Venture® Immunoaffinity Columns & Cartridges

GRACE

Sample Prep Made Easy

Applications

Mycotoxins
Food Nutrients
Acrylamide
Lactoferrin
Endocrine Disruptors
Pollutants
Steroid Hormones

Brochure #522

Grace Davison Discovery Sciences

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VENTURE® IMMUNOAFFINITY COLUMNS

Repetitive-Use, Silica-Based Immunoaffinity Columns

Currently, there is a increased demand for quick, easy, and inexpensive sample preparation procedures to isolate minor compounds from complex food, environmental, and biological matrices prior to analysis. Venture immunoaffinity HPLC columns provide an easy and quick way to purify and concentrate analytes from complex or dilute matrices or as an analytical tool for fast, accurate measurement.

Grace's Venture line of immunoaffinity columns and SPE cartridges use ICEtech™ (Inert Coating Enhancement Technology, US Patent No. 6,802,966) silica processing to eliminate non-specific binding on the silica surface. This enables Venture columns to be the first affinity matrix to take advantage of silica's rigid porous structure, making direct coupling of the columns with other modes of chromatography, such as reversed-phase HPLC feasible.

Benefits	*Features*
Rapid Analysis	• No laborious sample preparation procedures • Fast binding kinetics • Wide-pore silica support allows on-line coupling with HPLC for real time direct analysis
Excellent Accuracy and Precision	• Quantitative recovery of analyte from IAC column • High sensitivity due to high volume injection • Precision comparable with conventional HPLC methods
Highly Selective	• Specially treated wide-pore silica eliminates non-specific interactions • Secondary separation reduces errors due to cross-reactivity • Highly selective antibodies toward target analyte
Long Column Lifetime†	• Analyze over 200 samples on a single column without column deterioration due to the high ligand stability of the Venture IAC Columns
Analysis of Difficult Matrices	• Venture IAC columns isolate, concentrate and purify targeted sample components from dilute and complex matrices in one step
Significant Cost Reductions†	• Costs reduced by as much as 90% • Long column lifetime decreases material costs to less than $5.00 per analysis • Rapid analysis and automation reduces technician time • Significant reduction in solvent use Less solvent waste disposal costs
Automated Analysis	• HPLC format allows automation for high sample throughput

†See page 3 for more information.

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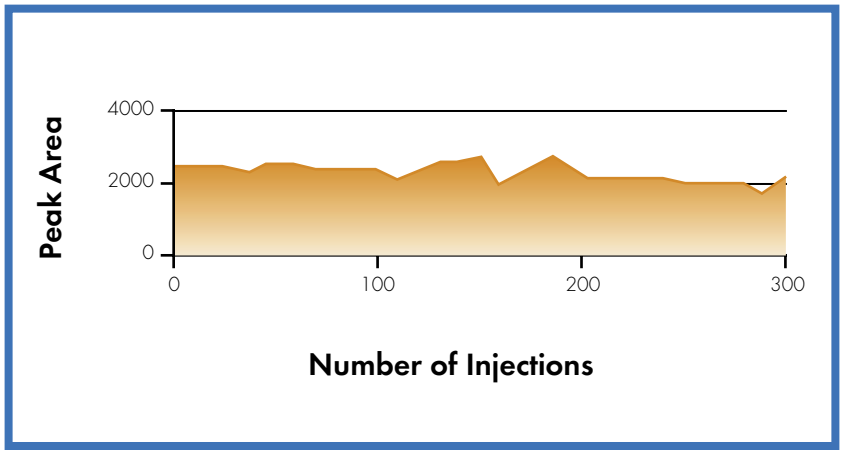
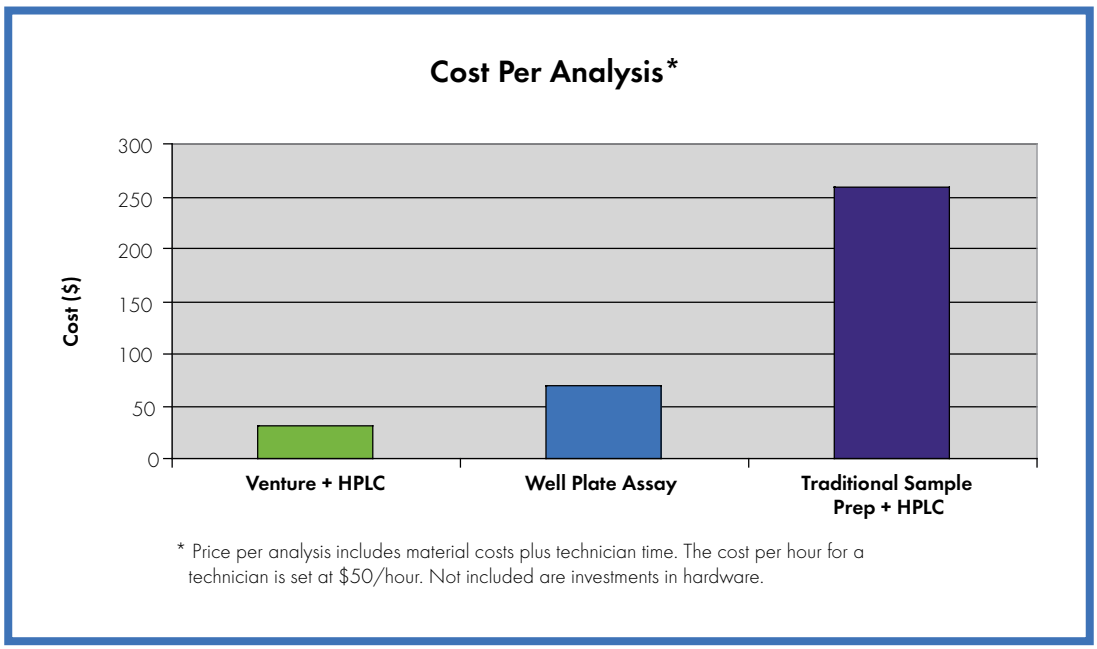
Long Column Lifetime

