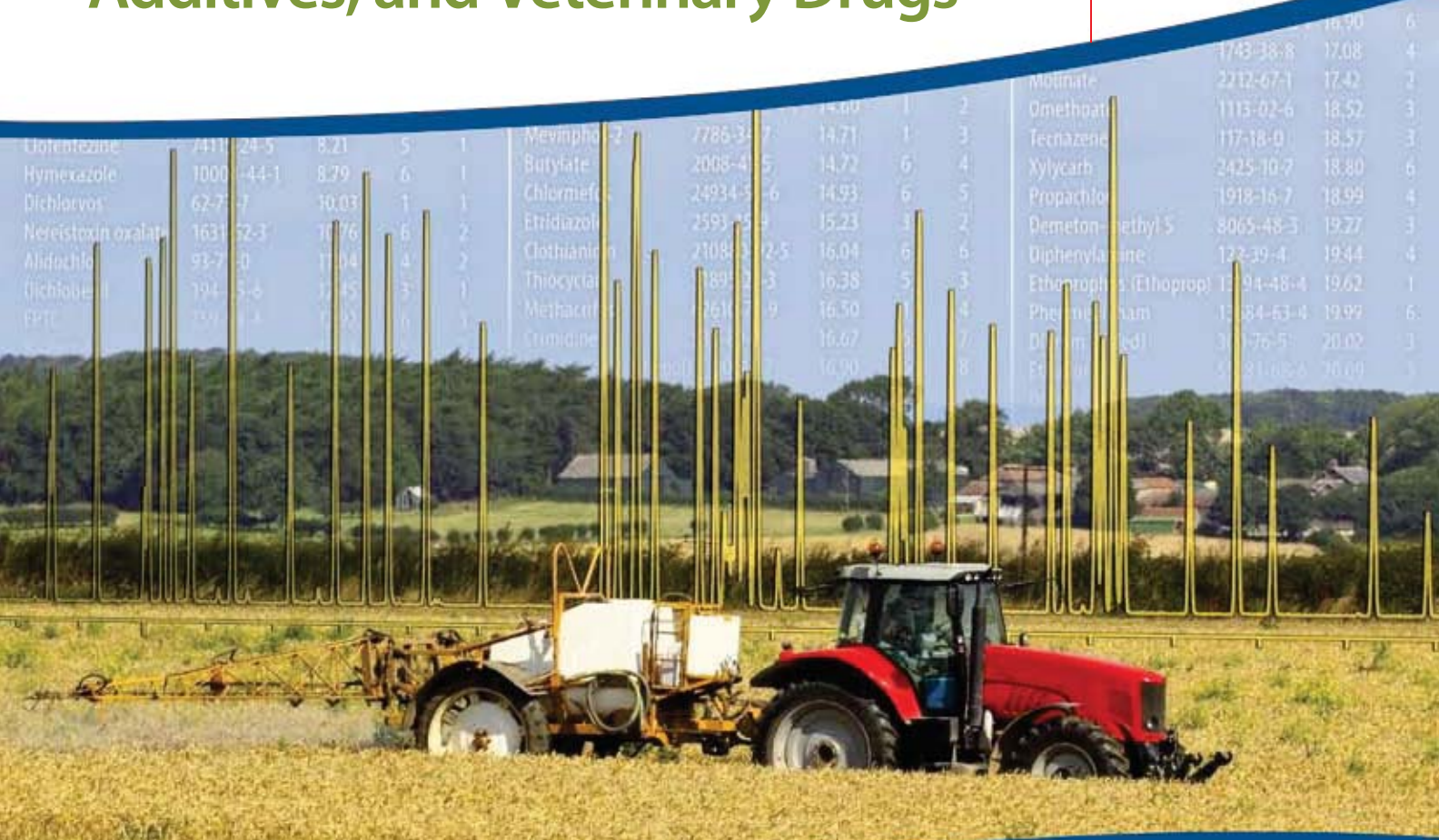


# Reporter

Volume 27.5



## GC-MS Analysis of Pesticides, Feed Additives, and Veterinary Drugs



*In May of 2006, the Japan Ministry of Health, Labour and Welfare (MHLW) introduced regulations for the levels of agricultural chemical residues allowed in plant-based and animal-based foods.*

- Liquid Chromatography
- Sample Handling
- Gas Chromatography
- Standards
- Accessories
- Chiral Chromatography



**Errol Fernandes**

*e-Business Product Manager*

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### Dear Colleague:

As we approach the end of 2009, it is an appropriate time to review the progress we have made over the year and to assess what remains to be completed before the year goes by.

With regard to the web, the noteworthy achievements of 2009 have been: enhanced in-site search capability, use of social networks for communication, and new content. Let's look briefly at each of these areas.

#### Enhanced In-site Search Capability

Last year our website was revamped with a new look, improved navigation, and an effective B2B platform – we hope those changes have been beneficial to you! This year, the in-site search feature, (which can be accessed from any page within our site), was upgraded to make it more helpful.

For example, in addition to the product list, you can now:

- Find related 'Applications', 'Site Content' and 'Technical Documents'
- Select up to 4 products from the results to compare their detailed specifications
- Use one of multiple search refinement categories to filter the search results

Our new user-friendly search has earned recognition in CIO business technology magazine's 2009 Top-100 honoree list, and we continue with more efforts to improve your browsing experience within our site.

#### Using Social Networks for Communication

The exponential growth in the number of people using social networks has pushed us to explore these media for sharing product news and event information. You will find Sigma-Aldrich on Facebook, Twitter, and YouTube. Follow us on Twitter to stay on top of the HPLC and chiral chromatography posts provided by Supelco's product managers:

<http://twitter.com/HPLCsessions>

<http://twitter.com/ChiralChrom>

#### New Content

From the numerous web updates completed this year, the following have received high interest:

- Chiral chromatography portal: access all chiral products and resources from one location
- Empore® Products: membrane-based SPE for pharmaceutical and environmental analysis
- Hot Topics: we present solutions for analytical challenges currently in the news

#### What Remains to be Done in 2009?

We are excited to be working on 3 additional web improvements, which we know you will appreciate:

- Applications search engine: perform a quick keyword search to look up chromatograms
- Reagents & solvents search engine: easily find the analytical reagent or solvent you need
- Environmental web portal: a user-friendly organization of analytical solutions for this industry

We hope to launch the two search pages about the time you receive this Reporter, so be on the lookout for them!

Wishing you success in completing all your key projects for 2009, and don't hesitate to send me any recommendations that you may have for improving our website.

Regards,

**Errol Fernandes**

*e-Business Product Manager*

[errol.fernandes@sial.com](mailto:errol.fernandes@sial.com)

# 'Positive List' Chemicals by GC-MS on the SLB-5ms

Katherine K. Stenerson, Takeyoshi Ide<sup>1</sup>, and Michael D. Buchanan  
mike.buchanan@sial.com

1. Sigma-Aldrich, Tokyo, Japan

## Introduction

In May of 2006, the Japan Ministry of Health, Labour and Welfare (MHLW) introduced regulations for the levels of agricultural chemical residues allowed in plant-based and animal-based foods. This list of chemicals includes pesticides, feed additives, and veterinary drugs. The goal of this regulation is to prevent the distribution of foodstuffs containing these residues at levels above specific limits. Products that exceed these limits cannot be sold in Japan. The analysis of these chemicals involves either liquid chromatography-mass spectrometry (LC-MS) or gas chromatography-mass spectrometry (GC-MS). In this article, the applicability of the SLB™-5ms capillary GC column was determined for the analysis of the chemicals listed in the GC-MS sections of the regulations.

## Experimental

A set of six standards covering the range of GC-amenable MHLW-regulated chemicals was located and obtained from a commercial source in Japan. Each mixture contained analytes at 20 µg/mL in acetone. An SLB-5ms column was selected because of several features, namely its low bleed characteristics and high inertness.

**Table 1. GC-MS Conditions for Figures 1-6**

Parameter	Setting	MHLW Methodology
column:	SLB-5ms, 30 m x 0.25 mm I.D., 0.25 µm (28471-U)	-
oven:	80 °C (2 min.), 5 °C/min. to 300 °C (4 min.)	50 °C (1 min.), 25 °C/min. to 125 °C, 10 °C/min. to 300 °C (10 min.)
inj.:	250 °C	-
MSD interface:	300 °C	-
scan range:	m/z 40-450	-
carrier gas:	helium, 1 mL/min. constant	-
injection:	1 µL, splitless (1 min.)	2 µL
sample:	each pesticide at 20 ppm in acetone	-

GC-MS run conditions were optimized to yield good chromatography for all analytes. These conditions are summarized in Table 1, and were used for the analysis of each of the six standards. Note that these conditions differ slightly from those stated in the MHLW methodology. Specifically, a higher initial oven temperature and a slower ramp rate were used to obtain better peak shape and spacing. Additionally, a smaller injection volume was used to ensure that the inlet liner contained the resulting expansion volume of the acetone solvent, and to minimize

band broadening. Table 1 lists the MHLW methodology condition if different from that used for this work.

## Results and Discussion

The resulting chromatography is shown in Figure 1 through Figure 6. Because each chromatogram was generated using identical GC-MS conditions, a composite list of analyte retention times in minutes could be assembled. This convenient list is shown in Table 2, and also includes the Chemical Abstracts Service Registry Number (CASRN) for each analyte. The corresponding Figure and Peak ID is integrated so users can easily locate individual analytes on one of the six chromatograms.

## Conclusion

The wide variety of functionality exhibited by this large list of analytes requires a column with both low bleed characteristics and high inertness. The SLB-5ms column was shown to be applicable for the MHLW 'positive list' methodology, able to produce good peak shape and resolution for most analytes. This work also led to the generation of a table of analyte retention times that should prove useful to food analysts who need a reference to help determine the elution order of their specific compound list on the SLB-5ms.

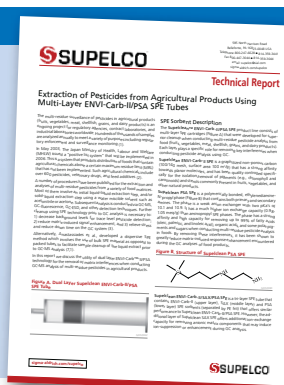
## References

- www.mhlw.go.jp/english, Japan Ministry of Health, Labour and Welfare web site.
- Analytical Methods for Residual Compositional Substances of Agricultural Chemicals, Feed Additives, and Veterinary Drugs in Food, Department of Food Safety, Japan Ministry of Health, Labour and Welfare.

(continued on page 4)

## Did you know...?

For information on the preparation of foodstuffs prior to LC-MS or GC-MS analysis, refer to Extraction of Pesticides from Agricultural Products Using Multi-Layer ENVI-Carb II/PSA SPE Tubes (T405060, HYA). This Technical Report contains detailed procedures for the preparation of a variety of foods, including fruits, vegetables, grains, beans, animal muscle, animal fat, seafood, milk, eggs, and honey.



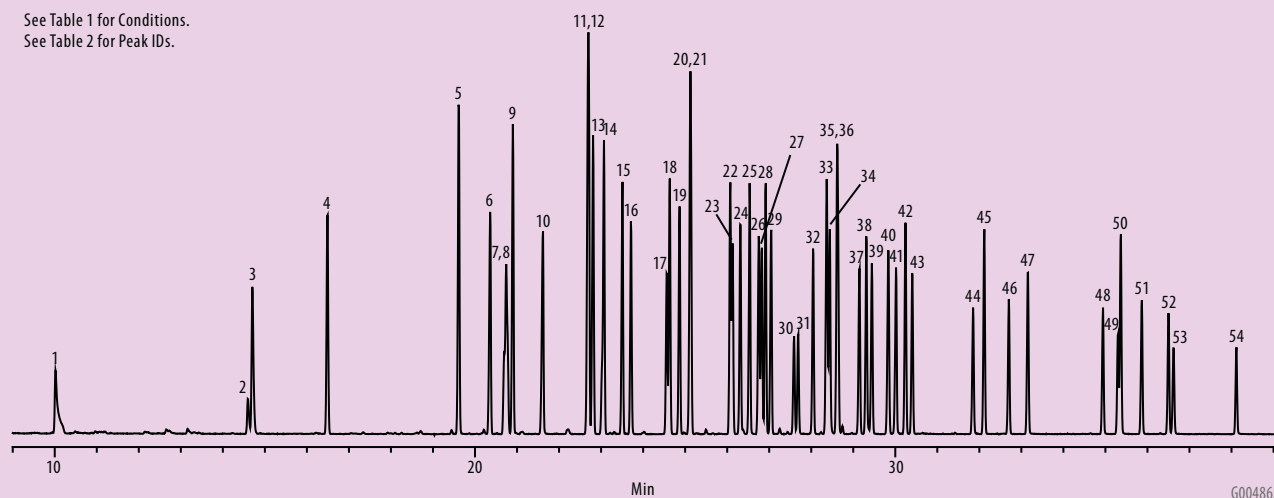
## Featured Products

Description	Cat. No.
SLB-5ms, 30 m x 0.25 mm I.D., 0.25 µm	28471-U

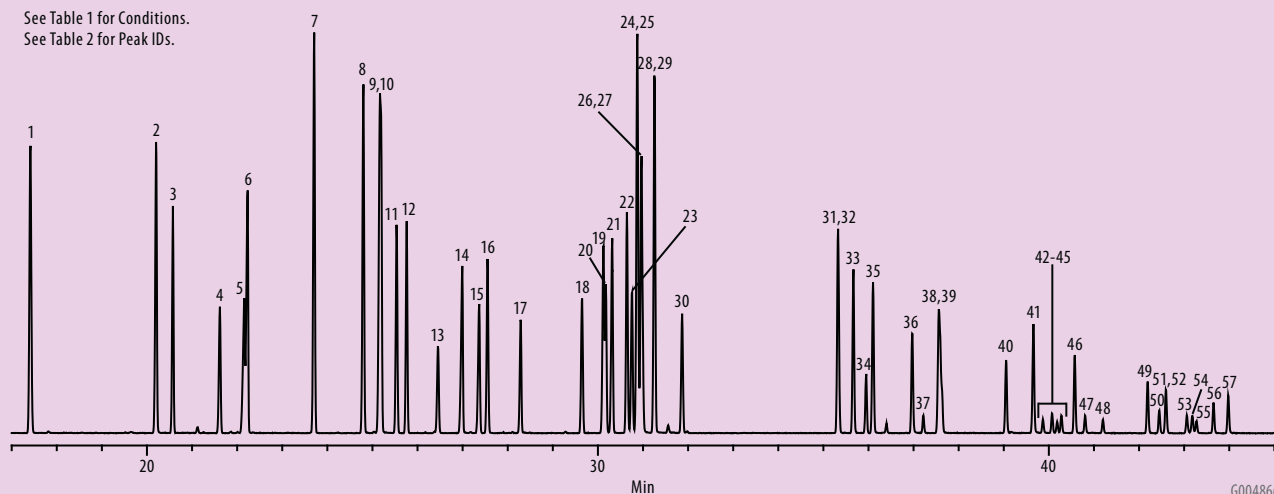
## Related Information

For more information on SLB-5ms capillary GC columns, visit [sigma-aldrich.com/slb](http://sigma-aldrich.com/slb)

