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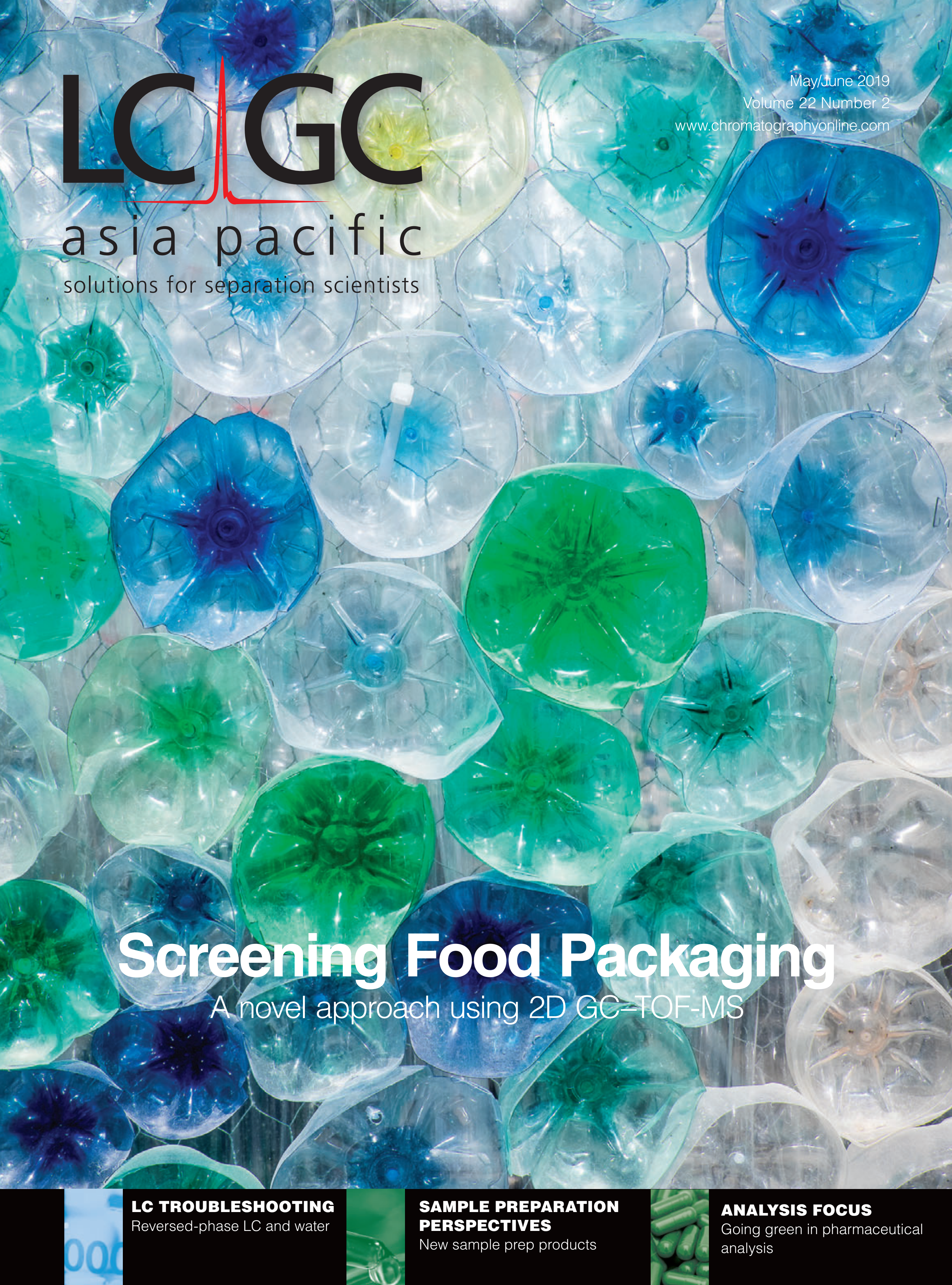

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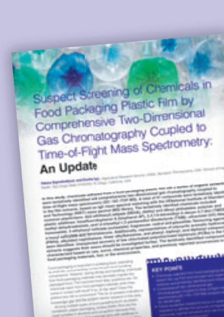
Yelena Sapozhnikova and Eunha Hoh
The occurrence of additives, such as plasticizers in plastic food packaging, has been documented, but information on their potential migration into foods is limited. In this pilot study, 2D GC–MS was used to identify chemicals extracted from a common stretch plastic film with a series of organic solvents. In total, 91 compounds were tentatively identified, including five PCBs.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Editorial Director
Laura Bush
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Editor-in-Chief
Alasdair Matheson
amatheson@mmhgroup.com

Managing Editor
Kate Jones
kjones@mmhgroup.com

Associate Editor
Lewis Botcherby
lbotcherby@mmhgroup.com

Associate Publisher
Oliver Waters
owaters@mmhgroup.com

Sales Executive
Liz Mclean
lmclean@mmhgroup.com

Senior Director, Digital Media
Michael Kushner
mkushner@mmhgroup.com

Webcast Operations Manager
Kristen Moore
kmoore@mmhgroup.com

Project Manager
Vania Oliveira
voliveira@mmhgroup.com

Digital Production Manager
Sabina Advani
sadvani@mmhgroup.com

Managing Editor Special Projects
Kaylynn Chiarello-Ebner
kebner@mmhgroup.com

Art Director
Dan Ward
dward@hcl.com

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Lloyd Drive,
Cheshire Oaks,
Cheshire,
CH65 9HQ, UK
Tel. +44 (0)151 353 3500
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Suspect Screening of Chemicals in Food Packaging Plastic Film by **Comprehensive Two-Dimensional Gas Chromatography Coupled to Time-of-Flight Mass Spectrometry**

Yelena Sapozhnikova[1] and Eunha Hoh[2], [1]Agriculture Research Service, USDA, Wyndmoor, Pennsylvania, USA, [2]School of Public Health, San Diego State University, San Diego, California, USA

In this study, chemicals extracted from a food packaging plastic film with a series of organic solvents were tentatively identified with comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC–TOF-MS). A total of 91 compounds were tentatively identified in the film extracts, based on high mass spectral matching with the US National Institute of Standards and Technology (NIST) mass spectral (MS) library. The tentatively identified chemicals included common plasticizers: di(2-ethylhexyl) adipate (DEHA), diethyl and dibutyl phthalates; polymer and plastic additives: hexafluorobisphenol A (bisphenol AF), 2,4,7,9-tetramethyl-5-decyn-4,7-diol (TMDD), methyl dehydroabietate, and 2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB); ultraviolet (UV) filters: homosalate, 2-ethyhexyl salicylate (octisalate); fragrances: cedrol and galaxolide; flavouring agents: *n*-hexyl salicylate and benzophenone. Additionally, representatives of polycyclic aromatic hydrocarbons (PAHs), alkylated naphthalenes, linear alkylbenzenes, and phenyl, biphenyl, and diphenyl compounds were identified. Unexpected discovery of low chlorinated polychlorinated biphenyls (PCBs) in the film extracts suggests their source should be investigated further. The tentatively identified compounds were characterized based on use, source, chemical properties, and previously reported occurrences in plastic food packaging materials, food, or the environment.

Food packaging is important in protecting food, extending its shelf-life, and providing consumers with food handling convenience. However, during storage and handling, chemicals from food packaging materials may potentially migrate into packaged food. The important food safety questions are: Do chemicals leach from food packaging materials when they come in contact with food? If so, at what rates? Does this present any risk to consumers? The lack of answers indicates a knowledge gap and the evident need for research in this area.

Among multiple types of food packaging (such as plastic containers, paper packaging, bottles), plastic films are the most complex in structure and composition because they are constructed of multiple laminate layers with additives in between to attain the desired properties. Stretch plastic films, also known as *cling films*, are one of the most widely used food contact materials in both commercial and consumer applications. The global market for flexible plastic packaging

KEY POINTS

- Chemicals extracted from food packaging plastic stretch film into FDA food simulant and organic solvents were tentatively identified and characterized.
- Comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC–TOF-MS) analysis was used for identification.
- Future studies are needed to focus on quantitative migration to estimate the amounts of migrating chemicals into food simulants and real foods to provide data for risk assessment.

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Table 1: Instrument conditions of GC×GC–TOF-MS for chemical analysis

1D Column	Rtx-5MS (30 m × 0.25 mm × 0.25 µm) with 5 m guard column
2D Column	Rtx-17 (0.79 m × 0.10 mm × 0.10 µm)
Injection Volume	2 µL/Splitless
Inlet Purge Time	36 s
Inlet Liner	Single Taper Inlet Liner
Inlet Temperature	300 °C
Carrier Gas	Helium
Carrier Gas Flow	1 mL/min (constant)
1D Oven Program	60 °C for 1 min, 6 °C/min until 300 °C (hold for 3 min), and 20 °C/min until 320 °C (hold for 15 min)
2D Oven Program	20 °C off from 1D Oven Program
Modulator Temp. Offset	15 °C from 2D Oven Program
Modulation Time	3.50 s
Hot Pulse Time	0.90 s
Cold Time	0.85 s
Transfer Line Temp.	285 °C
Solvent Delay	10 min
Ion Source Temperature	250 °C
Mass Range	50–1000 u
Acquisition Rate	150 spectra/s
Detector Voltage	1650
Electron Energy	70

reached 11.3 million tons in 2009, continuing to increase at 4% per year to 2016 (1). In terms of composition, polyethylene and polypropylene polymeric materials are most commonly used in plastic films, and other polymers, such as polyethylene terephthalate (PET), polyvinyl chloride (PVC), and polyamide (nylon), are used less frequently. These plastic polymers are generally inert, but chemicals added to polymeric films to improve their properties (such as antioxidants, light stabilizers, plasticizers, thermal stabilizers, lubricants, and antistatic agents), plus non-intentionally added substances referred to as *NIAS* (chemical impurities, contaminants, and chemicals formed during degradation or reaction of added chemicals) may migrate into packaged food. The occurrence of food packaging additives: antioxidants, ultraviolet (UV) stabilizers, and plasticizers (2); print-related contaminants (3); phthalates, polycyclic aromatic hydrocarbons (PAHs), photoinitiators, bisphenols, and polyfluorinated compounds (4) in plastic and paper food packaging has been previously documented. However, there is limited information on their potential migration in foods. A recent review outlined analytical challenges in identifying NIAS from food packaging materials (5), including lack of information on the composition of materials and added ingredients, complex structures, and the need for sophisticated analytical techniques. Recently, nontargeted studies utilizing high resolution mass spectrometry (HRMS) were conducted to identify compounds in polycarbonate food contact plastics (6) and nano-films (7).