The stability of the Venture immunoaffinity columns was demonstrated during routine use in certified laboratories and round-robin studies. Typically, more than 200 samples could be analyzed without deterioration of the Venture immunoaffinity columns. After 300 injections of a lactoferrin sample onto a Venture LTF column, the measured peak area had a %CV (coefficient of variation) of only 10%.



Significant Cost Reductions

Compared to alternative techniques, Venture columns can significantly reduce costs due to their long lifetime and ability to automate time consuming sample preparation procedures. The cost comparison below was calculated for the analysis of lactoferrin, and is representative of the cost advantage of the Venture line of immunoaffinity columns.



* Price per analysis includes material costs plus technician time. The cost per hour for a technician is set at $50/hour. Not included are investments in hardware.

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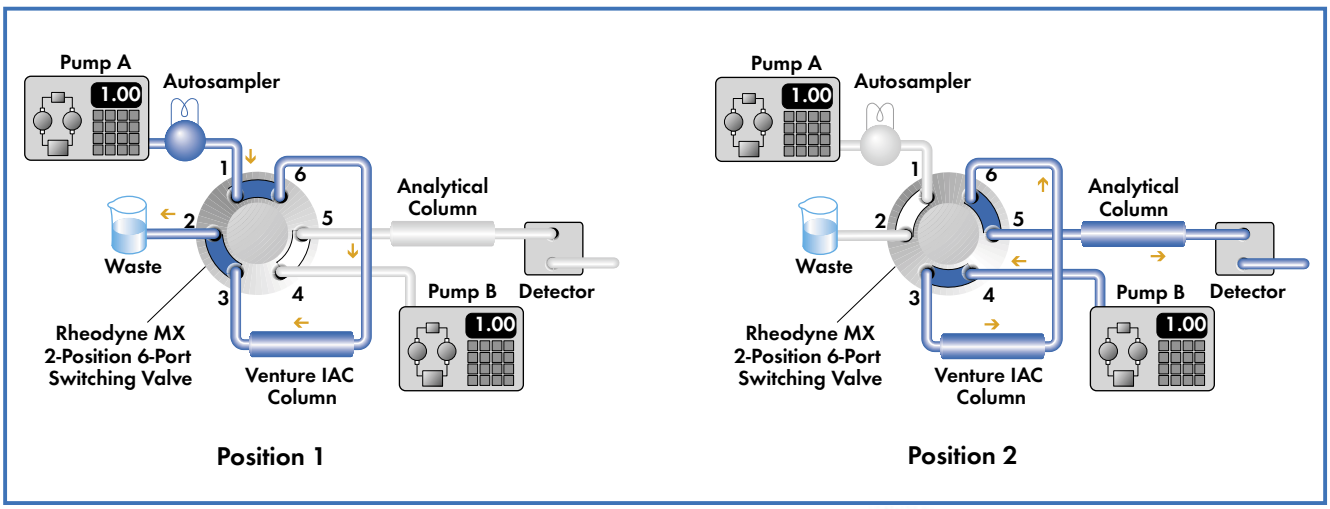
VENTURE® IMMUNOAFFINITY COLUMNS