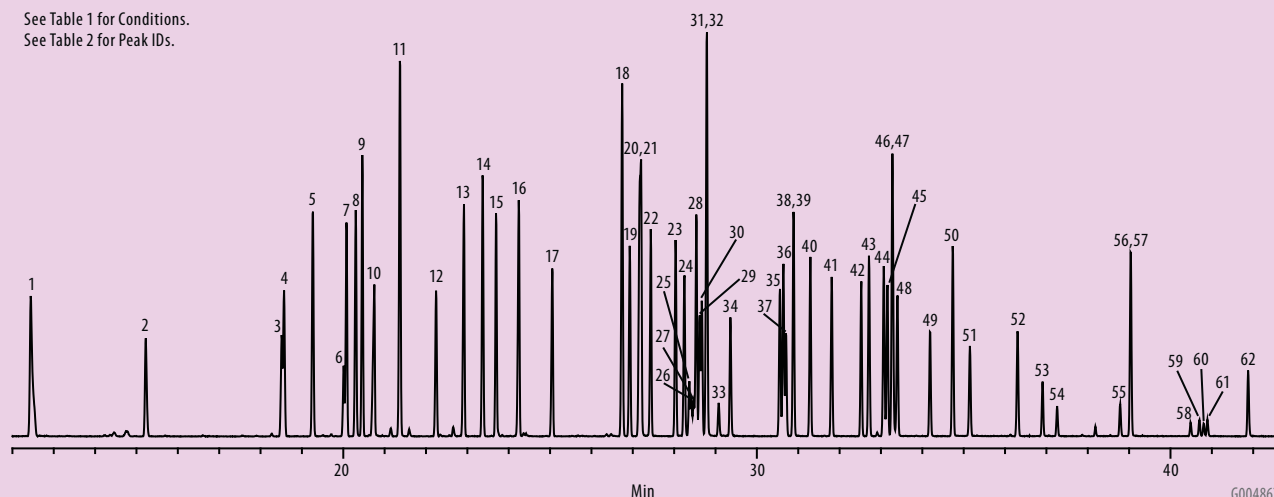
(continued from page 3)

**Figure 1. 'Positive List' Mix 1 on the SLB-5ms**See Table 1 for Conditions.  
See Table 2 for Peak IDs.

G004865

**Figure 2. 'Positive List' Mix 2 on the SLB-5ms**See Table 1 for Conditions.  
See Table 2 for Peak IDs.

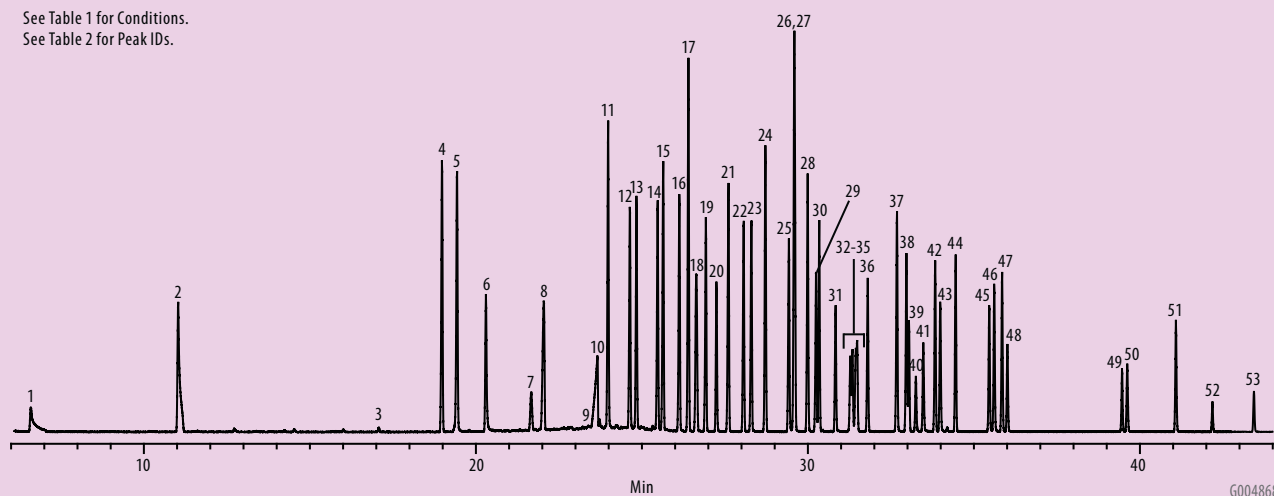
G004866

**Figure 3. 'Positive List' Mix 3 on the SLB-5ms**See Table 1 for Conditions.  
See Table 2 for Peak IDs.

G004867

Figure 4. 'Positive List' Mix 4 on the SLB-5ms

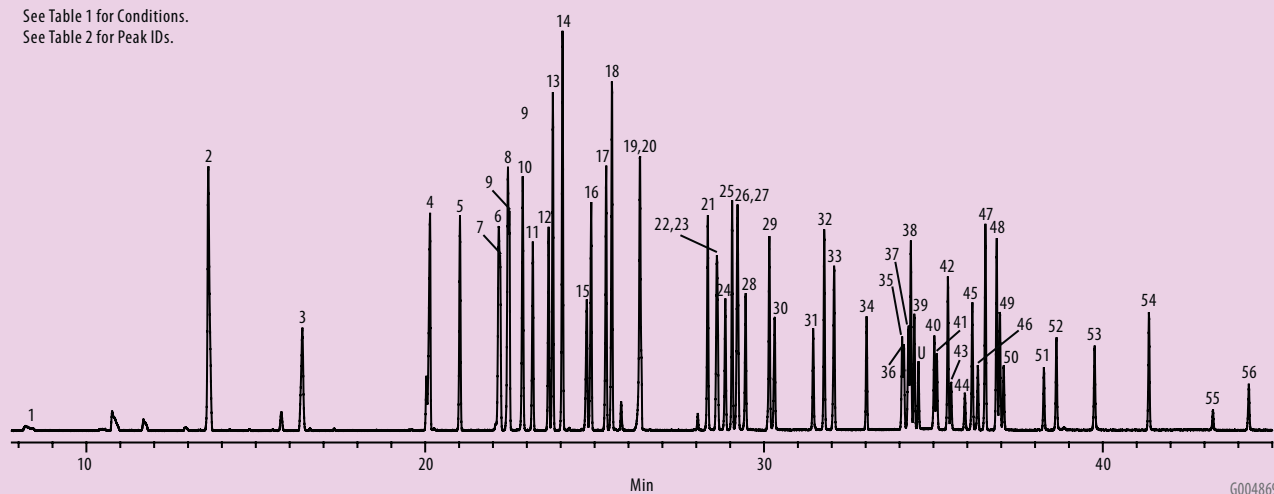
See Table 1 for Conditions.  
See Table 2 for Peak IDs.



G004868

Figure 5. 'Positive List' Mix 5 on the SLB-5ms

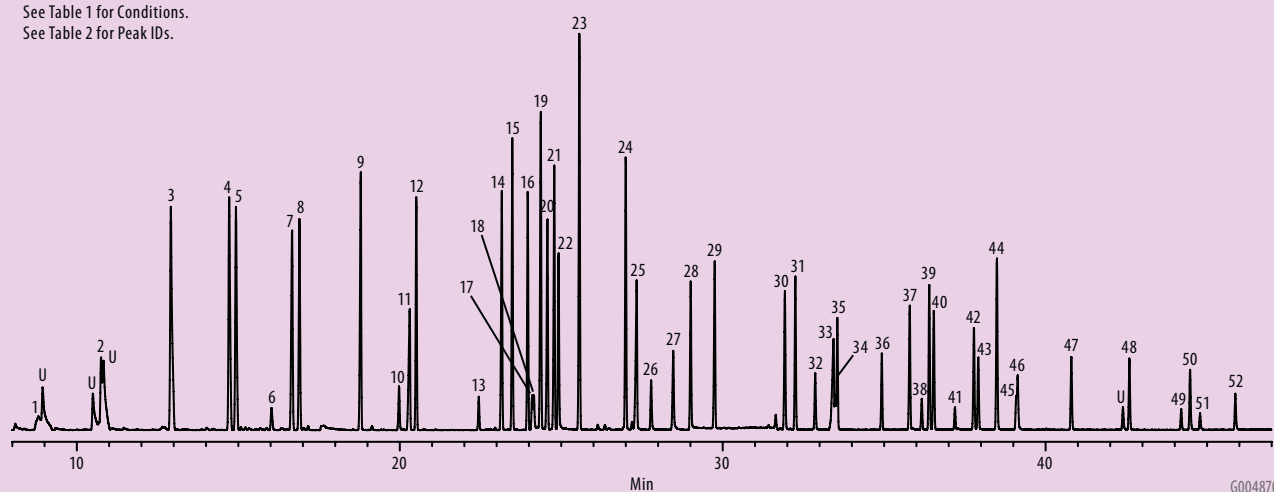
See Table 1 for Conditions.  
See Table 2 for Peak IDs.



G004869

Figure 6. 'Positive List' Mix 6 on the SLB-5ms

See Table 1 for Conditions.  
See Table 2 for Peak IDs.



G004870

(continued on page 6)

(continued from page 5)

Table 2. Retention Times of 'Positive List' Chemicals on the SLB-5ms

Analyte (synonym)	CASRN	t <sub>r</sub> (min.)	Figure	Peak ID	Analyte (synonym)	CASRN	t <sub>r</sub> (min.)	Figure	Peak ID
DCIP	108-60-1	6.60	4	1	Benfuresate	68505-69-1	24.58	6	20
Clofentezine	74115-24-5	8.21	5	1	Dichlofenthion	97-17-6	24.64	1	18
Hymexazole	10004-44-1	8.79	6	1	Dimethenamid	87674-68-8	24.65	4	12
Dichlorvos	62-73-7	10.03	1	1	Propanil	709-98-8	24.78	5	15
Nereistoxin oxalate	1631-52-3	10.76	6	2	Terbutcarb	1918-11-2	24.79	6	21
Alidochlor	93-71-0	11.04	4	2	Bromobutide	74712-19-9	24.81	2	8
Dichlobenil	194-65-6	12.45	3	1	Acetochlor	34256-82-1	24.84	4	13
EPTC	759-94-4	12.92	6	3	Chlorpyrifos-methyl	5598-13-0	24.87	1	19
Biphenyl	92-52-4	13.61	5	2	Metribuzin	21087-64-9	24.91	5	16
Mevinphos-1	7786-34-7	14.60	1	2	(Oxapocconazole) fumarate	174212-12-5	24.92	6	22
Mevinphos-2	7786-34-7	14.71	1	3	Vinclozolin	50471-44-8	25.06	3	17
Butylate	2008-41-5	14.72	6	4	Tolclofos-methyl	57018-04-9	25.13	1	20
Chlormefos	24934-91-6	14.93	6	5	Parathion-methyl	298-00-0	25.13	1	21
Etridiazole	2593-15-9	15.23	3	2	Simeconazole	149508-90-7	25.18	2	9
Clothianidin	210880-92-5	16.04	6	6	Alachlor	15972-60-8	25.18	2	10
Thiocyclam	31895-21-3	16.38	5	3	Simetryn	1014-70-6	25.35	5	17
Methacrifos	62610-77-9	16.50	1	4	Metalaxyl	57837-19-1	25.48	4	14
Crimidine	535-89-7	16.67	6	7	Ametryn	834-12-8	25.52	5	18
OPP (2-Phenylphenol)	90-43-7	16.90	6	8	Fenclorphos	299-84-3	25.54	2	11
Etobenzanid metabolite		17.08	4	3	Cinmethylin	87818-31-3	25.57	6	23
Molinat	2212-67-1	17.42	2	1	Prometryn	7287-19-6	25.65	4	15
Omethoate	1113-02-6	18.52	3	3	Dithiopyr	97886-45-8	25.77	2	12
Tecnazene	117-18-0	18.57	3	4	Pirimiphos-methyl	29232-93-7	26.08	1	22
Xylycarb	2425-10-7	18.80	6	9	Fenitrothion	122-14-5	26.14	1	23
Propachlor	1918-16-7	18.99	4	4	Terbutryn	886-50-0	26.14	4	16
Demeton-methyl S	8065-48-3	19.27	3	5	Dimethylvinphos-E	2274-67-1	26.32	1	24
Diphenylamine	122-39-4	19.44	4	5	Dichlofluanid	1085-98-9	26.35	5	19
Ethoprophos (Ethoprop)	13194-48-4	19.62	1	5	Bromacil	314-40-9	26.35	5	20
Phenmedipham	13684-63-4	19.99	6	10	Esprocarb	85785-20-2	26.41	4	17
Dibrom (Naled)	300-76-5	20.02	3	6	Quinoclamine	2797-51-5	26.46	2	13
Ethafuralin	55283-68-6	20.09	3	7	Malathion	121-75-5	26.54	1	25
Diclofluanid metabolite		20.15	5	4	Metolachlor	51218-45-2	26.65	4	18
Chlorpropham	101-21-3	20.21	2	2	Thiobencarb	28249-77-6	26.75	3	18
Dicrotophos	141-66-2	20.31	3	8	Chlorpyrifos	2921-88-2	26.76	1	26
Flusilazole metabolite		20.31	4	6	Dimethylvinphos-Z	2274-67-1	26.83	1	27
2,6-Dichlorobenzamide (BAM)	2008-58-4	20.31	6	11	Fenthion	55-38-9	26.92	1	28
Salithion (Dioxabenzofos)	3811-49-2	20.37	1	6	Chlorthal-dimethyl	1861-32-1	26.93	3	19
Trifluralin	1582-09-8	20.47	3	9	Diethofencarb	87130-20-9	26.93	4	19
Sulfotep	3689-24-5	20.52	6	12	Cyanazine	21725-46-2	27.00	2	14
Benfluralin (Balan)	1861-40-1	20.58	2	3	Fenpropimorph	67564-91-4	27.00	6	24
Cadusafos-1	95465-99-9	20.73	1	7	Parathion	56-38-2	27.05	1	29
Cadusafos-2	95465-99-9	20.75	1	8	Triademefon	43121-43-3	27.21	3	20
Monocrotophos	6923-22-4	20.76	3	10	Isocarbophos	24353-61-5	27.21	3	21
Phorate	298-02-2	20.91	1	9	Tetraconazole	112281-77-3	27.25	4	20
alpha-BHC		21.03	5	5	Carbetamide	16118-49-3	27.34	6	25
Thiometon	640-15-3	21.38	3	11	Phthalide	87-41-2	27.37	2	15
Dimethoate	60-51-5	21.62	1	10	Nitrothal-isopropyl	10552-74-6	27.44	3	22
Dicloran	99-30-9	21.62	2	4	Bromophos	2104-96-3	27.56	2	16
Desmediphan	13684-56-5	21.67	4	7	Fosthiazate-1	98886-44-3	27.60	1	30
Simazine	122-34-9	22.05	4	8	Diphenamid	957-51-7	27.62	4	21
Dimethipin	55290-64-7	22.17	2	5	Fosthiazate-2	98886-44-3	27.70	1	31
beta-BHC		22.18	5	6	Thiamethoxam	153719-23-4	27.79	6	26
SWEP	1918-18-9	22.18	5	7	Pendimethalin	40487-42-1	28.04	3	23
Atrazine	1912-24-9	22.24	2	6	Chlorfenvinphos-E	470-90-6	28.05	1	32
Quintozone	82-68-8	22.25	3	12	Cyprodinil	121552-61-2	28.07	4	22
gamma-BHC	58-89-9	22.45	5	8	Penconazole	66246-88-6	28.26	3	24
Dioxathion	78-34-2	22.45	6	13	Fipronil	120068-37-3	28.30	2	17
Tolyfluandit metabolite		22.49	5	9	Dimethametryn	22936-75-0	28.31	4	23
Terbufos	13071-79-9	22.70	1	11	Tolyfluandit	731-27-1	28.35	5	21
Cyanophos	2636-26-2	22.70	1	12	Pyrifenoxy-1	88283-41-4	28.37	3	25
Fonofos	944-22-9	22.82	1	13	Isofenphos	25311-71-1	28.38	1	33
Pyroquilon	57369-32-1	22.89	5	10	Allethrin-1	584-79-2	28.43	3	26
Propyzamide	23950-58-5	22.92	3	13	Chlorfenvinphos-Z	470-90-6	28.45	1	34
Diazinon	333-41-5	23.08	1	14	Allethrin-2	584-79-2	28.48	3	27
Pyrimethanil	53112-28-0	23.17	6	14	Ethyclozate	27512-72-7	28.48	6	27
Chlorthalonil	1897-45-6	23.18	5	11	Mecarbam	2595-54-2	28.55	3	28
Disulfoton	298-04-4	23.38	3	14	Captan	133-06-2	28.62	5	22
Prohydrojasmon-1	158474-72-7	23.49	6	15	Dicyclomet-1	139920-32-4	28.62	5	23
Tridemorph		23.50	4	9	Bioallethrin-1	584-79-2	28.62	3	29
Isazophos	42509-80-8	23.52	1	15	Phenthoate	2597-03-7	28.63	1	35
delta-BHC		23.65	5	12	Quinalphos	13593-03-8	28.63	1	36
Terbacil	5902-51-2	23.67	4	10	Bioallethrin-2	584-79-2	28.68	3	30
Tefluthrin	79538-32-2	23.70	3	15	Dimepiperate	61432-55-1	28.73	4	24
Triallate	2303-17-5	23.71	2	7	Procymidon	32809-16-8	28.80	3	31
Etrifos	38260-54-7	23.72	1	16	Triadimenol-1	55219-65-3	28.80	3	32
Phenothiol	25319-90-8	23.78	5	13	Folpet	133-07-3	28.87	5	24
Tebupirimfos	96182-53-5	23.97	6	16	Ferimzone E,Z	89269-64-7	29.02	6	28
Iprobenfos	26087-47-8	23.99	4	11	Methoprene	40596-69-8	29.07	5	25
Oxabetrinil	94593-79-0	24.06	5	14	Triadimenol-2	55219-65-3	29.08	3	33
Prohydrojasmon-2	158474-72-7	24.11	6	17	Methodathion	950-37-8	29.15	1	37
MCPB	94-81-5	24.16	6	18	Chinomethionate	2139-01-2	29.23	5	26
Formothion	2540-82-1	24.25	3	16	Dicyclomet-2	139920-32-4	29.23	5	27
MCPB-ethyl	10443-70-6	24.37	6	19	Propaphos	7292-16-2	29.32	1	38
Phosphamidon (E,Z)	13171-21-6	24.57	1	17	Pyrifenoxy-2	88283-41-4	29.37	3	34

