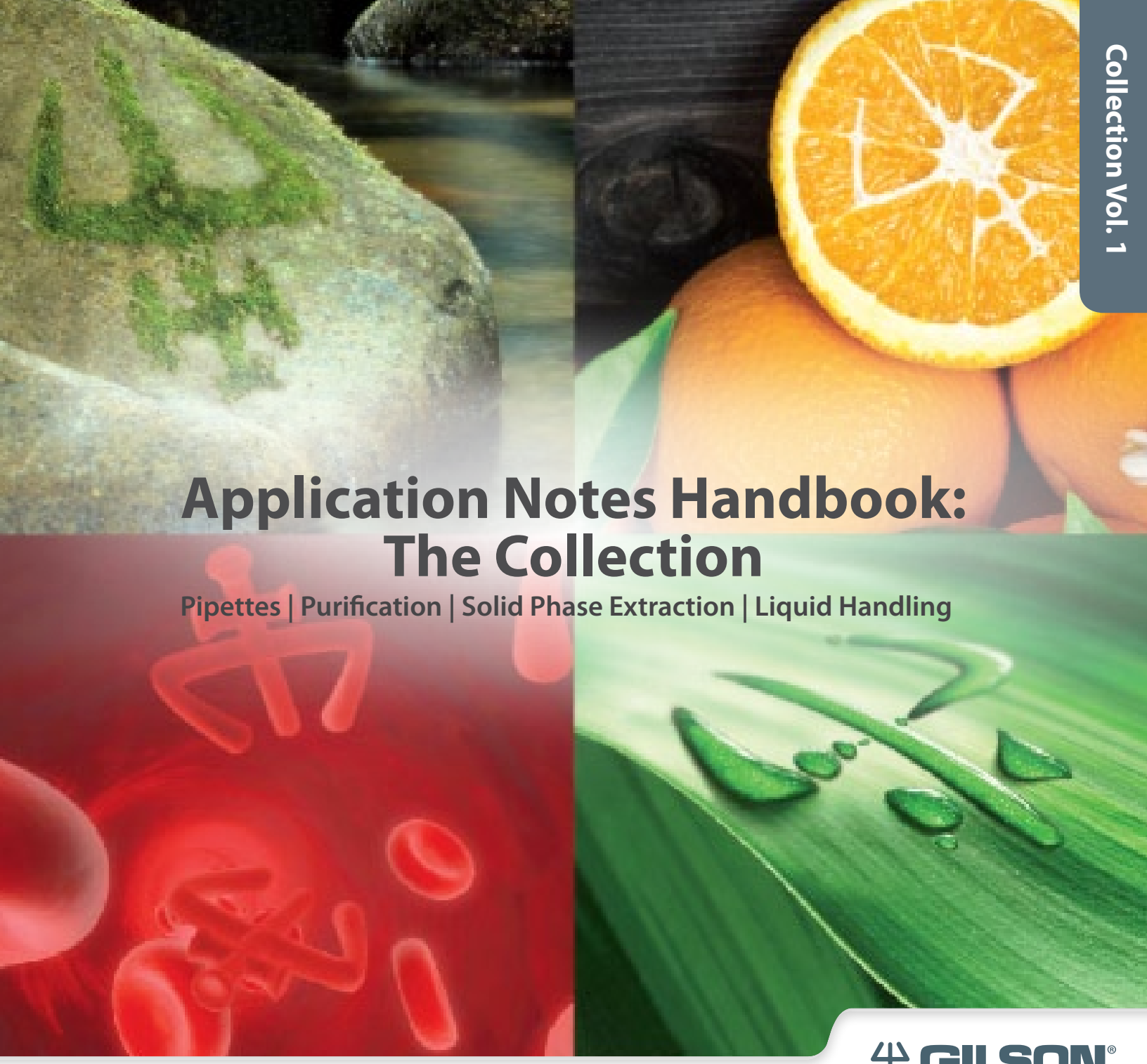

Collection Vol. 1

Application Notes Handbook: The Collection

Pipettes | Purification | Solid Phase Extraction | Liquid Handling

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Application Notes from Around the World!

GILSON®

Automated Solid Phase Extraction of Pharmaceuticals and Personal Care Products (PPCPs) from Water

This application note was part of a collaboration with Drs. Imma Ferrer and Michael Thurman at the Center for Environmental Mass Spectrometry at the University of Colorado-Boulder, Boulder, CO, USA.

Featured Product: GX-271 ASPEC™

Large quantities of pharmaceuticals and personal care products (PPCP) are sold and consumed each year throughout the world. A variety of PPCPs have been detected in low concentrations in surface water, ground water, and drinking water. Thus, there is a great deal of interest in measuring these compounds in water to determine their environmental impact. Solid phase extraction (SPE) is a common method for analyte pre-concentration and sample cleanup prior to determination of PPCPs from a water sample.

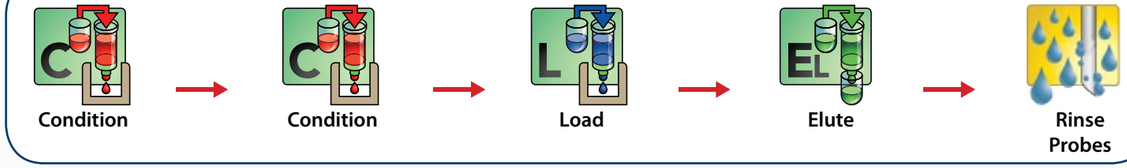
Experimental Conditions

Standard stock solutions of 17 PPCPs were prepared in LC-MS grade methanol at a concentration of 1 ppm. Water samples were ultra-pure water. No pH adjustment was performed on the water samples.

Solid Phase Extraction (SPE) Protocol

The SPE procedure used 6 mL Waters Oasis™ HLB (200 mg) Cartridges.The SPE protocol is entirely automated using the Gilson GX-271 ASPEC system. The SPE steps are summarized with the schematic provided in the GX-271 ASPEC control software, TRILUTION® LH (Figure 1).

Figure 1. TRILUTION® LH SPE and Liquid Handling Tasks for Extraction of PPCPs from Water



- Condition the cartridge with 4 mL of methanol at 1.0 mL/min.
- Condition the cartridge with 6 mL of water at 1.0 mL/min.
- Load 100 mL water sample at 7.5 mL/min.
- Move the Gilson Mobile SPE Rack over the collection tubes.
- Elute the analytes of interest with 10 mL of methanol.
- Evaporate to 0.5 mL with nitrogen at a temperature of 45° C in a water bath.

LC/TOF-MS Analysis

The separation of the PPCPs was carried out using an Agilent Series 1200 HPLC System equipped with a reverse phase C8 analytical column of 150 mm x 4.6 mm and 5 µm particle size (Zorbax Eclipse XDB-C8). The injected sample volume was 50 µL. Mobile phases A and B were acetonitrile and water with 0.1% formic acid, respectively. The flow rate was 0.6 mL/min.

The HPLC was connected to an Agilent 6220 MSD TOF equipped with a dual electrospray interface operating in positive ion mode. The data was processed with MassHunter® software.

Results

Figure 2. TIC of Standard Versus Spiked Water Sample

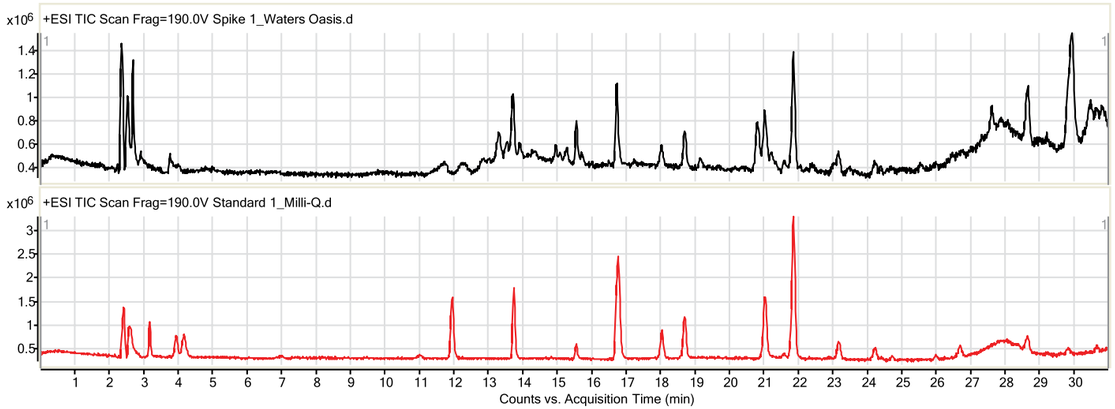


Table 1. Percent Recovery of Selected PPCPs in Water Using Oasis™ HLB Cartridge and 100 mL of Sample at a Loading Rate of 7.5 mL/min

PPCPs	% Recovery
Acetominophen	154
Albuterol	89
Atenolol	99
Caffeine	132
Carbamazepine	106
Cotinine	67
DEET	91
Dehydronifedipine	93
Diclofenac	100
Diphenhydramine	119
Gemfibrozil	125
Ibuprofen	97
Metoprolol	88
Sulfadimethoxine	95
Sulfamethazole	98
Triclocarbon	36
Trimethoprim	104

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Post-Extraction Gel Permeation Chromatography (GPC) Clean-up of Fish Tissue Prior to PCB and PBDE Analysis

This application note was part of a collaborative study with the Wisconsin State Lab of Hygiene, Madison, WI, USA

Featured Product: Automated GX-271 GPC Clean-up System

The determination of PCBs and PBDEs, as well as other environmental contaminants, in fish tissue, requires extensive sample clean-up prior to analysis by gas chromatography with an electron capture detector (GC/ECD) or GC/MS. The high fat content of fish tissue can cause buildup of nonvolatile materials on the GC injection port and the analytical column, giving poor analytical results and high instrument maintenance costs. GPC clean-up is often used for the clean-up of fish tissue extracts prior to analysis for halogenated compounds such as PCBs, PBDEs and chlorinated pesticides.

Experimental Conditions

GPC Standards were prepared according to USEPA Method 3640A. Stock solutions of PCB and PBDE congeners were obtained from Ultra Scientific.

Extraction Protocol

Weigh 10g of ground fish tissue into a beaker. Fortify with 1g of corn oil. Add 60g anhydrous sodium sulphate and 230 mL of dichloromethane. Spike with appropriate surrogate PCB/PBDE standards. Pour the mixture through a column containing Florisil® topped with a 1 mL layer of anhydrous sodium sulphate. Collect the eluent, and evaporate to near dryness using a gentle stream of nitrogen. Reconstitute in 5 mL GPC mobile phase.

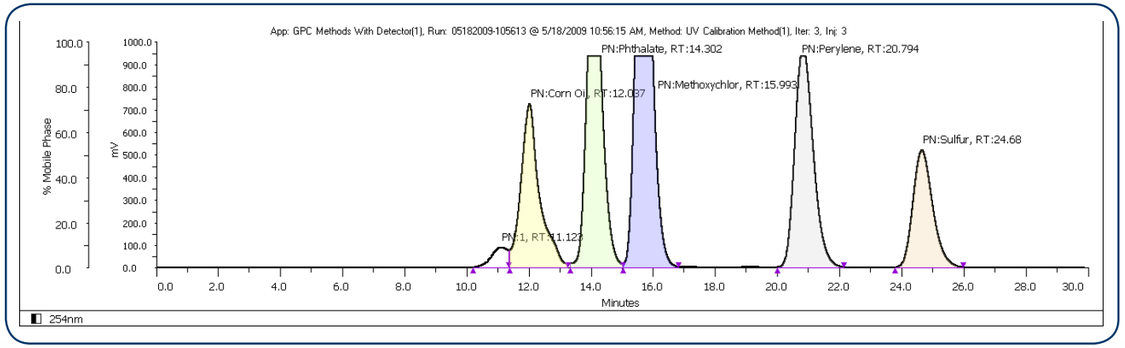
GPC Clean-up

Column calibration used a GPC calibration standard (as described on previous page), a Gilson 112 UV Detector set at 254 nm and Gilson TRILUTION® LC Software. Based on the UV trace, column eluent was collected just after bis(2-ethylhexyl) phthalate elution and stopped after perylene elution. For the columns listed in Table 1 below, the flow rate was 5 mL/min with an injection volume of 5 mL. The exception was with the OI Analytical Optima™ column, which used an injection volume of 1 mL.

Table 1. GPC Column Parameters for Fish Extract Clean-up

Column	Dump Volume (mL)	Collect Volume (mL)	Total Run Time (min)	Column Lipid Loading Capacity (g)
OI Analytical Glass, 1:1 DCM/CYX	100	110	60	1
EnviroSep-ABC, 1:1 DCM/CYX	75	48	32	0.5
EnviroSep-ABC, 100% DCM	75	43	30	0.5
Optima Column 1:1 CYX/ethyl acetate	45	60	28	0.2

Figure 1. Chromatogram of a USEPA Method 3640A Calibration Standard Using an EnviroSep-ABC Column with a Mobile Phase of 100% Dichloromethane

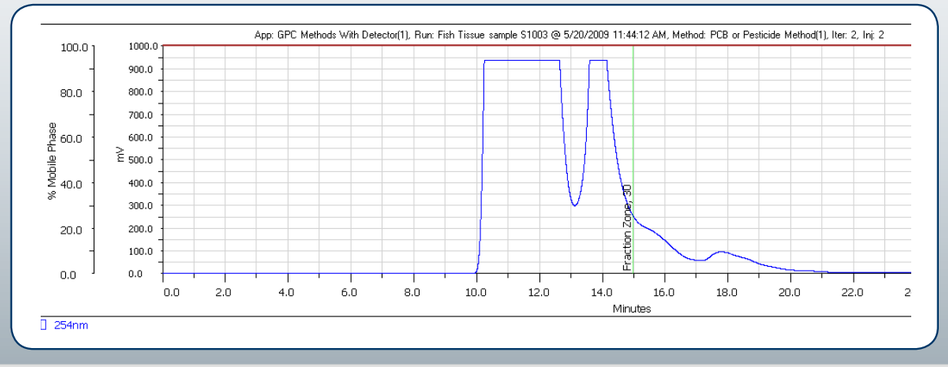


After GPC clean-up, collected fractions were put through further clean-up with silica gel to remove any pesticides and then concentrated with a gentle stream of nitrogen before they were reconstituted in appropriate solvent for GC analysis.

GC Analysis

PCBs and PBDEs were analyzed with an Agilent HP5890-II GC/ECD using a DB-5 column (60m x 0.25mm ID, 0.1 µM phase). PBDEs were confirmed using a DB-1 column (60m x 0.25mm ID, 0.1 µM phase).

Figure 2. Chromatogram of a Fish Tissue Extract During GPC Clean-up Using an EnviroSep-ABC Column with a Mobile Phase of 100% Dichloromethane and UV Detection at 254 nm



Results

Table 2. PBDE Recovery in Fish Using a Phenomenex EnviroSep-ABC Column with a Mobile Phase of 100% Dichloromethane; Fish Spiked at 2 ng/g (n=3)

PBDE#	28	47	66	85	99	100	138	153	154
% Recovery	127.5	77.5	97.8	97.0	70.5	76.5	107.8	100.0	93.0

Table 3. PCB Recovery in Fish (n=3)

Column	Mobile Phase	PCB BZ #14 % Recovery	PCB BZ #65 % Recovery	PCB BZ #166 % Recovery
OI Analytical Glass 60g Environbeads SX-3	1:1 Dichloromethane/ Cyclohexane	74.7	81.6	81.5
EnviroSep-ABC	1:1 Dichloromethane/ Cyclohexane	80.5	86.8	92.9
EnviroSep-ABC	100% Dichloromethane	87.9	86.7	91
OI Optima Column 1:1 CYX/EA	1:1 Ethylacetate/ Cyclohexane	90	89	117.5

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Determination of Veterinary Drug Residues in Fish Using Automated Solid Phase Extraction (SPE) Followed by HPLC

Featured Product: GX-271 ASPEC™ System with Direct Injection Module

Farm-raised seafood accounts for nearly half of the seafood production worldwide. As the aquaculture industry has grown, there have been increasing problems associated with infectious diseases in fish and shellfish. These diseases can cause major economic losses to seafood farmers. As a result, fighting infections has led to the increased use of antibiotics and other antimicrobials. The use of these products is highly regulated. Recent reports have found the presence of prohibited antibiotics in farm-raised fish and shrimp. Some of these compounds include malachite green (a carcinogen), fluoroquinolones, nitrofurans, chloramphenicol, and other antibiotics.

This application note describes a simple and automated procedure employing solid phase extraction (SPE) to extract and concentrate some representative illegal residues from fish tissue prior to HPLC analysis.

Experimental Conditions

All solvents were HPLC grade or higher. All reagents were ACS grade or higher. Malachite green, naladixic acid and tetracycline were obtained from Sigma-Aldrich (St. Louis, MO, USA). Fresh salmon was obtained from a local market.

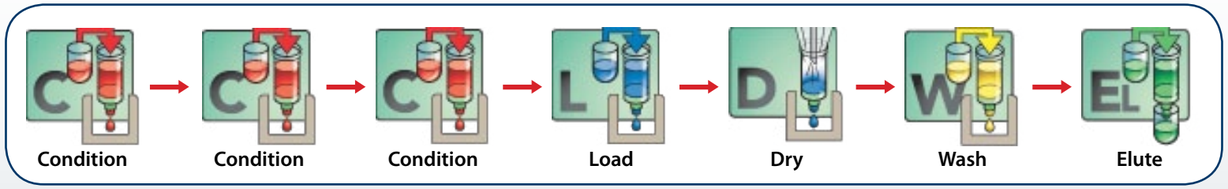
The following sample steps were performed prior to solid phase extraction:

- Mix 10g of ground salmon with 25 mL of ethyl acetate.
- Sonicate for 10 minutes, and transfer the ethyl acetate extract to a separate container.
- Add an additional 25 mL of ethyl acetate to the ground fish, and sonicate before removing the ethyl acetate extract to the same container as the last step.
- Repeat this process two more times. The total amount of ethyl acetate extract should be 100 mL.
- Mix the ethyl acetate extract and filter using smooth fluted, 313 folded filter paper. Add 0.5 mL of acetic acid to the filtrate and bring the volume to 100 mL with ethyl acetate.

Solid Phase Extraction (SPE) Protocol

The SPE procedure utilized 3 mL Macherey-Nagel Chromabond™ SA (500 mg) Cartridges. The SPE protocol is entirely automated using the Gilson GX-271 ASPEC System. The SPE steps are summarized with the schematic provided in the GX-271 ASPEC control software, TRILUTION® LH (Figure 1).

Figure 1. TRILUTION® LH SPE Tasks for Extraction of Select Residues in Fish



- Condition the cartridge with 2 x 3 mL n-hexane. Use an air push following the condition step to dry the cartridge.
- Condition the cartridge with 6 mL ethyl acetate containing 0.5% acetic acid. Do not dry the column.
- Load the sample extract onto the cartridge at a low flow rate (5 to 8 mL/min).
- Dry the cartridge with air for 10 minutes.
- Wash the cartridge with 10 mL of methanol.
- Move the Gilson Mobile SPE Rack over the collection tubes.
- Elute with 5 mL of 10% triethanolamine in methanol.
- The sample extract can now be analyzed immediately via HPLC using the Direct Injection Module.

HPLC Conditions

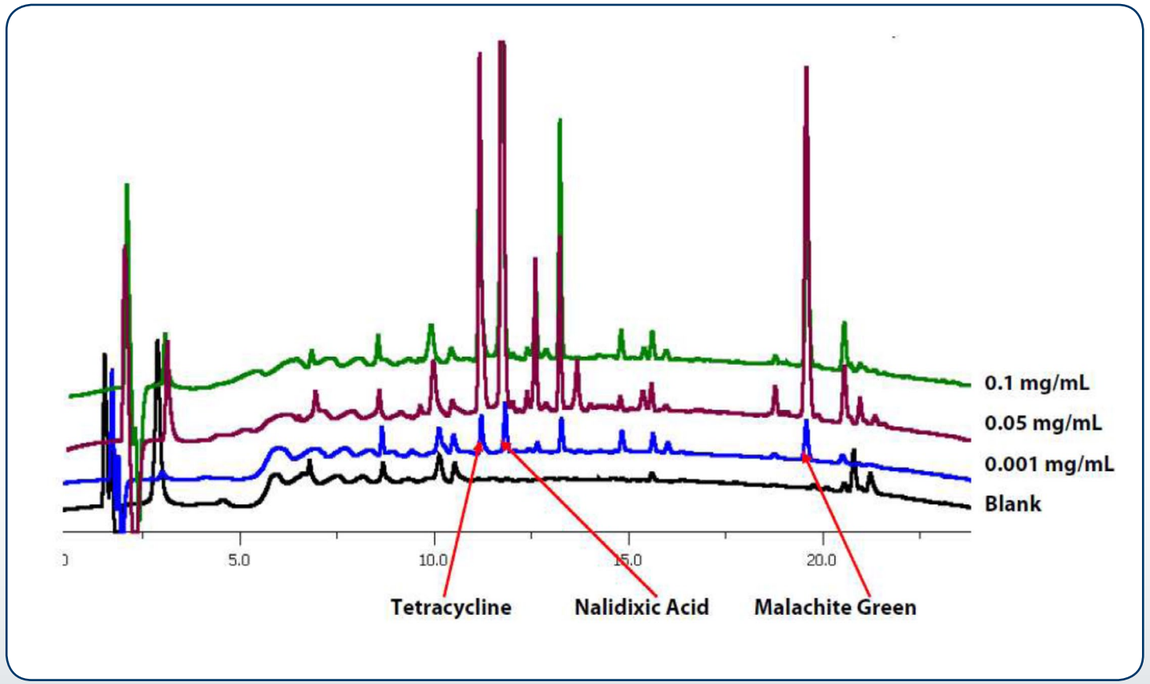
- Gilson GX-271 ASPEC with Direct Inject Module and 50µL Sample Loop
- Gilson 322 HPLC Pump with H2 Pump Heads
- Gilson 155 UV/VIS Detector, 210/254nm, Analytical flow cell (5 mm path length), Sensitivity Setting = 0.005/0.005
- Gilson TRILUTION® LC Software for HPLC Control and Data Acquisition
- Column: Waters Atlantis® dC18, 5µm, 4.6 x 150mm
- Flow Rate of 1.5 mL/min, injection volume = 50µL, Mobile Phase A; H_2O with 0.1% TFA, Mobile Phase B: Acetonitrile with 0.1% TFA

Figure 2. HPLC Gradient Conditions

Time (min)	% A (Aqueous)	% B (Organic)
0	95	5
1.3	95	5
26.5	5	95
30.0	5	95
31.5	95	5
34.0	95	5

Results

Figure 3. HPLC Chromatogram at Varying Concentrations



Recovery values ranged from 85% to 104% and were consistent within (n=3) and between days (n =3).

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Gel Permeation Chromatography (GPC) Clean-up of Plastic Additives from Edible Oils

Featured Product: Automated GX-271 GPC Clean-up System

Plastics are ubiquitous in our society and are extensively used in a large variety of packaged foods. Plastics are manufactured by combining monomers into polymers under heat and pressure (polymerization). A variety of additives are often added to plastics. Some commonly used additives include plasticizers (such as phthalates) to improve flexibility, UV filters for protection from sunlight, brightening agents, coloring agents and preservatives. Some of these additives may migrate into food products that have been packaged using plastics. There is a great deal of interest in measuring the migration of these compounds into food products.

Gel permeation chromatography (GPC) is a size exclusion clean-up procedure that uses organic solvents and a hydrophobic gel to separate macromolecules. It is a highly effective method for the removal of high molecular weight interferences such as lipids from a fatty food prior to analysis for the compounds of interest. GPC clean-up has been used to clean-up fatty foods prior to analysis for plasticizers and other plastic additives.

This application note describes the use of GPC clean-up to separate a variety of plastics additives such as phthalates, Chimassorb® 81(a UV absorber and stabilizer), Irganox® 1076 (an optical brightener) and Uvitex® OB (an antioxidant and thermal stabilizer) from a representative edible oil sample prior to analysis.

Experimental Conditions

All solvents were HPLC grade or higher. All reagents were ACS grade or higher. Phthalates and other plasticizer additives were obtained from Sigma-Aldrich (Table 1). Standards were prepared in 1:1 ethyl acetate/cyclohexane. Corn oil was obtained from a local market. GPC calibration standards were prepared according to USEPA Method 3640A in 1:1 ethyl acetate/cyclohexane (see Figure 1).

Table 1. Plasticizer Additives Used in the study

Compound	Trade Name/ Abbreviation	Formula Weight
Dimethyl phthalate	DMP	194.19
Diethyl phthalate	DEP	222.24
Dibutyl phthalate	DBP	278.35
2-ethyl hexyl phthalate	DEHP	390.56
Butyl benzyl phthalate	BBP	312.36
Diisobutyl phthalate	DIBP	278.35
Diisononyl phthalate	DINP	418.61
Diphenyl phthalate	DPP	318.33
Di-n-octyl phthalate	DNOP	390.56
Octadecyl 3- (3,5-di-tert-butyl-4-hydroxyphenyl) proprionate	Irganox® 1076	530.88
2-Hydroxy-4 (octyloxy)-benzophenone	Chimassorb® 81	326.4
2,5 - Bis (5-tert-butyl-2-benzoxazolyl) thiophene	Uvitex® OB	430.57

GPC Clean-up Protocol

GPC separation was achieved using the Automated GX-271 GPC Clean-up System and a Phenomenex EnviroSep-ABC GPC column with a mobile phase of 1:1 ethyl acetate/cyclohexane at a flow rate of 5 mL/min. The injection volume was 1mL. The column was calibrated using a GPC calibration standard (as described above), a Gilson 112 UV Detector set at 254 nm and Gilson TRILUTION® LC software (see Figure 1).