Typical Automation Sequence

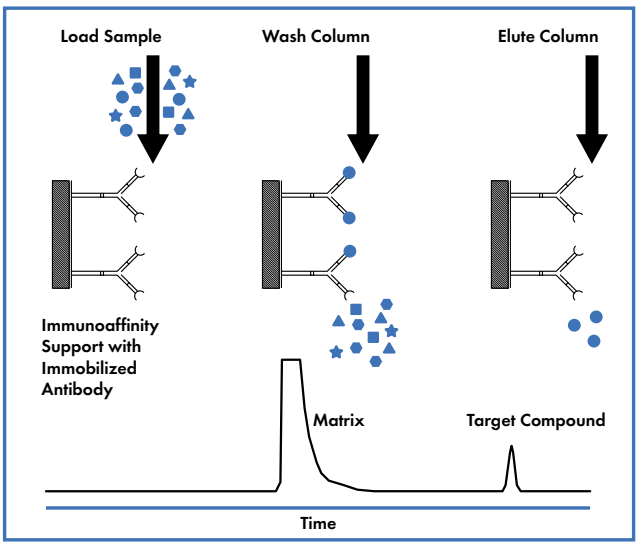
Method:

On-line sample clean up and enrichment uses a Venture IAC column connected to an analytical column using a switching valve. In enrichment, analytes are retained and concentrated on the IAC column while unwanted components bypass the analytical column to waste. When the switching valve is actuated, it backflushes analytes out of the IAC column onto the analytical column. (See illustration below.)



Rheodyne® MX™ 2-Position 6-Port Automated Switching Valves are the ideal choice for on-line sample cleanup. See back page for product specifications and ordering information.

Typical Immunoaffinity (IAC) Analysis



Immunoaffinity chromatography (IAC) combines the speed and selectivity of immunoassays with the superior precision of chromatography and can be accomplished in three easy steps:

1. A column with immobilized antibodies is loaded with sample; target analytes are bound to the column.
2. A mild washing step elutes all unbound material matrix; this eluate is discarded.
3. The analytes of interest are eluted under conditions to prevent denaturation of the antibodies and to enable reuse of the column. The eluate is suitable for direct analysis by any chromatographic technique.

Mycotoxins Analysis

Mycotoxins are highly toxic substances produced by mold that can contaminate agricultural crops. Regulations regarding the sampling and analytical testing of these foods have been established by government agencies to ensure food safety.

Traditional analytical methods use immunoaffinity chromatography with natural polysaccharide matrices as supports. Due to the limited mechanical strength of these polysaccharide matrices, sample clean-up must be performed off-line. By comparison, Grace's rugged silica-based immunoaffinity columns can withstand high pressures. This allows them to be placed in-line with reversed-phase HPLC analysis to take advantage of walk-away automation. The Venture column can be used to quickly and accurately measure the Aflatoxins (B1, B2, G1 and G2) and Ochratoxin A content of a wide variety of agricultural products.

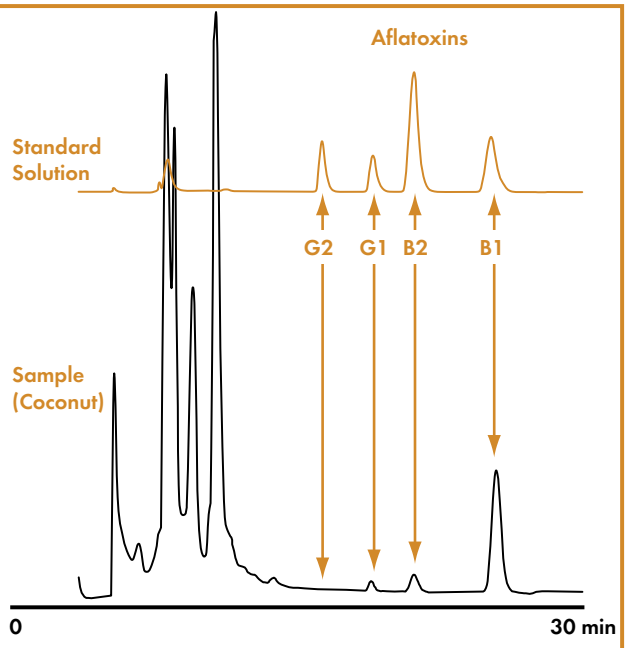
Typical performance characteristics (HPLC/Fluorescence)	
Precision:	90–95%
Sensitivity:	LOD 0.2–0.4µg/kg
Capacity:	≥100ng