Table 2. Retention Times of 'Positive List' Chemicals on the SLB-5ms (continued)

Analyte (synonym)	CASRN	t <sub>r</sub> (min.)	Figure	Peak ID	Analyte (synonym)	CASRN	t <sub>r</sub> (min.)	Figure	Peak ID
Paclobutrazole	76738-62-0	29.43	4	25	Bifenthrin	82657-04-3	35.33	2	32
Tetrachlorvinphos	961-11-5	29.45	1	39	Piperophos	24151-93-7	35.37	1	50
Trichlamide	70193-21-4	29.46	5	28	Tetramethrin	7696-12-0	35.44	5	42
Butachlor	23184-66-9	29.60	4	26	Fenoxycarb	79127-80-3	35.47	4	45
Fenothiocarb	62850-32-2	29.60	4	27	Bifenazate	149877-41-8	35.53	5	43
Endosulfan I	115-29-7	29.66	2	18	Etoazole	153233-91-1	35.62	4	46
Ditalimfos	5131-24-8	29.76	6	29	Fenpropathrin	39515-41-8	35.67	2	33
Butamifos	36335-67-8	29.84	1	40	Indanofan	133220-30-1	35.80	6	37
Napropamide	15299-99-7	30.00	4	28	Tebufenpyrad	119168-77-3	35.86	4	47
Fenamiphos	22224-92-6	30.03	1	41	Anilofos	64249-01-0	35.87	1	51
Chlorfenson	80-33-1	30.13	2	19	Bromuconazole-2	116255-48-2	35.94	5	44
Hexaconazole	79983-71-4	30.17	5	29	Bifenox	42576-02-3	35.96	2	34
Flutolanil	66332-96-5	30.18	2	20	Etoazole metabolite		36.02	4	48
Prothiofos	34643-46-4	30.25	1	42	Clomeprop	84496-56-0	36.11	2	35
Metominostrobin-E	133408-50-1	30.25	4	29	Furametryp	123572-88-3	36.16	5	45
Fludioxonil	13141-86-1	30.32	5	30	Phenothrin-1	26002-80-2	36.17	6	38
Isoprothiolane	50512-35-1	30.32	2	21	Tetradifon	116-29-0	36.31	3	52
Prethilachlor	51218-49-6	30.35	4	30	Iprodione metabolite		36.32	5	46
Profenophos	41198-08-7	30.41	1	43	Phenothrin-2	26002-80-2	36.40	6	39
Uniconazole P	83657-17-4	30.56	3	35	Phosalone	2310-17-0	36.51	1	52
Oxadiazon	19666-30-9	30.65	3	36	Pentoxazone	110956-75-7	36.54	5	47
Tribufos (DEF)	78-48-8	30.65	2	22	Leptophos	21609-90-5	36.54	6	40
Thifluzamide	130000-40-7	30.71	3	37	Azinphos-methyl	86-50-0	36.63	1	53
Myclobutanil	88671-89-0	30.76	2	23	Pyriproxyfen	95737-68-1	36.87	5	48
Flusilazole	88509-19-9	30.84	4	31	Cyhalothrin-1	91465-08-6	36.91	3	53
Buprofezin	69327-76-0	30.88	2	24	Mefenacet	73250-68-7	36.97	5	49
Diclobutrazole	75736-33-3	30.88	2	25	Cyhalofop	122008-78-0	36.98	2	36
Bupirimate	41483-43-6	30.89	3	38	Furametryp metabolite		37.08	5	50
Oxyfluorfen	42874-03-3	30.89	3	39	Amitraz	33089-61-1	37.20	6	41
Azaconazole	60207-31-0	30.97	2	26	Cyhalothrin-2	91465-08-6	37.27	3	54
Kresoxim-methyl	143390-89-0	30.97	2	27	Acinathrin	101007-06-1	37.57	2	37
Chlorfenapyr	122453-73-0	31.26	2	28	Fenarimol	60168-88-9	37.57	2	38
Cyflufenamid	180409-60-3	31.26	2	29	Pyrazophos	13457-18-6	37.57	2	39
Cyproconazole-1	94361-06-5	31.28	4	32	Azinphos-ethyl	2642-71-9	37.79	6	42
Isoxathion	18854-01-8	31.30	3	40	Dialifos	10311-84-9	37.92	6	43
Cyproconazole-2	94361-06-5	31.35	4	33	Pyraclufos	77458-01-6	38.12	1	54
Fenoxanil-1	115852-48-7	31.45	4	34	Fenoxaprop-ethyl	66441-23-4	38.27	5	51
Nitrofen	1836-75-5	31.46	5	31	Spirodiclofen	148477-71-8	38.50	6	44
Fenoxanil-2	115852-48-7	31.49	4	35	Bitertanol	55179-31-2	38.64	5	52
Chlorpropylate	5836-10-2	31.79	5	32	Permethrin-1	52645-53-1	38.79	3	55
Chlorbenzilate	510-15-6	31.81	4	36	Pyridaben	96489-71-3	39.05	3	56
Pyriminobac-methyl Z	136191-64-5	31.82	3	41	Permethrin-2	52645-53-1	39.05	3	57
Fensulfothion	115-90-2	31.86	1	44	Fluquinconazole	136426-54-5	39.06	2	40
Endosulfan II	115-29-7	31.87	2	30	Oxapocconazole	134074-64-9	39.12	6	45
Diniconazole	83657-24-3	31.93	6	30	Pyraclostrobin	175013-18-0	39.14	6	46
Oxadixyl	77732	32.08	5	33	Butafenacil	134605-64-4	39.47	4	49
Ethion	563-12-2	32.12	1	45	Etobenzanid	79540-50-4	39.63	4	50
Chlorthiophos, mixture	60238-56-4	32.26	6	31	Cafenstrole	125306-83-4	39.67	2	41
Fluacrypyrim	229977-93-9	32.53	3	42	Fenbucanazole	114369-43-6	39.76	5	53
Mepronil	55814-41-0	32.69	4	37	Cyfluthrin-1	68359-37-5	39.88	2	42
Triazophos	24017-47-8	32.71	1	46	Cyfluthrin-2	68359-37-5	40.08	2	43
Sulprophos	35400-43-2	32.72	3	43	Cyfluthrin-3	68359-37-5	40.19	2	44
Azamephiphos	35575-96-3	32.87	6	32	Cyfluthrin-4	68359-37-5	40.29	2	45
Benalaxyl	71626-11-4	32.98	4	38	Cypermethrin-1	52315-07-8	40.49	3	58
CNP	2275-14-1	33.03	5	34	Halfenprox	111872-58-3	40.58	2	46
Carfentrazone-ethyl	128639-02-1	33.05	4	39	Cypermethrin-2	52315-07-8	40.71	3	59
Carbofenthothion	786-19-6	33.08	3	44	Quizalofop-ethyl	76578-14-8	40.80	6	47
Edifenphos	17109-49-8	33.16	1	47	Cypermethrin-3	52315-07-8	40.81	3	60
Cyanofenphos	13067-93-1	33.16	3	45	Flucythrinate-1	70124-77-5	40.81	2	47
Propiconazole-1	60207-90-1	33.26	4	40	Cypermethrin-4	52315-07-8	40.90	3	61
Quinoxifen	124495-18-7	33.28	3	46	Etofenprox	80844-07-1	41.09	4	51
Trifloxystrobin	141517-21-7	33.28	3	47	Flucythrinate-2	70124-77-5	41.21	2	48
Pyriminobac-methyl E	136191-64-5	33.41	3	48	Silafuofen	105024-66-6	41.37	5	54
Lenacil	2164-08-1	33.44	6	33	Pyrimidifen	105779-78-0	41.89	3	62
Propiconazole-2	60207-90-1	33.48	4	41	Flumioxazin	103361-09-7	42.19	4	52
Chloridazon	1698-60-8	33.49	6	34	Fenvalerate-1	51630-58-1	42.20	2	49
Pyraflufen-ethyl	129630-19-9	33.56	6	35	Fluvalinate-1	69409-94-5	42.46	2	50
Tenylchlor		33.84	4	42	Fluvalinate-2	69409-94-5	42.60	2	51
Tebuconazole	107534-96-3	33.99	4	43	Fenvalerate-2	51630-58-1	42.60	2	52
Propargite-1	2312-35-8	34.08	5	35	Esfenvalerate	66230-04-4	42.60	6	48
Propargite-2	2312-35-8	34.14	5	36	Difenoconazole-1	119446-68-3	43.07	2	53
Diflufenican	83164-33-4	34.20	3	49	Difenoconazole-2	119446-68-3	43.19	2	54
Captafol	2425-06-1	34.27	5	37	Pyrazoxyfen	71561-11-0	43.26	5	55
Piperonyl butoxide	51-03-6	34.34	5	38	Deltamethrin-1	52918-63-5	43.28	2	55
Nitralin	4726-14-1	34.45	5	39	Indoxacarb	173584-44-6	43.44	4	53
Resmethrin	28434-01-7	34.46	4	44	Deltamethrin-2	52918-63-5	43.66	2	56
Pyributicarb	88678-67-5	34.74	3	50	Azoxystrobin	131860-33-8	43.99	2	57
Chlormethoxyfenyl (Chlormethoxyfen)	32861-85-1	34.93	6	36	Dimethomorph-1	110488-70-5	44.21	6	49
Pyridafenthion	119-12-0	34.95	1	48	Famoxadone	131807-57-3	44.32	5	56
Iprodione	36734-19-7	35.03	5	40	Tolfenpyrade	129558-76-5	44.48	6	50
Bromuconazole-1	116255-48-2	35.11	5	41	Dimethomorph-2	110488-70-5	44.79	6	51
Phosmet	732-11-6	35.16	3	51	Fluthiacet-methyl	117337-19-6	45.88	6	52
EPN	2104-64-5	35.31	1	49	Unknown				U
Bromopropylate	18181-80-1	35.33	2	31					

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# Recovery & Sample Cleanup of Pesticides in Spinach Using Supelclean ENVI-Carb-II/PSA SPE

Olga Shimelis and Katherine Stenerson  
olga.shimelis@sial.com

## Introduction

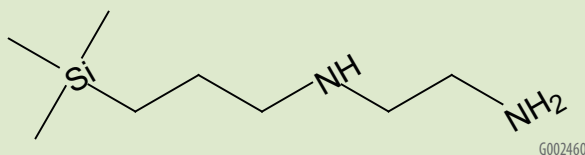
The multi-residue surveillance of pesticides in agricultural/food products is an ongoing project for regulatory agencies and industrial laboratories worldwide. Hundreds of thousands of samples are analyzed annually to meet a variety of purposes including regulatory enforcement and surveillance monitoring.

A number of procedures have been published for the extraction and analyses of multi-residue pesticides from a variety of food matrixes. Most of which involve an initial liquid-liquid extraction step, and/or solid-liquid extraction step using a water miscible solvent followed by GC analyses. Prior to GC analyses, further sample cleanup is necessary to decrease background levels for trace pesticide detection, reduce matrix-induced signal enhancement, and relieve stress on the GC system.