Results

Figure 1. Chromatogram of a USEPA Method 3640A Calibration Standard using a Phenomenex EnviroSep-ABC Column with a Mobile Phase of 1:1 Ethyl Acetate/Cyclohexane. Retention Times: Corn Oil = 12.6 min, DEHP = 15.6 min, Methoxychlor = 19.7 minutes and Perylene = 38.6 minutes

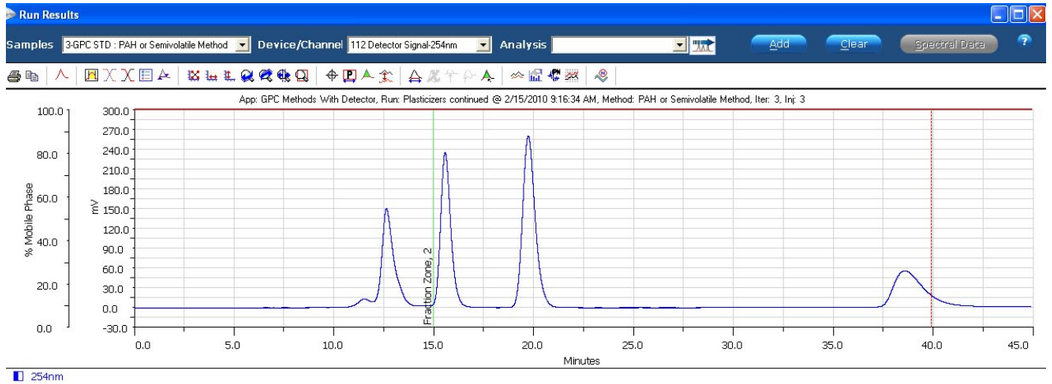


Figure 2. Chromatogram Showing Retention Time of Diisononyl phthalate (DINP)

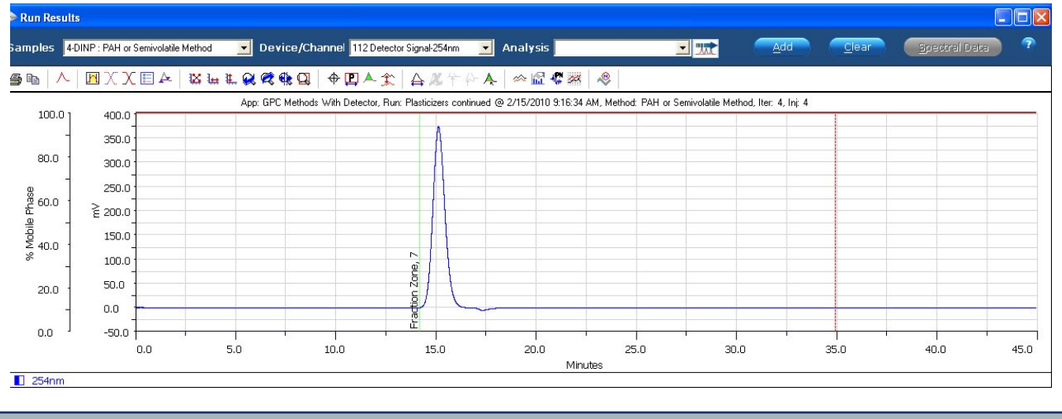
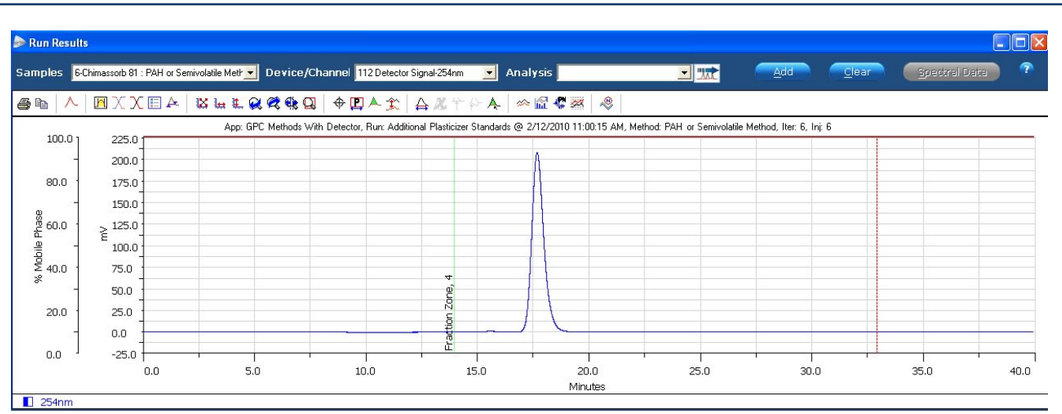


Figure 3. Chromatogram Showing Retention Time of Chimassorb 81



All plastic additives separated well from the representative edible oil (corn oil) as shown in Table 2.

Table 2. Retention Times for Plasticizer Additives, Corn Oil and GPC Standards
(For abbreviation key, see Table 1)

Additives	RT (min)
Corn Oil	12.6
Irganox® 1076	13.7
DINP	15.1
DNOP	15.5
DEHP	15.6
DIBP	17.1
Uvitex® OB	17.5
DBP	17.6

Additives	RT (min)
Chimassorb® 81	17.7
BBP	18.5
DEP	19.3
Methoxychlor (GPC Std)	19.7
DPP	19.9
DMP	21.0
Perylene (GPC Std)	38.6

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Comparison and Automation of Gel Permeation Chromatography (GPC), Solid Phase Extraction (SPE), and QuEChERS Extracting Organochlorine Pesticides from Olive Oil

This application, performed in collaboration with Julie Kowalski, Ph.D. at Restek in Bellefonte, PA, USA, investigates each of these separation techniques in separating pesticides from oil matrices and presents detailed information on the automation of each separation system.

Featured Products:

Gilson GPC Cleanup System

Figure 1. Gilson GX-271 Platform with TRILUTION® LC Used with a 307 pump and Gilson UV Detector for the GPC Cleanup method

Gilson SPE and QuEChERS System

Figure 2. Gilson GX-271 Preparative Liquid Handler Fitted with the Gilson Orbital Shaker for Liquid Liquid Extraction (LLE) Were Used for the SPE and QuEChERS Methods

Figure 1

Figure 2

Several techniques, such as GPC, SPE, or QuEChERS, are used today to investigate and isolate pesticides found in edible oils. Each technique provides strengths for its separation of pesticides. GPC has the ability to process large amounts of sample, SPE provides disposable cartridges with numerous sorbents to provide separation of the analyte from the matrix, and QuEChERS involves uncomplicated sample cleanup of pesticides in aqueous matrices.

GPC Cleanup Method

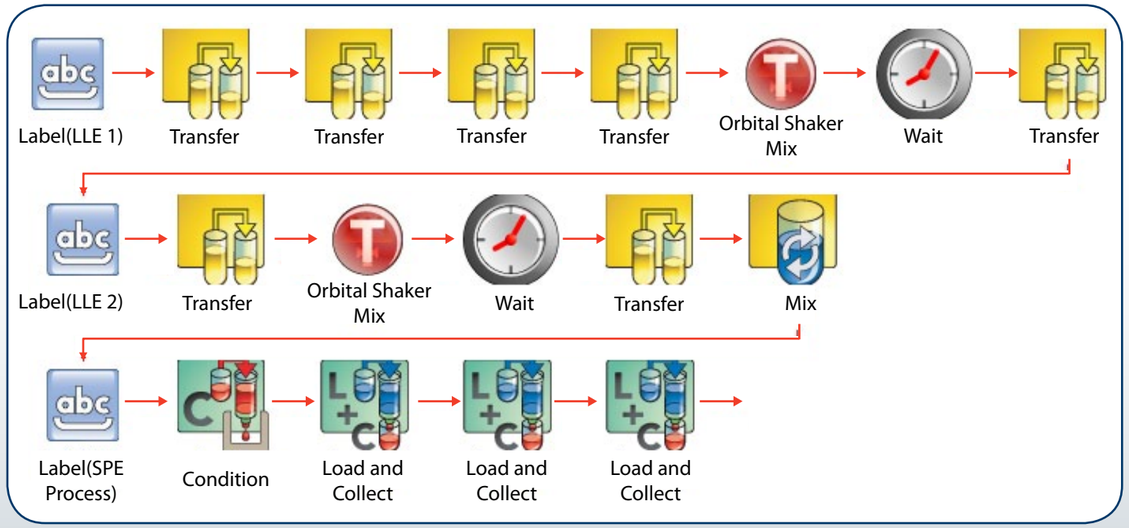
- Inject 1.5 mL of olive oil sample onto Gilson GPC Cleanup System using Phenomenex Envirosep ABC 350 x 21.20 mm GPC Column with 60x 21.20 mm GPC guard column
- Mobile phase: 50% dichloromethane : 50% hexane at 12 mL/min
- Start collecting fractions from end of oil peak (8 minutes) to 11 minutes; 6 fractions collected @ 6mL each
- Combine fractions
- Dry down eluent
- Add 200 µL of hexane
- Inject 1 µL sample onto GC outfitted with Rtx®-CL pesticides column
- Starting temp 150°C and ramp 3 degrees per minute until 300°C is reached then hold for five minutes

SPE and LLE Method

- Add 1.5 mL of olive oil sample to 1.5 mL of hexane
- 3 mL mixture is placed in test tube and 6 mL ACN is added
- Sample is mixed on an Orbital haker at 720 rpm for 30 minutes
- Wait 20 minutes for sample to separate
- Extract top layer (ACN)
- Repeat LLE with 6 mL of ACN
- Wait 20 minutes for sample to separate
- Mix ACN LLE fractions together
- Condition SPE with 5 mL ACN
- Extract 6 mL of ACN LLE mixture and place in SPE cartridge
- Elute with 6 mL ACN
- Collect eluent from sample load and elute

- Repeat ACN rinse and collect
- Dry down eluent
- Add 200 µL of hexane
- Inject 1 µL sample onto GC outfitted with Rtx®-CL pesticides column
- GC conditions - starting temp @ 150°C and ramp 3 degrees per minute until 300°C is reached; hold for five minutes

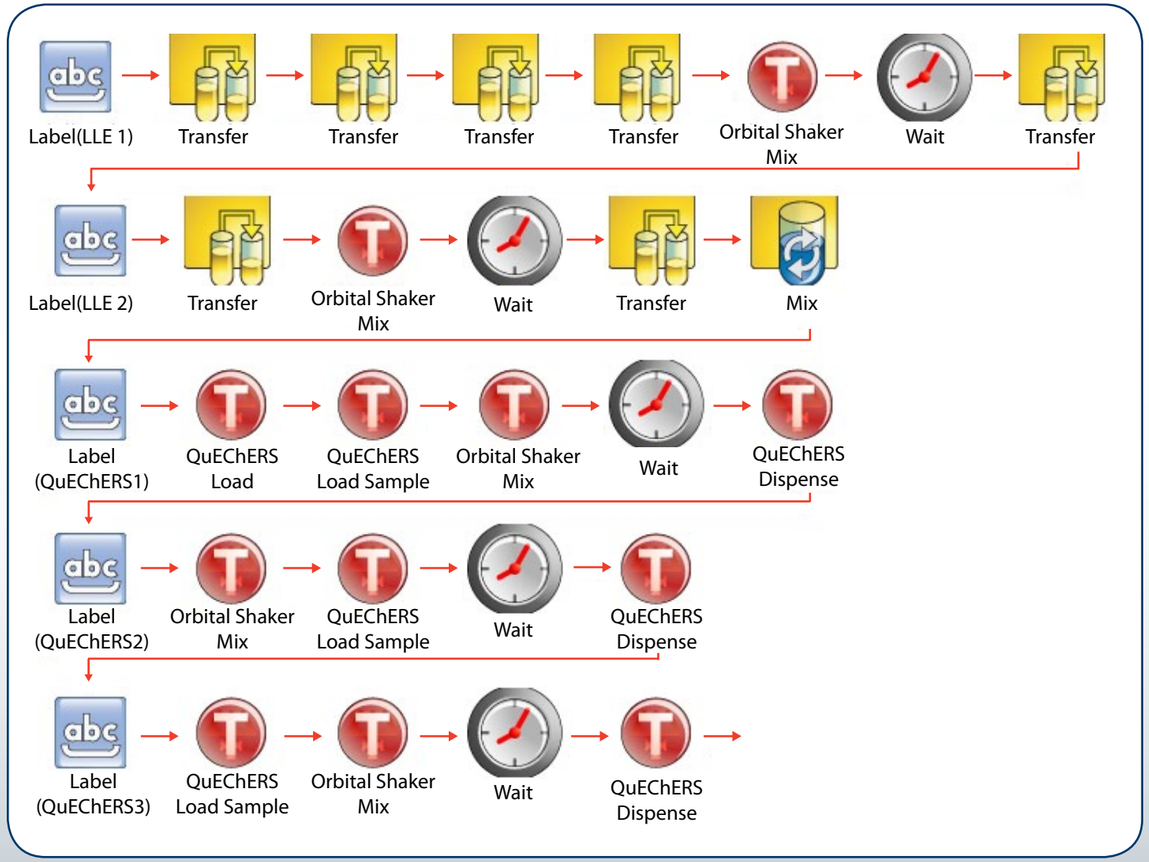
Figure 3. TRILUTION® LH Method for SPE with LLE Process



QuEChERS and LLE Method

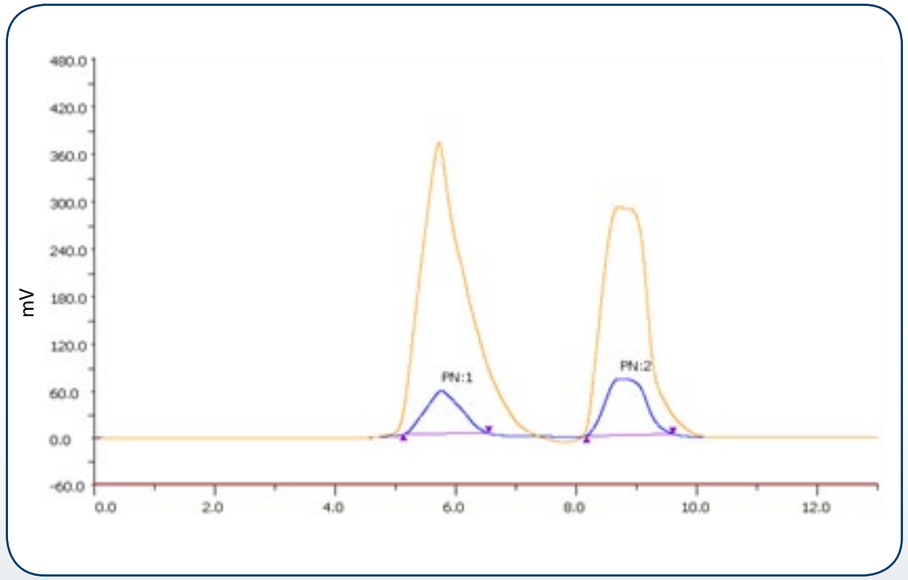
- 1.5 mL of olive oil sample added to 1.5 mL of hexane
- 3 mL mixture is placed in test tube and 6 mL ACN is added
- Mix sample on Gilson Orbital Shaker at 720 rpm for 30 minutes
- Wait 20 minutes for sample to separate
- Repeat LLE with 6 mL of ACN
- Wait 20 minutes for sample to separate
- Mix ACN LLE fractions together
- Condition SPE with 5 mL ACN
- Extract 1 mL of ACN mixture from the top layer and place in QuEChERS tube; shake on Orbital Shaker at 650 rpm for 2 minutes
- Push through filter tube and collect eluent
- Rinse with 2 mL ACN and mix for 5 minutes
- Push through filter tube and collect eluent
- Repeat ACN rinse and collect
- Dry down eluent
- Add 200 µL of ethyl acetate
- Inject 1 µL sample onto GC outfitted with Rtx®-CL pesticides column
- GC conditions - starting temp @ 150°C and ramp 3 degrees per minute until 300°C is reached; hold for five minutes

Figure 4. TRILUTION® LH Method for QuEChERS with LLE Process



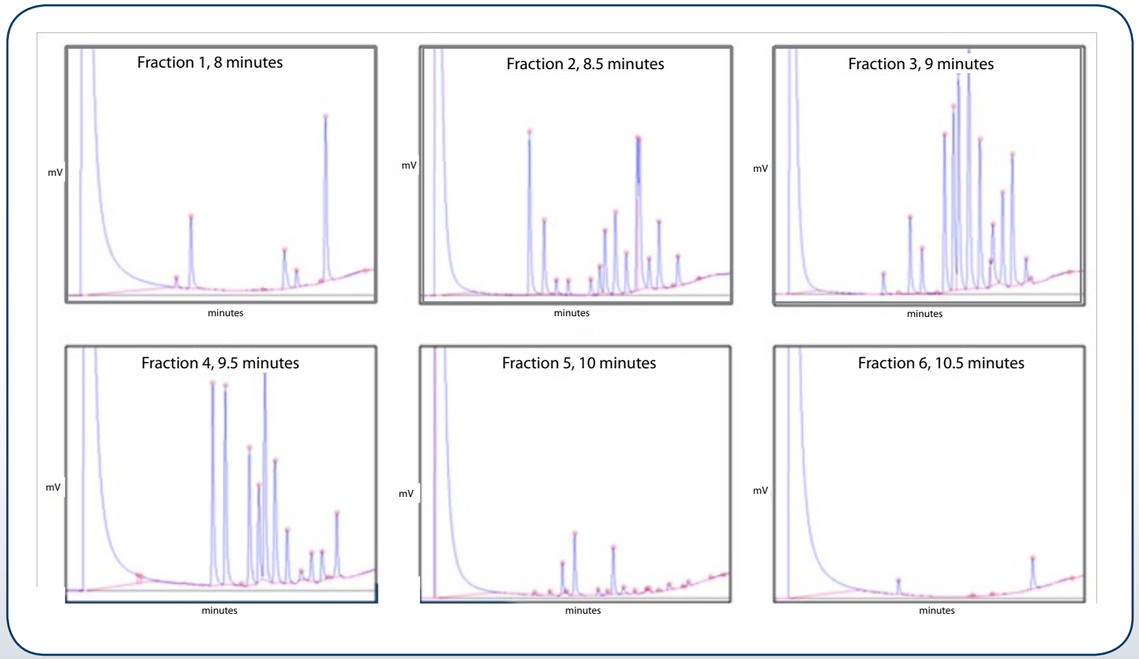
GPC Cleanup Results via UV

The first peak at 6 minutes is the olive oil and therefore was not collected. The second peak containing the pesticides was collected and subsequently analyzed via GC.



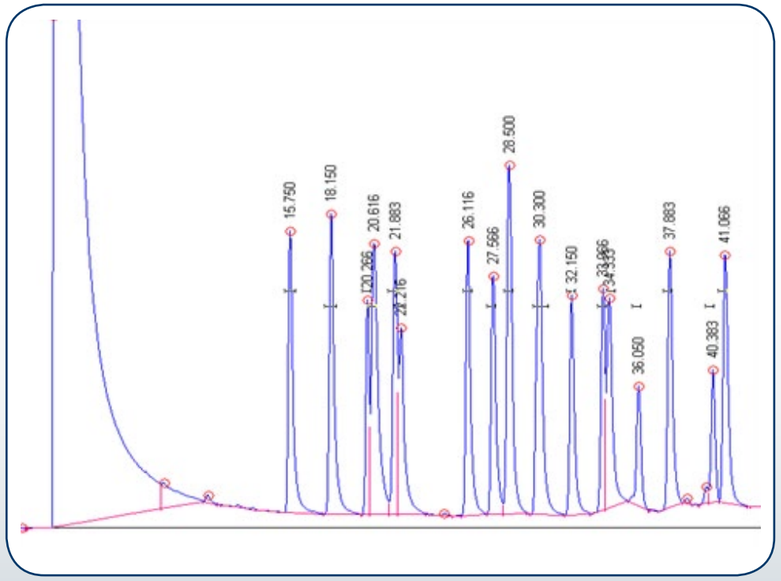
GPC Cleanup Results via GC

Each of the six fractions collected from 8 to 11 minutes (0.5 minute fractions) was analyzed individually to verify the collection region was appropriate prior to collecting another sample where fractions 1-6 were pooled and then analyzed by GC.



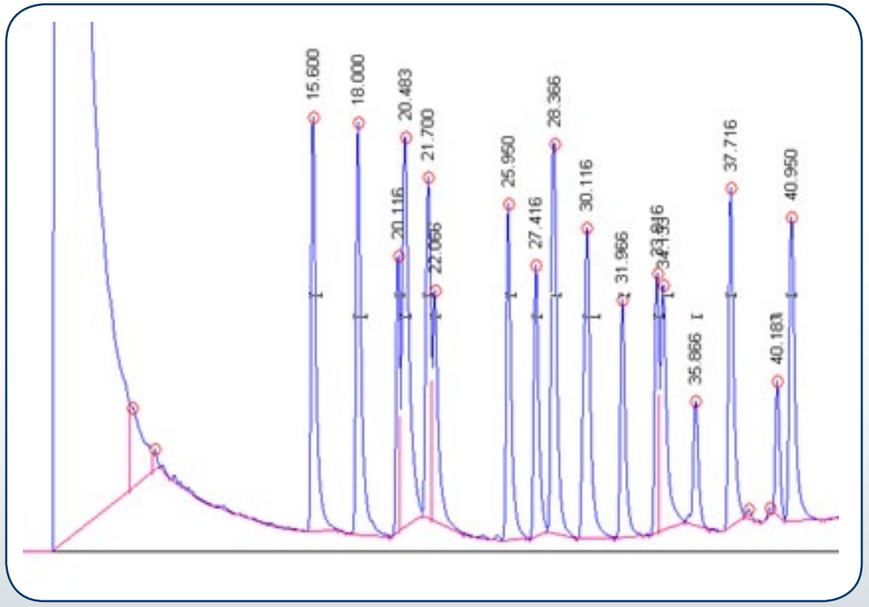
SPE Results via GC

The ability to perform method development of the solid phase extraction allowed for the recovery to be optimized. Sample was loaded to determine breakthrough of pesticides and several elution solvents were tested to determine the highest recovery percentage. To prevent the elution of interfering compounds, the SPE cartridge should not be over saturated with sample. Elution with a minimum of 10 mL of ACN provided highest recoveries.



QuEChERS Results via GC

In the optimization of the QuEChERS method the load of 1 mL was determined by the size of the cartridge and the amount of QuEChERS sorbent and magnesium sulfate within the cartridge. Several solvent amounts and types were tested rinsing the cartridge. Acetonitrile provided the highest recovery yield, and a rinse volume of 2 mL followed by another rinse of the same amount optimized this yield.



Conclusions

All methods attained levels of 25 µg/mL of each pesticide and extrapolated a possible low detectable limit of 5 µg/mL for QuEChERS, 1.7 µg/mL for SPE, and 400 ng/mL for GPC. GPC in this method attained the lowest detectable limits at 3.3 times that of SPE and 10 times that of the QuEChERS extraction method.

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Determination of Chlorinated Pesticides in Poultry Fat via AOAC 970.52, 984.21 and USDA-FSIS CHC3-19 Using GPC and Optional Alumina Clean-up with GC-ECD Detection

The data for this application note was in collaboration with Sean Linder, Ph.D. and Cheryl Fossler, M.S., Arkansas Livestock and Poultry Commission.

Featured Product: Automated Gilson GX-271 GPC Clean-up System

Poultry fat is routinely monitored for the presence of chlorinated pesticides. This insures that consumers are not exposed to unacceptable levels of these pesticides and that unauthorized use of these pesticides is detected. Determination of chlorinated pesticides in poultry fat requires post-extraction clean-up steps to effectively remove lipids and other co-extractives prior to analysis by gas chromatography (GC) or GC/MS. Failure to remove these compounds results in decreased column life, contamination of the ion source and decreased analytical performance. This application note describes the use of the Gilson Automated GX-271 GPC Clean-up System to perform the post-extraction clean-up of poultry fat followed by an optional alumina clean-up step using a special filter rack placed on-line to the GPC eluent.

Gel permeation chromatography (GPC) is a common tool for the post-extraction removal of high molecular weight interferents prior to pesticide analysis. GPC removes these interferents via a size separation mechanism that uses organic solvents and a hydrophobic gel (a cross-linked divinylbenzene-styrene copolymer) to separate the interferents from the lower molecular weight compounds of interest. The interferents are discarded to waste and the fraction containing the pesticides is collected for further clean-up and analysis. GPC clean-up may be followed by additional clean-up procedures such as adsorption chromatography using alumina, Florisil™, or silica.

Experimental Conditions

Materials – GPC Clean-up Procedure

This application utilized the Gilson Automated GX-271 GPC Clean-up System with a low pressure glass column filled with 60 g Envirobeads S-X3 resin. The isocratic mobile phase consisted of a 1:1 dichloromethane:cyclohexane. Evaporation flasks fitted with fritted filter with grooved 24/40 joint (Adams & Chittenden part no. BUCH30C24G) were required, along with Gilson's collection rack special 1340.

Sample Preparation

Poultry fat was prepared according to AOAC International Methods 970.52, 984.21 and USDA-FSIS Method CHC3-19. Poultry fat was spiked with 10 ppb of the following compounds: Lindane, Heptachlor, Aldrin, Heptachlorepoxide, Alpha-Chlordane, Methoxychlor, Internal Standard 1: TCMX, and Internal Standard 2: DCBP.