The goal of this pilot study was to perform a comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC–TOF-MS) nontargeted qualitative screening to identify and characterize compounds extracted from food packaging stretch film into FDA-approved food simulant and organic solvents with different polarities. We sought to create a list of potential migrants for future studies on migration of chemicals into fatty foods, particularly ground beef.

Figure 1: GC×GC chromatogram of plastic stretch film extracts in different solvents. The *x*-axis represents the 1st GC retention time (s) and the *y*-axis represents the 2nd GC retention time (s).

Table 2: Compounds tentatively identified in hexane, ethyl acetate (EtAc), acetonitrile, and 95% ethanol with 5% water food simulant (FSim) extracts of the stretch film

	Identified Compound	CAS	Hexane	EtAc	Acetonitrile	FSim
1	Acetic acid, nitro-, ethyl ester	626-35-7	---	x	---	---
2	Furan, 2-pentyl	3777-69-3	x	x	x	---
3	Hexanal, 2-chloro	762-39-0	---	x	---	---
4	Benzyl chloride (chlorotoluene)	100-44-7	---	x	---	---
5	2,5-Furandione, 3,4-dimethyl	766-39-2	x	x	x	---
6	1-Propyne, 3-phenyl-	10147-11-2	---	x	---	---
7	Acetophenone	98-86-2	x	x	x	---
8	3-Hydroxy-3-phenylbutan-2-one	3155-01-9	x	x	x	---
9	Mequinol	150-76-5	x	x	x	---
10	2,3-Dimethyl-4-hydroxy-2-butenoic lactone	1575-46-8	x	x	x	---
11	Benzoic acid	65-85-0	---	x	x	---
12	Ethanol, 2-phenoxy	122-99-6	x	x	x	---
13	Vanillin	121-33-5	---	x	---	---
14	Benzene-1,3-bis(acetyl)	6781-42-6	x	x	---	---
15	2,3,4,5,6-Pentamethyl acetophenone	2040-01-9	x	x	x	---
16	Benzene, 1,2,3-trimethoxy-5-(1-propenyl)-	5273-85-8	x	x	x	x
17	3,5-di-tert-Butyl-4-hydroxybenzaldehyde	1620-98-0	x	x	x	x
18	Benzothiazole	95-16-9	x	x	x	x
19	Phthalic anhydride	85-44-9	x	x	x	x
20	1,4-Dihyronaphthalene	612-17-9	---	x	---	---
21	Naphthalene	91-20-3	x	x	x	x
22	Naphthalene, 2-methyl-	91-57-6	x	x	x	x
23	Naphthalene, 1,7-methyl-	575-37-1	x	x	x	x
24	Naphthalene, 1-(2-propenyl)-	2489-86-3	---	x	---	---
25	Naphthalene, 1-methoxy-	2216-69-5	x	x	x	x
26	Naphthalene, 1-ethyl-	1127-76-0	x	x	x	x
27	Naphthalene, 2-(1-methylethyl)-	2027-17-0	x	x	x	x
28	Naphthalene, 1,4,5-trimethyl-	2131-41-1	x	x	x	x
29	Naphthalene, 1,4,6-trimethyl-	2131-42-2	x	x	x	x
30	Naphthalene, 1,2,3-trimethyl-4-propenyl-	26137-53-1	x	x	x	x
31	1,3-Diisopropylnaphthalene	57122-16-4	x	x	x	x
32	2,6-Diisopropyl naphthalene	24157-81-1	x	x	x	x
33	Naphthalene, 1-(chloromethyl)-	86-52-2	---	x	---	---
34	Acenaphthene	83-32-9	x	x	x	---
35	Fluorene	86-73-7	x	x	x	x
36	Dibenzofuran	132-64-9	x	x	x	x
37	9,10-dihydrophenanthrene	776-35-2	x	x	---	---
38	1H-Phenalene	203-80-5	---	x	---	---
39	Phenanthrene	85-01-8	x	x	x	x
40	4-Phenanthrenol, 1,2,3,4-tetrahydro-4-methyl-	77536-58-4	---	x	---	---
41	1,4-Ethenoanthracene, 1,4-dihydro-	27765-96-4	---	x	---	---
42	Fluoranthene, 1,2,3,10b-tetrahydro-	20279-21-4	---	x	---	x
43	Indeno[2,1-a]indene, 5,10-dihydro-	6543-29-9	---	x	---	---
44	Biphenyl	92-52-4	x	x	x	x
45	1,1'-Biphenyl, 4-methyl-	644-08-6	x	x	x	x
46	Diphenyl ether	101-84-8	x	x	x	x
47	Diphenylmethane	101-81-5	x	x	x	x
48	Cis-Stilbene	645-49-8	---	x	---	---

Table 2 (Continued): Compounds tentatively identified in hexane, ethyl acetate (EtAc), acetonitrile, and 95% ethanol with 5% water food simulant (FSim) extracts of the stretch film

	Identified Compound	CAS	Hexane	EtAc	Acetonitrile	FSim
49	Benzophenone	119-61-9	x	x	x	x
50	1,1'-Biphenyl,2,2',5,5'-tetramethyl-	3075-81-4	x	x	x	x
51	Benzyl benzoate	120-51-4	x	x	x	x
52	Naphthalene, 1,2,3,4-tetrahydro-1-phenyl-	3018-20-0	x	x	x	x
53	Benzene, 1,1'-(1,3-butadienylidene)	4165-81-5	---	x	---	---
54	Benzene, 1,3-dimethoxy-5-[(1E)-2-phenylethenyl]-	21956-56-9	x	x	x	---
55	1,1'Biphenyl, 2,2'-dichloro-	13029-08-8	x	x	x	x
56	1,1'-Biphenyl, 4,4'-dichloro-	2050-68-2	x	x	x	x
57	1,1'-Biphenyl, 2,2',6-trichloro-	35693-92-6	x	x	x	x
58	1,1'-Biphenyl, 2,2',5-trichloro-	37680-65-2	x	x	x	x
59	1,1'-Biphenyl, 2,3',5-trichloro	37680-68-5	x	x	x	x
60	Benzene, (1-butylhexyl)-	4537-11-5	x	x	x	x
61	Benzene, (1-methylundecyl)-	2719-61-1	x	x	x	x
62	Benzene, (1-butylheptyl)-	4537-15-9	x	x	x	x
63	Benzene, (1-propyloctyl)-	4536-86-1	x	x	x	x
64	Benzene, (1-ethylnonyl)-	4536-87-2	x	x	x	x
65	Benzene, (1-pentylheptyl)-	2719-62-2	x	x	x	x
66	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate (TXIB)	6846-50-0	x	x	x	x
67	Isopropyl myristate	110-27-0	x	x	x	x
68	Adipic acid, butyl pent-4-en-2-yl ester	N/A	x	x	x	x
69	Di(2-ethylhexyl) adipate (DEHA)	103-23-1	x	x	x	x
70	Adipic acid, 2-ethylhexyl nonyl ester	N/A	x	x	x	x
71	Diisooctyl adipate	1330-86-5	x	x	x	x
72	Triethylene glycol di(2-ethylhexcate)	94-28-0	x	x	x	x
73	Adipic acid, di(2-decyl) ester	N/A	x	x	x	x
74	Adipic acid, decyl 2-ethylhexyl ester	N/A	x	x	x	x
75	Cedrol	77-53-2	x	x	x	x
76	1H-Indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl-	3910-35-8	x	x	x	x
77	Homosalate	118-56-9	x	x	x	x
78	Cyclopenta[g]-2-benzopyran, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl- (Galaxolide)	1222-05-5	x	x	x	x
79	13-Isopropylpodocarpa-8,11,13-trien-19-al	24035-50-5	x	x	x	x
80	10,18-Bisnorabieta-8,11,13-triene	32624-67-2	x	x	x	x
81	Methyl dehydroabietate	1235-74-1	x	x	x	x
82	2,4,7,9-Tetramethyl-5-decyn-4,7-diol	126-86-3	x	x	x	x
83	4,4'-(Hexafluoroisopropylidene) diphenol	1478-61-1	---	x	x	x
84	Diethyl phthalate	84-66-2	x	x	x	x
85	n-Hexyl salicylate	6259-76-3	x	x	x	x
86	2-Ethylhexyl salicylate (Octisalate)	118-60-5	x	x	x	x
87	Butyl isobutyl phthalate	17851-53-5	x	x	x	x
88	Dibutyl phthalate	84-74-2	x	x	x	x
89	2-ethylhexyl methyl isophthalate	N/A	x	x	x	x
90	Glycidyl palmitate	N/A	x	x	x	x
91	4,8,12,16-Tetramethylheptadecan-4-olide	96168-15-9	x	x	x	x

Experimental

A commonly used stretch plastic film for food packaging, acquired from a local store, was sampled and extracted with US FDA approved food simulant: water–ethanol, 5%:95%, respectively and with three organic solvents with different polarities: hexane and ethyl acetate, mimicking lipophilic properties of fat-containing foods, and with acetonitrile, making it possible to extract both polar and relatively nonpolar chemicals. Approximately 4 g of film samples were completely immersed and extracted with 40 mL of solvents in thoroughly solvent-rinsed glass beakers using ultrasonic extraction for 30 min. Reagent blanks consisting of the representative solvents were processed alongside the samples to account for possible laboratory contamination from glassware, solvents, and laboratory equipment.