Available Columns	Target Analyte
Venture AF	Aflatoxin
Venture OT	Ochratoxin A

Detection of Aflatoxins in Coconut Waste

A 25 gram sample of coconut fiber was shaken with 5 grams of NaCl and 100mL of 80% v/v ACN in water. 1mL of this extract was diluted 10 fold with 9mL of PBS buffer. A sample of the extract was injected directly onto the immunoaffinity column. The chromatogram begins with elution of the immunoaffinity column onto the C18 reversed-phase column. Early peaks are system peaks as well as elution of small amounts of matrix components that partly precipitate when the extract comes in contact with the binding buffer. They do not affect Aflatoxin measurement.

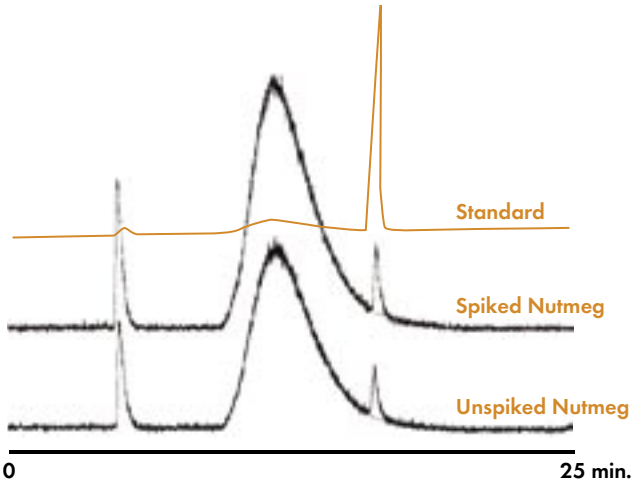
Column 1: Venture™ AF (2.1 x 50mm, 15-20µm)
Column 2: Genesis® C18 (4.6 x 250mm, 4µm)
Detection: Fluorescence (Excitation: 369nm, Emission: 422nm)
Binding: 0.10M Phosphate + 0.15M NaCl, pH 7.0
Elution: 20% v/v Acetonitrile in Water
Mobile Phase: 600mL MeOH + 80mL ACN + 200µL concentrated HNO_3 + 50mg KBr, adjusted to 1000mL with Water
Sample: 500µL



Determination of Ochratoxin A in Nutmeg

A 25 gram of nutmeg was incubated overnight at 4°C and then extracted with 60% v/v acetonitrile in water and sonification for 15 minutes. The extracts were diluted with PBS.

Column 1: Venture OT (2.1 x 50mm, 15-20µm)
Column 2: Genesis C18 (4.6 x 250mm, 4µm)
Detection: Fluorescence (Excitation 330nm, Emission 470nm)
Binding: PBS
Elution: 1% Acetonitrile in Water
Mobile Phase: 1% Water in Acetonitrile
Sample: 200µL



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Food Nutrients Analysis

Folic acid plays an important role in the biosynthesis of nucleic acids, while vitamin B_{12} is involved in blood cell synthesis, fatty acid metabolism, and the availability of folic acid. Deficiencies in folic acid influence cell division, which may lead to reduced growth rates, disorders in reproduction, and gastrointestinal symptoms. A deficiency of vitamin B_{12} may cause serious health complications, such as pernicious anemia or neurological symptoms. In order to assess the intake of both vitamins, it is essential to have a reliable, rapid, highly-selective, and quantitative method for the (combined) determination of folic acid and vitamin B_{12} in food samples. Compared with other methods, such as ELISA assays, Venture immunoaffinity columns directly coupled to HPLC offer these benefits:

- Rapid analysis (total analysis time under 1.5 hours)
- Elimination of errors due to cross-reactivity
- Superior reproducibility-precision = 99%.
- Detection limits up to 10µg/L.

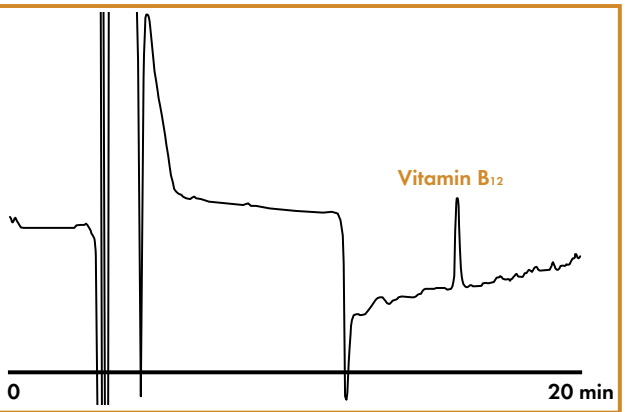
The Venture column can be used to quickly and accurately measure the amount of nutrients added to a variety of food products such as infant formula, pet food, and sports drinks.