Supelclean™ ENVI-Carb™-II SPE is a graphitized non-porous carbon (100/140 mesh, surface area 100 m<sup>2</sup>/g) that has a strong affinity towards planar molecules, and has been quality controlled specifically for the isolation/removal of pigments (e.g. chlorophyll and carotinoids) and sterols commonly present in fruits, vegetables and other natural products.

Supelclean PSA SPE is a polymerically bonded, ethylene-diamine-N-propyl phase (Figure 1) that contains both primary and secondary amines. The phase has a strong affinity and high capacity for removing up to 99% of fatty acids (oleic, palmitic and linoleic acid), organic acids, and some polar pigments and sugars when conducting multi-residue pesticide analysis in foods. By removing these interferences, it has been shown to greatly reduce matrix-induced response enhancement encountered during the GC-analyses of food products.

Figure 1. Structure of Supelclean PSA SPE

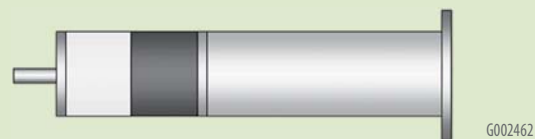


In this article, we offer quantitative data by demonstrating the high recovery and sample cleanup of 18 pesticides from spinach using Supelclean ENVI-Carb-II/PSA SPE and Equity®-1 GC-MS.

## Technology Description

Food samples are initially extracted/homogenized with a water miscible solvent (e.g. acetonitrile or acetone) in the presence of sodium chloride. The sodium chloride drives phase separation between the solvent and endogenous aqueous residues within the sample. Upon centrifugation/filtration to remove particulate matter, the acetonitrile layer of the supernatant/filtrate is removed and dried over anhydrous sodium sulfate or magnesium sulfate. At this point, the acetonitrile extract is unsuitable for further analysis due to the high levels of endogenous interferences co-extracted with the pesticides during initial solvent extraction. SPE is necessary for further cleanup prior to GC-MS.

Figure 2. Supelclean ENVI-Carb-II/PSA SPE Tube



Supelclean ENVI-Carb-II/PSA SPE is a dual-layer SPE cartridge (Figure 2). After the initial acetonitrile extract is concentrated, it is applied to a pre-conditioned ENVI-Carb-II/PSA SPE tube. The tube acts as a chemical filter in which pigments and sterols are retained on the ENVI-Carb-II layer (graphitized carbon), while fatty acids, organic acids, polar pigments, and sugars are trapped on the PSA (primary-secondary amine) layer. Pesticides are weakly retained on the tube and subsequently eluted with acetonitrile:toluene (3:1). Although the use of a vacuum manifold is recommended, the tubes are also amenable to gravity-driven applications.

## Experimental Approach

The performance of the SPE technology was evaluated by spiking spinach with a representative pesticide test mix. The mix represented a range of pesticide classes with varying physico-chemical properties. Included within this test mix were highly polar pesticides such as carbaryl, dichlorovos, acepate, and procymidone, which are primarily analyzed by LC.

Pesticides were spiked at the level of 0.2 ppm into 10 g fresh spinach. The mixture was carefully homogenized and extracted with acetonitrile:sodium chloride. The excess of acetonitrile was evaporated to ~1 mL under nitrogen at 40 °C, and the resulting extract was loaded onto the Supelclean ENVI-Carb-II/PSA SPE tube, 500 mg/300 mg/6 mL, preconditioned with 5 mL acetonitrile:toluene (3:1). Pesticide elution was facilitated with 20 mL acetonitrile:toluene (3:1). The SPE

eluate was evaporated and reconstituted with 1 mL hexane:acetone (1:1) for subsequent GC-MS analysis.

To compensate for 'matrix effects', matrix match standards were prepared by spiking pesticides into blank spinach extracts subjected to the SPE process.

### High Recoveries and Good Sample Cleanup

Recovery for the 18 pesticides tested averaged at 96%. Highly polar pesticides such as dichlorovos and methamidophos may be over-retained on the PSA sorbent due to secondary normal-phase (polar-polar) interactions resulting in poor recovery. At least 70% recovery was observed for all pesticides tested. In this application example with the exception of acephate (60% recovery). Table 1 lists recovery values for the pesticides tested.

**Table 1. Recovery of Pesticides from Spinach Using Supelclean ENVI-Carb-II/PSA SPE**

Peak ID	Compound	Pesticide Class	Pesticide Recovery (%)
1	Methamidophos	Organophosphorous	80
2	Dichlorovos	Organophosphorous	70
3	Acephate	Organophosphorous	60
4	Quintozene	Organochloride	92
5	Methyl parathion	Organophosphorous	97
6	Carbaryl	Carbamate	128
7	Methyl chloropyriphos	Organophosphorous	99
8	Vinclozolin	Organochloride	83
9	Procymidone	Dicarboximide	84
10	Imazalil	Imidazole	104
11	Chlorothiophos	Phosphosulfide	106
12	Tetrasul	Organochloride	87
13	Endosulfan sulfate	Organochloride	124
14	Acrinathrin	Organophosphorous	118
15	Bitertanol	Biphenol	108
16	cis-Permethrin	Pyrethroid	82
17	trans-Permethrin	Pyrethroid	82
18	Cypermethrin isomers	Organochloride	74
19	Deltamethrin	Organobromine	134

Excellent sample cleanup was observed signified by sharp peaks and low background (Figure 3).

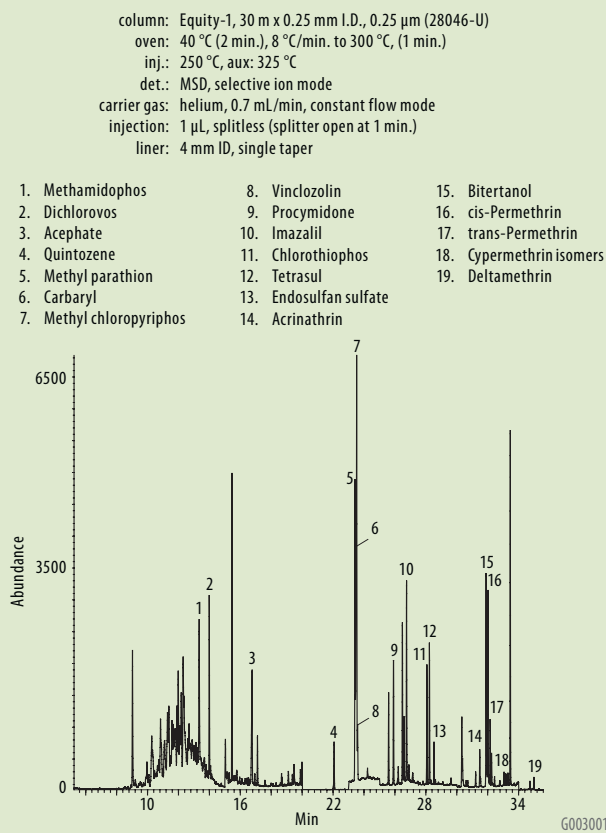
### Conclusion

Dual-layer SPE cartridges provide efficient sample cleanup of food samples, decreasing background interference levels during GC-MS analysis. Recoveries for most of the more challenging polar pesticides were 70% or greater. Table 2 describes the benefits of dual-layer SPE technology for pesticide analysis in food/agricultural products.

**Table 2. Benefits of Supelclean ENVI-Carb-II/PSA SPE**

- Decrease background levels for trace pesticide detection
- Reduce matrix-induced signal enhancement and suppression
- Relieve stress and reduce down time on the GC system
- Offers good recovery of pesticides including polar pesticides (e.g. methamidophos)

**Figure 3. Pesticides Spiked into Spinach Followed by Supelclean ENVI-Carb-II/PSA SPE**



### Featured Products

#### Supelclean SPE Tubes

Description	Qty.	Cat. No.
<b>Supelclean ENVI-Carb-II/PSA SPE Tube</b>		
500 mg/500 mg/6 mL	30	<b>54067-U</b>
500 mg/300 mg/6 mL	30	<b>55119-U</b>
<b>Supelclean ENVI-Carb-II/SAX/PSA SPE Tube</b>		
500 mg/500 mg/500 mg/12 mL	20	<b>52574-U</b>
<b>Supelclean SAX/PSA SPE Tube</b>		
250 mg/250 mg/6 mL	30	<b>52576-U</b>
500 mg/500 mg/6 mL	30	<b>52577-U</b>
<b>Supelclean PSA SPE Tube</b>		
200 mg/3 mL	54	<b>52578-U</b>
500 mg/6 mL	30	<b>52579-U</b>
<b>Supelclean ENVI-Carb SPE Tube</b>		
100 mg/1 mL	108	<b>57109-U</b>
250 mg/3 mL	54	<b>57088</b>
250 mg/6 mL	30	<b>57092</b>
500 mg/6 mL	30	<b>57094</b>

### Related Information

A complete listing of all Supelco SPE products for pesticide analyses, can be found on our website: [sigma-aldrich.com/spe-pesticide](http://sigma-aldrich.com/spe-pesticide)

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Sample Handling

**SUPELCO**  
Analytical

# An Improved BPE-DNPH Cartridge for the Simultaneous Determination of Ozone and Carbonyls

Jamie Desorcie and Shigehisa Uchiyama<sup>1</sup>  
jamie.desorcie@sial.com

1. National Institute of Public Health  
2-3-6, Minami, Wako City, Saitama 351-0197, Japan

The BPE-DNPH cartridge is a patent pending, dual-bed sampler for the simultaneous determination of airborne ozone and carbonyls. Each of the sampling beds consists of reagent-impregnated silica particles. The first bed contains *trans*-1,2-Bis(2-pyridyl)ethylene (BPE) while the second bed contains 2,4-dinitrophenylhydrazine (DNPH). In the sampling stream, the cartridge is configured such that air is first drawn through the BPE bed and then through the DNPH. Ozone reacts with BPE to form pyridine-2-aldehyde. Airborne carbonyls (aldehydes and ketones) pass unimpeded through the BPE to the second bed where they react with DNPH to form carbonyl DNPhydrazones. These chemical reactions are outlined in Figure 1. Following air sampling, the cartridge is eluted with solvent. It is during this stage that the pyridine-2-aldehyde (derived from ozone) comes into contact with residual DNPH to form its own carbonyl DNPhydrazone derivative. The resulting solution is analyzed by HPLC in order to measure all of the hydrazones and determine the amounts of captured ozone, aldehydes and ketones.

The BPE portion of the sampler provides the functions of both ozone measurement and ozone scrubbing. Thus, a traditional potassium iodide cartridge to remove potential interference by ozone is not needed.

## Improvements

In collaboration with a leading research group in Japan (1), we have developed and studied extensive improvements to the

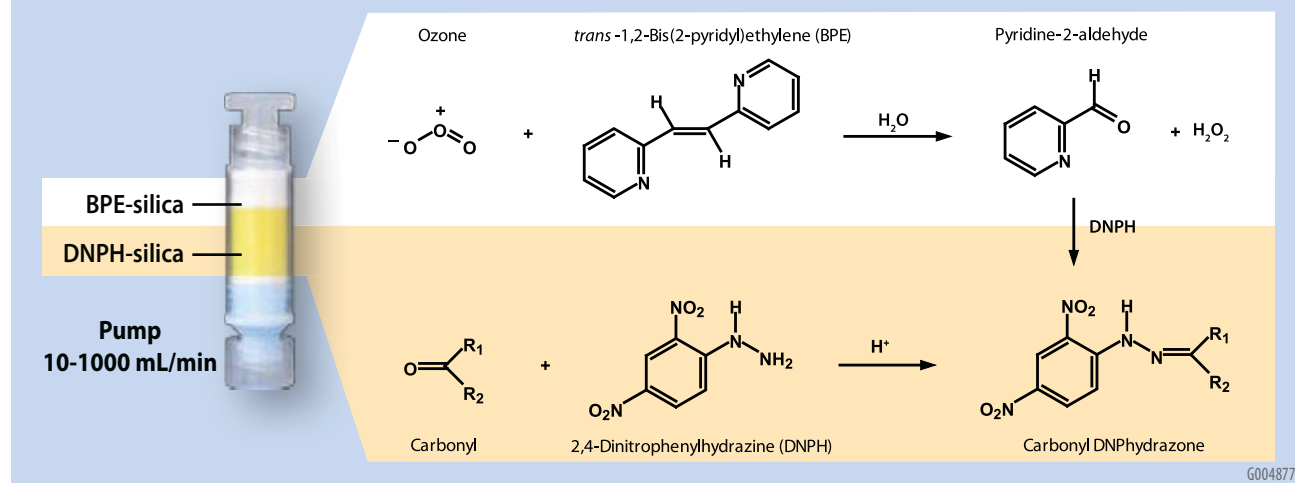
BPE-DNPH cartridge. Originally, *trans*-1,2-Bis(4-pyridyl)ethylene (4-BPE) was used as the ozone-capture reagent. However, we have found that the alternative isomer, *trans*-1,2-Bis(2-pyridyl)ethylene (2-BPE), offers several advantages. These include:

1. Better solubility of pyridine-2-aldehyde DNPhydrazone in acetonitrile/DMSO mixtures – More product derived from the reaction with ozone is eluted from the cartridge with less solvent.
2. Faster reaction with ozone and less dependence on atmospheric moisture – Implications are discussed below.
3. Faster reaction between pyridine-2-aldehyde and DNPH – Less wait time is required between cartridge extraction and HPLC injection. Addition of phosphoric acid to the extraction solvent increases the reaction rate even more.
4. Better HPLC peak shape of pyridine-2-aldehyde DNPhydrazone – Lower concentrations of ammonium acetate in the mobile phase are required.

*Advantage 2 is the most critical* and is presented here in more detail. In the laboratory, under conditions of controlled humidity, a 147 ppb concentration of ozone was measured using BPE-DNPH cartridges containing either 2-BPE or 4-BPE. The results are shown in Figure 2. From this data it is clear that 2-BPE reacts much more efficiently with ozone than 4-BPE. With 2-BPE, a consistent ozone concentration is measured following less than an hour of sampling. Alternatively, 4-BPE requires several hours of sampling before the measured ozone concentration no longer increases.