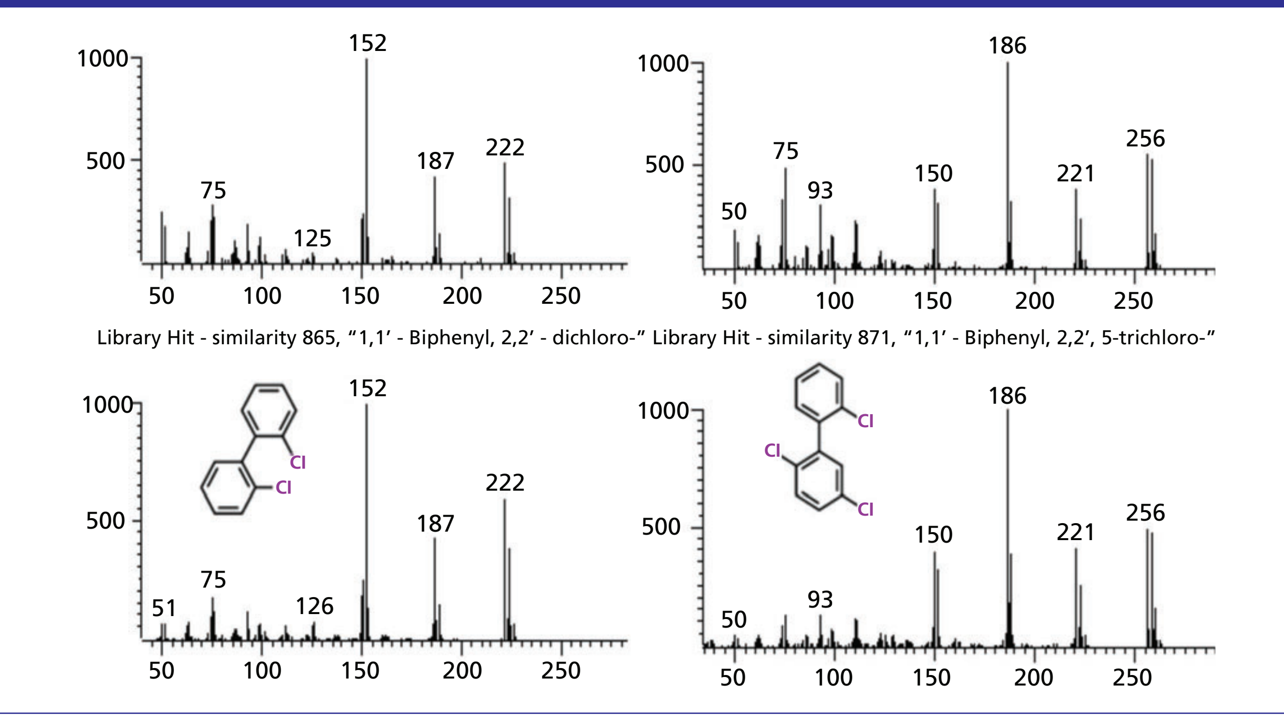
In the next experiment, we exposed ground beef samples, acquired from a local supermarket, and previously not exposed to any food packaging, to the plastic film: 1) during 2 min microwave defrosting of a frozen beef samples, and 2) during microwaving of non-frozen beef samples for 30 s (high power). After exposure, the beef samples were extracted with hexane at 1 g/mL ratio. Control beef samples (not covered with plastic film) were processed along with the exposed beef samples.

The extracts of the plastic film and ground beef were analyzed using a LECO Pegasus 4D GC×GC–TOF-MS to identify extracted compounds. The instrumental conditions are summarized in Table 1. The data generated by GC×GC–TOF-MS was processed by LECO ChromaTOF software (version 4.50.8.0 optimized for Pegasus). Peaks were searched at S/N = 100 or above, and an automatic library search was conducted to find the most similar mass spectrum for each detected compound in the 2014 National Institute of Standards and Technology (NIST) electron impact (EI) mass spectral library. Compounds with a similarity score of 700 or above (maximum score = 999) were selected, and the selected compounds were further manually reviewed for their similarity with matching mass spectra from the NIST EI mass spectral library. Multiple ions ($n > 3$) or unique features must be present for comparison. For example, phthalates contain mass (m/z) 149 at high abundance, and aromatic or cyclic compounds or halogenated compounds have unique patterns of mass fragments or isotope ratio patterns. After manual review, highly identifiable compounds were selected. The selected compounds were checked for their presence in each corresponding procedural blank sample. For final inclusion, the peak should not be present in the corresponding control sample. Otherwise the peak area (abundance) must be 10 times greater than the corresponding peak area in the control sample. Data analysis for the beef extracts was conducted similarly, with the only difference in the inclusion criterion, which was a ratio of three between peak abundance for exposed beef sample versus control. It should be noted that the compounds reported in this paper are identified tentatively, since they were not confirmed with their authentic standards, except 2,2',5-trichlorobiphenyl.

Results and Discussion

In the USA, food contact materials are regulated by the US Food and Drug Administration (FDA) under the Food, Drug, and Cosmetic Act. Title 21 of the Code of Federal Regulations (21 CFR) provides regulations for substances that may be safely used as "indirect food additives", generally recognized

Figure 2: Mass spectra identified as polychlorinated biphenyls (PCBs): the left shows a 86.5% match with 2,2'-dichlorobiphenyl and the right shows a 87.1% match with 2,2',5-trichlorobiphenyl from the US National Institute of Standards and Technology (NIST) library. The right spectrum was further confirmed by match to an authentic standard, 2,2',5-trichlorobiphenyl. For all figures shown, the *x*-axis is mass (*m/z*) and the *y*-axis is relative abundance.

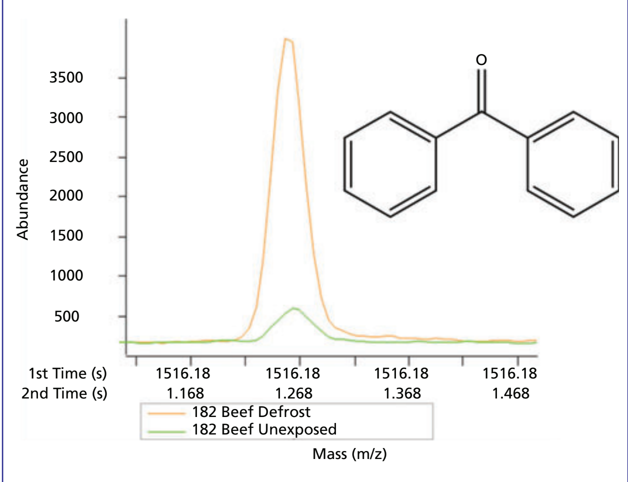


as safe (GRAS) and prior-sanctioned substances used under conditions of good manufacturing practice (GMP). Indirect food additives are substances in food packaging materials that may come in indirect contact with food.

In this study, we selected an FDA recommended food simulant for fatty foods (95% ethanol: 5% water, *v/v*) and organic solvents used for GC analysis (hexane, ethyl acetate, acetonitrile) for extracting organic chemicals from the stretch film. It was previously demonstrated that amounts of plasticizers, including adipates, citrates, and phthalates, were in good agreement when packaging films were extracted with ethyl acetate in Soxhlet extraction versus with 96% ethanol food simulant according to the European Union (EU) regulatory rules on migration experiments (8). Additionally, there was no difference in the amount of di(2-ethylhexyl) adipate (DEHA) migrated from films into 96% ethanol versus iso-octane.

The numbers of tentatively identified chemicals were 91 in ethyl acetate, 74 in acetonitrile and hexane, and 64 in ethanol–water extracts (Figure 1). Other migrated chemicals were not selected for identification due to their low mass spectral matching with the NIST database. The identified compounds with their CAS numbers and occurrence are presented in Table 2. Most of the identified chemicals were extracted from plastic films into all studied solvents. However, some migrated selectively into specific solvents. Thus, 14 compounds were identified in ethyl acetate extracts, but not found in other solvent extracts (Table 2). Two chemicals, benzene-1,3-bis(acetyl) and 9,10-dihydrophenanthrene (Table 2, #14 and 37), were found in hexane and ethyl acetate extracts only. Both chemicals are practically insoluble in water, and therefore most likely only dissolve and migrate to the least nonpolar solvents during extraction. Another interesting example of selective migration

Figure 3: Benzophenone identified in the ground beef exposed to plastic film during microwave defrosting (red line) versus control (unexposed) ground beef (green line) sample. The *x*-axis represents GC retention times and *y*-axis represents abundance of *m/z* 182.



was benzoic acid, which was identified in ethyl acetate and acetonitrile extracts, but not in ethanol-based food simulant and hexane. Benzoic acid is used as a food preservative, E210–E213. Lastly, 1,2,3,10b-tetrahydrofluoranthene was identified in ethyl acetate and ethanol–water extracts only, demonstrating a differential pattern of migration.

Overall, several classes of chemicals were identified among the extracted compounds: alkylated naphthalenes used as lubricants; PAHs including those on the US Environmental

Protection Agency (EPA) priority pollutant list; plasticizers, polymer additives, UV filters, flavouring agents and fragrances, surfactants, adhesives, products of thermal degradation, and polychlorinated biphenyls (PCBs).

Alkylated Naphthalenes

Several alkylated naphthalenes (Table 2) were identified in the extracts. These compounds are used as lubricants in many applications, including plastic film manufacturing. Migration of alkylated naphthalenes into food simulants and organic solvents has been previously reported for plastic baby bottles (9) and food contact paper (4). Among alkylated naphthalenes, 2,6-diisopropyl naphthalene was the most frequently identified compound leaching from food contact materials reported by others (4,9–11). Interestingly, this chemical is intended for use in the manufacturing of pesticides as a plant growth regulator. In our residual analysis of pesticides, 2,6-diisopropyl naphthalene is often detected in reagent blanks when plastic labware is used.