Figure 1. Using Evaporation Flasks With Fritted Filters on the Automated Gilson GX-271 GPC Clean-up System

General GPC Clean-up Procedure

- Isocratic mobile phase: 50:50 dichloromethane:cyclohexane
- Flow rate: 5 mL/min
- Start fraction collection: 24 min
- Fraction collection time: 26 min
- Total run time: 55 min
- Total injection of fat on column: 1 g
- Software: Gilson TRILUTION® LC with preinstalled GPC Clean-up Methods

Materials – Alumina Clean-up Procedure

A "keeper" (300 µL of 2% heavy paraffin oil in isooctane) was added to the GPC collection flasks prior to GPC collection (A keeper solution is a small volume of a high boiling solvent used to prevent full evaporation to dryness and the occurrence of significant losses during the dry-down step.). The collected GPC fraction passes over an alumina bed (2 g bed weight of Alumina, Neutral Brockman Activity 1; 60-325 Mesh; [1344-28-1]-Fisher A950-500) as it elutes from the GPC column. The alumina-cleaned extract is transferred and dried down using a RapidVap® N2 System. It is then reconstituted in the appropriate mobile phase prior to GC analysis.

Materials & General Procedure– Gas Chromatography (GC) Analysis

- Agilent® Series 5890 GC with ECD Detector
- Supelco Equity-5 column (30 m x 0.53 x 0.25)
- Splitless Injection, 3 µL
- Manual Flow Control

Results

Comparison analysis via GC-ECD was performed on poultry fat samples where no GPC Clean-up was performed, where GPC Clean-up was performed, and where GPC Clean-up + Alumina Clean-up was performed. The overlayed chromatograms displayed in Figure 5 represent the effectiveness GPC Clean-up + Alumina Clean-up has on reducing interferents from poultry fat samples. Recovery and reproducibility values for the pesticide compounds are presented in Table 2.

Figure 2. Overlayed Poutry Fat Analyses via GC-ECD

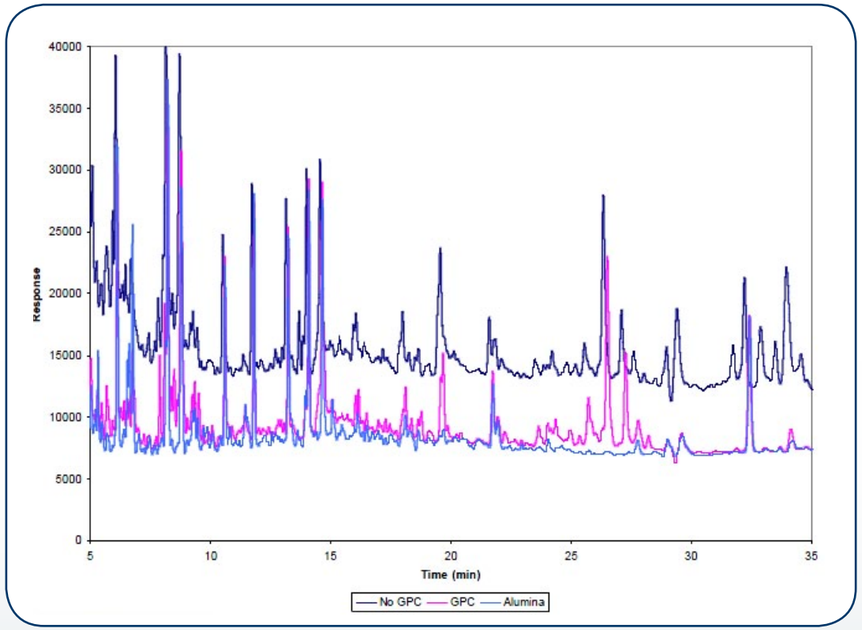

Table 1. Retention Times of Eluting Chlorinated Pesticides from GC-ECD Analysis

Compound Name	Retention Time (minutes)	Compound Name	Retention Time (minutes)
TCMX (IS 1)	6.17	Gamma-Chlordane	14.18
Lindane	8.29	Alpha-Chlordane	14.74
Heptachlor	10.68	Methoxychlor	21.87
Aldrin	11.89	DCBP (IS 2)	32.59
Heptachlorepoxide	13.32		

Note: Analysis was performed on different days with manual flow control so slight variations in retention times occurred.

Table 2. Observed vs. Expected Compound Recoveries and Reproducbility Values

Compound	Observed Recovery (%)	Observed Reproducibility %CV	CHC3 Expected Recovery (%)	CHC3 Reproducibility %CV
Lindane	108	3	70-120	20
Heptachlor	96	2	70-120	20
Aldrin	96	2	70-120	20
Heptachlorepoxide	98	1	70-120	20
Gamma-Chlordane	115	3	70-120	20
Edosulfan I	79	5	70-120	20
Alpha-Chlordane	101	2	70-120	20
Methoxychlor	93	3	70-120	20
2,4′-DDE	95	16	ND	ND
4,4′-DDE	97	14	70-120	20
2,4′-DDD	95	7	ND	ND
4,4′-DDD	93	6	70-120	20
2,4′-DDT	75	11	70-120	20
4,4′-DDT	93	10	70-120	20
TCMS (Internal Standard 1)	87	8	ND	ND
DCBP (Internal Standard 2)	103	4	ND	ND

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Using a DISTRIMAN® Repetitive Pipette to Screen for Chloramphenicol in Meat Products by ELISA

Featured Product: DISTRIMAN Positive Displacement, Repetitive Pipette

Chloramphenicol (CAP) is a broad spectrum antibiotic used in veterinary medicine. CAP is known to cause aplastic anemia in humans and is a potential carcinogen. The use of this antibiotic in foods is highly regulated and a zero tolerance level for CAP has been established in most countries. Recent reports of contamination in foods with chloramphenicol has increased the need for laboratories to determine its presence in foods.

The United States Department of Agriculture Food Safety Inspection Service (USDA-FSIS) has developed a method for screening for the presence of CAP in poultry, seafood, and catfish by ELISA (Enzyme Linked Immunoabsorbant Assay). This method is entitled "Screening for Chloramphenicol by ELISA, Method CLG-CAM1.01". Copies of this method are available on the USDA-FSIS website. This method specifies the use of the Gilson DISTRIMAN Pipette for certain procedures in the method.

Experimental Conditions

This method is based on a competitive-type ELISA for in vitro screening of chloramphenicol. The method uses the TRANSIA® PLATE Chloramphenicol Test Kit (part no. AB0299) from BioControl Systems, Inc., Bellevue, WA, USA. The solid support of the reaction is a microtiter plate with divisible strips coated with sheep anti-rabbit IgG antibodies. The method detects chloramphenicol in poultry, beef and catfish muscle tissue at levels > 0.01 ppb.

Materials

The TRANSIA PLATE Chloramphenicol Test Kit (Figure 1) contains the necessary microtiter plates, buffers, standards, substrate, conjugate, and antibody solution. Performing the method also requires an analytical balance, centrifuge, mechanical shaker, nitrogen evaporator, Distriman Repeater Pipette with 1250 µL mini syringes, micropipettes with a range of 2 to 250 µL, HPLC grade water, ethyl acetate, and n-hexane, as well as a 9Robot-Coupe® processor (Robot Coupe, USA), and a plate reader such as the BioTek Instruments Elx808 Ultra Microplate Reader.

Figure 1. BioControl TRANSIA PLATE Chloramphenicol Test Kit

Sample Preparation*

Samples are homogenized using the Robot-Coupe Processor prior to sample extraction. Sample extraction was accomplished with the following steps:

- Weigh 3g of tissue sample into centrifuge tube. Weigh blank samples for appropriate controls and fortify the positive control and recovery samples to a concentration of 0.25 ppb.
- Add 6 mL of ethyl acetate and mix on mechanical shaker for 10 min.
- Centrifuge at 4000 rpm and transfer 4 mL of volume for evaporation at 55 degrees C on a nitrogen evaporator.
- Dissolve residue in 1 mL of n-hexane followed by addition of 1 mL of sample dilution buffer 1X and vortex for 1 min.
- Centrifuge for 10 minutes at 4000 ppm.
- Collect the lower, aqueous phase for the ELISA.

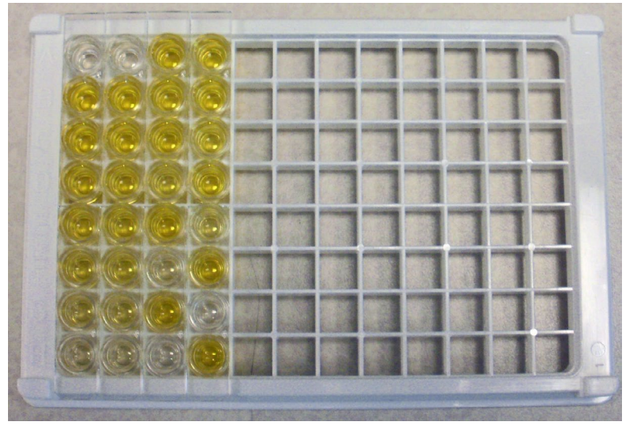
ELISA Procedure*

The Gilson DISTRIMAN Repetitive Pipette is used in any step that requires repetitive pipetting into the sample wells on the microplate. The ELISA procedure is accomplished with the following steps:

- Determine the number of controls, standards, blanks and samples that are needed for the analysis and attach the appropriate number of strips to the microplate.
- Place 100 µL of reconstitution buffer in first two wells as plate blanks; add 50 µL reconstitution buffer in the next two wells as a zero standard and then add 50 µL of additional standards in the next available wells.
- Transfer 50 µL aliquots of the samples, in duplicate. Randomly add positive controls throughout the set.
- Add 25 µL of reconstituted conjugate to every well except plate blanks using the Gilson DISTRIMAN Repetitive Pipette.
- Add 25 µL of reconstituted antibody solution to every well except plate blanks using the Gilson DISTRIMAN Repetitive Pipette.
- Manually shake the plate from side to side for 1minute and then incubate the plate in the dark at 2-8 degrees C for at least one hour.
- Wash each well on the the plate 3 times using 300 µL of washing buffer per well.
- Add 100 µL of the substrate to each well.
- Incubate the plate at room temperature for 30 minutes.
- Place 100 µL of stop solution using the Gilson DISTRIMAN repetitive pipette and shake plate for 1 minute to mix.
- Read the plate as soon as possible following the addition of the stop solution. The plate reader should be set to read at 450 nm.

* See kit insert for complete directions for use

Figure 2. Microplate Showing Color Reaction



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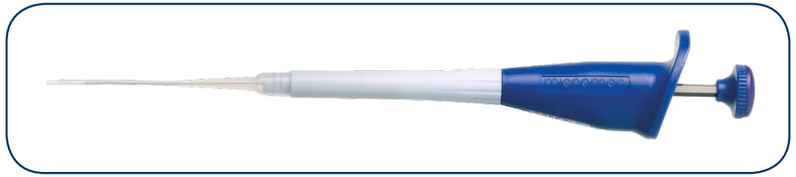
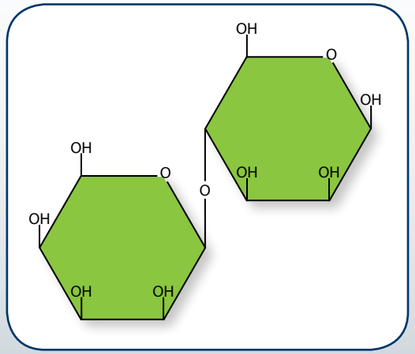
Using a MICROMAN® Positive Displacement Pipette to Determine the Lactose Content in Milk Via an Enzymatic Method

Featured Product: MICROMAN® Positive Displacement Pipette



Lactose is a disaccharide derived from the condensation of galactose and glucose (Figure 1). Its formal name is β-D-galactopyranosyl-(1 −4) D-glucose. Lactose is found in milk at levels of 2 to 8% and is often referred to as milk sugar.

Figure 1. Chemical Structure of Lactose.



The International Dairy Federation (IDF) has published a method for determining levels of lactose in milk and reconstituted milk (IDF 214:2010; ISO 26462:2010). The method is entitled "Milk – Determination of lactose content – Enzymatic method using difference in pH," and recommends the use of a positive displacement pipette, such as the Gilson MICROMAN®.

Experimental Conditions

In this method, β-galactosidase is added to milk in order to cleave lactose into glucose and galactose. At pH 7.8, glucose is phosphorylated by glucokinase, thereby releasing protons that induce a change in pH. The pH change varies as a function of the lactose content of the sample and is measured by using a differential pH analyzer such as the CL-10 Plus Analyzer (Figure 2) available from BioControl Systems, Inc. (Bellevue, WA, USA, www.biocontrolsys.com). Gilson MICROMAN® M25 pipettes are supplied with the CL-10 Plus Analyzer.**Figure 2.** CL-10 Plus Differential pH Analyzer.

Materials

The method calls for the use of an analytical balance, at least two positive displacement pipettes, a water bath, volumetric flasks and the differential pH analyzer. Reagents include a buffer solution at pH 7.8, a glucokinase enzyme solution, a β-galactosidase enzyme solution, a lactose standard solution, cleaning solution and regenerating solutions.

Sample Preparation

Warm the sample to 38° C in the water bath while mixing. Cool the sample to 20° C before preparing the test portion.

General Procedure

The Gilson MICROMAN® positive displacement pipette is used throughout the test procedure for the addition of 20 µL glucokinase enzyme solution, β-galactosidase enzyme solution, lactose standard solution or test sample into the instrument mixing chamber at the appropriate time during the assay.

The basic steps of the procedure are:

- Perform a blank determination by measuring the pH difference when both electrodes are filled with buffer: glucokinase versus one electrode containing buffer/glucokinase mixture and glucokinase versus a second electrode containing buffer/glucokinase/β-galactosidase mixture.
- Calibrate the instrument. First calibrate the difference in pH between the two electrodes by measuring the pH differential with lactose standard, glucokinase and buffer. Then add β-galactosidase, allow enzyme reaction to complete, and measure the offset differential pH between the two electrodes. Calculate slope of calibration curve.
- Check the calibration with 20µL of lactose standard solution.
- Analyze the samples for lactose levels.

All instrument manufacturer instructions should be followed throughout the procedure. This application note provides a brief summary of the method for determining lactose levels in milk and is not complete. A copy of the complete method can be purchased from The International Organization for Standardization at www.iso.org. Refer to Method ISO 26462:2010 (IDF 214:2010).

Results

Performance Characteristics for the EC® Milk Lactose Kit (Part No: MCP710):

Limit of Detection:	1.1g/L of lactose
Precision:	±0.54 g/L at 36 g/L
Accuracy (vs. reference method):	±0.7 g/L at 50 g/L

In addition to the above information, an interlaboratory collaborative study involving 11 laboratories was carried out on six test samples (milk) on a double blind basis. Refer to Method ISO 26462:2010 (IDF 214:2010) for details.

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An Automated Liquid Handling Strategy for Beverages Based on Sample Viscosity

Featured Product: GX-271 ASPEC™ System with 406 Dual Syringe Pump

Performing manual liquid handling procedures in the laboratory is commonplace. It is often necessary to automate these procedures when a laboratory faces an increase in the numbers of samples requiring processing. Transitioning from a manual to an automated process can be especially challenging for liquids of different viscosities (viscosity is defined as the resistance of a fluid to shear motion). Liquids with a higher or lower viscosity compared to water may require different liquid handing parameters in order to optimize the automated method.

Two of the most critical parameters for optimal transfer of liquids with different viscosities are the flow rate used to aspirate the liquid and the inner diameter of the probe used to transfer the liquid. This application note describes the impact of these two parameters when transferring liquids of varying viscosity such as polyethylene glycol (PEG), milk, orange juice and apple juice.

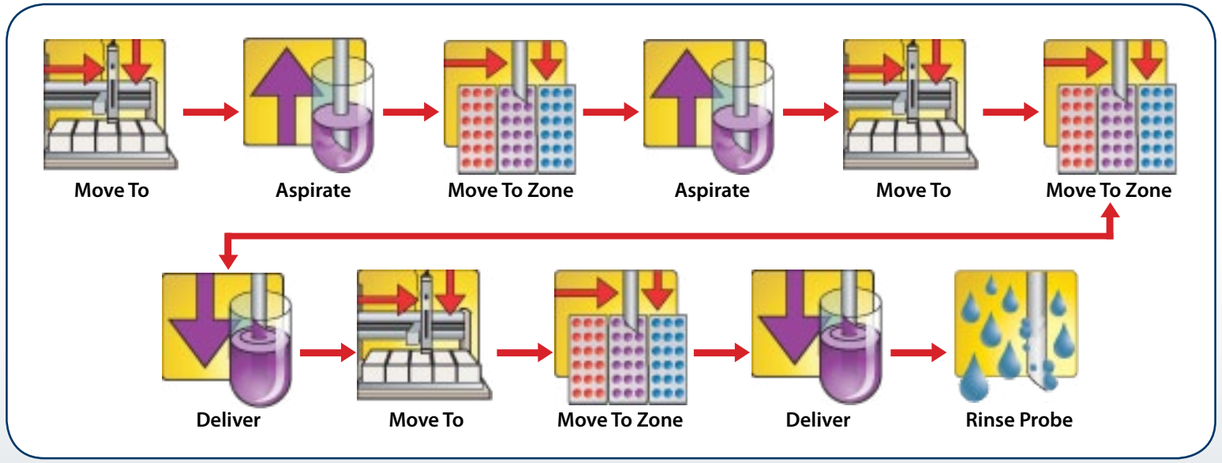
Experimental Conditions

Polyethylene glycol (PEG) 200 was obtained from Sigma-Aldrich (part no. P3015). Varying dilutions of PEG were prepared using HPLC grade water. Milk, orange juice (no pulp) and apple juice were obtained from a local supermarket.

Sample viscosity was measured in centistokes (cSt) using a Cannon-Fenske Viscometer according to established procedures (ASTM D445-09 Standard Test Method). All measurements were taken in triplicate.
Liquid Transfer Using Gilson TRILUTION® LH Software:

One milliliter of sample was aspirated and dispensed at different flow rates using an air gap setting of 50 µL. In each case, the probe was rinsed with an Inside Rinse of 3 mL water at a flow rate of 20 mL/min and an Outside Rinse with 2 mL of water at a flow rate of 20 mL/min.

Figure 1. Breakdown of the TRILUTION® LH Transfer Task for Aspirating and Dispensing Samples



All measurements were performed in triplicate. Clean and empty 13 x 100mm test tubes were weighed and the initial weights were recorded. One milliliter of sample was transferred to the empty tube using the GX-271 ASPEC with TRILUTION® LH. The test tube containing the sample was then weighed again and the weight of the sample was calculated. The sample weight was then converted to sample volume based on the density of the sample.

Results

The two main systematic parameters affecting sample recovery in a liquid handling method are the flow rate and the inner diameter (ID) of the probe. Based upon the sample's viscosity, these parameters can either have a significant effect or a rather limited effect on recovery. The higher the sample's viscosity, the more pronounced the effect becomes. Adding an equilibration time, where the probe stays in the sample while the pressure equilibrates, can help in minimizing the effect as well.

As you can see in Figure 2 and Table 3, with the larger 1.1 mm ID probe there is no significant change in recovery from the 5 mL/min flow rate to the 20 mL/min flow rate for these moderately viscous samples. With the smaller 0.4 mm ID probe, the recovery drops significantly from the 5 mL/min flow rate to the 20 mL/min flow rate. For these samples, it is the combination of the fast flow rate and small ID probe that impacts the sample recovery. Data for a larger range of viscosities can be found in the full application note at www.gilson.com. A summary flow chart of the complete data can be seen in Figure 3, which shows recommended flow rates for each probe inner diameter based on the sample viscosity.

Figure 2. Effects of Flow Rate on the Recovery of Selected Beverage Samples

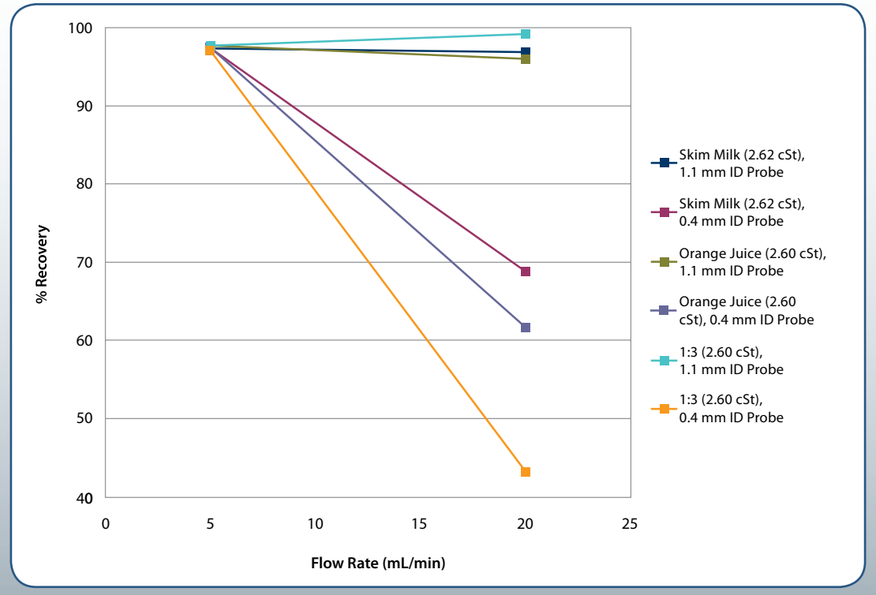
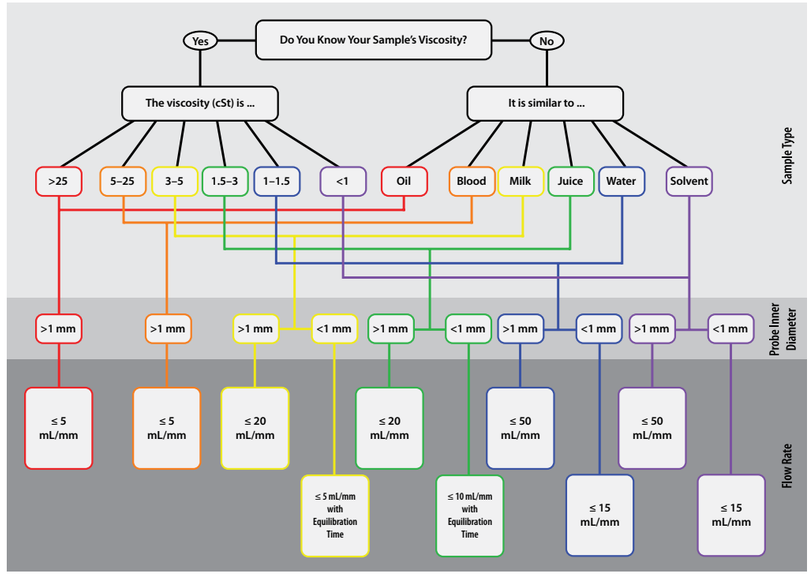


Table 3. Recovery and %CV Data for Selected Samples

		1.1 mm ID Probe (P/N 27067373) with Water as Reservoir Solvent				0.4 mm ID Probe (P/N 27067383) with Water as Reservoir Solvent			
Sample	**Viscosity**	**5mL/min**	**% CV**	**20mL/min**	**% CV**	**5mL/min**	**% CV**	**20mL/min**	**% CV**
2% Milk	4.05 cSt	96.87	0.04	95.44	0.07	96.88	0.06	61.66	1.74
Skim Milk	2.62 cSt	97.39	0.13	96.90	0.03	97.46	0.32	68.85	4.28
Orange Juice	2.60 cSt	97.76	0.05	96.14	0.12	97.48	0.09	61.69	4.39
Apple Juice	1.90 cSt	97.70	0.27	97.67	0.10	97.69	0.24	81.16	3.92
1:3 (PEG 200:Water)	2.60 cSt	97.73	0.16	99.25	0.26	97.12	0.64	43.23	2.91

Figure 3. Recommended Flow Rates Based on Viscosity and Probe Inner Diameter



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Identification of Color Additives in Powdered Drink Mix using Solid Phase Extraction and Automation of AOAC Method 988.13

Featured Product: GX-274 ASPEC™ System with Agilent 8453 UV-visible Spectrophotometer

The addition of synthetic color additives is regulated closely by the FDA and is examined from the manufacturing of the pigment itself, through to its use and appropriate product labeling. Color additives have come under scrutiny recently because of their potential adverse physical and mental health effects that may be linked to ingestion, especially in children.

AOAC method 988.13 qualitatively tests for the presence of eight synthetic color additives, one of which is now banned. These color additives are FD&C colors approved for use in food, drugs and cosmetics. Additives are extracted from the sample matrix using solid phase extraction (SPE), and then identified by spectrum analysis on a spectrophotometer.

In this application, AOAC method 988.13 was automated using a Gilson GX-274 ASPEC to perform the SPE process just prior to automated spectrum analysis using the Agilent 8453 UV-visible Spectrophotometer with the Agilent 8-position Multicell Transport. FD&C Yellow No. 5, FD&C Red No. 40 and FD&C Blue No. 1 were extracted from various powdered drink mixes. The full application note can be requested by emailing training@gilson.com.

Experimental Conditions

Tartrazine, Allura Red AC and Erioglaucine (Table 1) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Powdered drink mix (Kool-aid®) was obtained from a local supermarket.

Table 1. Alternate Names for FD&C Color Additives

FD&C Name	E Number	Common Name
FD&C Yellow No.5	E102	Tartrazine
FD&C Red No.40	E129	Allura Red AC
FD&C Blue No.1	E133	Erioglaucine

Sample Preparation

Kool-aid: 1 g powder dissolved in 100 mL of NanoPure water. Solution filtering was automated with the GX-274 ASPEC™ on-bed using 8 mL Grace Alltech® Extract-Clean™ Filter Columns.

Solid Phase Extraction (SPE) Protocol

The SPE procedure used 6 mL Phenomenex Strata® C18-E (1000 mg) cartridges. The parameters were optimized for each sample (Tables 2 and 3) from the original schema in AOAC method 988.13.

