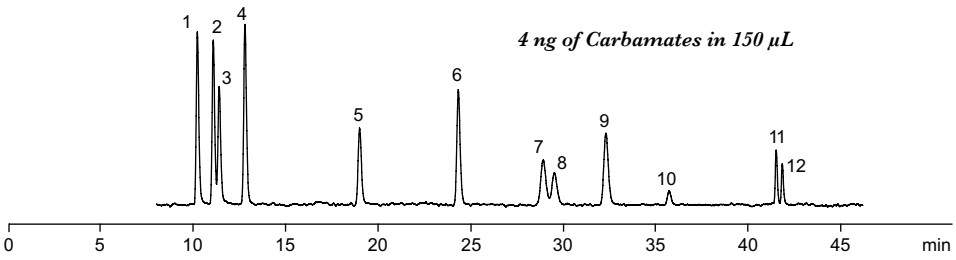
***Detection:***

*Fluorometer: $\lambda_{ex}$ 330 nm, $\lambda_{cm}$ 465 nm*

The recommended gradient conditions are subject to change without notice. This may happen because of lot changes in the columns, or improvements in the overall method.

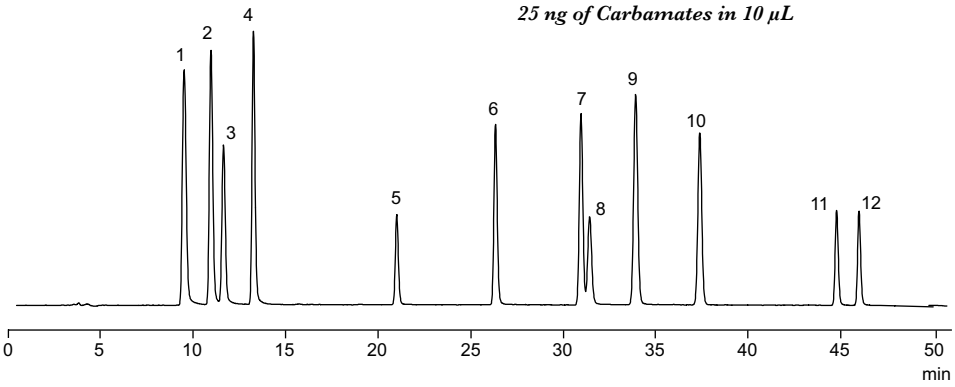
***The recommended gradient for the column will always be included in the column package, and it supersedes the information in this catalog.***

## CARBAMATE COLUMN 1846250 (4.6 X 250 MM), $C_{18}$, 5 µM




| CONDITIONS FOR AQUEOUS SAMPLES | | |
|---|---|---|
| TIME (MIN) | WATER % | MEOH % |
| 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 |

*FLOW RATE: 1 mL/min,*
*COLUMN TEMP.: 42 °C,*
*INJECTION VOLUME: Up to 400 µL*




| CONDITIONS FOR METHANOLIC SAMPLES | | |
|---|---|---|
| TIME (MIN) | WATER % | MEOH % |
| 0 | 85 | 15 |
| 1 | 85 | 15 |
| 44 | 25 | 75 |
| 44.1 | 0 | 100 |
| 49 | 0 | 100 |
| 49.1 | 85 | 15 |
| 57 | 85 | 15 |

*FLOW RATE: 1 mL/min,*
*COLUMN TEMP.: 42 °C,*
*INJECTION VOLUME: 10 µL*

| | | | | | |
|---|---|---|---|---|---|
| **1** | Aldicarb sulfoxide | **5** | 3-Hydroxycarbofuran | **9** | Carbaryl |
| **2** | Aldicarb sulfone | **6** | Aldicarb | **10** | 1-Naphthol |
| **3** | Oxamyl | **7** | Propoxur | **11** | Methiocarb |
| **4** | Methomyl | **8** | Carbofuran | **12** | BDMC (internal standard) |

# CLEAN-UP AND DETERMINATION OF AFLATOXINS IN PEANUT AND PEANUT BUTTER USING IMMUNOAFFINITY CLEAN-UP WITH HPLC - POST-COLUMN UVE PHOTOCHEMICAL DERIVATIZATION

*Wendy Rasmussen, Maria Ofitserova, PhD*

## BACKGROUND

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. The proposed method and instrumentation allows quick and efficient detection of Aflatoxins at the low ppb level.