PAHs

Another class of extracted substances were PAHs, and 11 compounds were identified: naphthalene, acenaphthene, fluorene, phenanthrene, dibenzofuran, 9,10-dihydrophenanthrene, 1H-phenalene, 1,2,3,4-tetrahydro-4-methyl-4-phenanthrenol, 1,4-dihydro-1,4-ethenoanthracene, 1,2,3,10b-tetrahydro-fluoranthene, and 5,10-dihydroindeno[2,1-a]indene. Naphthalene, acenaphthene, fluorene, and phenanthrene are included on the US list of priority PAHs frequently found in environmental samples, but not on the EU priority list based on toxicity (12). In a study of quantitative migration of these four PAHs from food contact paper, no acenaphthene was found, while the measured levels of phenanthrene were the highest, followed by fluorene and naphthalene (4). On the other hand, naphthalene was the most frequently identified compound migrating from various food contact materials into food simulants, food, or organic solvents. For example, naphthalene was reported in milk drinks contained in low density polyethylene (LDPE) bottles, and its migration levels increased with storage time and milk fat content (13). Similarly, naphthalene was shown to migrate into food simulants from baby bottles made of polypropylene, polyamide, and silicone (9,11).

Plasticizers

As expected, various plasticizers, including adipates and phthalates, were identified in film extracts. Plasticizers are added to polymers in plastic materials, including stretch films, to achieve the desired properties, such as stretching, flexibility, and durability. According to a migration study on food packaging films, plasticizers comprise 3 to 10% of the films (*w/w*) (8). The most common plasticizers are phthalate- and adipate-based compounds. Plasticizer migration generally occurs at their direct contact with foods, and is shown to be greater for fatty foods and increased temperatures (14). The most notable identified adipate-based plasticizers were diisooctyl adipate and di(2-ethylhexyl) adipate (DEHA). Both are approved by the US FDA for use in food contact substances as indirect additives (15), with DEHA approved for use only as a component of adhesives. Five adipic acid esters were also

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identified in the extracts. Diethyl and dibutyl phthalates are commonly used plasticizers, and both are included in the US FDA indirect additives for food contact substances. Dibutyl phthalate was banned in the EU for use in cosmetics, and restricted for use in children's toys in the EU and the USA. In a study on occurrence of common plasticizers, including DEHA and diethyl and dibutyl phthalates in Canadian food, only DEHA was detected in cling films and packaged cheese, beef, pork, chicken, and fish samples (16). Similarly, DEHA was reported migrating into cheese samples from food-grade film (17). Dibutyl- and diethyl phthalates were also detected leaching from food contact paper (4) and dibutyl phthalate from polypropylene baby bottles (9).

Fragrances and Flavouring Agents

Several fragrance and flavouring additives were identified in the extracts. For example, *n*-hexyl salicylate is a food additive flavouring agent (EU Food Improvement Agents) and an odour agent for fragrance. It is also widely used in personal care products, laundry and dishwashing, and air freshening and cleaning products. Cedrol is a flavouring agent and food additive (EU Food Improvement Agents); it was also detected in leachate from silicone baby bottles (9). Another flavouring agent, benzophenone, is approved as a food additive in the EU. Additionally, it is used in adhesives and sealants, paints, inks, toners, and colourant products, and personal care products.

Musk galaxolide is used as a fragrance in many consumer products, including cleaning, laundry and dishwashing products, personal care products, and plastic. Galaxolide was identified in post-consumer plastic packaging waste in Germany (18), but, surprisingly, this was the only study reporting its occurrence.

UV Filters

Two common UV filters were identified in the film extracts: 2-ethylhexyl salicylate (octisalate) and homosalate. Both are widely used in sunscreens and cosmetics as sunblocks. Many UV filters are added to plastic films to protect against sun exposure; oxybenzone, for example, is approved by US FDA as an indirect food additive.

Plastic and Polymer Additives: Several plastic and polymer additives, including hexafluorobisphenol A, 2,4,7,9-tetramethyl-5-decyn-4,7-diol (TMDD), methyl dehydroabietate, and 2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB), were identified. Hexafluorobisphenol A, also known as *bisphenol AF* (BPAF), is an alternative to bisphenol A, and is used in industrial applications of polymers and synthetic rubber, among others. TMDD is used as a non-ionic surfactant, adhesive, and plastic additive, and is an approved US FDA indirect food additive. TMDD occurrence is attributed to its use in printing inks, paints, and recycled paper, and was documented in wastewater and river water in Germany (19), but no reports in foods were found. 2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB) is a low-viscosity plasticizer, and is also approved by the US FDA as an indirect additive. Reports on TXIB migration from plastic baby bottles were previously published (9,11).

Linear Alkylbenzenes

Six linear alkylbenzenes (LABs) (Table 2, #60–65) were identified in the extracts. LABs are used in the manufacture of linear alkylbenzene sulfonates used as surfactants for household detergents, and are considered an indicator of human activities associated with sewage contamination. LABs have high octanol water partition coefficients (7–10), and have been reported in environmental samples (20).

PCBs

The discovery of the five PCB congeners (Figure 2) was unexpected. The identified PCBs contain two or three chlorines. Typical PCB profiles in environmental and biological matrices (for example, sediment or tissue) contain 30 to 50 congeners with a greater range of chlorination. PCBs were banned in the US in 1979 due to their carcinogenicity in humans and animals (21). However, due to their persistence, PCBs are still ubiquitous in the environment. Human health risk due to PCB exposure is actively monitored, for example, through sportfish consumption surveys. We suspect the PCBs identified in this experiment are by-products of the film manufacturing process. A recent study found that PCBs with one or two chlorines were detected at most abundance among the 50 PCB congeners in paint pigments (22), which are commonly used in several products, including plastics. The discovery of PCBs in the film extracts suggests their source should be investigated further.

Miscellaneous Others

We think that identified compounds 10,18-bisnorabieta-8,11,13-triene and 13-isopropylpodocarpa-8,11,13-trien-19-al, a derivative of PAH phenanthrene, could be thermal degradation products based on their structural similarity to 1-methyl-10,18-bisnorabieta-8,11,13-triene, which was identified as an intermediate compound formed during thermal degradation of adhesives in food packaging (5). Additionally, phenyl/biphenyl/diphenyl compounds were identified in the extracts.

Ground Beef Exposure

While the data analysis on identification of chemicals migrated from plastic film into beef samples is underway, we identified 182 and 102 chromatographic peaks with areas three times larger than in control in defrost and microwave exposed samples, respectively. Some of the tentatively identified chemicals included benzophenone (Figure 3) and 2,4,7,9-tetramethyl-5-decyne-4,7-diol, which were previously found in the film extracts. The latter is an approved FDA indirect additive. Interestingly, two compounds were tentatively identified in exposed beef samples, which were not found in the film extracts: 1,2,3,4-tetrahydro-1,1,6-trimethylnaphthalene, used in manufacture of polymers, and 1-phenyl-5-methylheptane, a precursor of biodegradable detergents. Possibly, the introduction of two additional variables, microwave heat and beef fat content, are responsible for this finding. Similarly, it was reported that migration of Irganox 1076, an antioxidant in food packaging polymers, had increased from plastics into foods and food simulants with increased temperature and fat content (23). This finding suggests the importance of studying migration into real food samples when possible, under controlled conditions.

Conclusions

In this study, we tentatively identified and characterized chemicals extracted from food packaging plastic stretch film into FDA food simulant and organic solvents: hexane, ethyl acetate, and acetonitrile. GC×GC–TOF-MS analysis based on ≥70% match similarity to the standard NIST mass spectral library and manual review of mass spectral matching was used for identification. Comparison of different solvents for extraction resulted in 91 identified compounds in ethyl

acetate, 74 in acetonitrile and hexane, and 64 in ethanol–water extracts, with some compounds showing selective migration into some solvents versus others. Overall, several classes of chemicals were identified among the extracted compounds: alkylated naphthalenes used as lubricants; PAHs including those on the US EPA priority pollutant list; common used plasticizers, polymer additives, UV filters, flavouring agents and fragrances, surfactants, adhesives, products of thermal degradation, and low chlorinated PCBs. Several compounds tentatively identified in our study were not previously reported as migrants from plastic materials by earlier studies, which may be attributed to the specific plastic film studied. Future studies should focus on quantitative migration to estimate the amounts of migrating chemicals into food simulants and real foods to provide data for risk assessment.

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Yelena Sapozhnikova is with the Agricultural Research Service of the USDA, in Wyndmoor, Pennsylvania, USA. Direct correspondence to: yelena.sapozhnikova@usda.gov
Eunha Hoh is with the School of Public Health at San Diego State University, USA.

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Jacqueline Robertson: +44 (0)1357 522961 | jacqueline@crawfordscientific.com

Reversed-Phase Liquid Chromatography and Water, Part 1—How Much is Too Much?

Dwight R. Stoll, LC Troubleshooting Editor

When can we use completely aqueous eluents with reversed-phase stationary phases, and what happens if we make a mistake?

Reversed-phase liquid chromatography (LC) is an incredibly powerful mode of separation that is applicable to a wide variety of applications ranging from the separation of small organic acids to 150 kDa proteins. Reversed-phase separations have limitations, however, with one of the most practically significant ones being low retention for compounds that are highly water soluble (that is, hydrophilic). Understanding the general trend for reversed-phase separations that retention increases as the fraction of water in the eluent increases, and encountering situations where retention is too low for an analyte of interest pushes us to use eluents with higher and higher levels of water. This, then, leads to the question,“How much water is too much?” Jumping to the end of this article, the short answer will be “It depends”.