Typical performance characteristics (HPLC/ UV-Vis)	
Precision:	>96%
Sensitivity:	LOD 10µg/L Folic Acid LOD 20µg/L Vitamin B_{12}
Capacity:	≥100ng Folic Acid ≥50ng Vitamin B_{12}

Available Columns	Target Analyte
Venture FA	Folic Acid
Venture B12	Vitamin B_{12}
Venture BF	Folic Acid & Vitamin B_{12}

Determination of Vitamin B_{12} in Milk

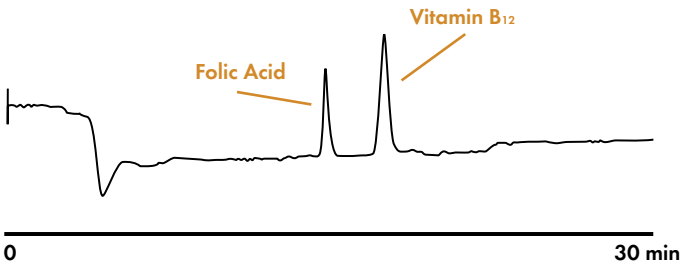
Column 1: Venture™ B12 (2.1 x 50mm, 15-20µm)
Column 2: Genesis® C18 (4.6 x 250mm, 4µm)
Detection: Absorbance, UV at 368nm
Flow Rate: 1mL/min
Binding: 0.01M Phosphate + 0.15M NaCl, pH 7.0
Elution: 0.01M HCl + 0.15M NaCl
Mobile Phase: 50% ACN/50% 0.01M HCl + 0.15M NaCl
Sample: 200µL



Determination of Folic Acid and Vitamin B_{12} in Yogurt

Simultaneous analysis of Folic Acid and Vitamin B_{12} was possible using the Venture BF column targeted to both compounds.

Column 1: Venture BF (2.1 x 50mm, 15-20µm)
Column 2: Genesis C18 (4.6 x 250mm, 4µm)
Detection: Absorbance, UV at 368nm
Mobile Phase: 0.8mL/min 1% v/v Water in Acetonitrile
Sample: 200µL of diluted yogurt after cleanup with IAC column



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Acrylamide Analysis

Acrylamide is a genotoxic compound recently discovered in carbohydrate-rich fried and baked foods. Venture Immunoaffinity chromatography columns provide a rapid and selective method for the analysis of the incidence and levels of this contaminant in food to ensure adequate food safety.

Typical performance characteristics (HPLC/Fluorescence)	
Precision:	>92%
Sensitivity:	LOD 5ng acrylamide absolute
Capacity:	≥500ng

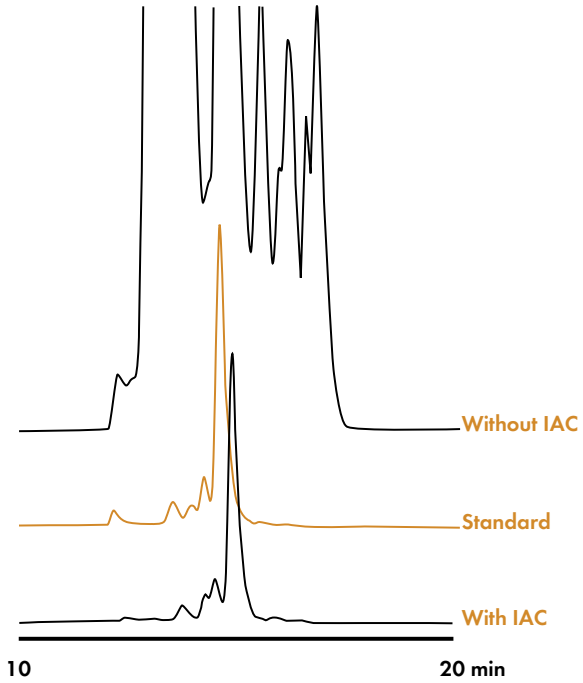
Available Columns	Target Analyte
Venture AC	Acrylamide

Determination of Acrylamide in Potato Chips

A 1.0g sample of potato chips was extracted by shaking with 10mL of water for 15 minutes. The extract was centrifuged for 15 minutes, filtered, and then purified with the Venture AC column. The sample eluate was derivatized before analysis by reversed-phase HPLC.