## PROJECT OVERVIEW

As part of a NIST study, we analyzed check samples of peanuts and peanut butter. The extracts were cleaned up using the AflaCLEAN™ (LCTech, Germany) Immunoaffinity columns for Aflatoxin B1, B2, G1, G2.

For the handling of the columns, we used the AcceCLEAN automated system that processes three samples simultaneously. We then analyzed the cleaned extracts using HPLC and post-column Photochemical derivatization using the UVE Photochemical Reactor.

The Immunoaffinity columns provide an easy, specific cleanup for Aflatoxins while the UVE photochemical reactor transforms B1 & G1 into stable fluorescent derivatives. The UVE derivatization module requires no additional reagents and is easy to install between the HPLC column and FLD detector.

## ISOLATION OF AFLATOXINS B1, B2, G1, G2

Blend 20g of sample at high speed with extraction solution (100 mL of Methanol/water 80/20, 50 mL of Hexane, 2 g NaCl) and filter through fluted paper. Dilute 14 mL of aqueous layer with 86 mL of PBS buffer (pH7.2), filter and apply 11 mL of solution on AflaCLEAN™ Immunoafinity column. The toxins are eluted with 2 mL of Methanol and analyzed using HPLC with photochemical derivatization.

***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 peanuts 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 |

## ANALYTICAL CONDITIONS

*Analytical Column:* Mycotox™ (Pickering Laboratories, Inc), $C_{18}$, 4.6x250 mm

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

*Flow Rate:* 1 mL/min

*Injection Volume:* 30 µL

*FLD:* Excitation 365 nm, Emission 430 nm

## RESULTS & DISCUSSION

The 6-point calibration curves were built in the 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 sample clean up using the Immunoaffinity columns, and using the AcceCLEAN, we were able to cleanup 30 samples automatically in just a few hours.

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

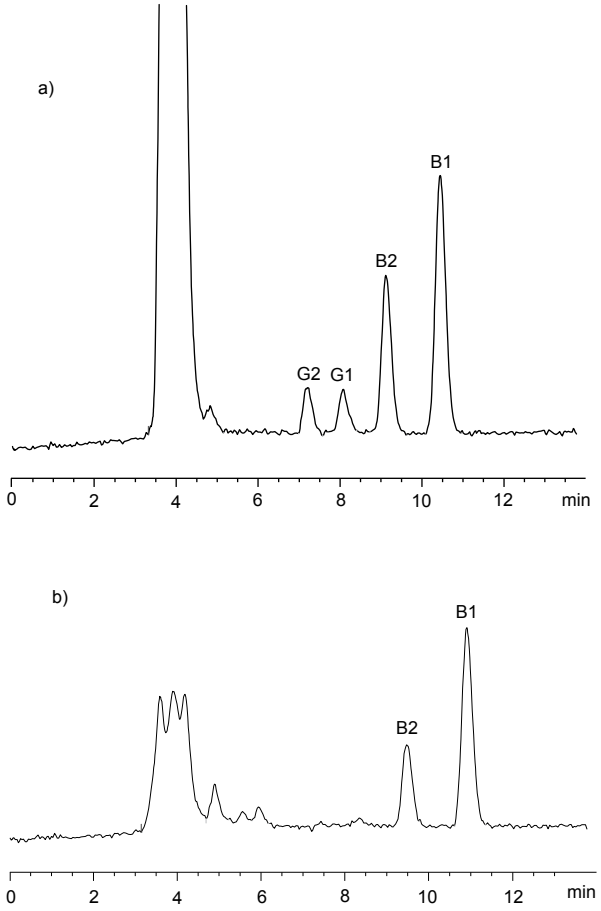



***Fig. 1 Chromatograms of a) Ground peanuts; b) NIST SRM2387 peanut butter sample. All samples are part of NIST Exercise E (April 2010).***

PICKERING
LABORATORIES
CATALYST FOR SUCCESS

Pickering Laboratories, Inc.
1280 Space Park Way / Mountain View, CA 94043

sales@pickeringlabs.com / support@pickeringlabs.com
800-654-3330 / 650-694-6700 / Fax: 650-968-0749