Table 2. SPE Parameters Used for the Separation of Grape Kool-aid

Step	Solvent	Volume (µL)	Air Push (µL)
Condition # 1	IPA	2000	1250
Condition # 2	1% Acetic Acid	2500	1750
Load	Kool-aid	2000	1200
Wash	2.5% IPA	3000	1500
Fractionate # 1	13% IPA	2000	1000
Fractionate # 2	20% IPA	3000	2000

Table 3. SPE Parameters Used for the Separation of Orange Kool-aid

Step	Solvent	Volume (µL)	Air Push (µL)
Condition # 1	IPA	2000	1500
Condition # 2	1% Acetic Acid	2500	2000
Load	Kool-aid	2000	1200
Fractionate # 1	2.5% IPA	4000	2500
Fractionate # 2	13% IPA	3000	2000

Automated SPE Fraction Preparation Protocol for Absorbance Reading

Each fraction collected from the SPE process was then prepared for identification:

- The fraction was transferred to a clean test tube; volume transferred was 250 µL less than the amount of solvent used for elution in the Fractionate step
- The fraction was diluted to 6 mL with appropriate IPA solution and volume
- 2 mL was transferred to each of two sets of clean tubes
- 1500 µL was transferred to the flow cells via the transfer ports, and an absorbance reading was taken on the neutral diluted fraction
- A drop (23 µL) of concentrated hydrochloric acid was added to the second set of test tubes and the solution was mixed
- 1500 µL was transferred to the flow cells via the transfer ports, and an absorbance reading was taken on the acidic fraction solution
- A drop (23 µL) of 50% sodium hydroxide solution was added to the third set of test tubes and the solution was mixed
- 1500 µL was transferred to the flow cells via the transfer ports, and an absorbance reading was taken on the basic fraction solution

A blank of the appropriate IPA solution was taken prior to each set of absorbance readings. The readings were taken from 190 to 1100 nm, however the spectra were only analyzed from 350 to 750 nm, as specified in AOAC 988.13. The flow cells and lines were rinsed with 5 mL NanoPure water after each reading to eliminate carryover between samples.

Results

Standards were prepared for each of the color additives. Using these standards, the Fraction Preparation Protocol described above was followed to obtain representative spectra of each color additive for comparison purposes (Figures 1-3).

Figure 1. Standard Spectra for FD&C Yellow No. 5

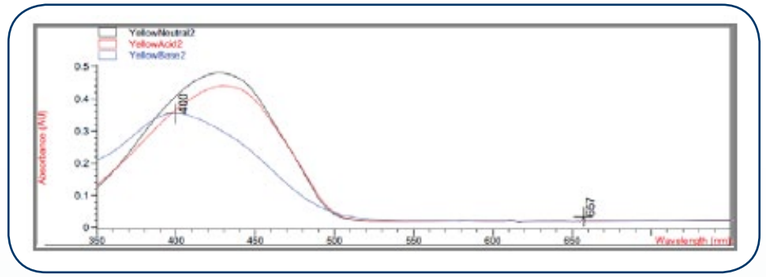


Figure 2. Standard Spectra for FD&C Red No. 40

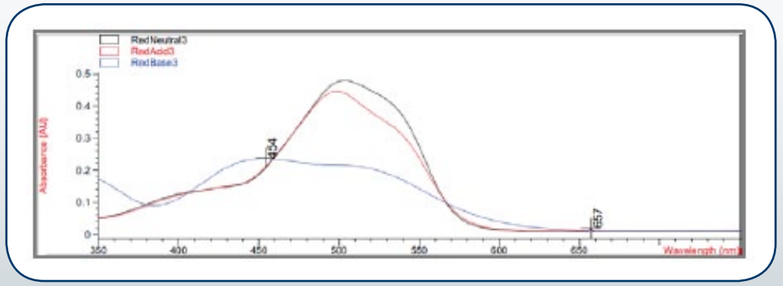
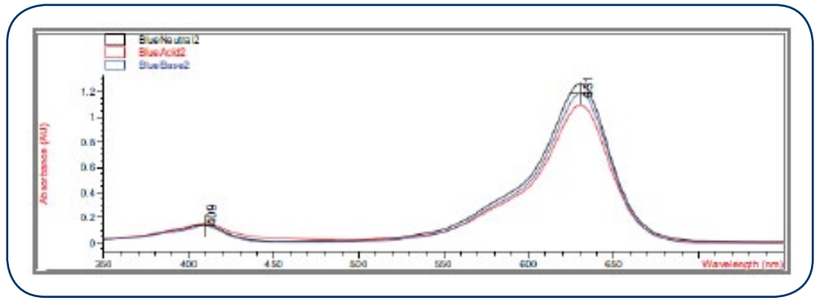


Figure 3. Standard Spectra for FD&C Blue No. 1



For the powdered drink mixes, the identifying features of the spectra are visible, though some overlapping of colors is apparent. The Grape Kool-aid® was confirmed to contain FD&C Red No. 40 (Figure 4) and FD&C Blue No. 1 (Figure 5). The average (n=4) absorbance values for the spectra can be found in Table 4. The %CV is relatively high due to extremely low absorbance values. This does not change the validity of the results for this qualitative method.

Figure 4. Representative FD&C Red No. 40 Spectra from Grape Kool-aid

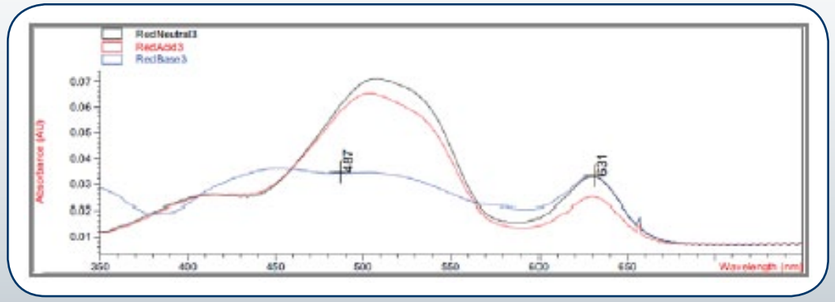


Figure 5. Representative FD&C Blue No. 1 Spectra from Grape Kool-aid®

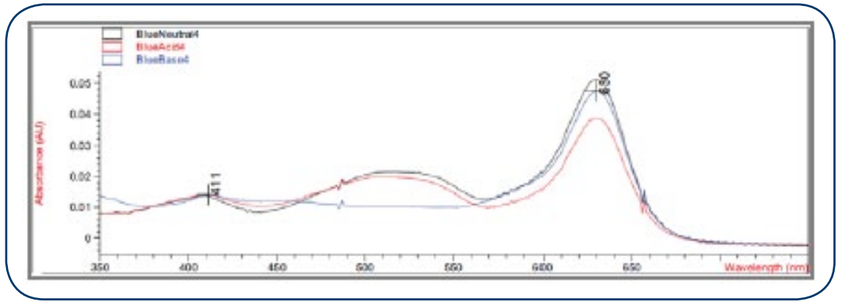


Table 4. Average (n=4) Absorbance Values for Grape Kool-aid

Sample	λ (Neutral)	AU (Neutral)	λ (Acid)	AU (Acid)	Acid/Neutral	λ (Base)	AU (Base)	Base/ Neutral
FD&C Red No.40 Average	507.75	0.07085	503.50	0.06345	0.89547	450.0	0.03364	0.47369
FD&C Red No.40 %CV	0.10	6.24	0.11	6.76	2.19	0.26	12.03	6.73
FD&C Blue No.1 Average	630.0	0.05582	630.0	0.04278	0.76587	630.0	0.05033	0.89392
FD&C Blue No.1 %CV	0.00	7.93	0.00	10.05	3.91	0.00	8.66	7.32

The Orange Kool-Aid® was confirmed to contain FD&C Yellow No. 5 (Figure 6) and FD&C Red No. 40 (Figure 7). Due to low concentration in the Orange Kool-aid, as well as the observance of some FD&C Yellow No. 5 remaining in the fraction, the absorbance for the FD&C Red No. 40 was not quantifiable, but could still be qualitatively identified. Average (n=4) absorbance values for the FD&C Yellow No. 5 spectra can be found in Table 5.

Figure 6. Representative FD&C Yellow No. 5 Spectra from Orange Kool-aid

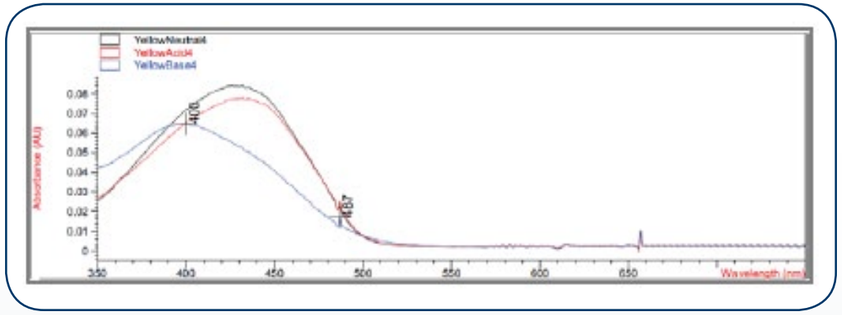


Figure 7. Representative FD&C Red No. 40 Spectra from Orange Kool-aid

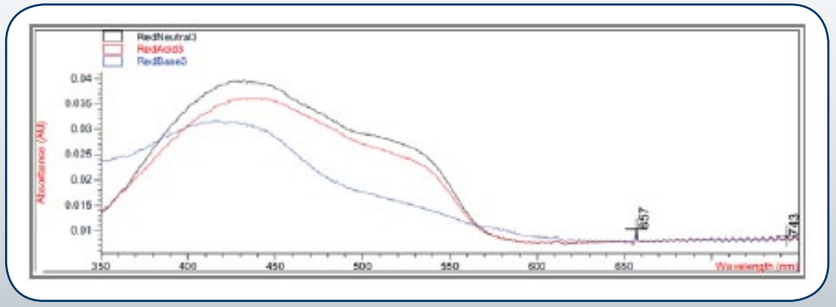


Table 5. Average (n=4) FD&C Yellow No. 5 Absorbance Values for Orange Kool-aid®

Sample	λ (Neutral)	AU (Neutral)	λ (Acid)	AU (Acid)	Acid/Neutral	λ (Base)	AU (Base)	Base/ Neutral
FD&C Yellow No.5 Average	430.5	0.08985	431.50	0.08329	0.92746	400.0	0.06807	0.75817
FD&C Yellow No.5 %CV	0.40	5.19	0.23	4.39	2.61	0.20	3.70	2.83

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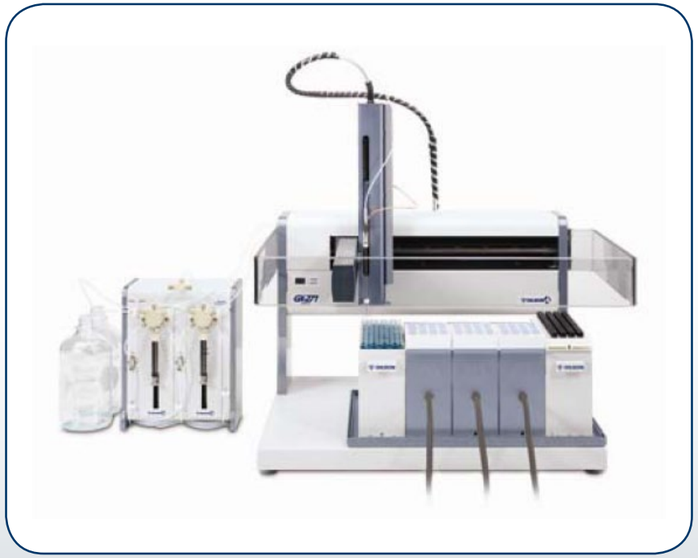
An Automated Method for the Selective Solid Phase Extraction of Ochratoxin A From Wheat Using Molecularly Imprinted Polymers

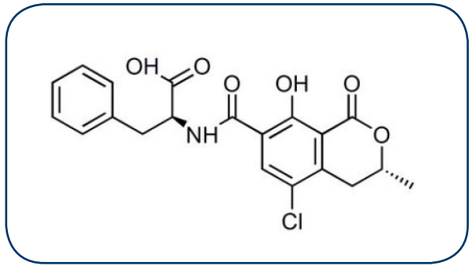
The data for this application note was performed in collaboration with Dr. Valerie Pichon's team at ESPCI, Paris, FRANCE and POLYINTELL Intelligent Polymers, Val de Reuil, FRANCE

Featured Product: Gilson GX-271 ASPEC™ System with 406 Dual Syringe Pump



Ochratoxin A (OTA) is a mycotoxin produced as a secondary metabolite of various Aspergillus and Penicillium fungi (see Figure 1). OTA has been detected in cereal products (wheat, maize, barley and oats), coffee and coffee beans, beer, wine, grapes, peanuts and cocoa products. OTA has also been found in animal tissue after exposure to OTA-contaminated feed (Pohland et al, 1992; Clark and Snedeker, 2006).

Figure 1. Chemical Structure of Ochratoxin A, CAS No. 303-47-9



Exposure to high levels of OTA or prolonged exposure to OTA have been linked to nephrotoxicity, hepatotoxicity and immunotoxicity in animals such as pigs and poultry. OTA is a teratogen and a suspected carcinogen. Humans are sensitive to OTA and ingestion has been linked to Balkan endemic nephropathy (Castegnaro et al., 1991). As a result, maximum limits for OTA contamination have been established in a number of countries. Member countries of the European Union have set maximum allowable levels of OTA at 5 µg/kg (5 ppb) in raw cereal grains, 3 µg/kg in cereal grains intended for human consumption and 2 µg/kg in wine (European Commission Regulation (EC) 1881/2006).

The analysis of OTA in agricultural products requires extensive extraction and post extraction clean-up of the sample prior to analysis by HPLC with fluorescence detection or detection by mass spectrometry. These steps remove matrix interferents and enhance sensitivity (Valenta, 1998; Zollner et al., 2000; Leitner et al, 2002). Molecularly Imprinted Polymers (MIPs) have been demonstrated to be very effective tools for the selective extraction of an analyte from a complex matrix such as a food product (Haginaka, 2009; Wei et al., 2007). This study describes the automated solid phase extraction (SPE) of OTA from wheat utilizing a Molecularly Imprinted Polymer SPE cartridge that is highly specific for OTA (AFFINIMIP™OTA, POLYINTELL) and the Gilson GX-271 ASPEC™ System.

Experimental Conditions

Materials

All solvents were distilled in glass suitable for GC, HPLC, pesticide residues analysis and spectrophotometry. All reagents and chemicals were ACS grade quality or better. Wheat certified as OTA free was obtained from LGC (Teddington, UK). Wheat blank is also available from Sigma-Aldrich (catalog number BCR471). Ochratoxin A standard was obtained from Sigma Aldrich (OEKANALR OTA solution in acetonitrile).

Preparation of Samples Prior to SPE with AFFINIMIP OTA Cartridge

Fifty grams of wheat grains were ground for 2 minutes in a blender to a powder. This powder was then mixed with 100 mL of acetonitrile/deionized water (60:40, v/v) for one minute to extract the OTA. Five milliliters of the extract was diluted with 5 mL of hydrochloric acid solution (HCl, pH=1). The solution was then filtered using filter paper and transferred to a test tube for MIP SPE extraction.

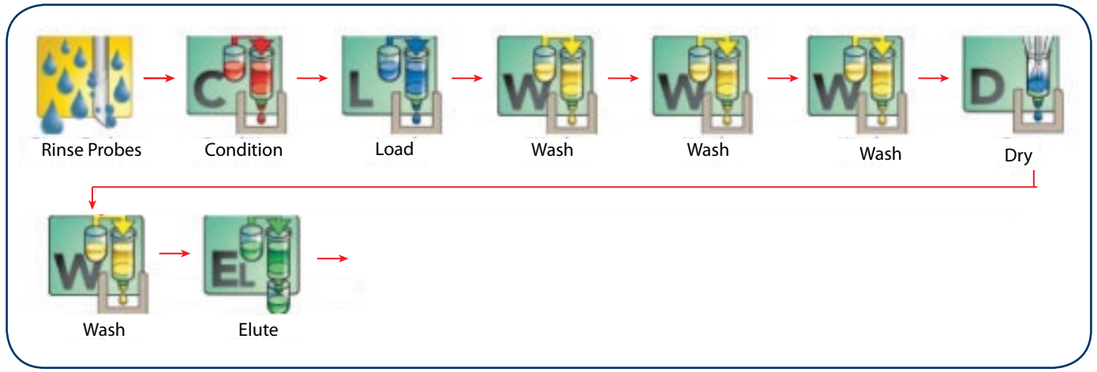
Solid Phase Extraction (SPE) Protocol

The SPE procedure used 3 mL POLYINTELL AFFINIMAP OTA Cartridges. The cartridges were sealed using Gilson 3 mL Sealing Caps. The SPE protocol is entirely automated using the Gilson GX-271 ASPEC™ system. The SPE steps are summarized with the schematic provided in the GX-271 ASPEC control software, Gilson TRILUTION® LH Software (Figure 2).

The details of each step are as follows:

- Initialization Step: Gilson Mobile SPE Racks are moved above the waste rack
- Rinse probe with deionized water
- Condition SPE Cartridge with 4 mL of deionized water at a flow rate of 5 mL/min
- Load 4 mL of sample extract at a flow rate of 0.8 mL/min
- Wash cartridge with 1 mL HCl solution (pH=1) at a flow rate of 5 mL/min
- Wash with 1 mL HCl (pH=1)/Acetonitrile (60:40, v/v) at a flow rate of 5 mL/min
- Wash with 10 mL of deionized water at a flow rate of 5 mL/min
- Dry column with nitrogen stream for 5 minutes
- Wash with 4 mL of acetonitrile-0.01% acetic acid at 5 mL/min
- Elute OTA with 2 mL of methanol-2% acetic acid at a flow rate of 0.8 mL/min

Figure 2. TRILUTION LH SPE Method for Extraction of OTA from Wheat Extract



The eluent was then evaporated and dissolved in HPLC mobile phase before injection into the HPLC system. An alternative to the evaporation step could be the dilution of the sample to a fixed volume prior to injection.

Analysis

HPLC Analysis was performed on a ThermoFinnigan Spectra System with a Thermo Hypersil GOLD™ polar endcapped C18 column (150 mm x 2.1 mm) with guard column (10 mm x 2.1 mm). Separation was accomplished using a mobile phase of methanol/water/1% acetic acid (60:39:1,v/v) at a flow rate of 0.2 mL/min. The detection system was a Jasco Model FP-2020 Fluorescence Detector set to excitation/emission wavelengths of 333 and 460 nm, respectively. The injection volume was 20 µL.

Results

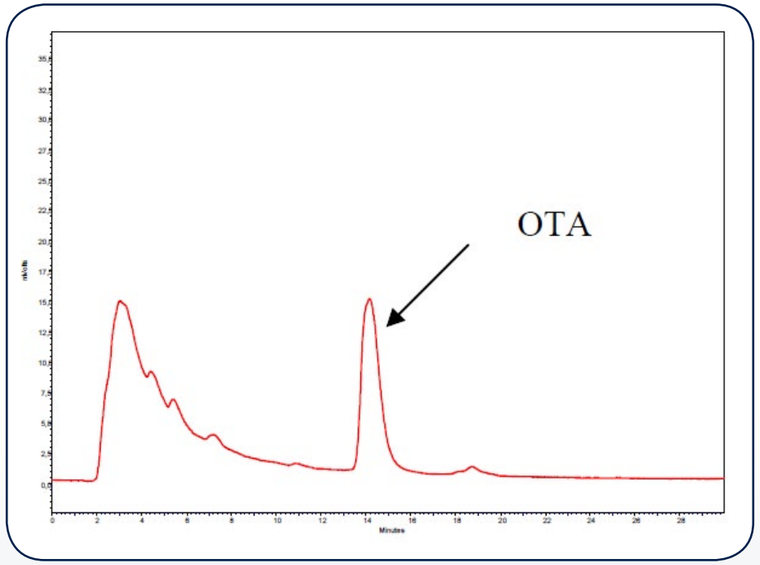
The use of MIP-based AFFINIMAP OTA SPE cartridges is simple, fast, sensitive and selective for the extraction of OTA from wheat samples. This cartridge readily lends itself to automation of SPE using the Gilson GX-271 ASPEC™ system. Automation of the SPE process improved reproducibility and increased sample throughput over the manual method. Sample throughput was approximately 30 minutes per sample. This could be improved using the Gilson GX-274 ASPEC for the processing of four sample extracts in parallel. Automation also allows one to easily optimize extraction conditions for different matrices and decreases the possibility of errors that can occur when using manual SPE methods.

Table 1. Recovery and Reproducibility of OTA (n=3) at a contamination level of 5 µg/kg in wheat after clean-up with AFFIMIP OTA Column. Manual versus automated (GX-271 ASPEC) clean-up were compared.

	% Recovery	% CV
Gilson GX-274 ASPEC™	90.5	1.7
Manual	90.4	2.5

This automated SPE method complies with the performance criteria for OTA analysis established by the European Commission Regulation (EC) 401/2006. This regulation requires recovery values for OTA in wheat of higher than 80% at 5 µg/kg. OTA recovery was 90%, with CVs of less than 2%. There was no OTA in any of the blanks tested and no carryover was observed between sample extracts. This method is well suited for the analysis of Ochratoxin A in wheat samples.

Figure 3. Chromatogram of OTA (5 µg/kg) in Wheat Extract After AFFINIMIP™ OTA Clean-up



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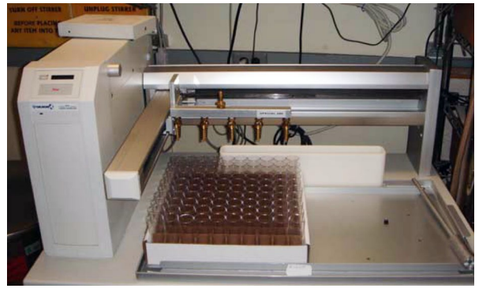
An Automated Method for Dispensing Fruit Fly (*Drosophila Melanogaster*) Food into Culture Vials and Bottles

Featured Product: Gilson 215 Liquid Handler Customized to Dispense Fly Food



The vinegar fly or fruit fly, *Drosophila Melanogaster*, has been utilized as an animal model in medical and biological research for over 100 years (see Figure 1). The fruit fly is currently being used as a genetic animal model to study the mechanisms underlying aging, immunity, diabetes, cancer and neurogenerative diseases.

Figure 1. *Drosophila Melanogaster*

A large number of *Drosophila Melanogaster* are usually grown in order to meet research demands. Fruit fly larvae eat and grow continuously over a four-day period before hatching. Preparing food for these large numbers of larvae requires the preparation of large quantities of fly food (or fly media). Fly food must be dispensed into a large number of culture tubes or other vessels which can be a very time-consuming activity. This note describes the use of a customized 215 Liquid Handler for filling large numbers of culture vials with fly food or fly culture media.

Preparing Fly Larvae Food

Fly food is a gel at room temperature. Fly food normally contains varying concentrations of water, agar, molasses, corn meal and an anti-fungal agent such as proprionic acid or Tegosept (contains methyl paraben). When heated to 65 degrees C, the gel becomes a liquid with a consistency of oatmeal that can be dispensed into tubes or other vessels.

The ingredients are mixed in a large heated pot with a large stainless steel stirrer (Figure 2). The fungicidal agent is added last after the solution has cooled to prevent any heat inactivation. The solution is then ready to dispense into appropriate vessels.

Figure 2. Heated Pot with Stirrer for Making Fruit Fly Larvae Food

Automated Dispensing of Fly Food

Automation of the dispense process utilizes a Watson Marlow 620 DI Pump with a 625L pump head (flow typically 1.4 mL/min) and a Gilson 215 Liquid Handler modified for dispensing fly food into 5 vials simultaneously. See Figures 3 through 5.

Figure 3. Typical Layout of a Fruit Fly Food Dispensing System
The System Can be Customized to Suit the Needs of the Lab

Figure 4. A Close-up of the 215 Fly Food Dispensing Head and Collection Vessels
The System Can Be Customized and Configured to Hold Many Different Bottles and Vials

Figure 5. Software Program for Operating the Automated 215 Fly Food Dispenser System with a Watson Marlow Pump

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Automated Solid Phase Extraction (SPE) of Illegal Drugs and Benzodiazepines from Human Plasma Prior to LC/MS-MS Analysis

This application note was part of a collaboration with C. Roy and N. Sadeg at the Centre Hospitalier René Dubos, Pontoise, France

Featured Product: GX-271 ASPEC™ with 406 Dual Syringe Pump

The identification and quantification of drugs of abuse in plasma has become very common in the forensic medicine laboratory. Liquid/liquid extraction (LLE) has been traditionally used for the extraction of amphetamines, cocaine and its metabolites and opiates in plasma. LLE can be time-consuming and difficult to automate compared to solid phase extraction (SPE). Poor reproducibility can also be a factor when using a manual method such as LLE. This note describes an automated SPE protocol for the simultaneous extraction of amphetamines, cocaine and metabolites, opiates and benzodiazepines from plasma prior to analysis by LC/MS-MS.

Experimental Conditions

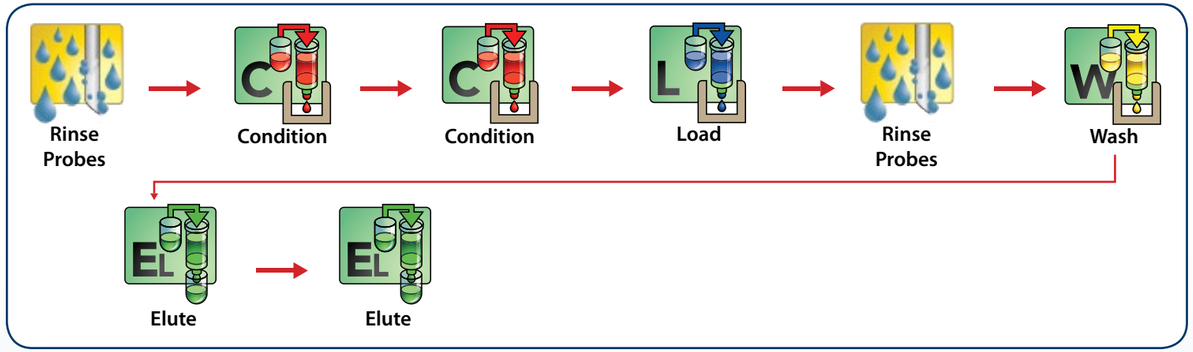
Plasma (0.5 mL) was spiked with appropriate drug standards (LGC Standards, Molsheim, France). The following Internal Standards were used:

- 100 ng benzoylecgonine-d3, internal standard used for cocaine metabolites and opiate quantification.
- 100 ng amphetamine-d5, internal standard used for amphetamine quantification.
- 50 ng clonazepam-d4, internal standard for benzodiazepine quantification.