In some cases, using completely aqueous eluents (that is, containing no organic solvent such as methanol or acetonitrile) may be completely acceptable and in fact can provide very useful separations of highly hydrophilic molecules. In other cases, completely aqueous eluents can cause some reversed-phase stationary phases to behave in undesirable ways and should be avoided. This is not a new topic by any means (1,2), but the options we have for handling such situations are constantly changing. It is worth taking the time here to refresh our perspective on the topic as column manufacturers introduce new stationary phase chemistries and particle morphologies, and our knowledge of what goes on inside the column improves through fundamental research. In this first segment on this topic, I will review the basic concepts that are important for reversed-phase separations in highly aqueous eluents, summarize recent advances in our understanding of what goes on inside the column, and provide examples of bad column behaviour and potential remedies.

Basic Concepts for Reversed-Phase Separations with Aqueous Eluents

It has been observed since the early days of LC that operating a typical C18-type reversed-phase column in a completely aqueous eluent can lead to gradual or sudden decreases in retention time (3). For at least a couple of decades, the prevailing idea seems to have been that the observed decrease in retention time was due to a change in the conformation of C18 chains bonded to the particle substrate in aqueous eluents from highly extended chains (that is, perpendicular to the substrate surface), to ones that laid down on themselves (that is, parallel to the surface). The latter state was commonly referred to as “phase collapse” (1–3). These observations also led to the notion that operating reversed-phase columns in completely aqueous eluents was generally a really bad idea, so much so that this idea made it into the Top 10 Myths of LC addressed by Ron Majors just five years ago (4). Around the late 1990s, however, experimental evidence led a number of groups to adopt the idea that the decrease in retention time was due instead to “dewetting” of the stationary phase (5). Specifically, the idea is that the high surface tension of an aqueous eluent in contact with a hydrophobic surface causes the eluent to extrude from the pores of the stationary phase particle. If there is no liquid in the pores of the particle, this effectively reduces both the column dead volume (that is, the volume of mobile phase inside the column, V_m) and the volume of stationary phase that is accessible to the analyte. Indeed, significant decreases in V_m have been measured for C18-type phases when switching from organic-rich to completely aqueous eluents, and these decreases are correlated with decreases in retention observed for analytes of interest (3,6). The extent to which this extrusion of the eluent occurs under actual separation conditions depends on a number of factors, including the particle pore size, stationary phase chemistry, column temperature, and operating pressure (through this last factor, we could say dewetting depends on particle size and flow rate, as well).

For over a decade, Siepmann, Schure, and coworkers have been using Monte-Carlo molecular simulations to study the microscopic details of mobile and stationary phases in an effort to better understand how reversed-phase

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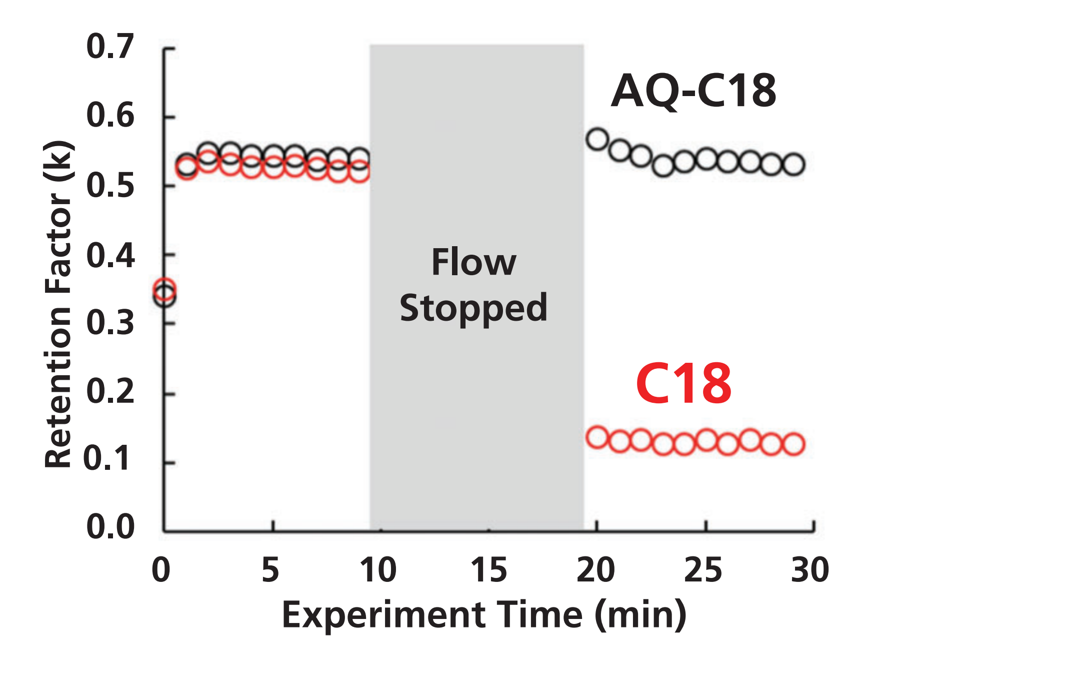
Figure 1: Retention factor for uracil measured before and after stopping the flow for 10 minutes. Chromatographic conditions: Columns, HALO AQ-C18 (black points) or HALO C18 (red points) (both Advanced Materials Technology, Inc.), 50 mm × 2.1 mm, 2.7-µm superficially porous particles; Eluent, 10 mM phosphoric acid in water; Flow rate, 0.40 mL/min; Temperature, 40 °C. Retention factors were calculated using thiourea as a dead time marker, and injections of the thiourea and uracil analyte mixture were made once per minute.



separations work (7). The results of these studies complement experimental work, and provide insights that cannot be obtained easily by experiment. Among a number of topics, they have addressed the question of what happens to reversed-phase stationary phases in aqueous eluents. They have found that the results of simulation support the idea that the observed decreases in retention must be due to loss of eluent from the particle pores, rather than physical collapse of the stationary phase chains onto themselves (8).

The equation of Young and Laplace is most commonly used to rationalize the effects of different chromatographic variables on the dewetting phenomenon (1,5,6,9). This equation, shown in equation 1 (10), provides a relationship between the pressure (P) required to force a liquid into a capillary, the contact angle of the liquid on the interior surface of the capillary (θ), the surface tension of the liquid (γ), and the radius of the capillary (r):

$$P = \frac{-2\gamma\cos\theta}{r} \qquad [1]$$

Since the contact angle of water on a hydrophobic surface, such as C18-modified silica, is greater than 90°, a positive pressure is required to force water into the pore of a particle. Under conditions typical of modern LC separations, the pressure required to push the eluent through the particle bed will exceed the pressure required to push the eluent into the pores of the particles at most points along the column length. However, when the flow is stopped (for example, if the LC is not operated overnight), an aqueous eluent can be spontaneously extruded from the pores of a hydrophobic stationary phase particle, and the retention behaviour will look very different the next time the column is used. With this framework in mind, we can think about how different chromatographic variables will affect this behaviour. As the stationary phase becomes less hydrophobic, the contact angle for water will decrease. When the angle is less than 90°, the pressure indicated by equation 1 becomes negative, meaning that the eluent will spontaneously be drawn into the pores of the particles. Among chromatographers, we refer to this as "wetting" of the pores. Equation 1 also suggests that the particle pore size ought to play a role (that is, through r), with the pressure required to force eluent into the pore increasing as the pore size decreases.

The Washburn equation provides a helpful framework for thinking about the effects of these parameters, but, of course, the pores of chromatographic particles are not ideal by any means. The pore structure itself is heterogenous, with a distribution of diameters and shapes, and the chemistry of the pore surface is locally heterogeneous with some unbonded silanol sites, stationary phase ligands (for example, C18), and endcapping functional groups (for example, trimethylsilyl groups). And so, we look to experimental results for the definitive answer to the question, "How much water is too much?" The chromatographic literature provides useful data that at least establish trends, even if the results are not exactly transferrable to a particular set of conditions of interest. For example, Bidlingmeyer and Broske showed results that speak to the effect of particle pore size, stationary phase chemistry, and column temperature on

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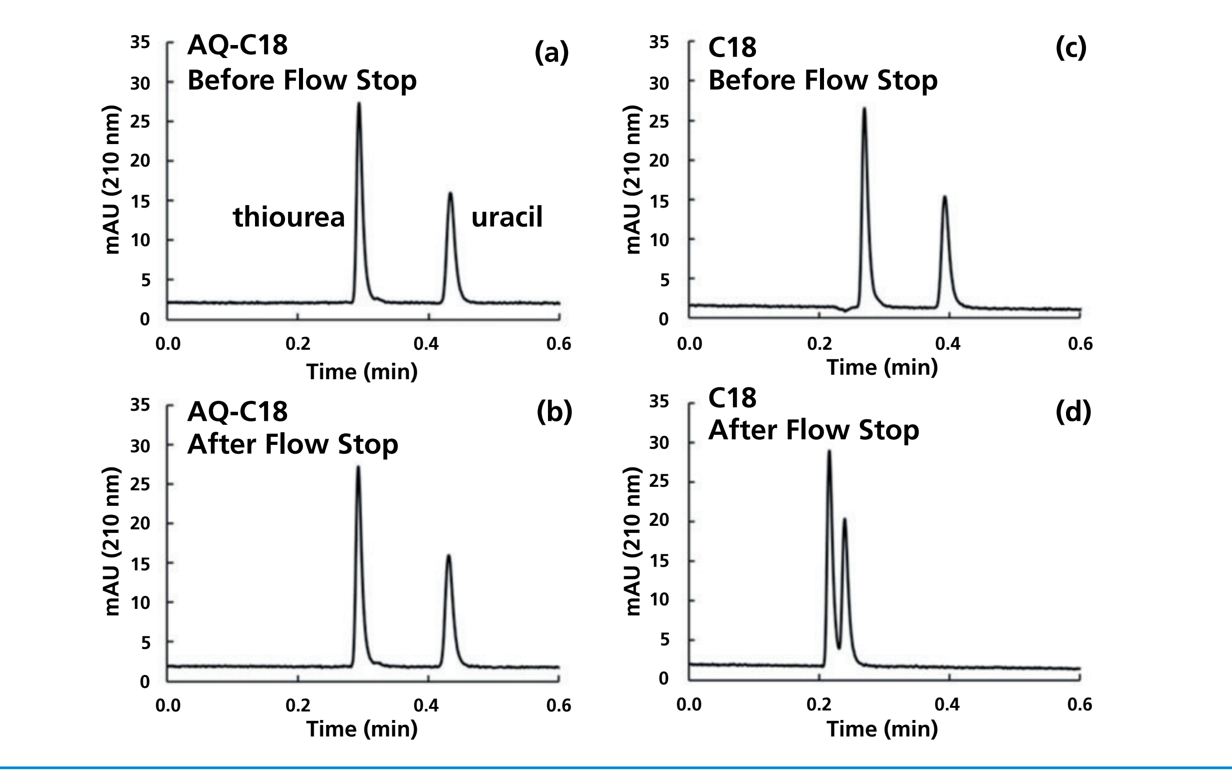
Figure 2: Representative chromatograms for the thiourea and uracil analyte mixture obtained before and after stopping the flow. Conditions are as described in Figure 1. (a) AQ-C18 before flow stop, (b) AQ-C18 after flow stop, (c) C18 before flow stop, (d) C18 after flow stop.