Due to disturbances by matrix components, quantitative analysis of Acrylamide in the extract is not possible without purification with immunoaffinity chromatography. The eluate purified with IAC produces a very clean chromatogram with one major peak descent from Acrylamide.

Column 1: Venture™ AC (2.1 x 50mm, 15-20µm)
Column 2: Genesis® C18 (4.6 x 250mm, 4µm)
Detection: Fluorescence (Excitation: 390nm, Emission: 480nm)
Flow Rate: 0.8mL/min
Mobile Phase A: Elution buffer
B: Acetonitrile



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Lactoferrin Analysis

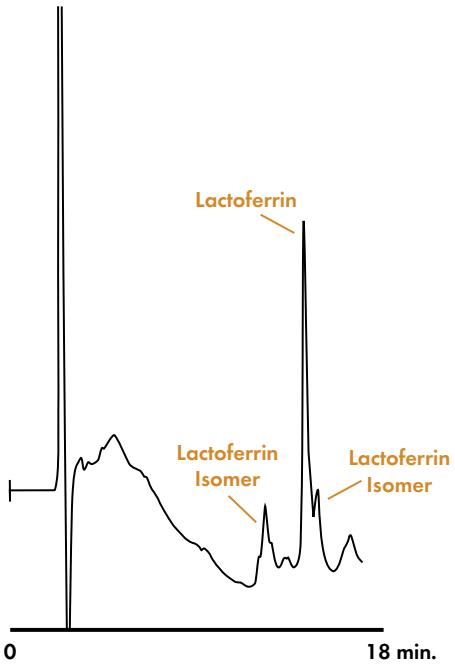
The US FDA regulates the level of milk-derived activated Lactoferrin used as an antimicrobial in beef, poultry and ready-to-eat foods. Immunoaffinity chromatography in combination with HPLC provides a fast and reliable method for the analysis of Lactoferrin in these complicated matrices. The total analysis time (sample prep and analysis) is 1.5 hours with on-line IAC/HPLC compared with 6.5 hours for ELISA and 5 hours for HPLC techniques. On-line IAC/HPLC has the added advantage of being able to distinguish among the three Lactoferrin isoforms, which is not possible with other available analytical techniques. Analysis with capture-elution has a sensitivity of 100µg/L while analysis in combination with on-line HPLC has a sensitivity of 20µg/L. Precision of this assay is greater than 96%.

Typical performance characteristics (HPLC/ UV-Vis)	
Precision:	>96%
Sensitivity:	LOD 20µg/L
Capacity:	≥25µg

Available Columns	Target Analyte
Venture LTF	Lactoferrin

Determination of Lactoferrin in Milk

Column 1: Venture™ LTF (2.1 x 50mm, 15-20µm)
Flow Rate 1: 0.5mL/min
Column 2: Supelcosil™ LC-318 (4.6 x 250mm, 5µm)
Flow Rate 2: 1.2mL/min
Detection: Absorbance UV at 280nm
Binding Buffer: 0.01M Phosphate + 0.15M NaCl, pH 7.0
Elution Buffer: 0.01M HCl + 0.15M NaCl
Mobile Phase: **A:** 2 v/v% Acetonitrile in 0.1% TFA
B: 2 v/v% Water in Acetonitrile



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Endocrine Disruptors Analysis

Endocrine disrupting chemicals (EDCs) have been found in the environment at concentrations in the low ng/L range, which is sufficient to induce estrogenic responses and alter the reproduction and development of aquatic wildlife.

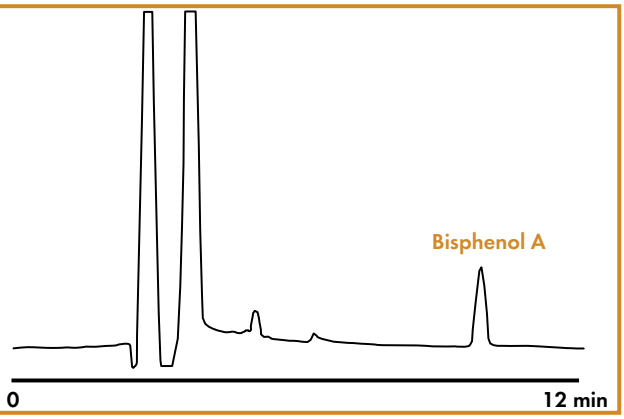
The analysis of EDCs often employs GC, LC/MS, or well-plate based assays, however sample preparation procedures are always the most decisive factor in the analysis of endocrine disrupting chemicals that have been found in low concentrations in the environment. Grace now offers a set of immunoaffinity columns (IAC) with high ligand stability that were developed and validated for the selective isolation, concentration and purification of most well known endocrine disrupting chemicals from surface waters and other complex environmental matrices. When coupled directly to HPLC, analysis is fast (total analysis time is only one hour) and errors due to cross-reactivity are eliminated.