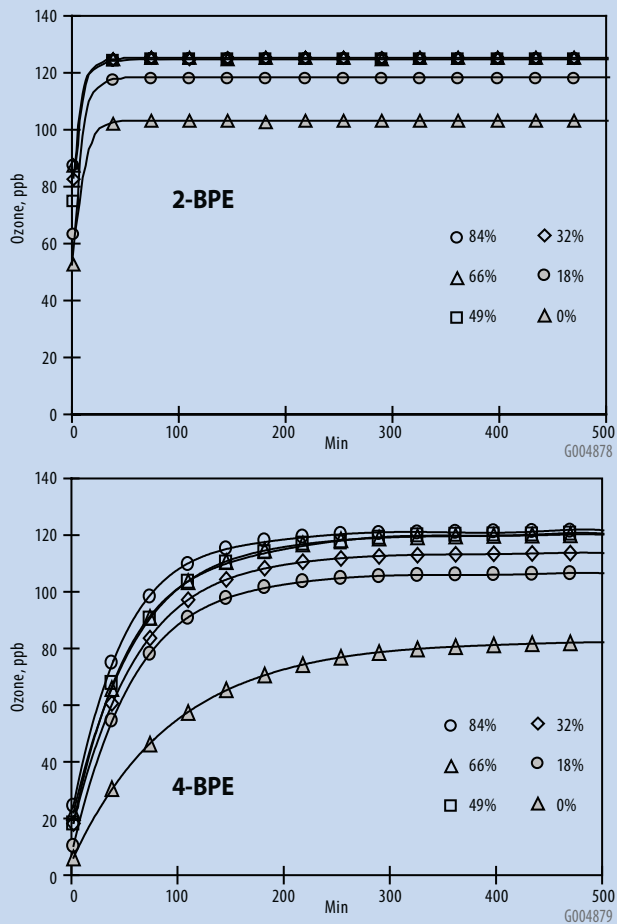
At 0% relative humidity (RH), the efficiency of the reaction with 2-BPE is 70% (103/147). Meanwhile, the reaction efficiency is only 55% (81/147) with 4-BPE at 0% RH. Both isomers of BPE exhibit greater reactivity toward ozone in the presence of higher levels of atmospheric moisture. 2-BPE exhibits a maximum reaction efficiency of 84% (123/147) at 32% RH and is clearly less dependent on atmospheric

Figure 1. BPE-DNPH Reaction Principle



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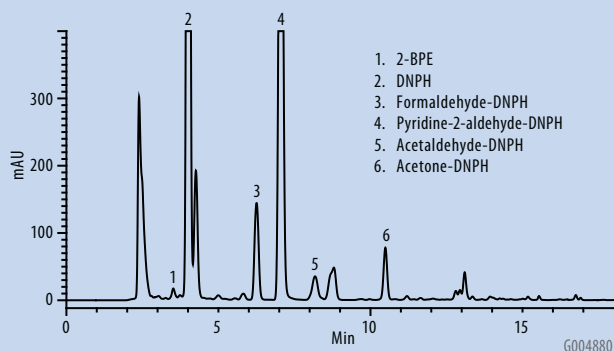
**Figure 2. Reactivity of Ozone with 2-BPE or 4-BPE at Various Relative Humidities**



**Figure 3. Pyridine-2-aldehyde and Other Carbonyl 2,4-DNPhydrazones from Laboratory Air Sample**

sampling tube: BPE-DNPH (54278-U)  
 extraction solvent: acetonitrile:DMSO (75:25) + 0.1% H<sub>3</sub>PO<sub>4</sub>  
 column: Ascentis® Express C18, 15 cm x 4.6 mm, 2.7 μm particles (53829-U)  
 mobile phase: A: 2mM ammonium acetate; B: acetonitrile  
 flow rate: 0.5 mL/min.  
 temp: ambient  
 det.: UV, 360 nm  
 inj.: 10 μL

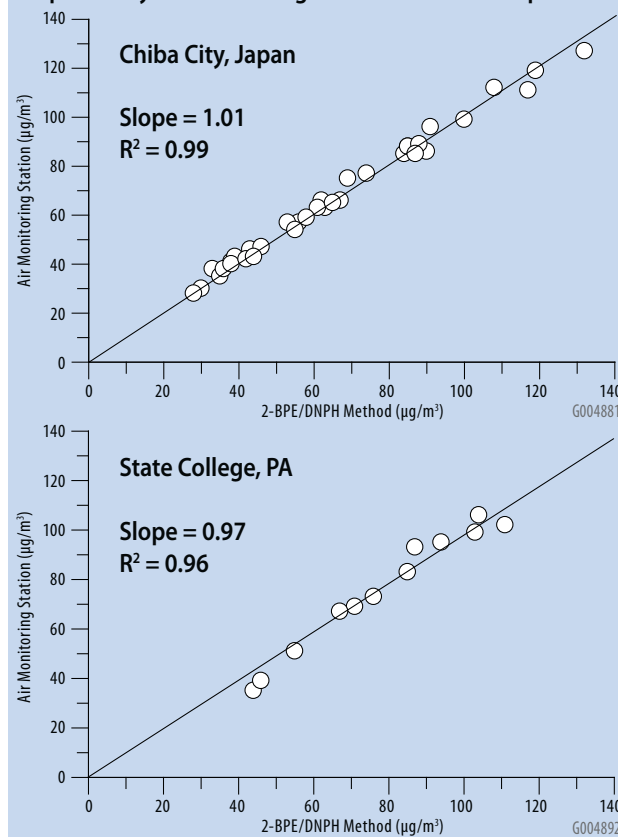
Gradient Profile		
Min	%A	%B
0	45	55
4	45	55
13	10	90
18	10	90



**Table 1. Conversion Factors for Ozone Measurements (Actual Ozone = BPE-DNPH Measured Ozone x Conversion Factor)**

% RH	Conversion Factor
0-10	1.39
10-20	1.30
20-30	1.22
>30	1.19

**Figure 4. Comparison of Outdoor Ozone Concentrations Measured Using the BPE-DNPH Cartridge and Data Reported by Air Monitoring Stations in US and Japan**



moisture than 4-BPE. Above 18% RH, the ozone reaction efficiency of 2-BPE is in the narrow range of 80-84%. Based on these findings, the conversion factors for the 2-BPE/DNPH cartridge listed in Table 1 are recommended.

### Method

The improvements to the BPE-DNPH cartridge culminate with the method and chromatogram depicted in Figure 3. Figure 4 demonstrates good correlation between outdoor ozone concentrations measured with the BPE-DNPH cartridge and data for the same time-period reported by local air monitoring stations.

### Reference

1. Uchiyama, S., Naito, S., Matsumoto, M., Inaba, Y., Kunugita, N., Anal. Chem. 2009, 81, 6552-6557.

# Extraction and Analysis of PAHs in Olive Oil using Molecularly Imprinted Polymer SPE and GC-MS

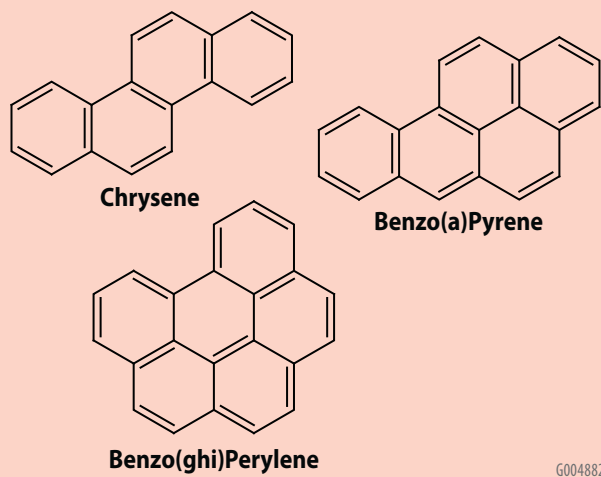
Olga Shimelis, Katherine Stenerson, Daniel Vitkuske  
Sanja Beyowich<sup>1</sup>, and Brian Boyd<sup>1</sup>

<sup>1</sup>. MIP Technologies AB, Scheelevägen 22, 220 07 Lund, Sweden

## Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) are environmental carcinogenic compounds that can be present in food from many different sources – either naturally or from pollution, packaging, or food preparation procedures such as heating and grilling (1). PAHs consist of fused aromatic rings. Due to the lipophilic nature of PAHs, fats and oils can be especially susceptible to PAH contamination. Previously, levels as high as 26 µg/kg of PAHs were found in virgin olive oils (2). The structures of representative PAHs are shown in Figure 1. In 2005 the European Union Commission Regulation (EC) No 208/2005 set maximum limits for PAHs in food. For oils and fats intended for direct human consumption, the maximum residue limit as benzo(a)pyrene on wet weight is 2 µg/kg (3). Edible oils are particularly difficult to analyze, because the oil matrix overloads HPLC columns.

Figure 1. Representative Structures of PAHs



SPE is one of the most popular methods for extraction of PAHs from oil. We have evaluated the recently developed molecularly imprinted polymer (SupelMIP® PAHs) for the extraction of PAHs from olive oil and subsequent analysis by GC-MS.

## Molecularly Imprinted Polymers

Molecularly imprinted polymers are a class of highly cross-linked polymer-based molecular recognition elements engineered to bind one specific target compound or a class of structurally related compounds with high

selectivity. The MIP material is designed with cavities that are sterically and chemically complementary to the target analyte(s). As a result, multiple interactions (e.g. hydrogen bonding, ionic, van der Waals, hydrophobic) can take place between the MIP cavity and the analyte.

## Extraction and Analysis of PAHs in Olive Oil

A standard mix of PAH compounds was prepared in methylene chloride and contained benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(123-cd)pyrene, dibenzo(ah)anthracene and benzo(ghi)perylene.

Store-purchased olive oil was spiked with PAHs in duplicate at the level of 2 µg/kg. Chrysene-d<sub>12</sub> was spiked as the internal standard at the level of 20 µg/kg.

0.5 g of spiked and blank oil sample was mixed with 0.5 mL cyclohexane and was extracted using the SupelMIP SPE procedure described in Table 1 and analyzed via GC-MS conditions detailed in Table 2.

Table 1. SupelMIP PAHs SPE Procedure

- SPE cartridge: SupelMIP SPE –PAHs, 50 mg/3 mL (52773-U)
1. Condition with 1 mL cyclohexane
  2. Load diluted oil sample
  3. Wash with 1 mL cyclohexane
  4. Elute with 3 x 1 mL ethyl acetate
  5. Evaporate SPE eluate under nitrogen at 40 °C and reconstitute in 0.2 mL ethyl acetate for analysis.

Table 2. GC-MS Conditions for PAH Analysis

- column: SLB-5ms, 30 m x 0.25 mm I.D., 0.25 µm (28471-U)  
instrument: Agilent® GC-MS  
oven: 60 °C, 25 °C/min. to 275 °C (5 min.),  
10 °C/min to 300 °C (1 min.)  
flow rate: helium, 2 mL/min., constant  
injector temp.: 300 °C  
MS detection: Autotune + EM offset of 300  
scan range: SIM  
injection: 1 µL, split

## Recovery

The recovery of PAHs was evaluated in the spiked olive oil samples. First, the blank sample of oil was analyzed for the presence of background contamination. The recovery values were corrected for the background levels found in the blank sample. The results are shown in Table 3.

Table 3. Analysis of Blank and Spiked Olive Oil Samples

Compound	Blank $\mu\text{g}/\text{kg}$	Spiked ( $2\mu\text{g}/\text{kg}$ ) Recovery
Benzo(a)anthracene	3.4	65%
Chrysene	9.4	70%
Benzo(b)fluoranthene	2.2	82%
Benzo(k)fluoranthene	1.4	84%
Benzo(a)pyrene	3.0	87%
Indeno(123-cd)pyrene	2.4	95%
Dibenzo(ah)anthracene	1.8	82%
Benzo(ghi)perylene	3.4	87%

PAH background contamination was found in the blank olive oil sample. The most abundant compound was found to be chrysene at almost  $10 \mu\text{g}/\text{kg}$ . This is consistent with previous studies that found light (2-4 rings) PAHs to be more predominant in the oils (2).

Using the SupelMIP SPE PAH approach, an average recovery of 82% was observed. All compounds contained at least 4 fused aromatic rings.

## Conclusions

In this article we described the extraction of PAHs from olive oil using SupelMIP SPE PAHs and analysis by GC-MS. The extraction procedure is very simple and includes only a 5-step SPE cleanup process. The procedure is amenable to larger PAH molecules. Extraction of smaller PAH molecules (2-3 rings) may be possible, however, lower recoveries may be expected.

## References

1. Purcaro G., Morrison P., Moret S., Conte L.S., Marriott P.J., J. Chromat. A 1161 (2007) 284-291.
2. Teixeira V.H., Casal S., Oliveira M.B.P.P. Food Chemistry 104 (2007) 106-112.
3. EU sets maximum levels of PAHs in food: [http://www.ihata.org/word\\_documents/EU\\_sets\\_maximum\\_levels\\_of\\_PAHs\\_in\\_food.doc](http://www.ihata.org/word_documents/EU_sets_maximum_levels_of_PAHs_in_food.doc)

## + Featured Product

Description	Cat. No.
SupelMIP SPE-PAHs, 50 mg/3 mL, pk. of 50	52773-U

## + Related Product

Description	Cat. No.
SLB-5ms, 30 m x 0.25 mm I.D., 0.25 $\mu\text{m}$	28471-U



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Sample Handling

SUPELCO Analytical

# Advantages of Reversed-Phase for Preparative Chiral Separations

*Astec CHIROBIOTIC columns operate in LC-MS- and prep-compatible reversed-phase mode*

**Jennifer E. Claus**  
jennifer.claus@sial.com

The use of reversed-phase mode in preparative separations provides a number of benefits over normal-phase methods. In addition to eliminating solubility issues often experienced in non-polar normal phase solvents, the utilization of reversed-phase chromatography uses less toxic solvents than those associated with normal-phase and provides timely sample recovery.

Unlike many cellulosic phases that are incompatible with reversed-phase modes, Astec CHIROBIOTIC™ phases possess multiple bonded chiral selectors, making them extremely robust and compatible with all commonly used HPLC solvents (1). The successful preparative separation of fluoxetine performed on a CHIROBIOTIC V2 in reversed-phase mode is illustrated in Figure 1. For rapid sample recovery following purification, fractions of the separated enantiomers may be run through a VersaPak® C18 flash cartridge. The analytes adhere to the cartridge and are washed with water to remove unwanted buffer salts and other mobile phase additives. This washing procedure ensures that the additives do not remain as contaminants in the pure final product. Each enantiomer may ultimately be eluted from the flash cartridge using a minimal amount of a polar organic solvent such as methanol or acetonitrile (100 mL or less). Removal of the organic solvent may be done quickly under vacuum, yielding the dried separated enantiomers.

Reversed-phase chromatography provides better solubility for polar analytes, uses nontoxic solvents, offers a method for removal of contaminants and mobile phase additives, and gives timely sample recovery with little solvent evaporation. In conclusion, the use of reversed-phase mode for preparative chiral separations is a safe and efficient way to execute successful chiral preparative separations.