Solid Phase Extraction (SPE) Protocol

The SPE procedure used 1 mL Waters Oasis™ HLB (30 mg) Cartridges. The SPE protocol is entirely automated using the Gilson GX-271 ASPEC system. The SPE steps are summarized with the schematic provided in the GX-271 ASPEC control software, TRILUTION® LH (Figure 1).

Figure 1. TRILUTION® LH SPE and Liquid Handling Tasks For Extraction of Drugs From Serum.



- Condition SPE cartridge with 1 mL of methanol followed by 1 mL of deionized water at 3 mL/min.
- Load sample at 1.0 mL/min.
- Wash cartridge with 2 mL deionized water at 6 mL/min.
- Move the Gilson Mobile SPE Rack over the collection tubes.
- Elute the analytes of interest with 2 x 0.5 mL of methanol at 1.5 mL/min.
- Inject 10 µL of eluate into the LC/MS-MS system at 0.2 mL/min.

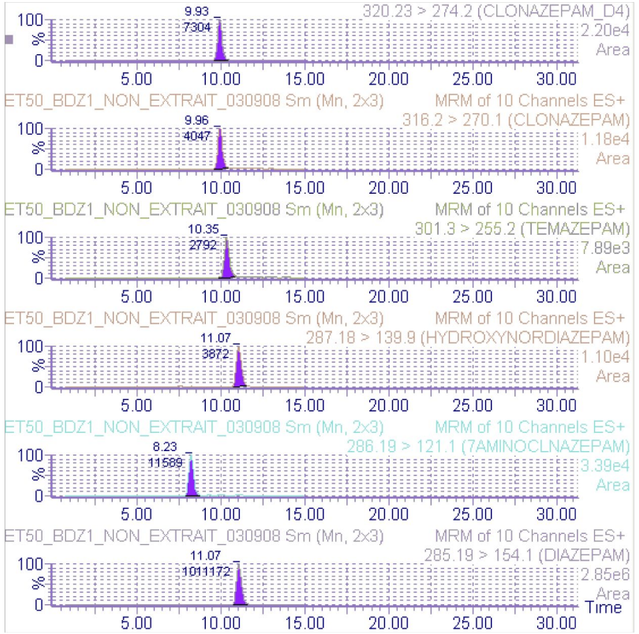
LC/MS-MS Analysis

HPLC analysis was performed on a Waters Alliance 2695 with a Waters Xterra C18 column. Separation was accomplished using a binary gradient of water and acetonitrile both containing 0.5% TFA. The detection system was a Waters QuatroMicro triple quadropole mass spectrometer.

Table 1. Recovery Values Obtained from Spiked Plasma Samples (n=3)

Analytes		Recoveries with plasma spiked at 50ng/mL (%)	Recoveries with plasma spiked at 150 ng/ml and 200 ng/mL for benzodiazepines (%)
Amphetamines	Amphetamine	100	100
	Methamphetamine	96	98
	MDA	72	79
	MDMA	88	96
	MDEA	80	75
	MBDB	100	95
	Ephedrine	86	90
Cocaine & Metabolites	Benzoylecgonine	84	75
	Ecgonine Methylester	100	87
	Cocaine	74	79
Opiates	Morphine	90	84
	6Monoacétylmorphine	100	92
	Codeine	88	80
	Pholcodine	84	80
	Ethylmorphine	100	100
Benzodiazepines	Diazepam	95	83
	Nordiazepam	87	75
	Hydroxynordiazepam	96	84
	Chlordiazepoxide	100	100
	Oxazepam	82	84
	Temazepam	78	79
	Clonazepam	95	83
	7-Aminoclonazepam	70	68

Figure 2. Assay of Plasma Extract Targeting Benzodiazepines



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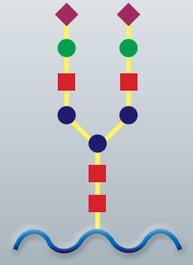
Processing Human Serum for Rapid and Reproducible N-Glycan Mass Profiling

The data for this application note was provided by Dr. Scott Kronewitter and Dr. Carlito Lebrilla at the Department of Chemistry, University of California Davis, CA, USA.

Featured Product: GX-274 ASPEC™ System with ASPEC System Organizer

N-Glycans are the most common glycans attached to proteins to form glycoproteins (Figure 1). N-glycans play a major role in regulating biochemical pathways and cellular communication. There is increasing interest in studying and researching the structure of N-glycans for use as disease biomarkers and ultimately for personalized medicine.

Figure 1. Example of Glycoprotein Structure



Biomarker discovery relies on the reproducible release and quantitative analysis of glycans from the glycoproteins. The first step is to release the glycans from the glycoproteins . The released products are then purified using solid phase extraction (SPE) followed by analysis using mass spectrometry. Automation of the SPE step using the GX-274 ASPEC improves sample throughput and reproducibility of results.

Experimental Conditions

Human serum samples were purchased from Sigma Aldrich and the Gynecological Oncology Group (GOG) Tissue Bank (Sacramento, CA). The pertinent processing parameters are shown in Table 1.

Table 1. Sample Breakdown. Sets A, B, and C Came From a Single Human Sample. Set D Consists of 48 Different Controls From Normal Individuals .

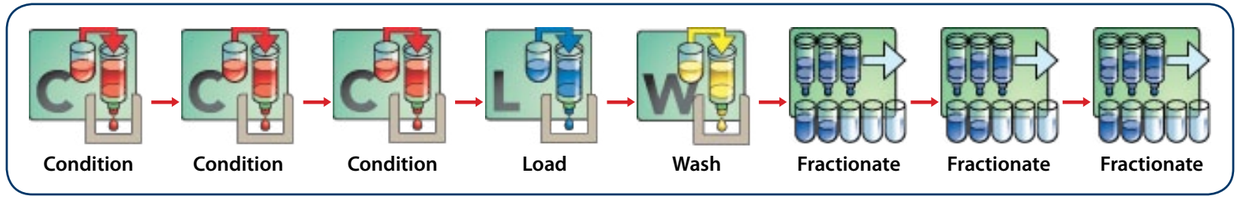
	Samples	Source	Release Method	Sold Phase Extraction
Sample Set A	4	Sigma	Water Bath	Vacuum Manifold
Sample Set B	4	Sigma	Microwave	Gilson GX-274
Sample Set C	1	Sigma	Microwave	Gilson GX-274
Sample Set D	48	GOG	Microwave	Gilson GX-274

Global enzymatic release of N-glycans was achieved using a CEM Microwave Reactor (Matthews, NC, USA) to enhance the enzymatic activity of PNGase F. Chilled ethanol was used to precipitate residual proteins prior to SPE purification.

Solid Phase Extraction (SPE) Fractionation Protocol

The fractionation procedure utilized 4 mL Alltech Carbograph™ (150mg) Cartridges (Grace Davison, Deerfield, IL, USA). The SPE protocol is entirely automated using the Gilson GX-274 ASPEC System. The SPE steps are summarized with the schematic provided in the GX-274 ASPEC control software, TRILUTION® LH (Figure 2).

Figure 2. TRILUTION® LH SPE Tasks For Fractionation of Glycans. Condition the cartridge with 6 mL of nanopure water



- Condition the cartridge with 6 mL of 0.1% TFA in 80% acetonitrile (ACN)/Water.
- Condition the cartridge with 6 mL nanopure water.
- Load glycan solution onto graphitized carbon cartridge at a low flow rate.
- Wash the cartridge with 12 mL nanopure water.
- Move the Gilson Mobile SPE Rack over the collection tubes.
- Elute the first fraction with 4.5 mL of 10% ACN/Water with 0.05% TFA.
- Move the cartridges to the next set of collection tubes and elute with 4.5 mL of 20% ACN/Water with 0.05% TFA.
- Move the cartridges to the next set of collection tubes and elute with 4.5 mL of 40% ACN/Water with 0.05% TFA.
- Evaporate the fractions using a centrifugal evaporator apparatus and reconstitute each sample in nanopure water prior to MS analysis.

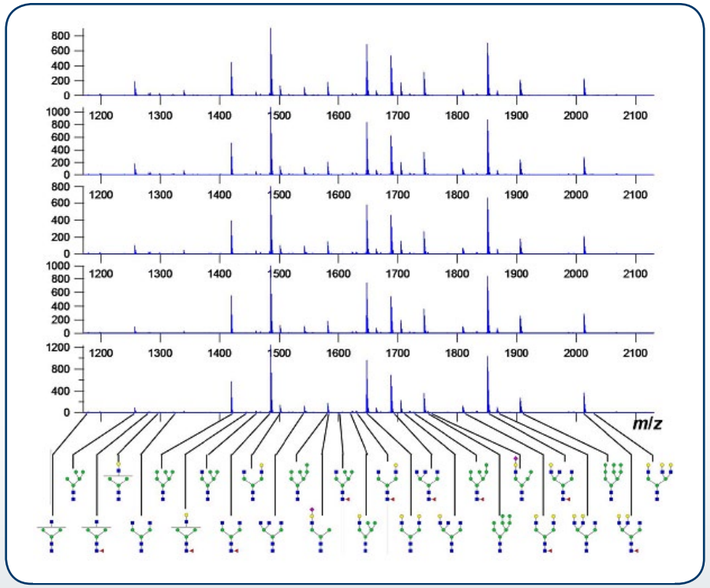
MALDI FT-ICR Mass Spectrometry

Mass spectra were recorded on an external source MALDI FT-ICR instrument equipped with a 7.0T superconducting magnet and a pulsed Nd:YAG laser at 355nm. A solution of 2,5-dihydroxybenzoic acid /NaCl was used as the matrix for all oligosaccharide analysis. For the negative mode analysis, a 0.01 M NaCl solution was used.

MALDI spotting was performed on disposable MALDI plates to prevent contamination from previous samples. Three to five spectra were collected from different parts of the MALDI spot and averaged together during data analysis. The coefficients of variation were calculated for the abundance of each glycan and averaged across several areas within the MALDI spot.

Results

Figure 3. Replicate Mass Spectra From a Single MALDI Spot From the 10% Elution Fraction. Notes: The relative distribution of the Glycans is highly conserved between the technical replicate spectra. Annotated putative structures are depicted based on common serum glycobiology.



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Fast and Reliable Immunoassay Test Results with the Qualigen FastPack® IP System Using the MICROMAN®

View the complimentary recorded web seminar discussing the FastPack IP System at: http://connectpro92894126.adobeconnect.com/p63868071/

The data for this application note was provided by John Middleton at Qualigen, Inc., Carlsbad CA, USA.

Featured Product: MICROMAN Positive-Displacement Pipette

The FastPack IP (Injection Port) System is a unique, fully automated In-office analyzer producing quantitative results in minutes (Figure 1). It is designed to be used at the point of care to analyze human plasma or serum for a number of different tests: thyroid stimulating hormone (TSH), free thyroxine (freeT4), human chorionic gonadotropin (hCG), total prostate specific antigen (tPSA), total testosterone, and alpha glutathione S-transferase (αGST). The FastPack tests are all heterogeneous immunoassay based.

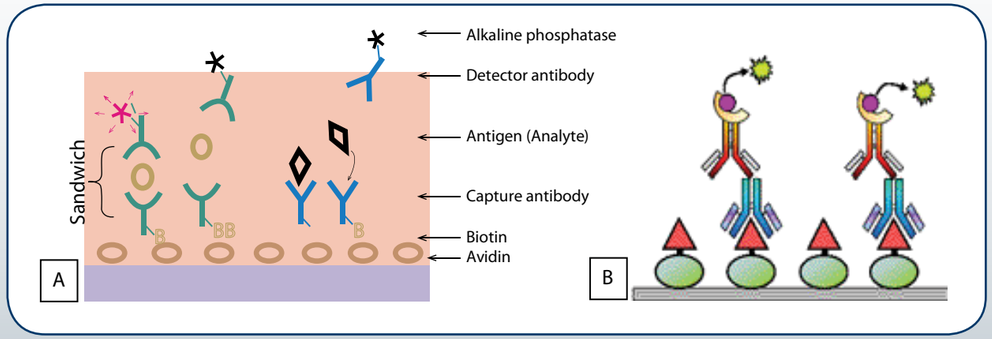
Figure 1. The FastPack IP System Analyzer (A) and Printer (B)

The FastPack® Immunoassay Methodology

The stationary phase for the FastPack system consists of magnetic beads coated with streptavidin. The noncompetitive immunoassay (Figure 2A) has an analyte-specific primary antibody linked to biotin. A secondary antibody has an alkaline phosphatase (ALP) enzyme attached. The analyte of interest binds to the primary antibody, and the secondary antibody binds to the analyte to create a sandwich. The biotin end of the sandwich binds to the streptavidin coated bead. After washing excess reagent from the magnetic beads to waste, a substrate is added which reacts with the ALP end of the bead/antibody/analyte complex. This chemiluminescent reaction generates light that is detected by the analyzer.

The competitive assay has analyte bound to the stationary phase and analyte-specific antibody free in solution (Figure 2B). Like the noncompetitive assay, the free antibody has an ALP enzyme attached. When the plasma or serum mixed with antibody solution is exposed to the magnetic bead solution, the sample analyte competes with the bound analyte for the free antibody. Any unbound antibody is removed to waste, and the remaining bound antibody is exposed to substrate, resulting in chemiluminescence.

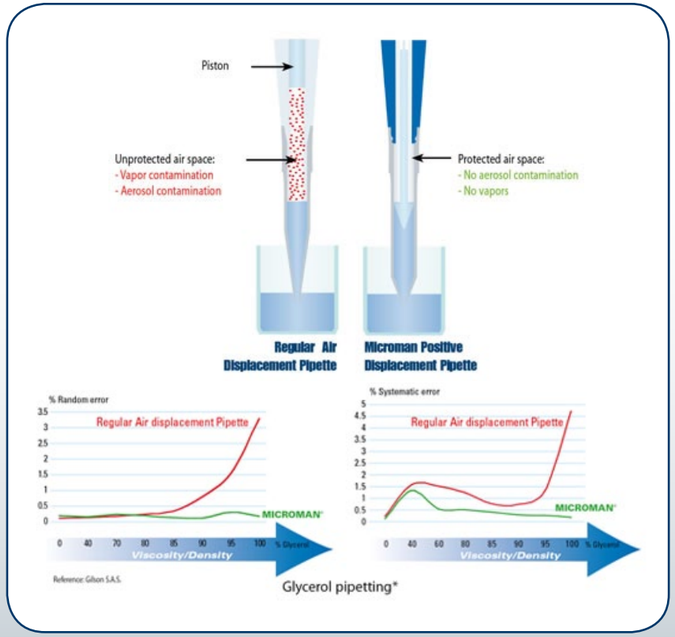
Figure 2. Noncompetitive (A) and Competitive (B) Immunoassays



Loading and Analyzing Patient Samples

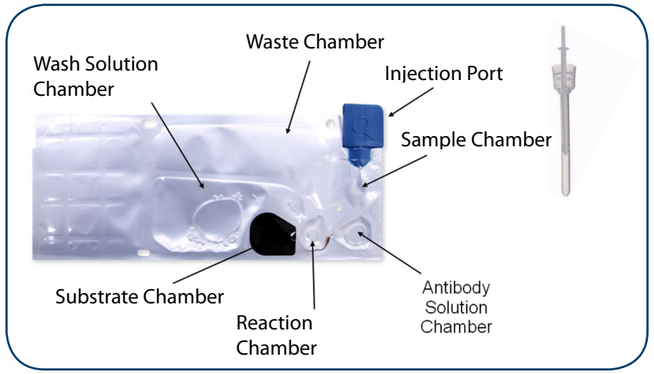
A fixed volume Gilson MICROMAN® positive-displacement pipette is used to load patient serum or plasma into the FastPack® pouch. The MICROMAN increases the accuracy of pipetting viscous liquids, thereby increasing the accuracy of test results. The disposable capillary piston and tip design increase safety by decreasing the potential interaction of biohazardous material with both the user and the pipette (Figure 3).

Figure 3. Preventing Aerosol Contamination and Increasing Accuracy With Viscous Biological Samples, using the MICROMAN



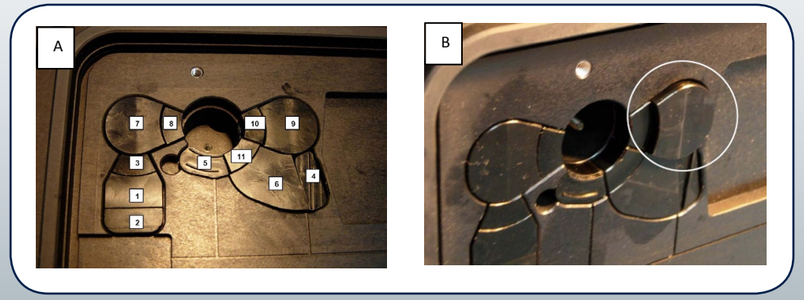
Patient serum or plasma is loaded into the FastPack pouch by inserting the MICROMAN disposable capillary piston and tip into the Injection Port (Figure 4). The tip and piston are ejected into the injection port creating a seal.

Figure 4. FastPack® Pouch with the MICROMAN® Disposable Capillary Piston and Tip



The FastPack pouch is then inserted into the analyzer, and the start button is pressed. The FastPack pouch contains chambers that are separated by chevron shaped seals. Pneumatic actuators within the analyzer break the chevron seals and move liquid from one chamber to the next (Figure 5). After the non-competitive or competitive reactions occur, magnetic particles are collected and washed in the primary reaction chamber. Light emitted from the chemiluminescent reaction at the end of the assay sequence is converted to signal data by a photomultiplier tube in the analyzer. This data is converted to analyte concentration according to the calibration for each test kit and is displayed on the front screen. The test results may also be printed onto a sticker on the pouch, which can be removed and placed in the patient's records.

Figure 5. Pneumatic Actuators within the Analyzer at Rest (A), and with a Single Actuator Engaged (B)



Results

Total PSA data was generated using the FastPack® IP System (Figure 6). The Total PSA is a non-competitive immunoassay, in which a higher concentration of analyte results in a higher signal. Testosterone data was generated using the FastPack IP System (Figure 7). The testosterone test is a competitive immunoassay, in which a higher concentration of analyte results in a lower signal. The Fastpack assay system using the Gilson MICROMAN® pipette is a fast and convenient way to generate diagnostically relevant patient results at the point of care.

Figure 6. Noncompetitive Immunoassay Data From tPSA Kit

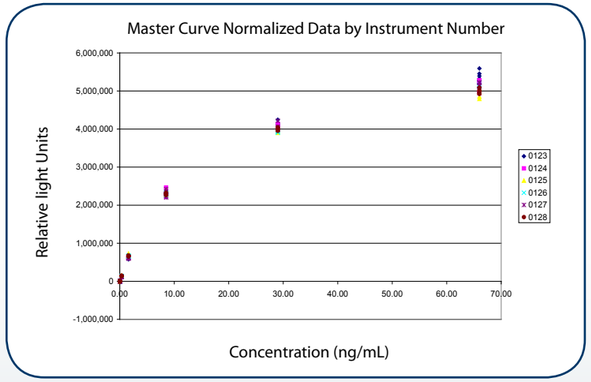
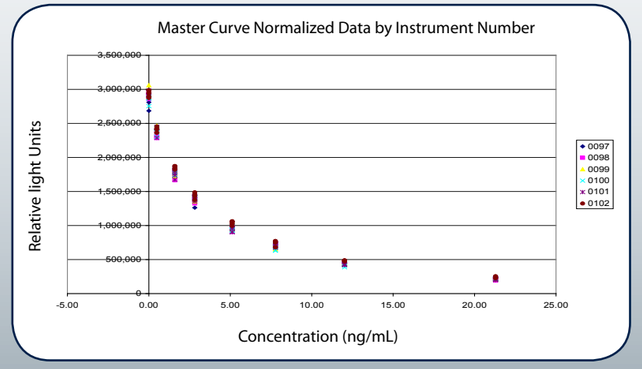


Figure 7. Competitive Immunoassay Data From Testosterone Kit



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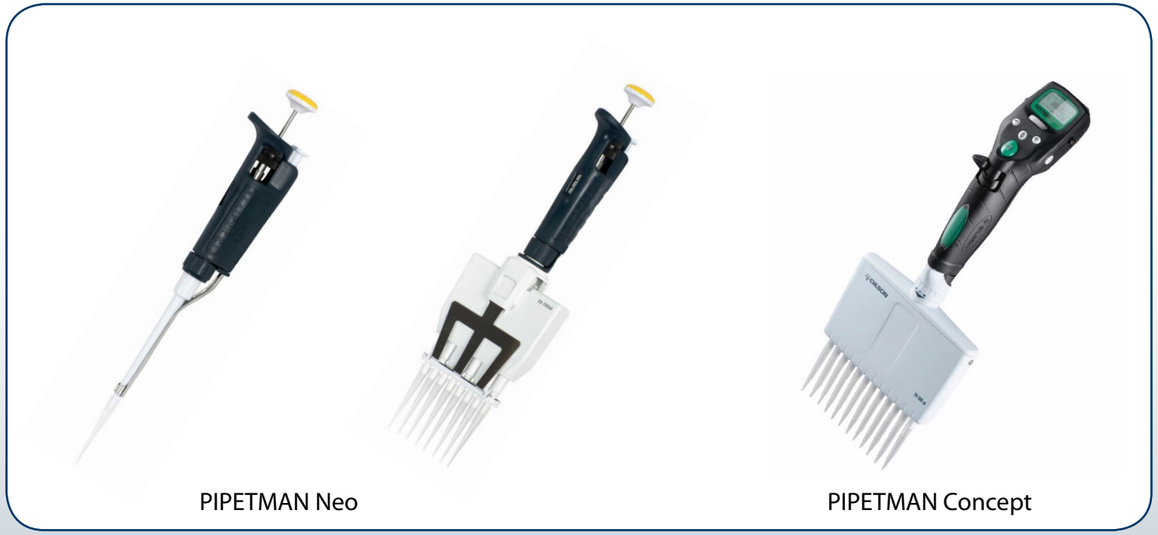
mPrep™ System for Specimen Preparation in the Electron Microscopy Laboratory

View the complimentary recorded web seminar discussing the mPrep™ System at: http://connectpro92894126.adobeconnect.com/p63868071/

The data for this application note was provided by Dr. Steven Goodman, Chief Scientific Officer of Microscopy Innovations, Marshfield WI, USA. Products and procedures described here are also applicable to some applications in light microscopy.

Featured Products: PIPETMAN Neo® (Single or Multichannel) or PIPETMAN Concept®



PIPETMAN Neo PIPETMAN Concept

Specimen preparation for electron microscopy is a complicated, multi-step process. Preparing a biological tissue specimen may require 50-100 fluid exchanges and manual transfers. The process is time-consuming and prone to errors. Documentation required at each step increases both the effort required and error potential. In addition, reagents used are often toxic, hazardous, or expensive.

The mPrep™ System for microscopy sample preparation is an end-to-end solution for processing microscope specimens from point of acquisition through archival storage. Virtually all processing steps and storage take place within the innovative mPrep capsules. These precision micro-molded, polypropylene capsules not only act like a pipette tips for fluid transfers, but also eliminate the need for other processing containers and storage devices. Reagent consumption is reduced by 75% or more.

The System consists of mPrep/s™ (capsules for fixation and embedding steps) and mPrep/g™ (capsules for staining and archiving TEM (transmission electron microscopy) grids). Each specimen is placed into its own capsule and given a unique barcode or human-readable identifying label. Specimen "touches" are greatly reduced, and sample tracking is simplified.

The likelihood of losing, damaging or mixing up specimens is reduced with the mPrep System. Documentation steps are effortless and costly re-work virtually eliminated. User cost savings result from: a) greatly reduced reagent volumes, b) using less disposable labware, and c) significant labor savings in both processing and documentation.

The system utilizes either a single- or multichannel PIPETMAN Neo®, depending on the number of samples to be processed. A PIPETMAN Concept® may be used to store a user-defined custom protocol for sample preparation, further decreasing the chance of error. Other PIPETMAN models may also be utilized.

Method for Fixing and Embedding Samples

Fixing and embedding takes place in the mPrep/s capsule. It has a screen-like bottom and a removable insert screen for retaining the specimen. Specimen orientation may be achieved in several ways, including: a) sandwiching the specimen in place, b) temporarily "gluing" the specimen to the insert screen using "glues" appropriate to the specimen, or c) using the micro-clamp function of the insert to pinch the back of the specimen (Figure 1). If orientation does not matter, the specimen may simply be dropped into the capsule and trapped with the insert.

With the specimen sandwiched between the insert and the capsule bottom, the capsule is then labeled and attached to the PIPETMAN Neo just like a standard pipette tip. The bottom of the capsule allows liquid to enter and fixation to occur through multiple fluid exchanges in the capsule.