Typical performance characteristics (HPLC/ UV-Vis)	
Precision:	>96%
Sensitivity:	LOD 15–70µg/L depending on Endocrine Disruptor tested
Capacity:	≥100ng

Available Columns	Target Analyte
Venture EE2	17α-Ethynyl Estradiol
Venture E2	17β-Estradiol
Venture E1	Estrone
Venture BPA	Bisphenol A

Determination of Bisphenol A in Wastewater by IAC Combined with Reversed-Phase HPLC

Column 1: Venture™ BPA (2.1 x 50mm, 15-20µm)
Column 2: Genesis® C8 (4.6 x 250mm, 4µm)
Detection: Absorbance, UV at 230nm
Flow Rate: 1mL/min
Binding: 0.01M Phosphate + 0.15M NaCl, pH 7.0
Elution: 35% Acetonitrile/65% Water
Mobile Phase: 35% Acetonitrile/65% Water



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Pollutants Analysis

Multi-residue methods, such as GC/MS or LC/MS are used to examine food and environmental samples for residual levels of herbicides, pesticides, and fungicides. However, the sample preparation steps used affect the total analysis time and the number of pesticides recovered. For example, polar pesticide groups are often lost during multi-step extraction.

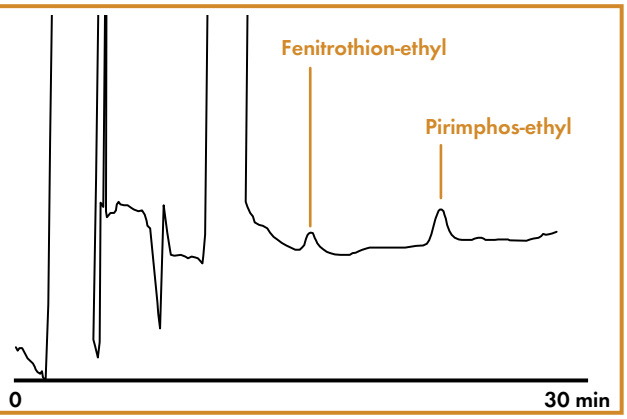
To increase analysis speed and sensitivity, Grace offers several immunoaffinity columns highly selective for the rapid isolation of several groups of pesticides prior to analysis. When coupled directly to an HPLC column, highly specific quantitative results for the target analyte group and each individual compound of the group is obtained in less than 2 hours.

Typical performance characteristics (HPLC/ UV-Vis)	
Precision:	>94%
Sensitivity:	LOD 0.04–5µg/L depending on pesticide group
Capacity:	250–500ng depending on pesticide group

Available Columns	Target Analyte
Venture CPA	Chlorophenoxy Acetic Acid Herbicides
Venture PVH	Phenylurea Herbicides
Venture OPP	Organophosphorus Pesticides
Venture VCZ	Vinclozolin Fungicides

Determination of Organophosphorus Pesticides by IAC Combined with Reversed-Phase HPLC

Column 1: Venture™ OPP (2.1 x 50mm, 15-20µm)
Column 2: Genesis® C8 (4.6 x 250mm, 4µm)
Detection: Absorbance, UV at 230nm
Flow Rate: 1.2mL/min
Binding: 0.01M Phosphate + 0.15M NaCl, pH 7.0
Elution: 35% Acetonitrile/65% Water
Mobile Phase: 65% Acetonitrile/35% Water



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Steroid Hormones Analysis

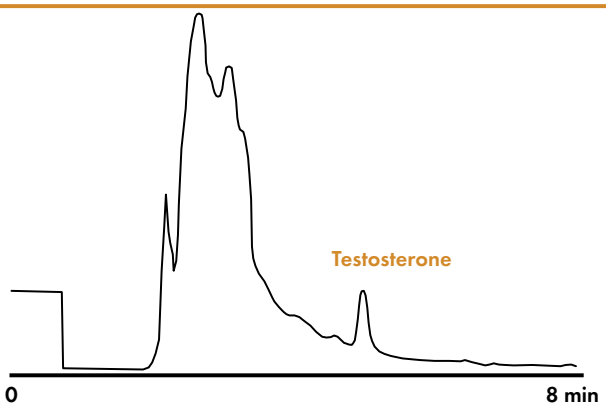
Grace has developed highly selective immunoaffinity columns for the rapid analysis of testosterone and nortestosterone in a variety of matrices. Anti-testosterone and anti-nortestosterone antibodies are immobilized on Grace proprietary passivated silica producing columns that are highly specific and eliminate errors due to cross-reactivity. When analysis is performed in combination with on-line HPLC, repeatability/precision is 99% and sensitivity (LOD) is 20µg/L testosterone and 10µg/L nortestosterone.