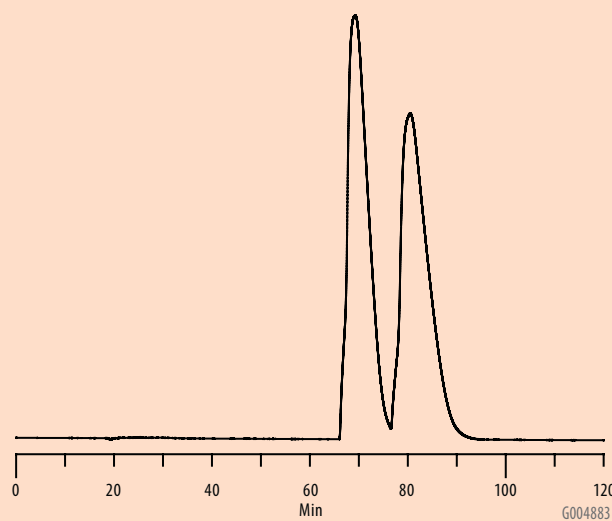
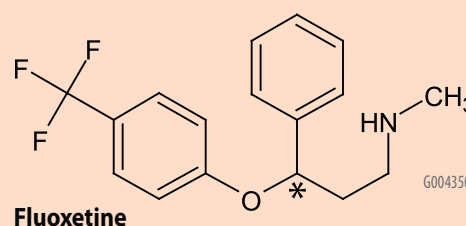
For the complete listing of our chiral chromatography products, please visit [sigma-aldrich.com/chiral-chromatography](http://sigma-aldrich.com/chiral-chromatography). The VersaPak cartridges we describe and the entire VersaFlash® system can be found at [sigma-aldrich.com/versaflash](http://sigma-aldrich.com/versaflash).

## Reference

1. CHIROBIOTIC Handbook (T408131) (Available from Supelco.)

**Figure 1. Chiral Preparative Separation of Fluoxetine on Astec CHIROBIOTIC V2 in Reversed-Phase Mode**

column: Astec CHIROBIOTIC V2,  
25 cm x 21.2 mm I.D., 5 µm particles  
mobile phase: 70:30, 20 mM ammonium acetate (pH 4.0):acetonitrile  
flow rate: 3.2 mL/min.  
temp.: 10 °C  
det.: UV at 230 nm  
injection: 88 µL  
sample: racemic fluoxetine, 50 mg/mL in mobile phase



## Featured Products

Description	Cat. No.
Astec CHIROBIOTIC V2, 25 cm x 21.2 mm I.D., 5 µm particles	15044AST
Astec CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles	15024AST
VersaPak C18 Cartridge, 23 mm x 110 mm I.D.	97760-U

# Supelco Preparative HPLC products for Pharmaceutical Development and Production

Wayne K. Way  
wayne.way@sial.com

There is an increasing need for purified materials in the development and production of pharmaceutical products. FDA and other regulatory agencies continue to require more stringent purity requirements on active pharmaceutical ingredients (API), including most recently chiral purity. Studies are often executed concurrently with impurities, degradants, and metabolites to determine if their potency or toxicity is a concern for the API in development. As the demand for purification increases, tools beyond classic crystallization are often needed. Some choices include filtration, distillation, solid phase extraction, thin-layer chromatography (TLC), low-pressure liquid chromatography (LPLC), and high-performance liquid chromatography (HPLC). In many instances, preparative HPLC is the most powerful and versatile method for the challenging purification tasks in the pharmaceutical industry.

## Stationary Phase Selection

The first important criterion for preparative chromatography is that the proper stationary phase is selected. A consideration beyond phase applicability is the commercial availability in both analytical and preparative dimensions as well as bulk packing material. Worldwide availability may be a consideration depending on final intended use. Typically, packing materials with 10 micron or greater particle size are used for preparative columns. Supelco offers three major product lines for preparative applications that are summarized in Table 1.

Although >80% of analytical HPLC separations are performed on reversed-phase silica phases, a majority of small molecule preparative chromatography separations are performed on normal-phase silica phases. Normal phase methods are often the first choice because:

- Removing organic solvents typically used in normal-phase are easier and more cost effective than removing aqueous solutions used in reversed-phase chromatography
- Easier method transfer from normal-phase TLC
- Cost of bare silica for normal-phase is typically less than the cost of C18 phases for reversed-phase

## Preparative HPLC Scale-Up

Analytical conditions are typically developed on a 25 cm x 4.6 mm I.D. column with 5 micron particles. If excessive resolution is available, a 10 cm x 4.6 mm I.D. column should be considered. Once chromatographic conditions are

Table 1. Summary of Supelco HPLC Product Lines for Preparative Applications

Product Line	Application	Particle Size (µm)	Multiple Phases	Bulk Available
Ascentis	Small Molecule	3, 5, 10	Yes	Yes
Astec	Chiral	5, 10, 16	Yes	Yes
Discovery® BIO	Peptide/Protein	3, 5, 10	Yes	Yes

optimized, a loading study on the analytical dimension is recommended. At this point, successful scale-up from analytical to preparative work is quickly and reliably obtained by using two simple formulas. The formulas are displayed in Table 2.

Table 2. Formulas for Scaling Analytical to Preparative Applications

**Loading Capacity:**  $I_p = I_a \times (D_p/D_a)^2 \times L_p/L_a$

Where:

$I_p$  = Injection load of preparative column

$I_a$  = Injection load of analytical column

$L_p$  = Length of preparative column

$L_a$  = Length of analytical column

$D_p$  = Internal diameter of preparative column

$D_a$  = Internal diameter of analytical column

**Flow Rate:**  $F_p = F_a \times (D_p/D_a)^2$

Where:

$F_p$  = Flow rate of preparative column

$F_a$  = Flow rate of analytical column

$D_p$  = Internal diameter of preparative column

$D_a$  = Internal diameter of analytical column

## Ascentis for Small Molecule Purification

The Ascentis HPLC product line has several features that make it the ideal platform for small molecule HPLC purification. The benefits are outlined below.

Feature	Advantage
High surface area (450 m <sup>2</sup> /gram)	High loading
Stable bonding, dense endcapping	Low column bleed, high recovery
Available in C18, C8, bare silica, and other phases	Suitable for normal-phase or reversed-phase with unique selectivities
3, 5, and 10 micron particles	Predictable scaling

Shown in Figure 1 are the results of a loading experiment. The analytes used were quinidine and dihydroquinidine, two closely-related compounds that are typical of the type of preparative separation in which Ascentis might be utilized.

(continued on page 16)

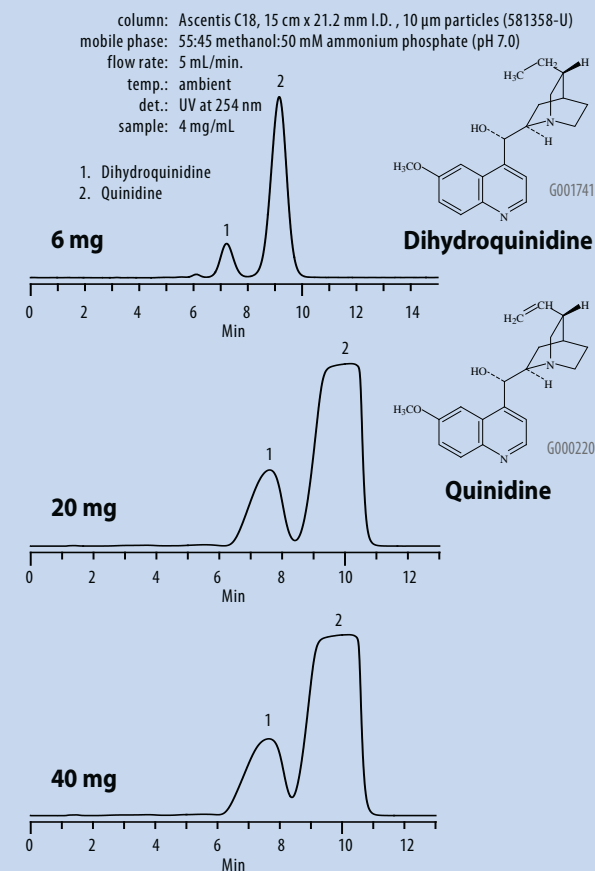
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(continued from page 15)

This study utilized a 15 cm x 21.2 mm I.D. column packed with 10 micron particles. The separation was performed in the reversed-phase mode with C18 as the stationary phase. The high surface area Ascentis columns provided maximum loading capacity for this small molecule application.

**Figure 1. High Loading Capacity**

Stable, highly selective bonded phases with the loading capacity necessary to carry out challenging preparative separations.

### Astec CHIROBIOTIC for enantiomeric purification

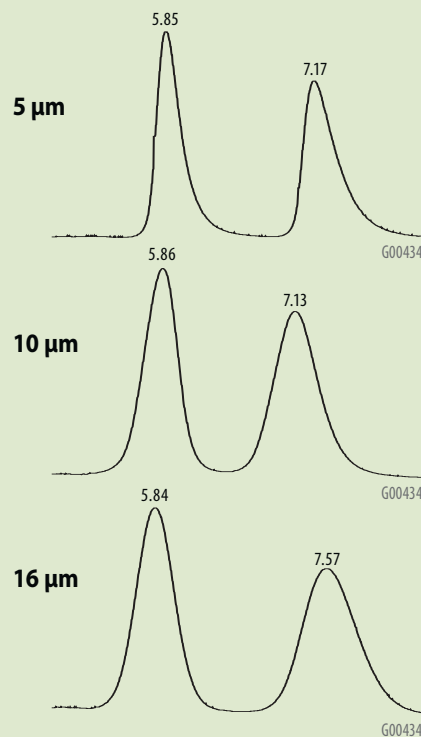
The Astec CHIROBIOTIC product line has several features that make it useful for enantiomeric purification. The benefits are outlined below.

Feature	Benefit
Immobilized/bonded phases	Can't accidentally wash off phase with wrong solvent
Suitable for all solvents	Performs in the solvents you need
Complimentary selectivity to cellulosics	First choice if cellulosics fail
5, 10, and 16 micron particles	Predictable scaling

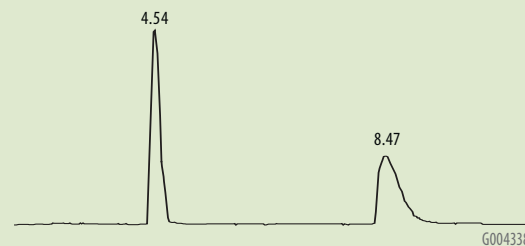
CHIROBIOTIC columns can be used in all preparative HPLC techniques, including simulated moving bed (SMB), supercritical fluid chromatography (SFC), and mass-directed prep.

**Figure 2. Scalability Across CHIROBIOTIC CSP Particle Sizes**

column: CHIROBIOTIC T, 25 cm x 4.6 mm  
mobile phase: 50:50 ethanol:water  
flow rate: 0.9 mL/min.  
det.: UV at 220 nm

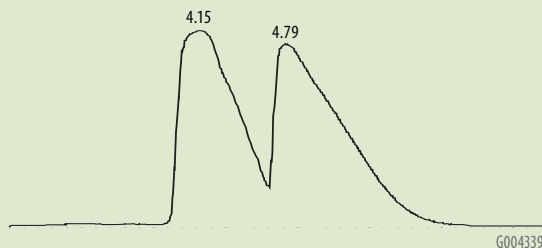
**Figure 3. Sample Solubility Considerations in Preparative Analytical Scale**

column: CHIROBIOTIC V, 25 cm x 4.6 mm, 5  $\mu$ m particles (11024AST)  
mobile phase: CH<sub>3</sub>OH  
det.: UV at 293 nm  
flow rate: 1 mL/min.  
analyte: thalidomide



### Prep Scale

column: CHIROBIOTIC V, 25 cm x 21.2 mm, 5  $\mu$ m particles (11044AST)  
mobile phase: 80:20 CH<sub>3</sub>OH:dioxane  
det.: UV at 313 nm  
flow rate: 20 mL/min.  
load: 70 mg in 12 mL  
analyte: thalidomide



Prep separations on CHIROBIOTIC are reproducible and scalable, as shown in Figure 2. Figure 2 shows the separation of a racemic mixture of phenylalanine on columns packed with 5, 10, and 16 micron particles of CHIROBIOTIC T.

Another advantage of CHIROBIOTIC for preparative applications is that the mobile phase flexibility can be utilized to optimize sample solubility. Figure 3 shows the analytical and preparative separation of thalidomide on CHIROBIOTIC V. The analytical scale method showed a high selectivity with a 100% methanol mobile phase. However, since thalidomide is poorly soluble in pure methanol, it was possible to add 20% dioxane to the mobile phase to increase solubility three-fold while still achieving the necessary separation.

### Discovery BIO for Peptide and Protein Purification

The Discovery BIO HPLC product line has several features that make it the ideal platform for peptide and protein purification. The benefits are outlined below.

Feature	Benefit
300 Å pore size	The standard for protein separations
Available in C18, C8, C5 phases	Selective phases optimized for proteins and peptides
Advanced bonding techniques	Good peak shape
3, 5, and 10 micron particles	Predictable scaling

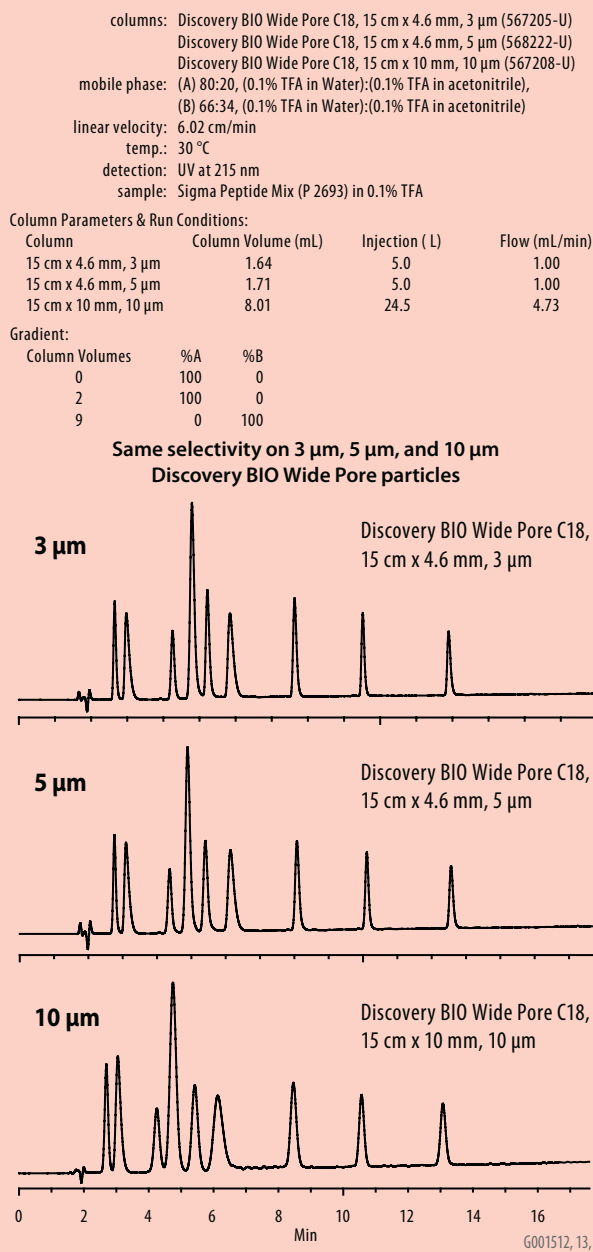
Reversed-phase chromatography is often used in the final polishing steps of oligonucleotides and peptides, and is ideal for scaling from analytical to preparative purification. While reversed-phase chromatography can be used for protein purification, it is typically not recommended if recovery of activity or correct tertiary structure are required, since many proteins are denatured when exposed to reversed-phase solvents. Discovery BIO HPLC columns are available in three different phase hydrophobicities (C18, C8 and C5) as well as three different particle sizes (3, 5 and 10 micron).