Figure 1. Specimen Orientation and Fixation in mPrep/s™ Capsules
(mPrep tools used to position and manipulate the insert and attach specimens are not shown)

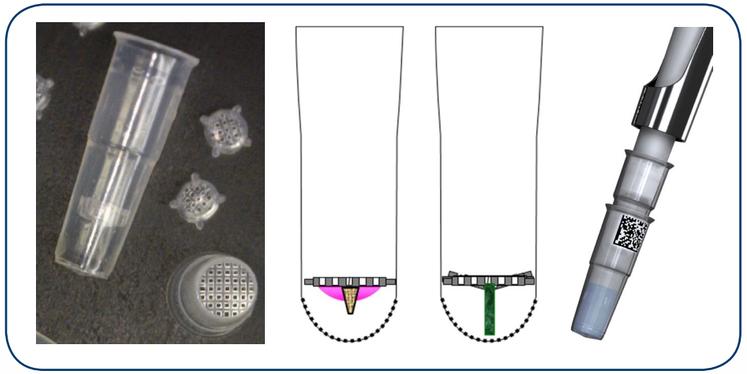
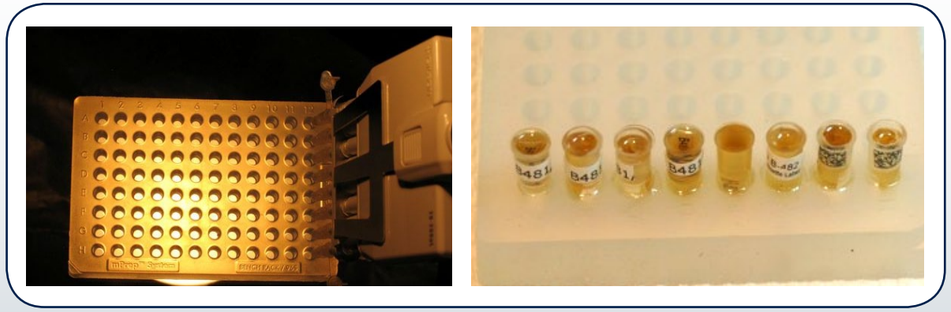


Figure 2. mPrep/bench™ Model 96S



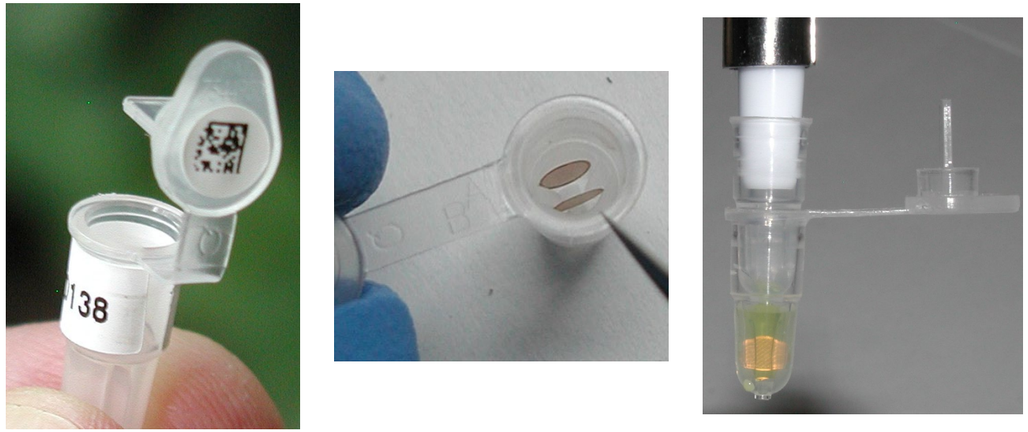
Following these steps, the specimen can be embedded within the mPrep/s capsule. The capsule itself becomes the embedding mold. For polymerization, the liquid resin filled capsules may be detached from the PIPETMAN and placed in the mPrep/bench Model 96S. This silicone holder provides a liquid-tight seal around the capsules during oven curing or other processes, such as microwave processing (Figure 2). With the sample embedded in resin, the capsule itself may be placed in a microtome and sectioned. Sections are then placed on TEM grids, while the specimen block stays in the labeled mPrep/s capsule.

Method for Staining and Archiving TEM Grids

The mPrep/g™ capsule is designed to eliminate most manual handling required to process TEM grids. Fragile and difficult to handle, grids are small metal foil screens (3.05 mm dia. x ~40 microns thick). Keeping track of grids is also difficult, because there is no way to label the grid itself.

Using a forceps, one or two grids are inserted into each mPrep/g capsule for staining and archiving (Figure 3). Grid-Tite™ technology holds the grids securely in their labeled slots until removal for TEM imaging. Two grids can be stained with only 35µL of reagent per step, greatly reducing reagent consumption and disposal costs as compared with standard droplet staining. After TEM imaging, the prepared grids are returned to the capsule for archival storage inside the supplied storage box. There the grids remain clearly identifiable with a barcode or alphanumeric label on each capsule, allowing for easy tracking of specimens.

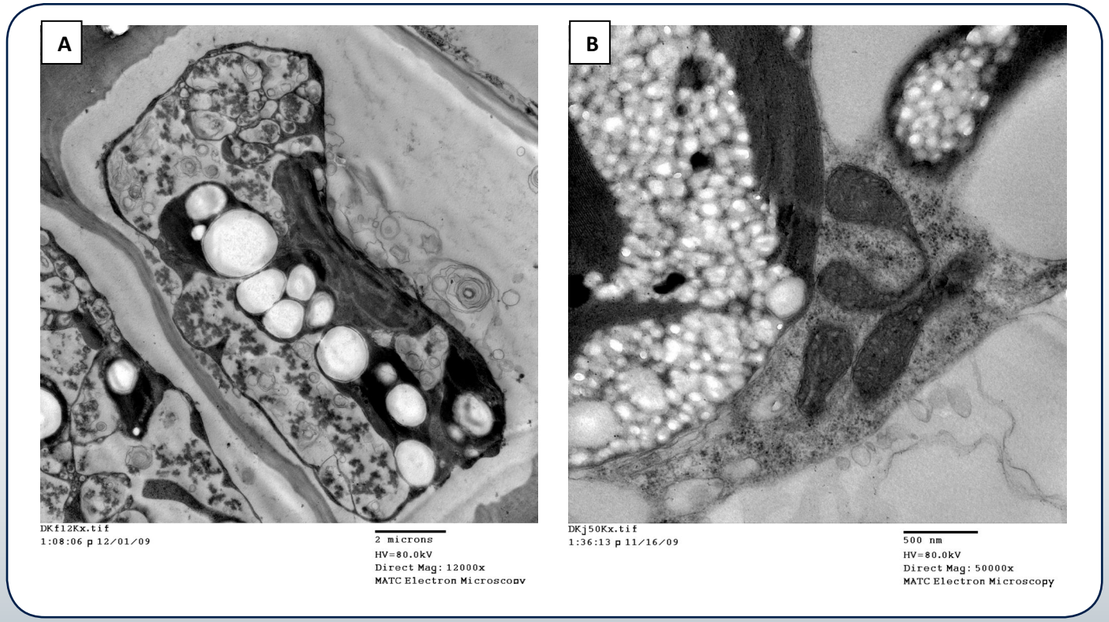
Figure 3. Grid Staining and Archival Storage in mPrep/g Capsules



Results

The mPrep™ System produces excellent stain quality, even with difficult to stain plant tissues (Figure 4). The images shown here are from *Dieffenbachia* (Dumb Cane) prepared using the mPrep/g capsules. The specimen was fixed by Karnovsky's method, containing 2.5% Paraformaldehyde, 1.5% Glutaraldehyde in 0.15 M PO_4 buffer pH 7.0. Sections were post stained using 2.5% Uranyl Acetate in 50% ETOH followed by Lead Citrate; both for 7 minutes. Images were taken on a JEOL 1200 EX at 80kV using an AMT 2K side mount camera. [Courtesy of Electron Microscopy Program - Madison Area Technical College, Madison WI, USA]

Figure 4. *Dieffenbachia* at 12000x (A) and 50000x (B).



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Manual Automation of Rapid N-Glycan Sample Preparation – Reliable Results from Error Free Sample Tracking and Accurate Pipetting

Featured Product:

Gilson SD & MD TOOLKITs N-Glycan
SD – Single Channel Diagnostic TOOLKIT
MD – Multichannel Diagnostic TOOLKIT

View the Gilson TOOLKIT N-Glycan in Action!
Click HERE!

Rapid sample preparation for N-Glycan analysis allows glycobiologists an efficient venue to speed the laborious manual process historically used for glycoanalysis. N-linked glycans are the most common glycans bound to proteins in eukaryotic cells (Apweiler, R. et al., 1999; Kronewitter, S.R., 2010). Simply defined, N-Glycans are carbohydrates linked to proteins (a.k.a. glycoproteins) that become freed glycans upon completion of the ProZyme GlykoPrep™ protocol.

Characterization of glycans is becoming increasingly important as researchers try to understand the various structures and resulting functions that glycans play for future personalized medicine and disease biomarkers (Sheridan, C. et al., 2007). The GlykoPrep protocol uses innovative AssayMAP® cartridges, a microliter-scale analytical bind/elute chromatography, to perform sample preparation in a matter of 2-3 hours versus days.

Figure 1. AssayMAP® Cartridge

AssayMAP® technology scales standard chromatography practices to the micoliter range, enabling high-throughput preparation and analysis of samples with known, workable resin chemistry in microchromatography cartridges (see Figure 1). Bind-and-elute chromatography methods are used to both purify and quantitate samples. This application discusses benefits of manual automation of the GlykoPrep protocol using the Gilson SD TOOLKIT (Single channel Diagnostic) and Gilson MD TOOLKIT (Multichannel Diagnostic) to provide automatic sample preparation tracking and electronic protocol display using the TRACKMAN™.

Experimental Conditions

Materials – Samples

Fetuin, from Fetal Calf Serum (Sigma, P/N F3385)
IgG from Human Serum, Technical Grade, >80% SDS-PAGE (Sigma, P/N 18640)

Materials – Analytical HPLC System

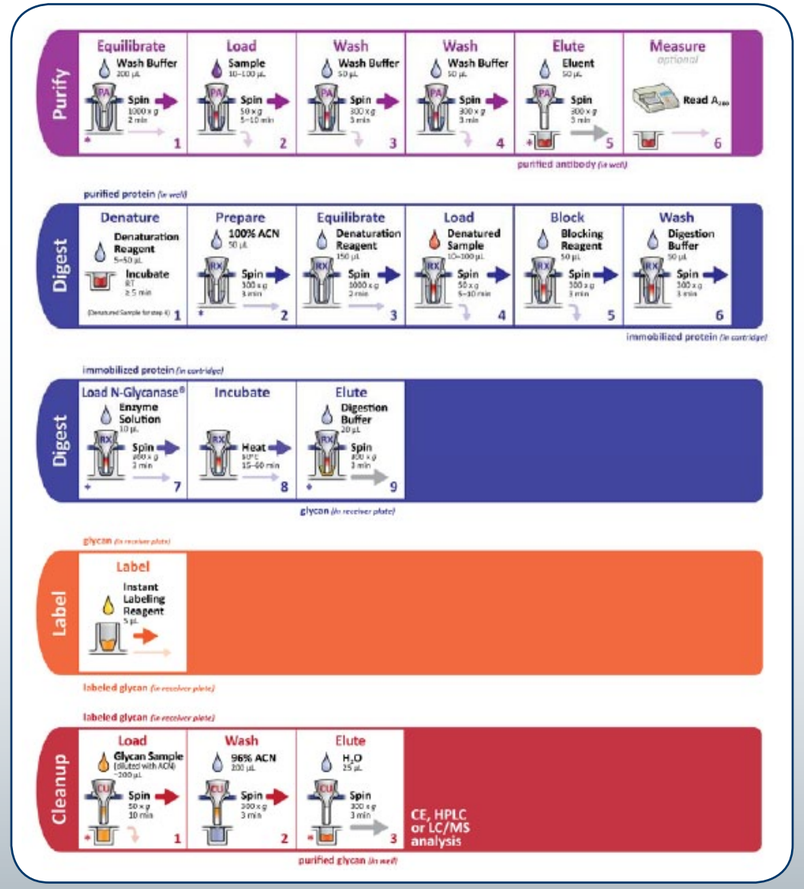
Gilson GX-271 Liquid Handler with 402 Dual w/ Tee Dilutor (10 mL and 100 µL syringes)
Gilson 306 Mobile Phase Pumps (5 SC pump heads)
Gilson 811D Dynamic Mixer with 1.5 mL Analytical Mixing Chamber
Gilson 805 Manometric Module
Jasco FP-2020 Plus Intelligent Fluorescence Detector
ProZyme GlycoSep™ N-Plus Column (P/N GKI-4730) and Guard Column @ 50°C

Figure 2. Gilson GX-271 Analytical HPLC System with Jasco FP-2020 Plus Intelligent Fluorescence Detector

General Sample Cleanup Procedure

The Gilson TOOLKIT N-Glycan program follows the ProZyme GlykoPrep™ protocol steps:

Figure 3. Rapid N-Glycan Preparation ProZyme GlykoPrep™ Protocol



Prior to starting the GlykoPrep protocol (Figure 3), samples of hIgG were mixed with bovine fetuin to simulate impure antibody sample loading for the Purify step. The purified hIgG antibodies were taken through the remaining GlykoPrep protocol steps 2 through 4 (see Figure 3). The Gilson TRACKMAN fitted with the GlykoPrep protocol was used throughout steps 1 through 4 to both track via lighting and notify via individual text screens of each protocol action between the solvent plate and the cartridge receiving plate.

The TRACKMAN GlykoPrep protocol eliminated the need to manually track each step or remember which AssayMAP cartridge was loaded for 1 to 4 or 8, 16, 24 samples. This provided additional time savings as a result of eliminating a manual tracking step. Additional time savings is gained by having confidence that each sample was treated equally. The TRACKMAN also consistently held the AssayMAP cartridges in place during the pipetting process which simplified the sample and solvent loading steps.

Using HILIC RP-HPLC (Hydrophilic Interaction Reverse Phase High Pressure Liquid Chromatography), labeled free glycan samples and hIgG standards were separated and analyzed using total loop injection on a Gilson GX-271 Analytical HPLC System fitted with a Jasco FP-2020 Plus Intelligent Fluorescence Detector set to Excitation at 278 nm and Emission at 344 nm. Separation was performed on GlycoSep HPLC column and guard column from ProZyme. Using TRILUTION® LC version 2.1, Gilson binary mobile phase pumps were programmed to run an Acetonitrile and buffer gradient over 48 minutes.

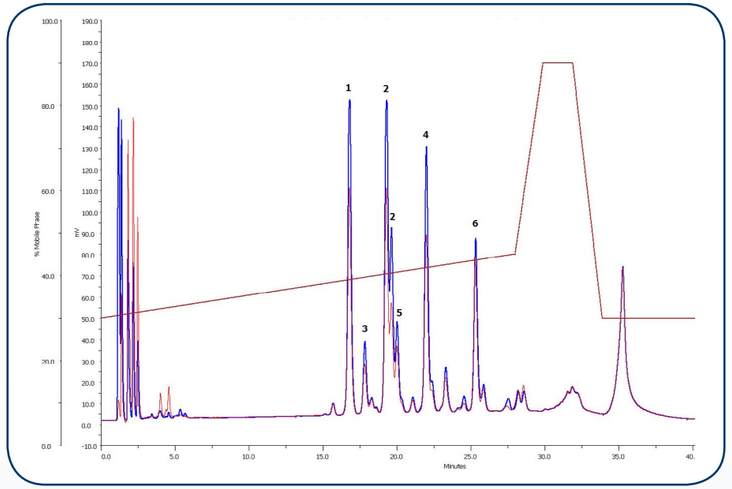
Results

Chromatographic results show effective separation and sensitivity of the main free glycans between 12 and 23 minutes in both the independent hIgG standard and purified hIgG sample injections (Figure 4). The six major N-Glycan peaks are evident in both standard and sample injections. Overlayed standard and sample chromatograms show reproducible retention times.

Table 1. Identification of Peak Names from hIgG Chromatogram (Figure 4)

Peak Number	Peak Name
1	G0F (NGA2F)
2	G1F (NA2G1F) isomers
3	G0FB (NGA2FB)
4	G2F (NA2F)
5	G1FB (NA2F1FB)
6	G2FS1 (A1F)

Figure 4. Independent Standard Injection (Blue Trace) Overlayed with Mixed Impure hIgG + Fetuin Sample Injection (Red Trace)



Reproducibility data was generated for a set of 3 samples run through the entire TRACKMAN GlykoPrep™ Protocol using the PIPETMAN M to pipette 50 uL of hIgG mixed with 10 µL Fetuin as the starting impure sample. Percent mean peak area, standard deviation, and %CV were calculated for each of the six peaks for the set of three samples. Table 2 displays the peak area reproducibility data showing all peaks with relative standard deviation results at or less than 3%.

Rapid N-Glycan sample preparation of the GlykoPrep Protocol from ProZyme was made even more efficient, consistent, and error-free with the use of the Gilson SD TOOLKIT and Gilson MD TOOLKIT. The TRACKMAN GlykoPrep protocol allowed for accurate pipetting and effective tracking throughout the multi-step protocol using text screens and lighted wells. Chromatographic results showcased accurate sample purification with Protein A and preparation when compared with the independent standard. The use of the Gilson TOOLKITs eliminated the hassle of manually checking after each step in the protocol and further increased efficiency by letting the TRACKMAN keep track of which cartridges were loaded and processed. This saved valuable time and decreased the total time to perform the GlykoPrep protocol.

Table 2. N-Glycan Peak Area Reproducibility (n=3)

Peak Number	% Mean Area	Standard Deviation	%CV
1	21.2609	0.3101	1.458
2	34.6972	0.1869	0.538
3	4.7334	0.1335	2.820
4	17.2219	0.3089	1.793
5	9.0760	0.2529	2.786
6	13.1012	0.3977	3.056

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Automated Determination of VMA, HVA, 5-HIAA in Urine Using the Gilson GX-274 and Gilson ASPEC™ XL

The data for this application note was provided by Martin Knirsch from Recipe, Chemicals + Instruments GmbH, Munich, Germany; www.recipe.de.

Featured Product: Gilson GX-274 with 402 Dual Syringe Modules

The catecholamines epinephrine, norepinephrine and dopamine fulfill a number of vital functions within the central and periphere nervous system. The analysis of catecholamines and their metabolites is important for tumor diagnosis within the sympathoadrenal system (responsible for the regulation of many homeostatic mechanisms). Diagnosed tumors are responsible for an elevated catecholamine biosynthesis and secretion. As a result, significantly enhanced concentrations of catecholamines and metabolites are found in plasma and urine.

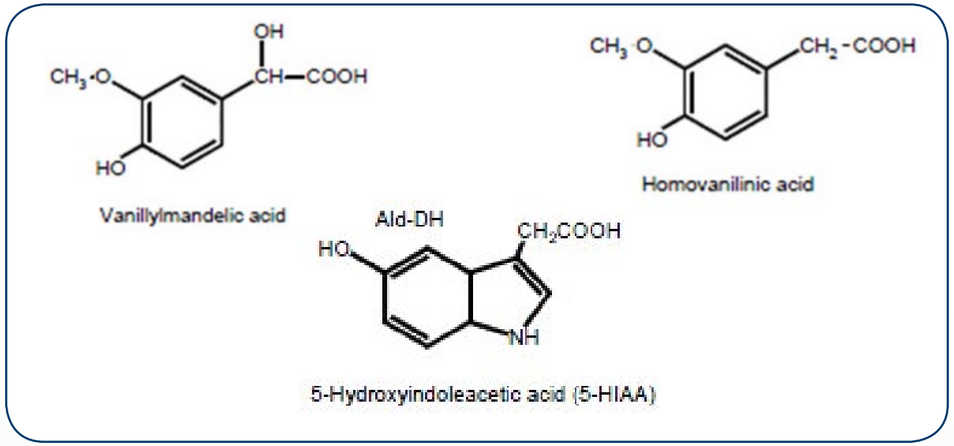
Sample clean-up and preparation are essential prior to the analysis of biological samples in order to remove proteins and other interfering compounds prior to analysis of HVA, VMA, and 5-HIAA (see figure 2). Recipe has utilized both the Gilson ASPEC™XL (ClinRep® HPLC complete kit, see Figure 1) and Gilson GX-274 with 402 Dual Syringe Pumps to automate the sample clean-up and preparation process.

Figure 1. Recipe ClinRep® HPLC Kit Consumables

Diseases are known to be a result of an increased or decreased activity of the catecholamine metabolism. VMA is a metabolite of epinephrine and norepinephrine, HVA a metabolite of dopamine. Serotonin, another biogenic amine, is mainly located in the enterochromaffine cells of the small intestine. Malignant growth in the enterochromaffine cells of the intestine results in increased production of serotonin and hence an increased excretion of 5-HIAA.

In general, sample preparation has to be performed prior to the injection of samples onto the analytical system. Sample preparation serves to provide both sample clean-up and conversion of the analytes into a detectable form, respectively. Sample preparation is performed fully automated by the Gilson ASPEC™ XL or the Gilson GX-274. After sample injection, a special reversed-phase column is used for the HPLC separation of the analytes. The analytes are measured by EC detection and, using the internal standard method, are quantitatively evaluated via peak areas.

Figure 2. Chemical Structures of VMA, HMA, and 5-HIAA



Experimental Conditions

HPLC Conditions:

Mobile Phase Flow Rate: 0.9 mL/min.
Injection Volume: 20 µl
Run Time: 15 minutes
Gilson System Washing Solution: 10 % Methanol : 90% Water
Analytical Column: Heated at 30 °C
Detector: RECIPE ClinLab® EC Detector, Model EC3000
Retention times:
VMA: ~ 3.7 minutes
Internal Standard: ~ 5.5 minutes
HVA: ~ 7.9 minutes
5-HIAA: ~ 11.6 minutes

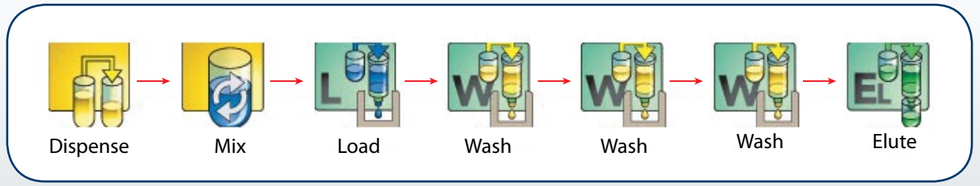
GX-274 with two 402 Dual Syringe Modules Automated Sample Preparation

The samples are automatically processed by the GX-274 with two 402 Dual Syringe Modules or the ASPEC XL. An overview of the automated SPE sample preparation with the GX-274 with two 402 Dual Syringe Modules is provided below:

1. Dispense 50 µL urine sample and 1 mL internal standard (IS) to vial
2. Mix dispensed urine and IS
3. Load 1 mL mixed sample to SPE column
4. Wash with 1 mL Ammonia Solution A
5. Wash with 2.5 mL Boric Acid B
6. Wash with 2.5 mL Boric Acid B
7. Elute 2 mL Eluting Reagent E

Note: Mix the eluted fraction from the Gilson GX-274.
Using the HPLC system, inject 20 µL eluted fraction after mixing.

Figure 3. TRILUTION® LH Solid Phase Extraction Method Using the GX-274

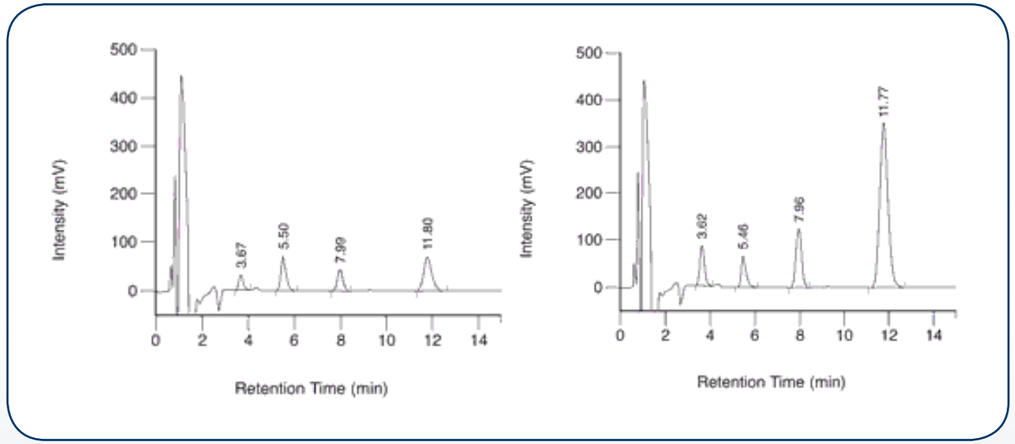


Results

Analyzing catecholamines and their metabolites is important for tumor diagnosis in both urine and plasma samples. Automated sample preparation and solid phase extraction extraction for patient urine samples is easily performed with the Gilson ASPEC™ XL or Gilson GX-274 with 402 Dual Syringe Pumps. Resulting SPE fractions are analyzed via HPLC for analytes VMA, HVA, and 5-HIAA using a highly sensitive, specific, and selective electrochemical detection (ECD) method.

Automating the sample preparation process and solid phase extraction extraction process increases laboratory efficiency and reduces laboratory error. Using the internal standard, recovery from integrated analytical runs can be calculated to verify the overall SPE process is consistent and within expected limits. Typical recovery rates for this assay range from 75 – 89%.

Figures 4 & 5. Example Chromatogram of ClinChek® Urine Control, Level I & II



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Automated Solid Phase Extraction of Allantoin From Cosmetics and Topical Pharmaceuticals Prior to HPLC Analysis

Featured Product: GX-271 ASPEC™

Allantoin is a heterocyclic organic compound derived from purine. Allantoin has a long history of use in a variety of topical pharmaceuticals and cosmetics for skin care due to its keratolytic, moisturizing, soothing and anti-irritant properties. Allantoin is typically used in these products at a level of 0.1 to 2.0%. Solid phase extraction (SPE) is often used as a purification step prior to analysis of allantoin in these products due to the complex nature of the matrices.