Typical performance characteristics (HPLC/ UV-Vis)	
Precision:	>98%
Sensitivity:	LOD 20µg/L Testosterone LOD 10µg/L Nortestosterone
Capacity:	≥100ng of Testosterone ≥50ng of Nortestosterone

Available Columns	Target Analyte
Venture TT	Testosterone
Venture NT	Nortestosterone

Determination of Testosterone in Urine

Column 1: Venture™ TT (2.1 x 50mm, 15-20µm)
Column 2: Genesis® C18 (4.6 x 250mm, 4µm)
Detection: Absorbance, UV at 230nm
Flow Rate: 1.2mL/min
Binding: 0.01M Phosphate + 0.15 M Sodium Chloride, pH 7.0
Elution: 75% Methanol/25% Water
Mobile Phase: 75% Methanol/25% Water
Sample: Urine, 200mL



Don't See the IAC Column for Your Analysis?

Please fill out the survey on the web at www.discoverysciences.com/venture.
The information you provide helps guide our new product development.
We appreciate your feedback.

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Ordering Information

Venture Immunoaffinity Columns			
Product Name	**Target Analyte**	**Column, 2.1mm i.d. x 50mm**	**SPE Cartridge, 0.5 mL**
Mycotoxins Analysis			
Venture AF	Aflatoxin	**5120313**	**5119654**
Venture OT	Ochratoxin A	**5135954**	**5135955**
Food Nutrients Analysis			
Venture FA	Folic Acid	**5135952**	**5135958**
Venture B12	Vitamin B_{12}	**5120201**	**5119662**
Venture BF	Folic Acid & Vitamin B_{12}	**5135951**	**5135959**
Acrylamide Analysis			
Venture AC	Acrylamide	**5135953**	**5135957**
Lactoferrin Analysis			
Venture LTF	Lactoferrin	**5120400**	**5119661**
Endocrine Disruptors Analysis			
Venture EE2	17α-Ethynyl Estradiol	**5120404**	**5119650**
Venture E2	17β-Estradiol	**5120403**	**5119651**
Venture E1	Estrone	**5120405**	**5119652**
Venture BPA	Bisphenol A	**5120406**	**5119653**
Pollutants Analysis			
Venture CPA	Chlorophenoxy Acetic Acid Herbicides	**5120407**	**5119606**
Venture PVH	Phenylurea Herbicides	**5120408**	**5119607**
Venture OPP	Organophosphorus Pesticides	**5120410**	**5119608**
Venture VCZ	Vinclozolin Fungicide	**5120411**	**5119609**
Steroid Hormones Analysis			
Venture TT	Testosterone	**5120401**	**5119663**
Venture NT	Nortestosterone	**5120402**	**5119664**

To learn more about the full Grace line of Chromatography Products request our catalogs.

Rheodyne® MX™ Automated Switching Valves

The Rheodyne® MX™ two-position, six-port motorized valves can be operated either by push button, allowing them to function as a "manual" valve, or by contact closure for complete remote control. For multiple automated valve applications, MX Valves have a unique "snap'n'stack" system allowing units to be stacked vertically or connected horizontally to conserve valuable bench space and reduce connection volumes.

Rheodyne MX Automated Switching Valve Specifications	
Maximum Pressure:	5,000psig (345 bar)
Flow Passages *Nano*:	0.10mm (0.004") diameter, <25nL port-to-port Volume
Flow Passages *Analytical*	0.25mm (0.010") diameter
Connections:	M4 Nano, 10-32 Analytical
Power Requirements:	100–240 VAC, 50/60 Hz
Communication:	One-line contact closure
Operating Temperature:	4–40°C
Dimensions:	10.2cm H x 7.6cm W x 12.7cm D

Rheodyne® MX™ Automated Switching Valves			
Description	**Version**	**Bore**	**Part No.**
2-Position Valves			
6-Port	Duralife	Analytical	**447900**
6-Port	Duralife	Nano	**447980**
6-Port	PEEK	Analytical	**449900**

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