Shown in Figure 4 is the separation of a peptide mix on all three available particle sizes. The same selectivity is obtained on all three particles making scale up quite easy and predictable. Although peaks were not identified for this study, the peptide mix contains *bradykinin*, *bradykinin fragment 1-5*, *substance P*, *[Arg8]-vasopressin*, *luteinizing hormone releasing hormone*, *bombesin*, *leucine enkephalin*, *methionine enkephalin* and *oxytocin*.

### Conclusions

Sigma-Aldrich offers a complete line of HPLC purification products for small molecule, chiral, and peptide/protein separations. Beyond HPLC purification methods, Sigma-Aldrich offers other purification products such as Flash, bulk silica, TLC, glass columns, and solvents.

**Figure 4. Matched Selectivity from Analytical to Preparative on Discovery BIO Wide Pore C18**



### Featured Products

Description	I.D. (mm)	Length (cm)	Particle Size (µm)	Cat. No.
Ascentis C18	4.6	25	5	581325-U
Ascentis C18	10.0	25	10	581355-U
Ascentis C18	21.2	25	10	581359-U
Astec CHIROBIOTIC V2	4.6	25	5	15024AST
Astec CHIROBIOTIC V2	10.0	25	5	15034AST
Astec CHIROBIOTIC V2	21.2	25	5	15044AST
Astec CHIROBIOTIC T	4.6	25	5	12024AST
Astec CHIROBIOTIC T	10.0	25	5	12034AST
Astec CHIROBIOTIC T	21.2	25	5	12044AST
Discovery BIO Wide Pore C18	4.6	25	5	568223-U
Discovery BIO Wide Pore C18	10.0	25	10	567209-U
Discovery BIO Wide Pore C18	21.2	25	10	567212-U

ordering: 800-247-6628 (US only) / 814-359-3441 technical service: 800-359-3041 (US and Canada only) / 814-359-3041

# Ascentis Express Family of Selectivities

Ascentis Express HPLC columns with Fused-Core™ technology provide fast separations on both conventional and UHPLC instruments. Separations on Ascentis Express meet the efficiency of sub-2 μm particles, but at back pressures similar to 3 μm particles. Even higher efficiencies than are possible on sub-2 μm particles can be achieved on Ascentis Express using longer columns on ultra high-pressure (UHPLC) instruments.

While efficiency is valuable to chromatographic resolution, it does nothing to help resolve perfectly co-eluting peaks or change the elution order. To do either of these requires changing the types of molecular interactions between the analytes and the stationary phase, which is accomplished primarily by changing the bonded phase chemistry.

## Chromatographic Selectivity and Resolution

The resolution equation (Eqn. 1) shows that chromatographers can change column efficiency ( $N$ ), retention ( $k$  or capacity factor) and selectivity ( $\alpha$ ) to improve a separation.

$$\text{Eqn. 1: } R = \frac{\sqrt{N}}{4} \cdot \frac{k}{k+1} \cdot \frac{\alpha-1}{\alpha}$$

Of the three parameters,  $N$ ,  $K$  and  $\alpha$ , selectivity ( $\alpha$ ) has the greatest affect on resolution. Also, its affect does not have a limit, as do the affects of efficiency and retention. Changing the mobile phase and the stationary phase, and sometimes temperature, can change selectivity. However, chromatographers usually find changing the column is the easier approach.

## Ascentis Express: Leveraging the Power of Stationary Phase Chemistry

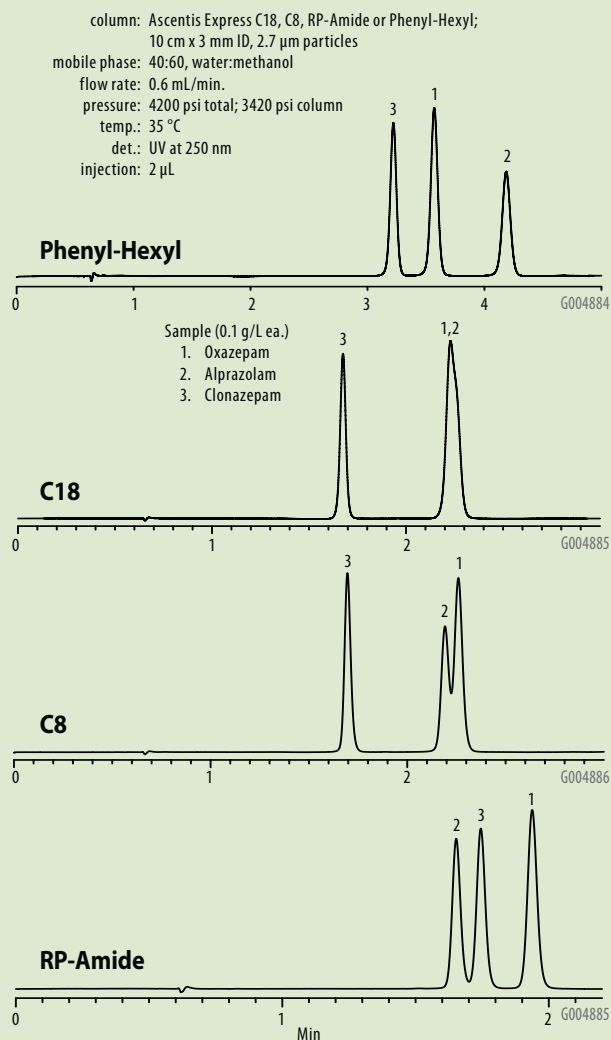
The Ascentis Express reversed-phase family comprises four chemistries: the workhorse C18, the alternate C8, the polar-embedded RP-Amide and the aromatic Phenyl-Hexyl. Ascentis Express HILIC is available for normal phase. The different phases provide selectivity choices that enable rapid method development and optimization. The accompanying figure shows the range of selectivities the reversed-phases offer. Under the same mobile phase conditions the four Ascentis Express phases give different elution patterns and peak order reversals. In this example, the Phenyl-Hexyl provided the ideal peak spacing. For other separations, the right column would depend on the analytes and goals of the method: whether to increase or decrease retention or peak spacing, or change the elution order.

## Product Listing

Following is a partial listing of Ascentis Express dimensions. Please consult our web site or call Technical Services for the complete Ascentis Express offering, including guard columns and high-performance hardware.

Phase	Dimensions	Cat. No.
<b>Ascentis Express Columns</b>		
C18	15 cm x 4.6 mm I.D.	53829-U
C18	10 cm x 4.6 mm I.D.	53827-U
C18	10 cm x 3 mm I.D.	53814-U
C18	5 cm x 2.1 mm I.D.	53822-U
C8	15 cm x 4.6 mm I.D.	53838-U
C8	10 cm x 4.6 mm I.D.	53837-U
C8	10 cm x 3 mm I.D.	53852-U
C8	5 cm x 2.1 mm I.D.	53831-U
RP-Amide	15 cm x 4.6 mm I.D.	53931-U
RP-Amide	10 cm x 4.6 mm I.D.	53929-U
RP-Amide	10 cm x 3 mm I.D.	53918-U
RP-Amide	5 cm x 2.1 mm I.D.	53911-U
Phenyl-Hexyl	15 cm x 4.6 mm I.D.	53353-U
Phenyl-Hexyl	10 cm x 4.6 mm I.D.	53352-U
Phenyl-Hexyl	10 cm x 3 mm I.D.	53345-U
Phenyl-Hexyl	5 cm x 2.1 mm I.D.	53334-U
HILIC	15 cm x 4.6 mm I.D.	53981-U
HILIC	10 cm x 4.6 mm I.D.	53979-U
HILIC	10 cm x 3 mm I.D.	53970-U
HILIC	5 cm x 2.1 mm I.D.	53934-U

**Figure 1. Selectivity Differences in Ascentis Express Phases**



# LC-MS CHROMASOLV Solvents, Blends and Additives

## High Purity for Accurate Analysis

**Shyam Verma**

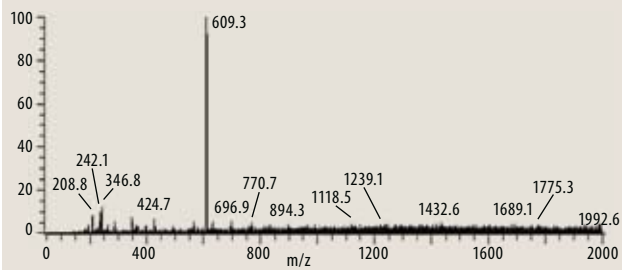
shyam.verma@sial.com

Solvent impurities are the most common cause of extraneous peaks and unstable LC-MS baseline. Solvent-derived impurities do not condition out over time. Most common contaminants include inorganic ions, decomposition products, microbes and their excretion products and particulate matter. These impurities can interfere in the analysis in multiple ways, such as: 1) collect on the head of an HPLC column and elute as a distinct peak or as baseline rise, 2) cause general elevation in baseline, lowering sensitivity of analysis, 3) foul or damage sensitive instrument components, and 4) cause cluster ion formation that prevents reliable identification and quantification.

The LC-MS CHROMASOLV® solvents undergo 34 distinct and relevant tests to ensure solvent requirements of sensitive LC-MS analyses. Some of the most important features are:

- Application-tested for LC-MS using the reserpine tests (Figure 1)
- Very low level of inorganic and metal ions for high sensitivity spectra
- Particle/non-volatile compound-free to maintain system integrity
- Low gradient baseline with your own optimized protocols
- Significantly reduced level of phthalate contaminants

**Figure 1. Reserpine Test: reserpine spectrum measured in Methanol (Cat. No. 34966); no signals should be greater than  $[M+H]^+=609$  (100 ppb reserpine; ESI, positive mode)**



### Pre-Blended LC-MS Solvents

The mobile phase composition plays a critical role in the success of an LC-MS experiment. Precise formulations provide accurate and reproducible results. Sigma-Aldrich offers pre-blended solutions of most commonly-used LC-MS mobile phases prepared with precision and unsurpassed attention to quality. Using the precisely blended solvents eliminates time-consuming mobile phase preparation, and can eliminate lost sample information and instrument downtime caused by impure mobile phase. A special formulation assures that no precipitation or decomposition of the additive occurs under normal laboratory conditions. These pre-blended solvents offer: 1) time saving, 2) accurate composition, 3) minimized baseline and artifacts, and 4) high quality.

### Additives

For minimizing the background and artifacts in LC-MS analysis, highly-specified solvents are spiked with ultra pure salts and acids. These additives improve the chromatographic peak shape and optimize ionization in the MS interface.

### Flush Solution

LC-MS Flush Solution is convenient for keeping your LC-MS equipment clean and operational for reliable analysis. In order to maintain minimum baseline and reduce extraneous peaks, the LC-MS system should be flushed at regular intervals depending upon the nature of the samples. A commonly employed flush solution is 50% isopropanol in water that solubilizes both inorganic and moderately hydrophobic contaminants.

It is critical that the purity of the flush solution be such that it does not add contaminants to the LC-MS system. Our CHROMASOLV Flush Solution (Cat. No. 34689) comprises a 50% v/v mixture of 2-propanol (isopropanol) in water. Both components are our highest quality LC-MS CHROMASOLV solvents. The mixture has been tested for GC and LC-MS purity, water content by Karl Fischer titration, organic and non-volatile impurities, UV transmittance and levels of sixteen inorganic ions. To ensure your flush solution is not a source of impurities, use CHROMASOLV Flush Solution. The convenience of the preblended mixture and the high purity make it an ideal solution for meeting challenges of both routine and high-throughput LC-MS analyses.

### Featured Products

Description	Cat. No.
<b>LC-MS CHROMASOLV Solvents</b>	
Water	39253
Acetonitrile	34967
Methanol	34966
2-Propanol	34965
Ethyl acetate	34972
<b>LC-MS CHROMASOLV Solvent Blends</b>	
Acetonitrile with 0.1% TFA	34976
Methanol with 0.1% TFA	34974
Acetonitrile with 0.1% formic acid	34668
Acetonitrile with 0.1% ammonium acetate	34670
Acetonitrile with 0.1% formic acid and 0.1% TFA	34676
Water with 0.1% TFA	34978
<b>LC-MS CHROMASOLV Mobile Phase Additives</b>	
Trifluoroacetic acid, puriss p.a.	40967
Formic acid, puriss p.a.	56302
Acetic acid, puriss p.a.	49199
Ammonium formate, puriss p.a.	55674

ordering: 800-247-6628 (US only) / 814-359-3441 technical service: 800-359-3041 (US and Canada only) / 814-359-3041

Liquid Chromatography

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# LC-MS CHROMASOLV Water

Sigma-Aldrich offers LC-MS CHROMASOLV Water with quality suitable for both gradient HPLC and MS applications. This product offers tremendous advantages over other quality grades. It can be used in both UV and MS detection methods, without any compromise. Figure 1 clearly shows differences between LC-MS CHROMASOLV Water and a non-gradient grade water.

The water quality mentioned above also applies to all its blends with other mobile phase CHROMASOLV additives. These pre-blended solvents offer accurate composition with minimized baseline and artifacts, and significant time savings.