the extent to which dewetting occurs in aqueous eluents (11). They found that, for one type of C18 stationary phase, there was a retention loss of 80% for particles with a pore diameter of 80 Å, but, with a diameter of 150 Å and the same stationary phase, there was no measurable retention loss. On the other hand, they found that very little retention loss was measured for a phenyl-type stationary phase, even for particles with 80 Å pores. Walter and coworkers observed similar trends, and also described results for the dependence of retention loss on stationary phase bonding density, the concentration of methanol in the eluent, and use of a post-column restrictor to increase pressure inside the column (5).

Testing for Dewetting, and Some Remedies

One good way to assess whether or not a reversed-phase stationary phase is susceptible to dewetting under a particular set of conditions is by first equilibrating the column under conditions where the stationary phase is highly solvated, or fully wetted. For most reversed-phase stationary phases, this could be an eluent high in methanol or acetonitrile content. Flushing the column at a modest flow rate for a time equivalent to about 20 column volumes should be more than enough. Then, we switch to the aqueous eluent (or whatever eluent is useful for the application at hand), begin injecting a mixture of two or three probe compounds that have reasonable retention under these conditions, and monitor the change in retention factor (*k*) as the column equilibrates. One could simply wait for 20 column volumes to pass first before injecting the test sample, but, if we start injecting right away, we also learn something about how quickly the column equilibrates when switching from the organic-rich to the aqueous eluent. Then, once the retention has stabilized after this initial equilibration step, turn the flow off, wait 10 minutes, then turn the flow back on, and start injecting the test mixture again. If there is a significant difference between the retention before and after stopping the flow, dewetting is likely to be a serious problem under these conditions. Retention will vary from day to day, depending on the post-column flow restriction in the system, and peak shapes may deteriorate and become variable.

Figures 1 and 2 show representative results from such a test, conducted with two C18-type stationary phases that are otherwise very similar (that is, they use the same base silica), but one (AQ-C18) is designed for use in highly aqueous eluents. In other words, the AQ-C18 is engineered to avoid the dewetting phenomenon in completely aqueous eluents. Figure 1 shows the retention factor for uracil measured on these two columns in a completely aqueous eluent, injecting sample once per minute, before and after stopping the flow for 10 minutes. For the very first injection, the retention is slightly lower than the rest of the points, because the column has not equilibrated from the 50:50 organic–water flushing solvent to the completely aqueous eluent. After equilibration, the retention of uracil on the two phases is remarkably similar. After turning the flow off for 10 minutes, we observe that the two phases behave very differently. For the AQ-C18, there is no statistically significant change in the

retention of uracil. On the other hand, the retention of uracil on the C18 column decreases by about 75%.

Representative chromatograms for the two columns before and after stopping the flow are shown in Figure 2. The chromatograms for the AQ-C18 column are indistinguishable, as expected. In the chromatograms for the C18 column, though, we see that there is not only a change in the retention factor of uracil, but also a 23% decrease in the measured dead time. This is consistent with the idea discussed above, that eluent is extruded from the pores of the stationary phase particles when dewetting happens, effectively decreasing the dead volume of the column.

The good news is that dewetting does not have to be a death sentence for reversed-phase columns. As is the case when recovering a column that has dried out during storage (12), columns that have dewetted can be recovered by flushing with several column volumes of organic-rich eluent. Figure 3 shows a comparison of chromatograms obtained for the analyte butyrophenone before

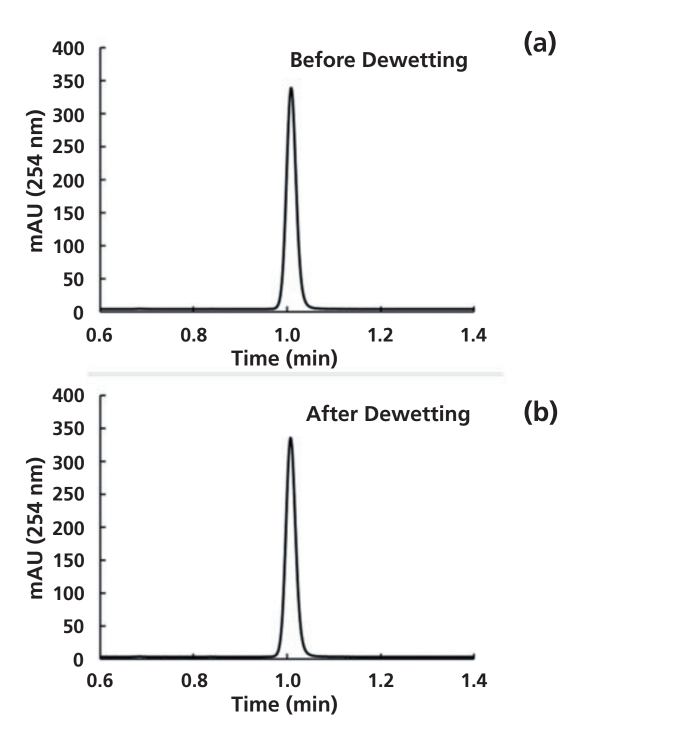


Figure 3: Comparison of chromatograms obtained for the analyte butyrophenone in 50:50 acetonitrile–10 mM phosphoric acid in water using the C18 (a) before, and (b) after the dewetting experiment. All other conditions are the same as in Figure 1.

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and after the dewetting experiment summarized in Figures 1 and 2. In this case, simply pumping 50:50 acetonitrile–water through the column at 0.4 mL/min for 5 minutes (pressure drop across the column was about 100 bar) was enough to restore both the retention and peak shape for this column to a “like new” state.

Summary

In this instalment of “LC Troubleshooting”, I have described the phenomenon that has become known as “dewetting” in reversed-phase chromatography. I have discussed the basic principles that explain why and when this occurs, so they can be used as a guide during method development. Since the extent of dewetting depends on a number of factors, including particle pore size, stationary phase chemistry, and operating conditions, it is a good idea to test for dewetting using the stop-flow during method development of applications involving an alkyl bonded phase (for example, C8 or C18), if they will be used with eluents containing less than 5% organic solvent. This piece has been restricted to isocratic conditions (that is, the use of completely aqueous eluents with reversed-phase stationary phases). In a future instalment, I will discuss the implications of using solvent gradient elution involving these highly aqueous eluents.

Acknowledgements

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Dwight R. Stoll is the editor of “LC Troubleshooting”. Stoll is a professor and co-chair of chemistry at Gustavus Adolphus College in St. Peter, Minnesota, USA. His primary research focus is on the development of 2D-LC for both targeted and untargeted analyses. He has authored or coauthored more than 60 peer-reviewed publications and three book chapters in separation science and more than 100 conference presentations. He is also a member of *LCGC*’s editorial advisory board. Direct correspondence to: LCGCedit@mmhgroup.com

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New Sample Preparation Products and Accessories

Douglas E. Raynie, Sample Preparation Perspectives Editor

This yearly report on new products introduced since March 2018 covers sample preparation instrumentation, supplies, and accessories.

In late 2018, the *LCGC* editorial staff submitted a survey to vendors of sample preparation products. Responses to this survey are compiled in this review, as are other new product introductions observed during the past 12 months. Note that I, for only the second time since 1990, did not attend Pittcon this year, so the additional information usually gained during the exposition is lacking from this year's review.

New sample preparation technologies introduced in the past year were somewhat passive. Sorbents and accessories for solid-phase extraction (SPE) led the way, as did a multiplatform integration of sample preparation with gas chromatography (GC).

This review is presented in three sections. First, we discuss GC-specific sample preparation platforms and accessories. Next, new solid-phase sorbents and sorbent-based products are presented. Finally, we turn to other sample preparation accessories and supporting technologies. To assist the reader with some of the details behind these new products, each section presents a tabular summary of the associated products. In all cases, the new products we uncovered are presented in the annotated table, while the text highlights particularly noteworthy products.

Figure 1: Showing (a) high-split (50 mL/min) gas chromatographic analysis of off-flavours in wine using immersive adsorption with the HiSorb probe. In the (b) low-split (5 mL/min) bottom chromatogram, the recollected sample is injected with a low split flow to increase sensitivity. (Courtesy of Markes International).