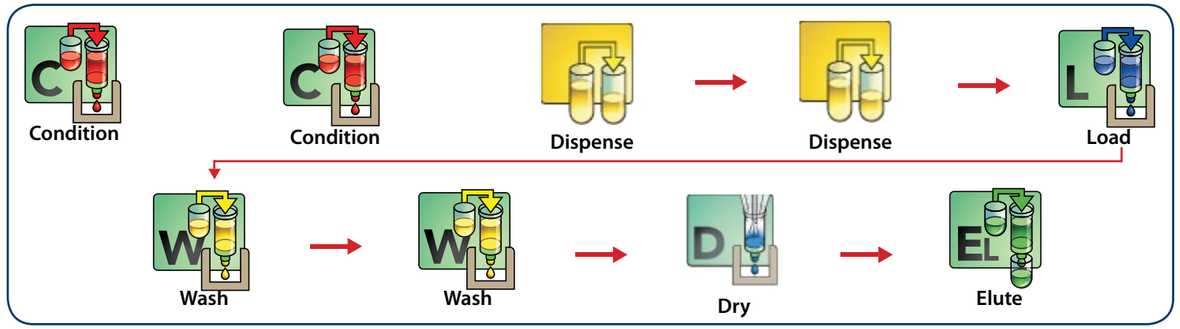
Experimental Conditions

Mix one gram of sample containing allantoin with 100 mL of ultra-pure water. Allantoin-free cosmetics/ topicals were spiked with 5 mg allantoin.

Solid Phase Extraction (SPE) Protocol

The SPE procedure used 3 mL Macherey-Nagel CHROMABOND®HR-XA (60mg) Cartridges. The SPE protocol is entirely automated using the Gilson GX-271 ASPEC system. The SPE steps are summarized with the schematic provided in the GX-271 ASPEC control software, TRILUTION® LH (Figure 1).

Figure 1. TRILUTION® LH SPE and Liquid handling Tasks for Extraction of Allantoin



- Initialization Step: Gilson Mobile SPE Racks are moved above the waste rack.
- Condition the cartridge with 1 mL of methanol at 0.5 mL/min.
- Condition the cartridge with 1 mL of ammonia, w(NH3) = 5% at 0.5 mL/min.
- Dispense 4 mL of sample (1g in 100 mL water) into a tube at 5 mL/min.
- Dispense 400 µL ammonia, w(NH3) = 26% at 0.5 mL/min into the same tube as step above.
- Load 1.1 mL of the sample mix created above onto the SPE cartridge at 0.5 mL/min.
- Wash cartridge with 1 mL of ammonia, w(NH3) = 5% at 0.5 mL/min.
- Wash cartridge with 1 mL of methanol at 0.5 mL/min.
- Dry with 5 mL air, 3 mL/min.
- Move the Gilson Mobile SPE Rack over the collection tubes.
- Elute with 2X 600uL Hydrochloric acid, HCl, 0.1 mol/L at 0.5 mL/min.
- Eluent can be injected directly into the HPLC system.

HPLC Analysis

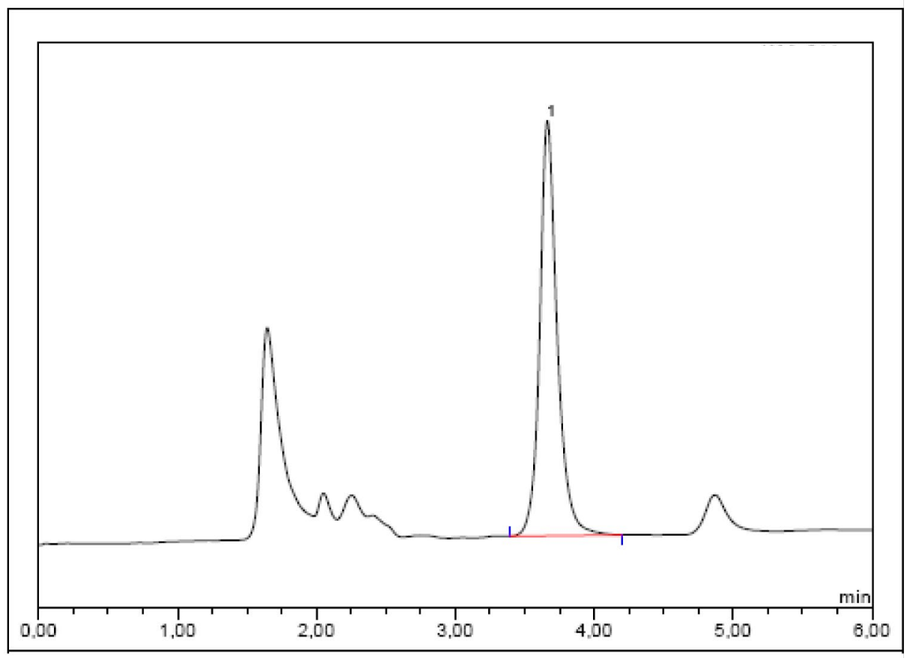
Column: Macherey-Nagel EC 125/3 NUCLEODUR® 100-3 HILIC (Part no. 760 531.30)

Conditions:		
Eluent A:	10 mmol/L Ammonium chloride, pH 3.0	20
Eluent B:	Acetonitrile	80
Flow Rate:	0.3 mL/min	
Temperature:	Ambient	
Injection Volume:	20 µL	
Concentration:	β(Allantoin) = 5 µg/mL Eluent	

Detection : UV, 214 nm

Figure 2. Chromatogram of Allantoin from Cosmetic Product Following SPE Extraction

The recovery of Allantoin (3.66 minute retention time) from the cosmetic product was 85.5% (n=3).



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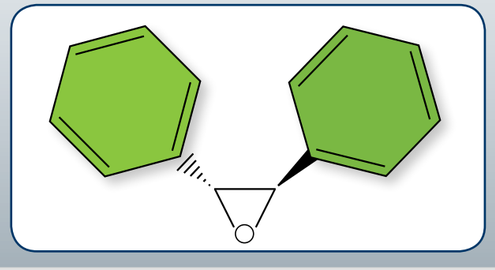
Optimizing the Purification of a Chiral Compound Utilizing a Benchtop Semi-Preparative to Preparative HPLC System

Featured Product: Gilson PLC 2020 Personal Purification System

There are often significant differences in the effectiveness and toxicity of drug enantiomers in biological systems. This has led to the need for the separation of chiral drug candidates into their respective enantiomers. Semi-preparative and preparative chromatography has become a common tool for the separation of chiral enantiomers. This application note describes the chiral separation of *trans*-Stilbene oxide (Figure 1) using a multi-purpose, benchtop, preparative chromatography system that is capable of separating compounds by normal-phase or reverse-phase HPLC at flow rates of up to 100 mL/min.

Figure 1. Chemical Structure of *trans*-Stilbene oxide



Experimental Conditions

Samples and Solvents

trans-Stilbene oxide was obtained from Sigma-Aldrich (part no. S4921-25G). HPLC grade hexane and isopropyl alcohol were obtained from Burdick and Jackson.

HPLC Conditions

Column: Phenomenex Lux 5 micron Cellulose-2, 250 mm X 10mm (part no. OOG-4457-NO)

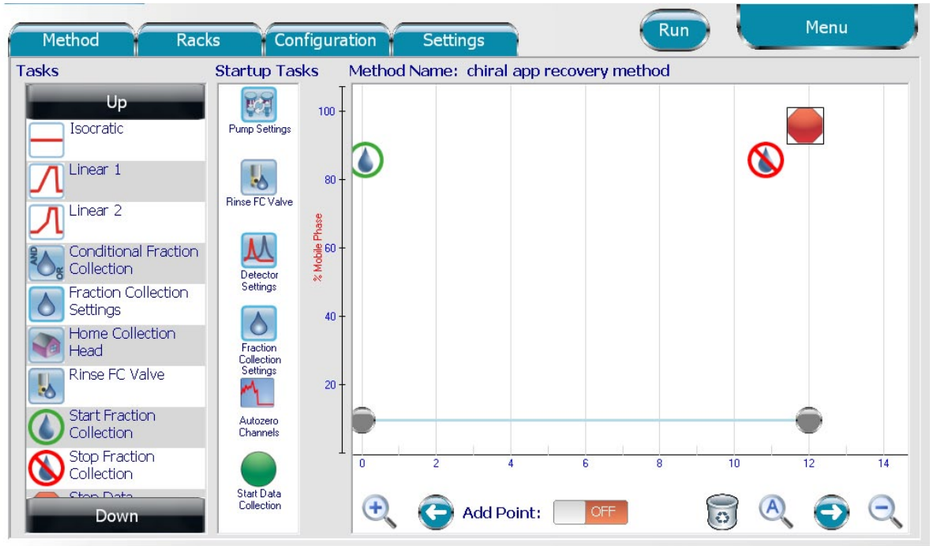
Apparatus: Gilson PLC 2020 Personal Purification System

- 5 mL/min using 50SC Pump Heads
- 100 uL injection loop, Total Loop Injection
- Detector Flow Cell: Preparative, 0.2mm Path Length, 0.7µL Volume, Quartz

Table 1. PLC 2020 Method Conditions

	Mobile Phase Solvents	Mobile Phase Gradient % A	Fraction Collection Conditions	Run Time (Minutes)	Flow Rate (mL/min)	UV Detection (nm)
Normal Phase	A = Hexane B = Isopropyl Alcohol	0 - 12min = 90	Front Slope = 65 Back Slope = 65 Maximum Collection Volume per Tube = 20 mL	12.0	5	220 & 254

Figure 2. PLC 2020 Software Method Screen for Chiral Separation of *trans*-Stilbene oxide

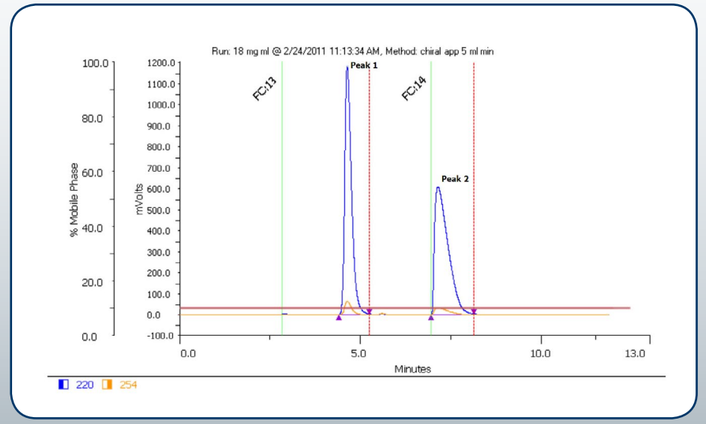


Results

Table 2. Resolution of *trans*-Stilbene Oxide with increasing sample loading Chiral purification can be optimized by performing a resolution study to determine the amount of compound that can be loaded onto a specific column as noted in Table 2. In this case, 1.8 grams was optimal at a resolution of 1.15.

	trans-Stilbene oxide Peak 1		*trans*-Stilbene oxide Peak 2		
Column Loading Study (mg in 90:10 Hexane:IPA)	**Retention Time (minutes)**	**Peak Width (minutes)**	**Retention Time (minutes)**	**Peak Width (minutes)**	**Resolution**
0.5	4.60	0.67	7.06	0.83	3.26
0.6	4.55	0.73	7.05	0.84	3.19
0.7	4.40	0.75	6.70	0.91	2.77
0.8	4.66	0.77	7.27	0.98	1.88
1.5	4.64	0.82	7.17	1.15	1.39
1.8	**4.65**	**0.91**	**7.16**	**1.22**	**1.15**

Figure 3. Chromatogram of *trans*-Stilbene Oxide at Resolution = 1.15



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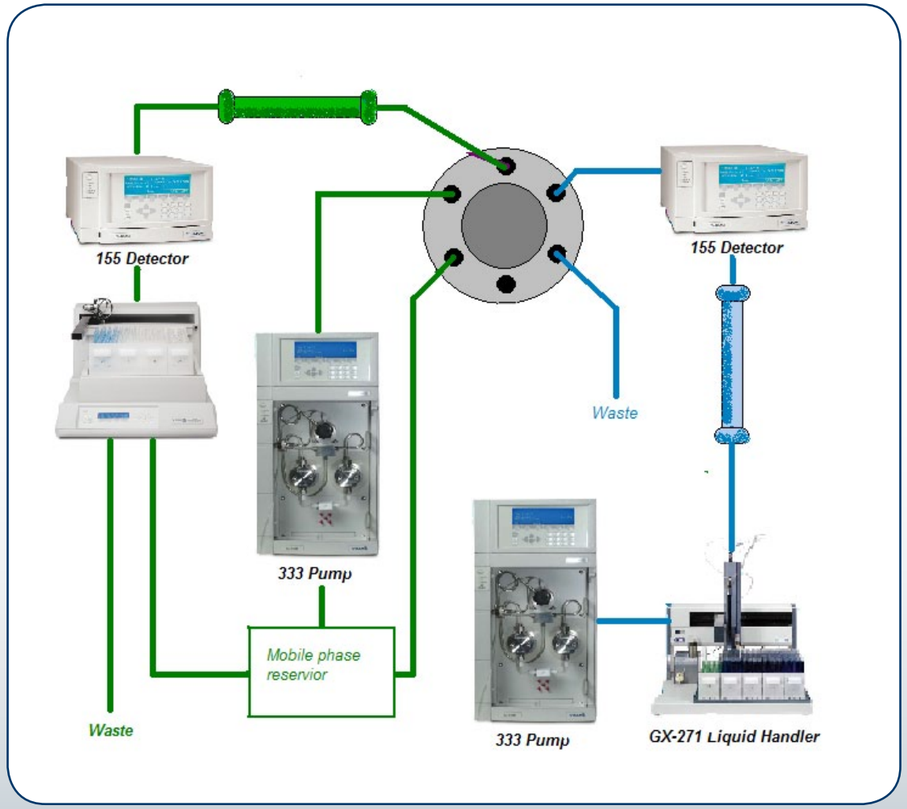
Optimizing Chiral Separations and Purification of Enantiomers through Selective Sample Slicing and Column Switching

Featured Product: GX-271 Preparative HPLC System (as outlined in Figure 1 on page 20)

Current stringent restrictions on chiral drug investigations have pushed the separation of chiral compounds to the forefront of semi-prep separations. Pharmaceutical laboratories are compelled to provide requisite separations of all chiral compounds to facilitate the testing of each enantiomer individually. The cost and fragility of chiral sorbents force chemists to clean samples completely before injection impeding the efficacy of the separation method. Therefore, to eliminate exhaustive sample cleanup, a complete automation of the analyte purification and chiral separation is presented. Samples are injected onto a primary column and individual peaks are cleaved and transferred onto a secondary chiral column for separation. Automated and complete purification of analytes with high resolution of the separated enantiomers provides speedy purification and separation of chiral analytes, eliminating the need for extensive sample cleanup.

Figure 1. Gilson GX-271 Preparative HPLC System & Flow Paths



Experimental Conditions:

- Two separate pumps are required to control the mobile phase flow on each column if an isocratic gradient is being used
- A Gilson GX-271 with direct injection module and preparative solvent system performed the liquid handling tasks
- A primary separation column from Keystone (20 mm x 150 mm normal phase column) was used for the initial separation (blue column image)
- Two Chiral Technologies (21.2 mm x 50 mm OD) columns were used in series to perform the chiral separation (green column image)
- Two Gilson 155 UV/VIS detectors were used for monitoring of compound peaks; one for the primary separation column and one for the chiral columns in series
- A Gilson FC204 Fraction Collector was used for enantiomer peak collection
- A Gilson VALVEMATE® II performed the valve switching

Gilson Chiral Switching Method:

- Mobile phase consisted of 70% Hexane : 30% Ethanol @ 20 mL/min for the chiral columns and 75% Hexane : 25% Ethanol @ 15 mL/min for the normal phase column
- UV detection was set at 210 nm
- Injections were from 100 µl to 1000 µL
- A sample of guaifenesin in cold remedy tablets with ephedrine and acetaminophen components was injected after being dissolved in ethanol. The final concentration was 40 mg/mL guaifenesin in 30% ethanol : 70% hexane.
- The Gilson FC204 controlled the switch of the VALVEMATE II via a contact that was pulsed when the guaifenesin peak reached a height of 5 mV. Simultaneous to the valve switch, the mobile phase flow on the primary pump was diverted to the chiral column. Once the peak was collected on the chiral column, flow was then diverted back to waste, and the chiral column was switched to the secondary pump.

Results

The guaifenesin peak was transferred to the chiral columns without loss of product. Resolution allowed for a clean cleavage of the peak from the mobile phase stream. Any foreign compounds that were transferred along with the guaifenesin were separated from the enantiomer peaks. The peak resolution on the chiral chromatogram was 1.9. Mobile phase that was passed through the chiral column was recycled back to the reservoir.

Using two columns allows sample to be injected immediately following the separation of the compounds on the normal phase column. The target peak is transferred to the chiral column and injection of the new sample can begin while the enantiomers are separated creating an efficient and automated purification process.

Figure 2. Initial Overlayed Guaifenesin Standard Chromatogram of Slice and Separation

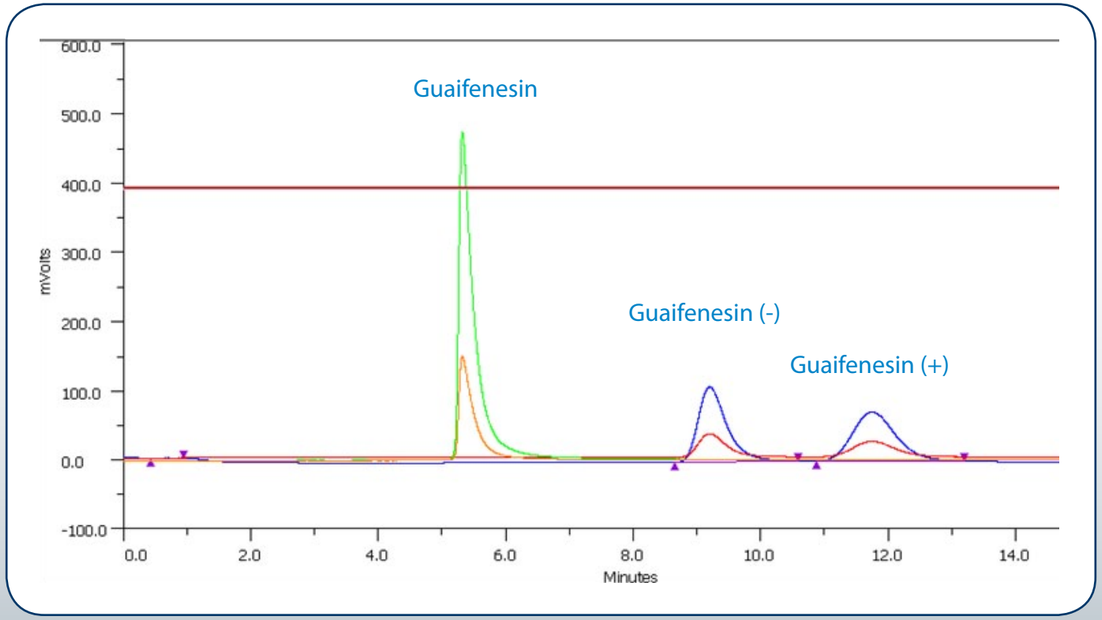
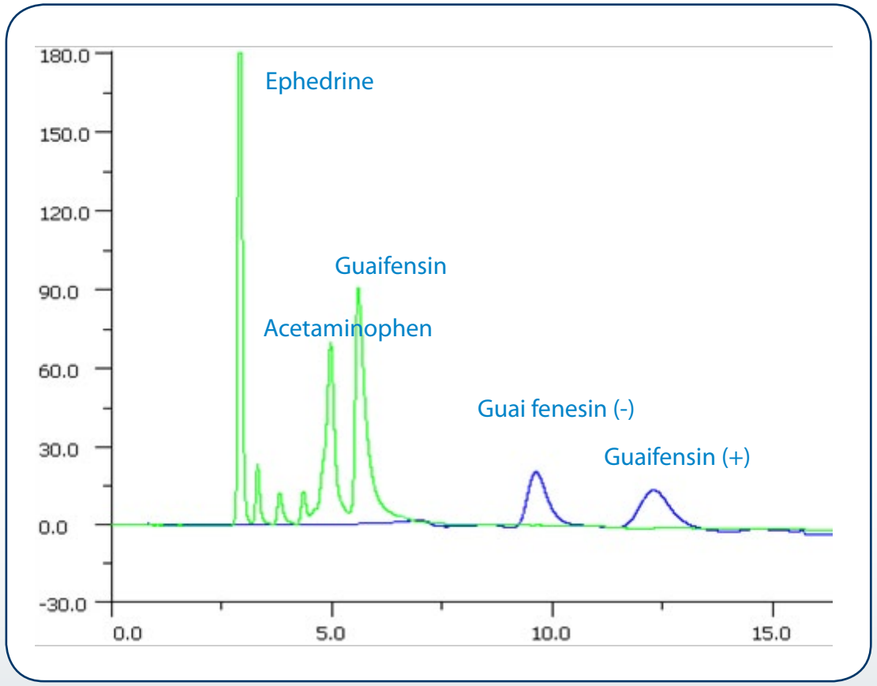


Figure 3. Dual Overlayed Chromatogram of Sample Slice and Separation



Conclusions

Dual column switching allows for maximum throughput via normal phase separation to occur while enantiomers are purified. Flexibility in method development and optimization of methods are provided using flow rate and mobile phase ratios for each column. Automation of this dual column switching method provides accurate column switching via a two-position valve and collection of enantiomers once separated on the chiral column.

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The Automated Extraction and Isolation of Silymarin Flavonolignans from Milk Thistle Seed via Normal Phase SPE

The study of natural medicines has grown in recent years. Many of the natural medicine regimens have been investigated to validate the effectiveness of their treatments. Milk thistle has been used medicinally for poisoning and treatment of liver diseases as part of a natural medicine regimen. This has promoted further investigation of the constituents within milk thistle (silymarin). Anti-inflammatory and anticarcinogenic properties as well as scavenging abilities make silymarin a likely candidate for further scientific studies. This necessitates SPE extraction and analytical processing of the extract to provide information on its components. Automation of the extraction provides an efficient process to compliment investigation of silymarin flavonolignans.

Featured Products:

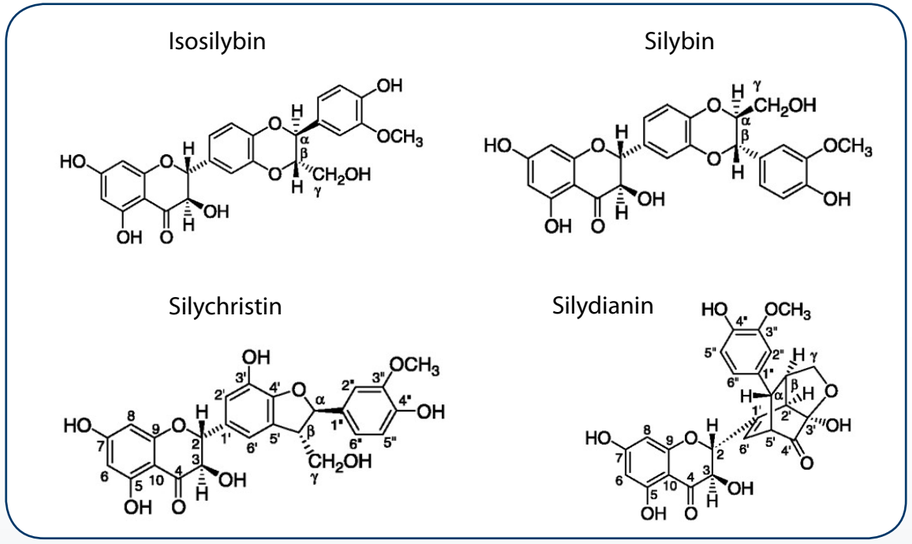
Figure 1: GX-271 ASPEC™ with direct injection module was used for the SPE method using 3 mL Phenomenex Strata X C cartridges and subsequent injection of the eluent using TRILUTION® LH software control.

Analysis @ 1 mL/min was performed using the Gilson HPLC system consisting of 305 and 306 pumps, 811D Mixer, and 155 UV/VIS Detector with a Waters Atlantis 4.6 x 150 mm C18 column and TRILUTION® LC software control.