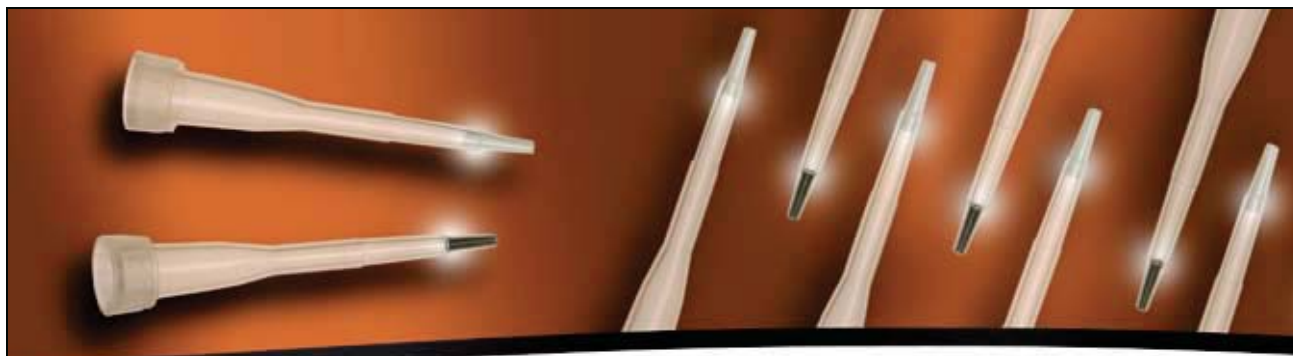
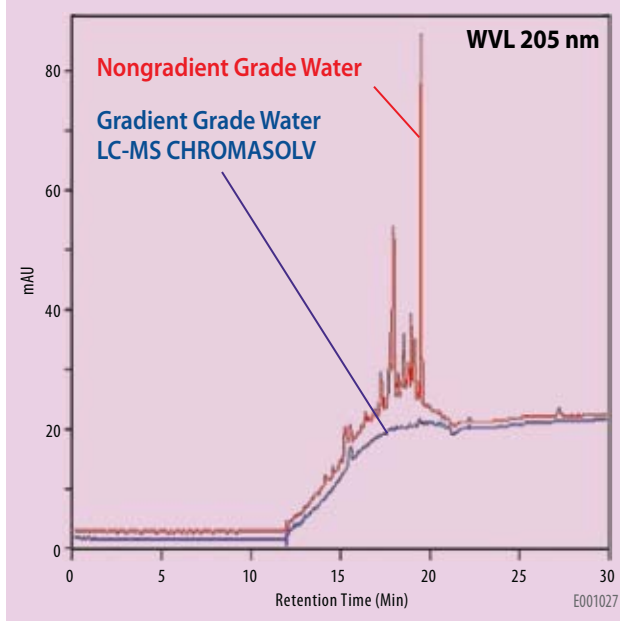
## Featured Products

Description	Qty.	Cat. No.
Water	1 L	39253
Water with 0.1% trifluoroacetic acid (TFA*)	2.5 L	34978
Water with 0.1% formic acid and 0.01% TFA	2.5 L	34677
Water with 0.1% ammonium acetate*	2.5 L	34674
Water with 0.1 % formic acid**	2.5 L	34673
Water with 0.1% acetic acid**	2.5 L	34675

\*LC-MS CHROMASOLV

\*\*Other blends available. Please inquire.

Figure 1. UV Gradient at 205 nm, LC-MS Water (39253) and Nongradient Grade Water



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**20% off the list price**

Mention promotion code **987**  
when placing your order

(Offer valid until 12/31/09)

Description	Qty.	Cat. No.
Supel-Tips Zr Pipette Tips, 10 µL	96	54266-U
Supel-Tips Ti Pipette Tips, 10 µL	96	54263-U
Supel-Tips C18 Pipette Tips, 10 µL	96	TPSC18-96EA
	960	TPSC18-960EA
Supel-Tips Carbon Pipette Tips, 10 µL	96	54227-U

For complete listing of our products or to request a free sample, please visit [sigma-aldrich.com/pipette-tips](http://sigma-aldrich.com/pipette-tips), or contact our Technical Service at 800-359-3041 (US and Canada) or 814-359-3041 or [techservice@sial.com](mailto:techservice@sial.com).

## NEW! Oil & Grease Standards for US EPA Method 1664

Vicki Yearick,  
vicki.yearick@sial.com

Our new NSI Solutions Oil and Grease PAR (Precision and Recovery) Surrogate standards are designed to meet the requirements of USEPA Method 1664, n-Hexane Extractable Material (HEM; Oil and Grease) and Silica Gel Treated N-Hexane Extractable Material (SGTHEM; Non-polar Material) by Extraction and Gravimetry. NSI Oil and Grease PAR (Precision and Recovery) Surrogate standards are packaged in easy-to-use, inert, thermally sealed PTFE tubes,



SNIPS. Simply snip off the top of the tube and pour the entire pre-measured contents into your extraction vessel. Oil and grease control samples packaged in bottles and ampulized recovery standards are also offered.

Each oil and grease standard purchase includes a certificate of traceability listing certified concentrations and acceptance limits.

These products are available only in the USA at this time. Order online at [sigma-aldrich.com/nsisolutions](http://sigma-aldrich.com/nsisolutions) or by calling Customer Service at 800-811-5352.

### Benefits:

**Convenient** – no measuring or weighing

**Consistent** – precise and accurate volumes delivered each time

**Cost effective** – 25 SNIPS for the price of 20!

Description	Pkg. Size	Cat. No.
<b>Surrogate Standards</b>		
Method 1664 Oil and Grease PAR Surrogate (0.2%) <i>0.2% n-hexadecane, 0.2% stearic acid in acetone delivers 40 mg total oil &amp; grease per tube</i>	25 x 10 tubes	<b>NSI-QC-003LSNIP</b>
Method 1664 Oil and Grease PAR Surrogate (0.4%) <i>0.4% n-hexadecane, 0.4% stearic acid in acetone delivers 80 mg total oil &amp; grease per tube</i>	25 x 10 tubes	<b>NSI-QC-003LSNIP</b>
<b>Control Samples</b>		
Oil and Grease, Ready-to-use bottles	500 mL 4 x 500 mL	<b>NSI-QCI-069</b> <b>NSI-QCI-069C</b>
<i>Works with freon and hexane extraction. Oil and grease supplied as 20 - 100 mg/L. Minimum of 10 units available per year.</i>		
Oil and Grease, Supplied in Boston Round bottle	1 L 4 x 1 L	<b>NSI-QCI-069-33,400</b> <b>NSI-QCI-069-33,400C</b>
<i>Designed for use with SPE. Oil and grease supplied as 20 - 100 mg/L. Minimum of 10 units available per year</i>		
<b>Recovery Standards</b>		
Oil and Grease Method 1664 Recovery Standards (0.4%) <i>0.4% (w/v) n-Hexadecane and 0.4% (w/v) Stearic acid in acetone</i>	25 mL 10 x 25 mL	<b>NSI-QC-003</b> <b>NSI-QC-003TP</b>
Oil and Grease Method 1664 Recovery Standards (0.2%) <i>0.2% (w/v) n-Hexadecane and 0.2% (w/v) Stearic acid in acetone</i>	25 mL 10 x 25 mL	<b>NSI-QC-0003L</b> <b>NSI-QC-003LTP</b>

## Quantitative Pesticide Calibration Mixtures Available from Sigma Aldrich



Analysts screening or monitoring pesticides and pesticide metabolites can choose from a wide selection of Supelco brand calibration mixtures for their specific applications. These quantitative mixtures eliminate

the time and money associated with sourcing individual raw materials, preparing the mixture, and then disposing of unused hazardous materials. Supelco brand mixtures also meet the needs of commercial testing laboratories seeking a reference standard from a second source.

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If your application requires a custom tailored pesticide mixture, we can help. Our custom standard chemists will quickly formulate, test, and /or package a mixture to your exact specifications. We will also discuss stability and solubility concerns with you and make suggestions where needed to improve the quality of your purchase. If you are interested in using our custom standard services, please feel free to contact us by e-mail at [customstandards@sial.com](mailto:customstandards@sial.com), or use the quote request form on our website [sigma-aldrich.com/standards](http://sigma-aldrich.com/standards).

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Standards

**SUPELCO**  
Analytical

# PNA/PONA/P-I-A-N-O Calibration Standards for Detailed Hydrocarbon Analyses

Vicki Yearick and Steve Cecil

techservice@sial.com

Detailed hydrocarbon analysis (DHA) applications are widely used in the petroleum industry for the analysis and characterization of light petroleum fractions having boiling points up to 450 °F (225 °C). These applications comply with ASTM methods D 5134, D 6729, D 6730, and D 6733 in which single column gas chromatography (GC) technology is used to group the hydrocarbon components by structure.

Petroleum streams analyzed by DHA include naphtha, alkylate, reformer feed, reformate, isomerate, gasoline and compressed liquids. DHA characterizes these petroleum streams based on the Kovats Index, and differentiates the composition and concentrations into five groups that are collectively called **PIANO**: Paraffins, Isoparaffins, Aromatics, Naphthenes and Olefins.

Precise calibration of the chromatographic system is a critical requirement for running DHA applications. Calibration utilizes a set of six complex, quantitative analytical standards known as the PIANO standards. These standards are difficult for most laboratories to prepare due to their complexity and the lack of commercial sources for obtaining the many branched chain hydrocarbons present in the mixtures.

Sigma-Aldrich is addressing this need by offering Alphagaz™ PIANO standards. These standards are available in kit form, as well as individual mixtures. The kit includes a 139 component, quantitative multi-group PIANO mix, plus the five individual quantitative PIANO group mixes. Utilizing these six high-quality analytical standards insures your GC system is optimized for proper performance of the DHA analyzer.

Our Alphagaz PIANO standards are accurately prepared by weight to three decimal places. Each mixture is shipped with a comprehensive analytical data sheet listing components by weight percent, mole percent, liquid volume percent, retention times, and retention indices for each

component. A chromatogram of each mixture ran on a 100-meter capillary column is also provided. Standards are supplied in crimp-top vials with hole caps and septa.

Routine quality assurance checks are equally important, since DHA methods require high precision from your gas chromatography system. Regular analysis of the Supelco 139-component PIANO Mix reveals when it is necessary to perform maintenance on your GC system, including the replacement of the GC column.

For optimal DHA system performance, the GC column should be a high-quality, petrochemical-specific column. The 100-meter Supelco Petrocol™ DH column is specifically manufactured for the analysis of complex hydrocarbon mixtures and is ideally suited for DHA systems.

## + Featured Products

Description	Qty.	Cat. No.
<b>Varied concentration, lot specific</b>		
n-Paraffins Mix	0.1 mL	44585-U
Isoparaffins Mix	0.1 mL	44586-U
Aromatics Mix	0.1 mL	44587
Naphthenes Mix	0.1 mL	44588
Olefins Mix	0.1 mL	44589
P-I-A-N-O Mix	0.1 mL	44593-U

*A single quantitative mix containing 139 paraffins, isoparaffins, aromatics, naphthenes, and olefins*

P-I-A-N-O Kit	6 x 0.1 mL	44594-U
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*Contains 1 each of 44585-U, 44586-U, 44587, 44588, 44589, and 44593-U.*

Visit [sigma-aldrich.com/piano](http://sigma-aldrich.com/piano) for complete product compositions.

## + Related Products

**Petrocol DH** – A highly reproducible column displaying more than 40,000 theoretical plates, designed for detailed analysis of complex petroleum products, including PNA, PONA, and PIANO-type analyses.

Description	Cat.No.
Petrocol DH, 100 m x 0.25 mm ID, 0.50 µm df	24160-U

## Booth 2714 2009 AAPS Annual Meeting and Exposition

**November 8-12, 2009,**

Los Angeles Convention Center  
Los Angeles, California

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**3 Poster Presentations in West Exhibit Hall A:**

**Tuesday, November 10, 2009: 8 am – 12 pm**

*Increased Bioanalytical Throughput Utilizing Fused-Core™ Particles with Selective Phospholipid Depletion Biocompatible, Solid Phase Microextraction for Facilitated Sample Preparation in Bioanalysis*

**Thursday, November 12, 2009: 8 am – 12 pm**

*Retention Mechanisms in Chiral Chromatography: LC-MS Analysis Using Macrocyclic Glycopeptide Chiral Stationary Phases*

# QSertVial Kits - The Ultimate Microsampling Device



E001074

QSertVial™ and closure kits eliminate all the problems associated with using microvolume sample inserts. No more searching for the proper inserts. No more worry about insert sealing and alignment. The QSertVial contains a 300 µL glass conical insert fused to a 12 x 32 mm vial forming a precise integral unit. The rim of the insert is positioned slightly above the top of the vial so the septum can make a secure seal with the closure, eliminating evaporation and cross contamination. The insert is centered into the vial, 2 mm from the bottom, allowing for consistent sample recovery and eliminating problems caused by variation in insert depth.

QSertVial kits are available in 9 mm screw thread finish, 11 mm crimp top, and a snap ring design. The 9 mm vial kits include vials with fused inserts and closures with bonded septa to retain the liner and decrease contamination.

The crimp top vial kits contain one-piece septumless polyethylene caps that seal the vial without using rubber or silicone liners. These special caps, also known as the Pico Pure Plus+™ closures, are designed specifically for LC-MS applications. Extractable impurities from these caps are well below the picogram range

The snap top vial kits are offered in clear glass with a PTFE liner, a PTFE/silicone liner, or a pre-slit PTFE/silicone liner. The polypropylene snap top cap allows the closure to be attached to the vial without the use of a crimper.

## Benefits of the QSertVial Kits

- Evaporation and cross contamination between insert and vial are eliminated.
- Higher sample recovery with either bottom loading or side loading needles (point style 2 and 5). Residual dead volume can be as low as 4 µL, depending on precision adjustment of the instrument.
- Consistent sample recovery from vial to vial.
- Wide-mouth opening has larger target area reducing the possibility of bent needles.
- Compatible with most major autosamplers.
- White matte-finish marking panel allows for easy sample identification.

The QSertVial products for microsampling compliment the already extensive line of chromatography vials and accessories currently being offered by Supelco Analytical. For more information on any of the kits, email our Technical Service Department at [techservice@sial.com](mailto:techservice@sial.com) or visit us on the web at [sigma-aldrich.com/vials](http://sigma-aldrich.com/vials).

Description	Cat. No.
<b>QSertVial Kits, 300 µL, 9 mm thread, pk. of 100</b>	
Clear glass, bonded PTFE/silicone septa	29391-U
Clear glass, bonded PTFE/silicone septa with slit	29392-U
Clear glass, bonded PTFE/silicone/PTFE septa with slit	29394-U
Amber glass, bonded PTFE/silicone septa	29398-U
Amber glass, bonded PTFE/silicone septa with slit	29401-U
Amber glass, bonded PTFE/silicone/PTFE septa with slit	29402-U
<b>QSertVial Kits, 300 µL, Snap Top, pk. of 100</b>	
Clear glass, PTFE liner	29427-U
Clear glass, PTFE/silicone liner	29428-U
Clear glass, pre-slit PTFE/silicone liner	29429-U
<b>QSertVial Kits, 300 µL, Crimp Top, pk. of 100</b>	
Clear glass, cap with septumless liner	29406-U
Amber glass, cap with septumless liner	29416-U

## Seminars at The Eastern Analytical Symposium

Booth  
**318**

**November 17, 2009**

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Somerset, New Jersey

Visit Supelco/Sigma-Aldrich  
at Booth 318

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**9:45 - 10:45** – *Strategies for Chiral HPLC Method Development*

**11:00 - 12:00** – *Fast HPLC with Ascentis® Express Fused-Core™ HPLC Columns... extend the value of traditional HPLC instruments*

**1:00 - 2:00** – *SPME Fibers for Bioanalytical Sample Prep*

**2:15 - 3:15** – *HybridSPE™-Precipitation: An Innovation in Sample Prep Through Selective Phospholipid and Protein Removal*

**Seminars hosted by Supelco/Sigma-Aldrich.**

To register for any or all of the above seminars, visit [sigma-aldrich.com/eas](http://sigma-aldrich.com/eas)

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