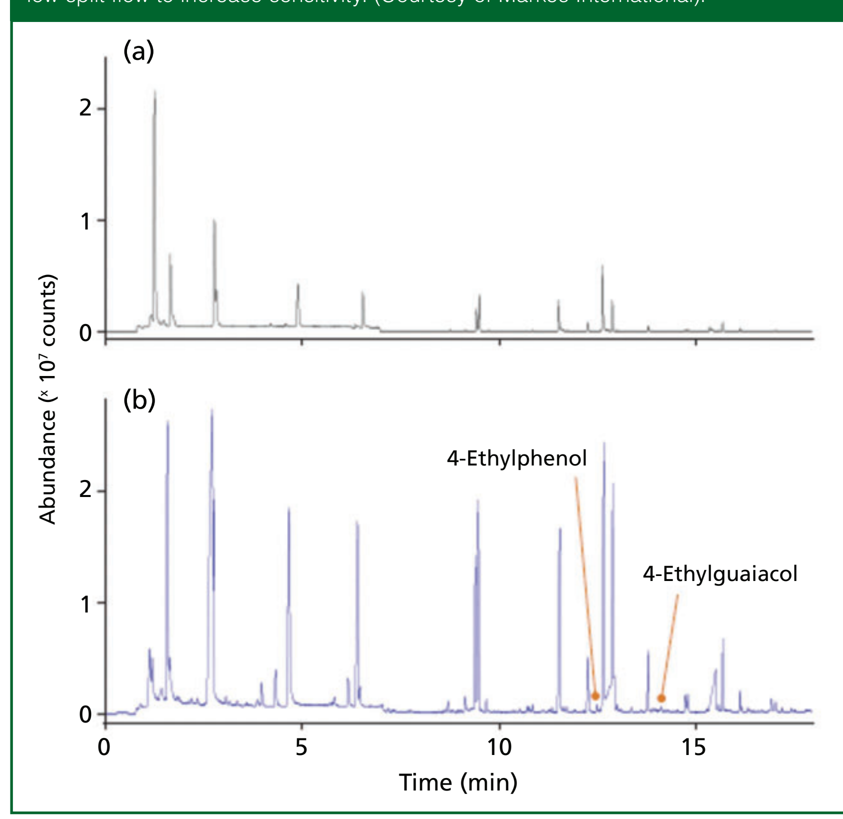

Table 1: Sample preparation instrumentation

Product Name	Supplier	Application	Main Use	Important Feature	Comments
Centri	Markes International www.markes.com	Multiple sampling modes for gas chromatography	GC sampling and sample injection	Recollection trap for enhanced sensitivity	Cryogen and solvent-free operation

Gas Chromatography–Specific Sample Preparation

A multiplatform sampling and concentration system for GC,

Table 2: Solid-phase sorbent products

Product Name	Supplier	Product Type	Mode	Base Material	Functional Group	Dimensions	Comments
Strata-X Drug B Plus	Phenomenex	96-well plate	Strong cation	Polymer	Proprietary	96 wells with 10 mg or 30 mg per well	Sample clean-up of basic drugs of abuse from urine
SiliaPrep PAH	SiliCycle Inc.	SPE cartridge	Dual mode: reversed and ion exchange phases	Silica	Proprietary	Range from 30 mg/1 mL–100 g/276 mL	Extract polycyclic aromatic hydrocarbons from wastewater
bioZen N-Glycan Clean-up	Phenomenex	Microelution 96-well plate	Sample clean-up	Silica	Proprietary	96-well plate	Clean-up of labelled, released n-glycans before liquid chromatography
β-Gone Plus β-Glucuronidase Removal	Phenomenex	96-well plate	Enzymatic clean-up	Proprietary	Proprietary	96-well plate	Drugs of abuse from urine
SiliaFast FaPEx	SiliCycle Inc.	QuEChERS	Dispersive solid-phase extraction	Silica	Proprietary	7.0 × 1.5 cm	Pesticide residue analysis
Strata-X Peptide Screening Microelution 96-Well Plate	Phenomenex	Microelution 96-well plate	Mixed sorbent: weak cation exchange and strong anion exchange	Polymer	Ion exchange	2 mg/well	Small volume peptide extraction
BioPureSPN Graphite	Nest Group	Cartridges, 96-well plates	Adsorption	Graphite	Activated charcoal	20 mg, 50 mg, 100 mg spin columns and plates	Desalting glycans, phosphopeptide enrichment
BioPureSPN HIL-PSA	Nest Group	Cartridges, 96-well plates	HILIC, anion exchange, ERLIC	Silica	Amine	20 mg, 50 mg, 100 mg spin columns and plates	Proteomic fractionation
HisSep Kit	Nest Group	Spin column	Sample clean-up	Proprietary	Proprietary	10–50 mg and 200–500 mg capacities	Histidine removal from antibody formulations
BioPureSPN TARGA C18	Nest Group	Cartridges, 96-well plates	Water-wettable reversed phase	Silica	C18	20 mg, 50 mg, 100 mg spin columns and plates	Desalting
Phthalate-Free SPE Cartridges	Applied Separations	Cartridge SPE	Various	Various	Various	1, 3, 6, 12, 20, 35, and 60 mL	Trace analysis EPA Method 525.2

Centri, was introduced by Markes International. A combination of robotics and analyte trapping is employed to accommodate a variety of sampling modes, including sorptive extraction, headspace sampling, solid-phase microextraction (SPME), and thermal desorption. The Centri's HiSorb sorbent extraction uses a large volume of polydimethylsiloxane adsorbent on a robust metal tip, for robustness and enhanced sensitivity in either the headspace or immersive modes, for the extraction and characterization of volatile and semivolatile organic compounds. Another feature of the Centri is a recollection trap. High split flows can be used in the initial GC separation of volatile samples, to minimize sample overload. The recollected sample may be injected with lower split ratios for high sensitivity, as illustrated in Figure 1. Thermal desorption with the Centri can be in the passive sampling, pumped sampling, dynamic headspace sampling, or direct desorption modes. Additional details of the Centri product are found in Table 1.

Solid-Phase Sorbents and Products

Although solid-phase extraction (SPE) has matured, developments continue in a variety of modalities to improve the ease of method development, application range, and other pending criticisms of the various formats of the technique. This year, the developments seem much more

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Table 3: Solid-phase extraction and sample preparation accessories

Product Name	Supplier	Application Area	Product Type	Suggested Application	Comments
BioChromato Slit Seal Well Plate Seal	BioChromato	Bioanalysis	96-well plate cover	Used with automated handling of 96-well plates	PET and silicone construction to prevent solvent volatilization
Presston 1000 Positive Pressure Manifold	Phenomenex	SPE	SPE manifold	Processing of 96-well plates for SPE and similar extraction formats	Fully pneumatic positive-pressure manifold for consistent well-to-well sample processing
EZpress 144 EXP Positive Pressure Processor	Orochem Technologies	SPE	SPE manifold	Processing of 96-well plates for SPE and similar extraction formats	Can process up to 48, 96, or 144 cartridges in three separate zones
EZpress 96 EXP Positive Pressure Processor	Orochem Technologies	SPE	SPE manifold	Processing of 96-well plates for SPE and similar extraction formats	Can process up to 48 or 96 cartridges in three separate zones
EquaVap	Analytical Sales & Service	Bioanalysis	Well-plate evaporator	24-, 48-, 54-, and 96-well plates	Equal and consistent drying time among all wells
Smart Evaporator	BioChromato	Sample concentration	Solvent evaporation	Small volume solvent evaporation	Patented technology for use with high boiling point solvents
Pulverisette 11 Knife Mill	Fritsch Milling and Sizing	Particle-size reduction	Knife mill	Range of sample types: moist, oily, fatty, dry, and fibrous	Blending, homogenizing, and SOP capable
Vial Centrifuge	MicroSolv Technology	Chromatography samples	Centrifuge	Small volume centrifugation of GC, LC, and other samples	Used with 12 × 32 mm vials
Milli-Q IQ 7003/05/10/15	MilliporeSigma	Liquid chromatography	Water purification	Preparation of high-purity LC mobile phases	Compact design, mercury-free UV technology, more precise total organic carbon measurement
SolvFil 1000 Nylon Bottletop Solvent Filter	Chrom-Supply	Mobile phase filtration	Solvent filtration	Mobile phase preparation	Design prevents solvents from absorbing oxygen during decanting
MicroLiter Certified ULTRAPure Septa	Wheaton	Autosampler vial caps	11-mm crimp caps	Chromatography samples	Multiple syringe penetration. Minimal siloxane contamination

limited than in previous years, and are primarily oriented towards bioanalysis. Phenomenex, SiliCycle, and Nest each introduced a family of new sorbents. The range of new sorbent-based product introductions is given in Table 2.

Phenomenex, primarily under the Strata-X family, developed the Strata-X Drug B Plus, bioZen N-Glycan Clean-Up, β-Gone Plus β-Glucuronidase Removal, and Strata-X peptide screening microelution 96-well plate products. The market for these products centres on analysis of drugs of abuse or enzyme hydrolysis. Each of the enzyme-hydrolysis products is in the microelution 96-well plate format, and allow in-well hydrolysis and filtrations. The extractions are cleaner than dilute-and-shoot samples, with low elution volumes, and shorter analysis times.

Meanwhile, the SiliCycle suite of products was developed for the

environmental market. The SiliaFast FaPEx is an enhanced QuEChERS-type cartridge for determination of a wide spectrum of pesticide residues in a faster method than more traditional approaches. The SiliaPrep PAH uses a proprietary endcapping with irregular silica of 40–63 µm, 60 Å, 500 m^2/g 13% C, and a 2.0–10.0 pH range. This exact silica is available in bulk, SPE cartridge, and flash cartridge formats.