Sample Preparation of Seed Samples and Tablets

- Milk thistle seed samples were prepared as follows:
  - 10 grams of milk thistle seed was mixed with 200 mL of ethanol
  - Mixture was shaken for 2 hours to extract flavonolignans from the milk thistle seed into the ethanol solution
- Milk thistle extract tablets were dissolved by placing 1 tablet into 40 mL of ethanol and shaken for 15 minutes

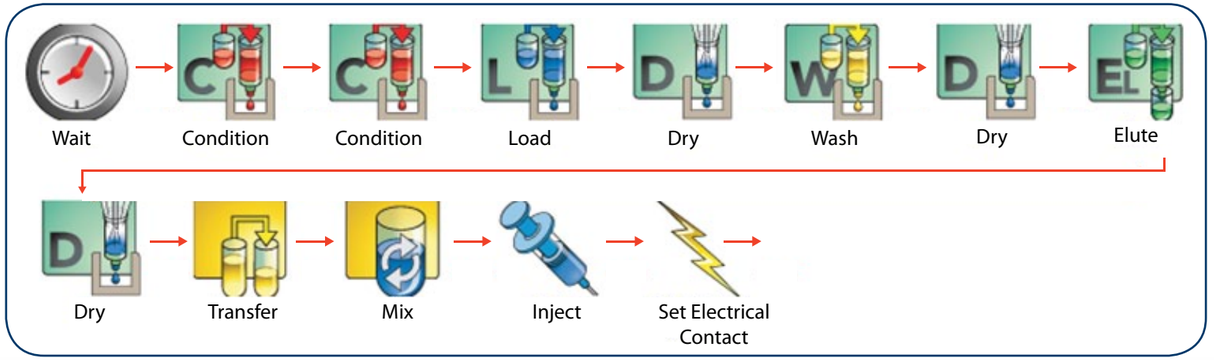
Figure 2. Flavonolignans Extracted



Automated SPE Method

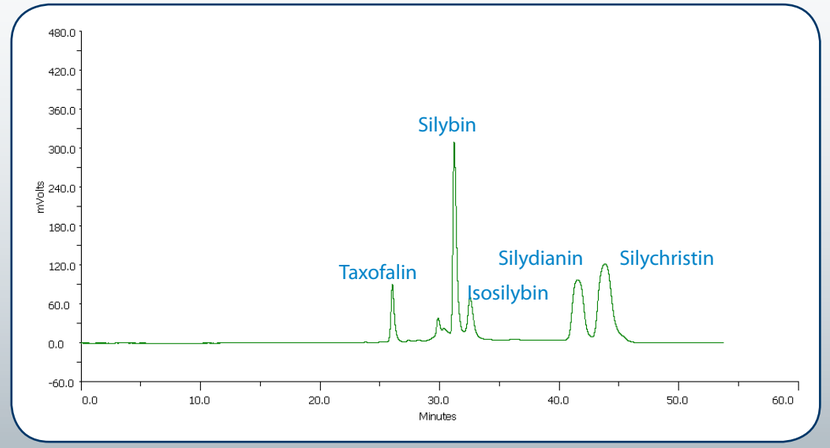
- Wait for contact signal from TRILUTION® LC
- Condition 3 mL Strata X C cartridges with 5 mL ethanol
- Load 0.5 mL of milk thistle seed extract onto SPE cartridge
- Dry SPE cartridges thoroughly with nitrogen
- Wash cartridges with 5mL heptane
- Dry SPE cartridges thoroughly with nitrogen
- Elute with 4 mL 1% NH_4OH in ethyl acetate
- Dry eluent down with nitrogen and bring back up in 1 mL 50% ethanol : 50% water solution
- Mix sample prior to 50 µL injection
- Send contact to TRILUTION LC to begin data collection

Figure 3. Automated SPE and Injection Method Using TRILUTION® LH Control Software



Results

Figure 4: Optimization of SPE Wash and Elution Conditions Allowed for Adequate Separation and Identification of Five Flavonolignan Compounds, with Recovery Percentages Averaging 99% for Each Flavonolignan



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Rapid Isolation of Geraniin from Nephelium lappaceum Rind Waste Using the Gilson GX-281 Preparative HPLC Purification System

The data for this application note was performed by Uma D. Palanisamy, Jeffery Cheah School of Medicine and Health Sciences, Monash University Sunway Campus, Malaysia; Lai Teng Ling and Thamilvaani Manaharan, Faculty of Medicine with the Department of Physiology from the University of Malaysia; David Appleton, Faculty of Medicine with the Department of Pharmacology from the University of Malaysia.

Featured Product: Gilson GX-281 Preparative HPLC Purification System

Natural product purification of geraniin from N. lappaceum (see Figure 1) rind waste using the Gilson GX-281 Preparative HPLC Purification System was performed. Results of this application note strongly support the use of a geraniin-standardised N. lappaceum extract in the management of hyperglycemia. Nephelium lappaceum L. is native to Southeast Asia, and it is part of the sub-tropical fruit family of Sapindaceae. This fruit is a commercial crop in Asia, where it is often eaten fresh. Dried fruit rind is used in traditional medicine, cooking, and in the manufacture of soap. The roots, bark, and leaves have various uses in medicine and in the production of dyes.

Figure 1. Nephelium lappaceum L.
(Source: Encyclopedia of Life (www.eol.org))

The ability of ethanolic Nephelium lappaceum L. rind extract to act as an anti-hyperglycemic agent has been confirmed. Geraniin, an ellagitannin, was identified as the major bioactive compound isolated from the ethanolic Nephelium lappaceum L. rind extract. In addition to its extremely high anti-oxidant activity and low pro-oxidant capability, geraniin is seen to possess in vitro hypoglycemic activity, aldol reductase inhibition activity; and has the ability to prevent the formation of advanced glycation end-products having potential to be developed into an anti-hyperglycemic agent.

Experimental Conditions

Materials

Chemicals and reagents were obtained from various scientific suppliers. All solvents used were HPLC grade or higher. All reagents were ACS grade or better.

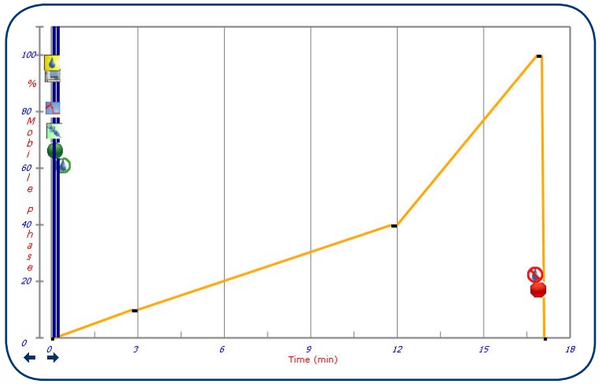
Gilson Purification System:

System: GX-281 Preparative HPLC System (GX-281/322/156)
Mobile Phase: 0.1% Formic Acid in Acetonitrile and 0.1% Formic Acid in Ultra Pure Water at a flow rate of 18 mL/min (see Figure 2)
0–10% Acetonitrile for 3 min
10–40% Acetonitrile for 12 min
100% Acetonitrile for 5 min (column recondition)
Column: Waters Xterra Prep RP18 OBD (19 x 50 mm)

HPLC–LCMS/MS Analysis of Geraniin:

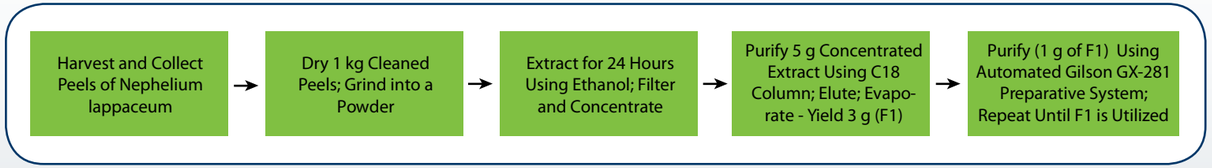
System: Shidmazu Prominence UFLC-LCMSIT-TOF (Pos and Neg)
Mobile Phase: 0.1% Formic Acid in water and 0.1% Formic Acid in Acetonitrile at 0.5 mL/min
Column: Waters Xterra MS C18 (2.5 x 20 mm, 2.5 um) IS column heated to 40^{0} C

Figure 2. Example Gilson TRILUTION® LC Preparative Gradient Method



Sample Preparation and Geraniin Isolation Procedure

Figure 3. Sample Preparation & Geraniin Isolation Procedure



Results

The rind of N. lappaceum, apart from being a highly efficient anti-oxidant, was shown to be effective in inhibiting carbohydrate hydrolysing enzymes and enzymes involved in the polyol pathway. In addition, N. lappaceum was found to prevent the formation of advanced glycation endproducts. Geraniin was found to be the major compound isolated from the rind of an ethanolic extract of N. lappaceum (see Table 1). A rapid and mid-scale purification of geraniin was achieved as described in Table 1 within this application note.

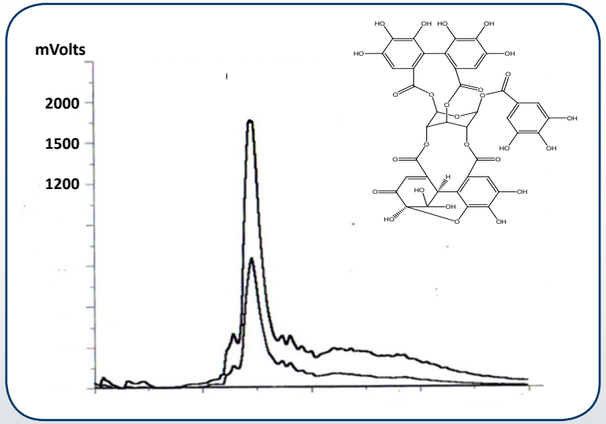
Geraniin is an ideal candidate for the management of hyperglycemia in diabetic individuals. In addition, there is support for the use of a geraniin-standardised N. lappaceum extract, as an herbal formulation in the management of hyperglycemia.

Table 1. Quantification of Geraniin in the Rapid Purification Method

Sample/Fraction	Extraction Method	Yield (%)	Geraniin in Sample* (%)
N. lappaceum rind	Ethanol Extraction	30.58	3.79
Ethanolic Extract	LiChroprep RP-18	60.00	12.68
F1	Gilson Preparative HPLC	21.15	21.13

** Calculation of the content of geraniin (%) is based on the assumption that 3 g of F1was in final purification step.*

Figure 4. Example GX-281 Purification Geraniin Chromatogram @ 13 minutes – Major Compound in Ethanolic Nephelium lappaceum Rind Extract; Geraniin Peak 1 = 210 nm; Geraniin Peak 2 = 275 nm



Reference

Uma D. Palanisamy, Lai Teng Ling, Thamilvaani Manaharan, David Appleton, Journal of Food Chemistry, 127 (2011) 21-27

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A Practical User Guide for the Determination of Optimal Purification Gradients for the Gilson PLC 2020

The data for this application note was performed by Janice Chin, Courtney Cullis, Kenneth Gigstad, Matthew Jones, and He Xu from Millennium Pharmaceuticals, Inc., Cambridge, MA, USA

Featured Product: Gilson PLC 2020 Personal Purification System

See the PLC 2020 Software in Action!
Click HERE!

This application note provides a practical guide on the efficient use of the Gilson PLC 2020 Personal Purification System along with an experimentally derived correlation table for the selection of 'optimal' solvent gradients or 'isolation' gradients to be used for routine purifications of pharmaceutical organic compounds. Simple touch screen control allows for quick modifications of preparative methods based on retention times obtained from standard HPLC analytical methods.

The flexibility to set preparative solvent gradients for purification injections using method modifications or variables caters to an 'open access' laboratory environment where the main focus is to quickly elute products of interest at an 'optimal' time off the column, with minimal interference, and with reduced solvent waste. The following guidelines were compiled to ensure optimal preparative methods were obtained for each sample.

Experimental Conditions

Materials

All solvents used were HPLC grade or higher. All reagents were ACS grade or better.

Analytical System:

System:	Agilent 1100 HPLC System with Diode Array Detector
Mobile Phase:	Acetonitrile:H2O solvent gradients with 0.1% formic acid
Column:	Waters Symmetry, C18, 3.5 micron, 4.6 x 100 mm

Preparative System:

System:	Gilson PLC 2020
Mobile Phase:	Acetonitrile:H2O solvent gradients with 0.1% formic acid
Column:	Waters SunFire™ C18 OBD Column, 5 micron, 19 x 150 mm

Methods – Sample Preparation

Effective sample preparation aids in maintaining the system in good working order and helps to increase the longevity of the column. Ensure that a thorough work up has been carried out on the sample of interest prior to running on the HPLC system.

- To prepare the sample, dissolve compound (30 mg crude) in 2ml of Dimethyl Sulfoxide (DMSO), Methanol (MeOH), or water (or a combination thereof). Do not use any solvents that are incompatible with the mobile phase or could potentially damage the column.
- Ensure complete sample dissolution and filter before injecting the solution onto the system.
- Run an analytical analysis on your sample (see Figure 1) using a standard gradient to obtain a retention time and λ max for the peak of interest.

The following analytical system gradient was established as a general method with a flow rate of 1 mL/min.

Table 1. Standard Analytical System Gradient

Time (min)	%A 99% Water: 1% Acetonitrile: 0.1% Formic Acid	%B 95% Water: 5% Acetonitrile: 0.1% Formic Acid
0.00	95	5
10.00	0	100
12.50	0	100
13.00	95	5

Figure 1. Simulated Analytical System Chromatography Using Standard Gradient

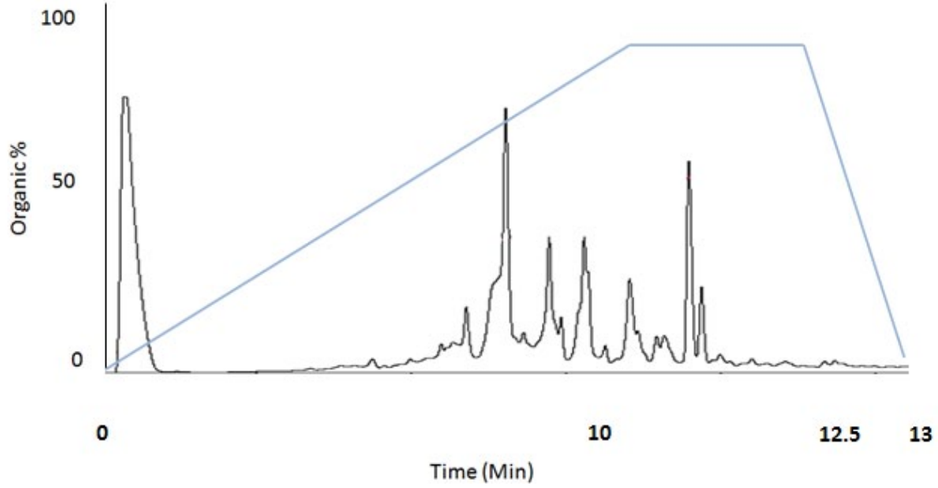
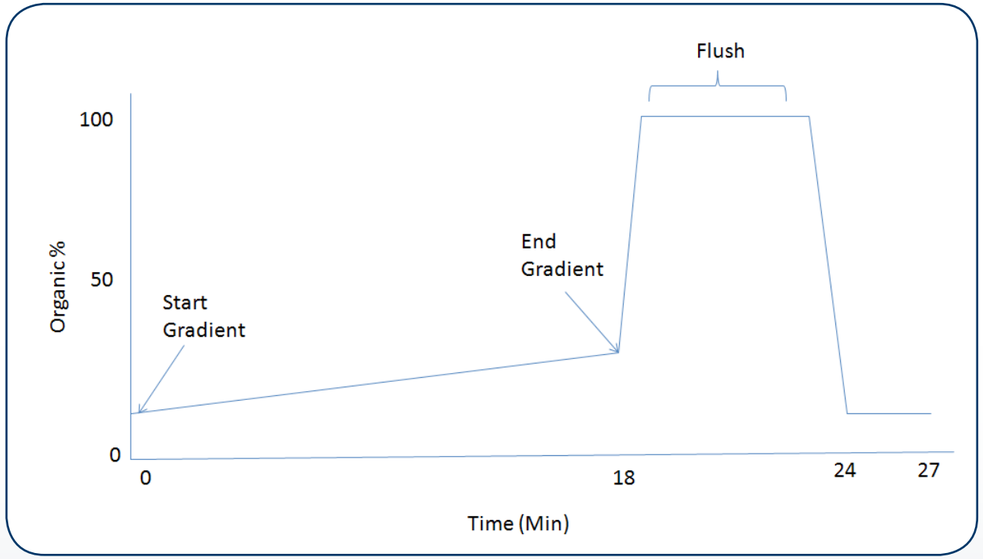


Figure 2. Simulated Gradient View of a 'General or Isolation Gradient Method'



Based on the simulated standard analytical gradient, the slope or shallow gradient window remains a constant; only the start and end % organic change according to the retention time of the peak of interest. The new preparative isolation gradient (see Figure 2) optimizes the separation of the peak from other interfering compounds through the use of this very shallow gradient window (consisting of the start gradient and end gradient). The flush time eliminates any late eluting compounds from interfering with subsequent injections.

Methods – Building the New Preparative Isolation Gradient

- To begin, go to the main screen in the PLC 2020 touch screen software.
- Choose Method Builder, then Menu, then Open. Select to open the Preparative Isolation Gradient.

- A screen similar to the simulated gradient view (Figure 2) will be displayed where the first mobile phase point (start gradient) shows a starting solvent mixture of CH3CN/H2O and a line ramping to the ending CH3CN/ H2O mixture (end gradient). The subsequent time points provide a wash and re-equilibration sequence (Figure 3).

Figure 3. PLC 2020 View of Method: Preparative Isolation Gradient

To choose the appropriate gradient, use the observed retention time from the standard analytical method to locate the start and end values for the preparative column gradient from the Analytical to Preparative Conversion Table (Table 2).

Table 2. Analytical to Preparative Conversion Table

Analytical Retention time (min)	Prep column gradient	Approx retention time of product peak on Prep column
2.6	2-20	5.9
3.4	2-30	8.1
3.5	2-30	8.5
3.6	2-40	7.0
4.4	5-27	11.7
4.7	5-30	11.9
4.8	5-35	10.7
5.0	5-35	11.0
5.1	5-40	12.8
5.3	15-45	11.0
5.4	15-45	13.0
5.6	20-45	11.8
6.1	25-50	8.2
6.3	25-50	9.8
6.5	25-50	9.3
7.3	30-60	10.3
8.4	35-75	11.6
8.9	30-75	12.8
9.2	40-90	11.7
10.1	50-80	11.0
11.0	55-90	10.5
11.5	60-90	12.0
13.0	75-95	13.3

For example - the retention time of the peak of interest of a hypothetical sample is found to have a retention time of 8.5 minutes on the standard analytical method (see Figure 1). According to Table 2, a compound with an 8.4 minute retention time indicates a 35-75% CH3CN/H2O gradient could be used. This should result in the elution of the compound on the prep system at ~11.6 min.

Figure 4. Simulated Determination of Preparative Gradient from Analytical System Standard Gradient

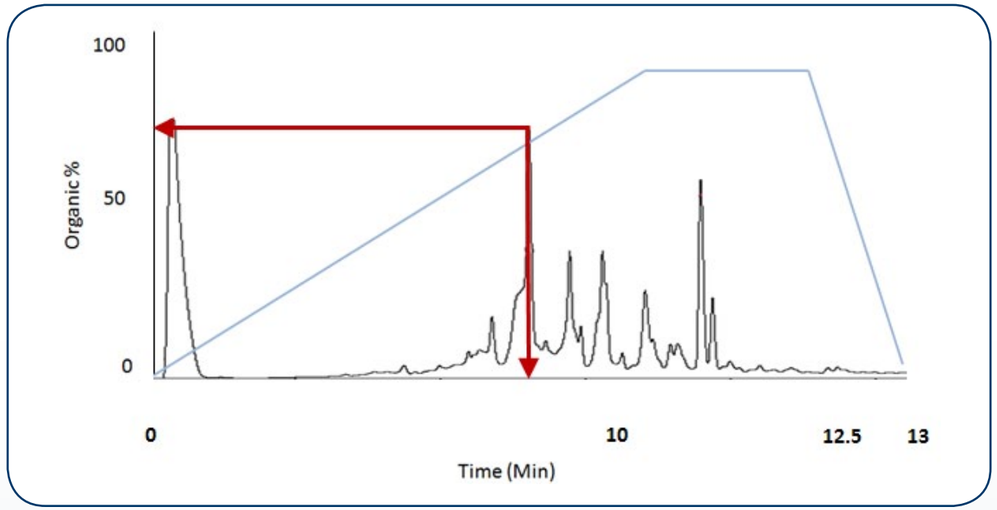
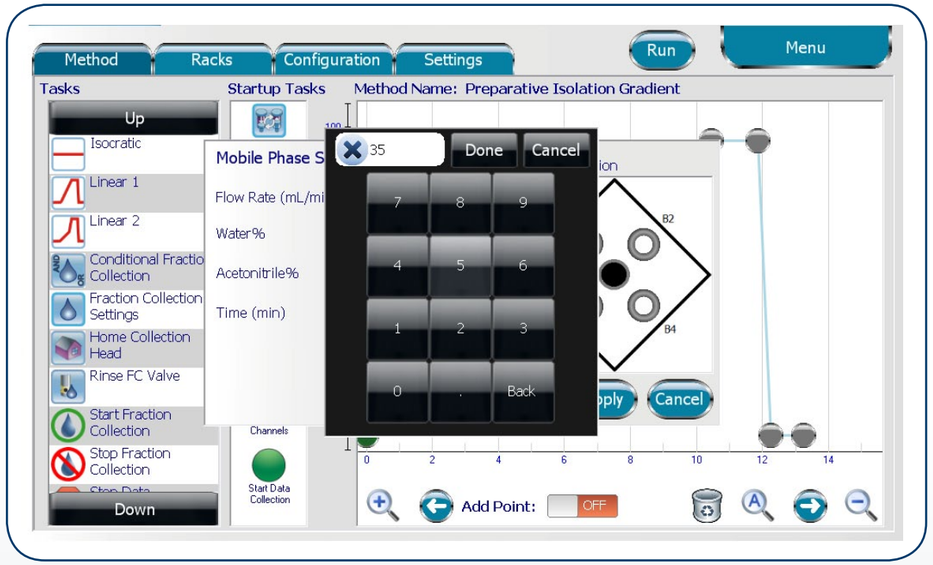


Figure 5. Using the PLC 2020 Touchscreen to Set the Acetonitrile Value for the New Preparative Gradient

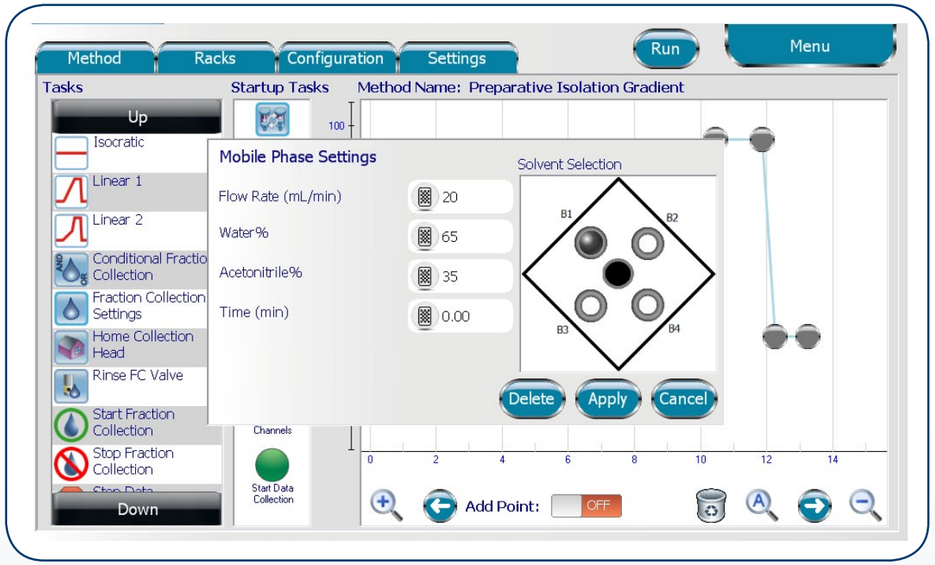


Alternatively, a compound with an 8.9 minute retention time (listed in Table 2) suggests using a 30-75% CH3CN/H2O gradient to elute material at 12.8 minutes. Either of these choices will probably work for the compound of interest. Factors (purity, amount, close elution of impurities, etc) may play a role in selection or modification of gradients selected.

To set the preparative gradient of 35-75% CH3CN/H2O, double-click on the first mobile phase node to set the start gradient. Touch the keypad icon next to Acetonitrile. Enter a value of 35, touch the Done button, and then touch the Apply button (see Figures 5 and 6). Deselect the start gradient node (touch in the white space).

Set the End Gradient by following the same process. Double-click on the node, touch the keypad icon next to Acetonitrile, and enter a value of 75. Touch the Done button, and then touch the Apply button.

Figure 6. PLC 2020 Mobile Phase Settings for the New Preparative Gradient



Notes:

- It is possible to add mobile phase nodes to a run, just remember to change the flow rate for each node that you add, since the default flow rate may not match the flow rate chosen for the run.
- Alternatively, mobile phase variables may be used so the changes can be made directly in the run screen.
- Once finished setting the method, select 'Menu', then 'Save As' to appropriately label the method for future recall. Select 'Run' from the menu to proceed to the next section of operation.

Figure 7. Modified New Preparative Gradient

Results

Purification of pharmaceutical organic compounds with the Gilson PLC 2020 using retention time criteria from analytical injections allows for quick elution of the compounds of interest at an 'optimal' time off the column, with minimal interference, and with reduced solvent waste. A practical guide to efficient use the PLC 2020 with an experimentally derived correlation table for the selection of 'optimal' solvent gradients or 'isolation' gradients offers simple, routine purification of organic compounds.

The simple PLC 2020 touch screen control allows for quick modifications of preparative methods based on retention times obtained from standard HPLC analytical methods. The flexibility to set preparative solvent gradients for purification injections using method modifications or variables caters to an 'open access' laboratory environment.

The enclosed guidelines represent the simplicity of establishing preparative compound purification conditions based on an analytical standard gradient injection.

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SHARE the SCIENCE in the Community...

The **University of Leicester's Genetics Department** produced two informative short films showing how to properly use a Gilson PIPETMAN® and how to run an agarose gel (using a Gilson PIPETMAN to load the gel). These short films were created to support the undergraduate Biological Science students, and they are all available on YouTube **HERE.**

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Jeff Hall

Gilson Golden PIPETMAN® Award Winner.

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Programs fall into three main categories: (1) field trips, workshops and courses for middle and high school students and their teachers; (2) scientific courses for undergraduate and graduate students, postdoctoral candidates, university faculty, industry scientists and the general public; and, (3) conferences, including the Wisconsin Stem Cell Symposium and the International Bioethics Forum, both held annually.

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G.E.T. LIVE Tips & Techniques	SPE or LH Tips for TRILUTION LH Topics at www.gilson.com	LIVEEDUCQLH (multi-user access)	1 hour
	Purification Tips for TRILUTION LC Topics at www.gilson.com	LIVEEDUCQLC (multi-user access)	
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	Pipetting Maintenance	LIVEEDUCPIPETM	

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