Products from the Nest Group are available in either µ-spin cartridge or 96-well plate formats. Desalting is a feature of these products, and the BioPureSPN HIL-PSA product used for proteomic fractionation provides a salt-free first dimension for two-dimensional analysis, and can capture polyphosphorylated peptides. The HisSep Kit is designed for histidine removal from antibody formulations prior to polyacrylamide gel electrophoresis, and features 20–50 or 75–150 µL capacities. A water-wettable reversed-phase offering (BioPureSPN TARGA C18) is capable of loading 100% water to desalt glycans and phosphopeptides. Although novel sorbent phases or chemistries were not developed, Applied Separations launched a phthalate-free SPE cartridge available in a range of sizes that can be packed with any of their sorbents. For those using 96-well plates, BioChromato presented the BioChromato Slit Seal well plate seal, which features a three-layer polyethylene terephthalate and silicone construction for easy syringe penetration and prevention of solvent evaporation.

Constant, positive-pressure manifolds for SPE processing was the theme of products from Phenomenex and Orochem. Use of positive pressure, instead of vacuum manifolds, allows constant flow across each position in the manifold. The Presston 1000 manifold from Phenomenex has both high- and low-pressure gauges for use at a broad range of pressures. The Orochem EZpress is developed as two products to accommodate up to 96 or 144 one-millilitre cartridges, processed in two or three zones.

Sample Preparation Accessories and Related Products

Sample preparation accessories, including those previously discussed, are summarized in Table 3. Two new solvent evaporation systems were introduced in the past year. The EquaVap series from Analytical Sales & Services provides solvent evaporation from plates of 24, 48, 54, and 96 wells. The blowdown evaporators have an internal flow equalizer for equal output across all needle ports. The BioChromato Smart Evaporator accommodates single sample or up to 10 vials. A patented Spiral Plug technology allows evaporation of dimethylsulfoxide, dimethylformamide, and other high boiling solvents by placing solvents in a vacuum vortex under atmospheric pressure to eliminate bumping. This technology is diagrammed in Figure 2.

Figure 2: Diagram of patented vacuum-assisted vortex concentration technology used with BioChromato Smart Evaporator (Courtesy of BioChromato).

Other sample preparation accessories spanned a number of areas. Fritsch introduced the Pulverisette 11 knife mill for use with a range of sample types, from moist, oily, and fatty samples to dry and fibrous samples. The Vial centrifuge from MicroSolv Technology is utilized for small-volume samples associated with chromatography. The next generation Milli-Q system (IQ 7003/05/1015) from MilliporeSigma includes removal of colloids, particles, ions, and free chlorine, reverse osmosis, UV bacteria inactivation, and UV oxidation in a system with a smaller footprint than previous ultra-high purity water purifiers. When preparing mobile phases, a large membrane solvent filtration SolvFil 1000 Nylon Bottletop Solvent Filter is available from Chrom-Supply. A next generation of Wheaton vial caps (Microliter certified UltraPure septa) allows multiple syringe penetration, with minimal siloxane carryover to retard sample evaporation.

Conclusion and Future Directions

The trend, if any, emerging the past year seemed oriented towards bioanalysis, particularly using SPE technology. Whether the trend in coming years is aimed at tool-building or problem-solving for food, environmental, and related analysis, is the question.

"Sample Prep Perspectives" editor **Douglas E. Raynie** is a department head and Associate Professor at South Dakota State University, USA. His research interests include green chemistry, alternative solvents, sample preparation, high-resolution chromatography, and bioprocessing in supercritical fluids. He earned his Ph.D. in 1990 at Brigham Young University under the direction of Milton L. Lee. Raynie is a member of *LCGC*'s editorial advisory board. Direct correspondence about this column to LCGCedit@ mmhgroup.com

Going Green in Pharmaceutical Analysis

LCGC Asia Pacific spoke to Yong Liu and Adam Socia from MSD about the cost-saving benefits of implementing green chromatography in the pharmaceutical sector, the importance of analytical method volume intensity (AMVI), and effective practices to reduce solvent consumption and replace harmful solvents, including supercritical fluid chromatography (SFC), fast chromatography, and "cocktail chromatography".

Interview by Alasdair Matheson, Editor-in-Chief, *LCGC Asia Pacific*

Q. What is the definition of green chromatography?
Yong Liu: Green chromatography is an important part of green analytical chemistry and originates from the 12 green chemistry principles developed to reduce the environmental impact of chemical synthesis and analysis (1). Green chromatography incorporates practices to reduce the amount of solvent consumption and waste generation mainly through separation and sample preparation.

Q. What green approaches are being used to reduce the amount of solvents being used in liquid chromatography (LC)?
YL: From our perspective, the first step is to provide a metric tool for measurement of the "greenness" of a liquid chromatographic method. Hartman, Helmy, and coauthors developed analytical method volume intensity (AMVI), a simple metric that offers quick measurement of total solvent consumption for a high performance liquid chromatography (HPLC) method (2). AMVI enhances the awareness of green chromatography and, more importantly, makes this concept an integral part of method development for bench analytical scientists.

From a technical point of view, the review article by Welch, Wu, and coauthors offered an excellent summary of major green approaches in chromatography (3). The first is solvent reduction through fast chromatography by using conventional HPLC with elevated pressures, ultrahigh-performance liquid chromatography (UHPLC) or conventional HPLC systems with minor modifications, such as smaller diameter columns for greener separation or new column technologies, such as fused-core particle columns. The second approach is to use microflow and capillary HPLC. The third strategy is solvent replacement, which is achieved by using ethanol to replace acetonitrile, super-heated water chromatography, and carbon dioxide-based chromatography. Recently, a work by Welch, Regalado, and coauthors demonstrated that HPLC and LC–mass spectrometry (MS) experiments can be performed using distilled alcohol spirits, such as cachaça, rum, vodka, and aguardiente, as well as other common household items, including vinegar and ammonia, as mobile phases and additives (4). The former has been nicknamed "cocktail chromatography". This methodology provides a low cost and environmentally sustainable alternative in LC that does not depend on the use of hazardous organic solvents (such as acetonitrile). Supercritical fluid chromatography (SFC) uses pressurized carbon dioxide in the subcritical or supercritical state as a chromatographic mobile phase and has been adopted for both preparative separation and analytical purposes.

Q. You recently published an article on a greener chromatography method for dissolution testing on solid pharmaceutical formulations. What is novel about your approach and what benefits does it offer the analyst?
Adam Socia: Green chromatography has been practiced to support small molecule drug substance synthesis and release in the pharmaceutical industry for more than 10 years. However, the application of green chromatography in formulation development for drug products is not so common. Our work represents one of the first applications of green chromatography in drug product development (5). We provided an improved chromatographic protocol combining the utilization of smaller internal diameter (i.d.) columns, superficially porous column technology, injection cycle time for gradient re-equilibration, system dwell volume understanding, and basic separation concepts for optimization for a greener, faster, and robust way to conduct dissolution testing. This methodology provides 70–80% reduction in solvent consumption and waste generation, as well as run times with equivalent accuracy, precision, and robustness based on current LC instruments commonly used in our industry.

Q. Have you applied this approach to "real life" samples?
AS: Yes, we have implemented this approach to several phase I programmes. We strongly believe this approach should be adapted at the earliest point possible in drug product development to achieve the greatest savings.

Q. Are there any other practical examples of green chromatography you have developed in your laboratory that illustrate the benefits in terms of sustainability and cost-effectiveness?
YL: Yes. From a sample preparation perspective, we—with work led by Nowak and Regalado—developed a method that significantly reduced organic solvent use in standard preparation for residual solvent analysis, a test required by regulatory agencies for a drug substance developed in the pharmaceutical industry (6). Typically, the analyst makes fresh residual solvent

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standard in volumetric flasks and only uses a small portion of solution (an HPLC vial, for example) for every new sample submission. We showed that a multisolvent standard mixture can be stored in the crimped HPLC vials at -10 °C for at least 31 months with excellent recovery for all 25 solvents (over 97% with overall relative standard deviation [RSD] below 5%). The multisolvent standard in crimped HPLC vials are made in a large quantity and are provided to analysts to support almost all the projects in our department. This practice greatly reduces the repetitive standard preparation and organic solvent consumption and waste generation to support residual solvent analysis for in-process samples.

Q. What green chromatography techniques are being adopted in gas chromatography (GC)?
YL: At MSD, small bore and short GC columns were adopted to reduce the analysis time for fast chromatography and bring the benefit of lower instrument energy and gas consumption (6). The improved instrument output also offers the possibility to use fewer instruments. The other practice adopted for GC is in standard sample preparation, which we mentioned earlier. Another green chromatography effort in GC is to replace the carrier gas helium with renewable clean gas, such as hydrogen. Bernardoni *et al.* recently published a GC–flame ionization detection (FID) method using hydrogen as carrier gas for the analysis of 30 common solvents in pharmaceutical synthesis (7).

Q. SFC was widely touted as a greener technique. Is this approach being used more in the pharmaceutical sector?
YL: SFC uses pressurized carbon dioxide to replace hexane or heptane, which are commonly used, nonpolar eluents in normal phase chromatography. SFC was first widely adopted for preparative purification of chiral molecules (8). Recently, analytical SFC has become more popular for chiral separation to support synthetic process development, monitor chiral purification, and even as an analytical release assay to support regulatory filing (9–11). At MSD, SFC became the “go-to” analytical tool for chiral separation for bench analysts several years ago in the area of process chemistry development (12).

Q. Are there any other examples in your organization that illustrate the benefits of green chromatography in the pharmaceutical sector?
AS: Purity and content analysis, primarily performed by HPLC, is a required test for drug products in development and release, regardless of formulation type. Green chromatography is still relatively new and we are working on implementing existing best practices, such as AMVI and fast chromatography, into this area.

Q. Green chromatography seems to go in and out of fashion. Why is this and is it back in fashion?
YL and AS: Being green and protecting our environment by doing good science should always be a social concern for an analytical scientist. Science and technology advances always offer new opportunities for greener chromatography, such as the invention of UHPLC, analytical-scale SFC, and microflow and capillary HPLC to name just a few examples. Chromatography is widely used in different areas of drug discovery and development in the pharmaceutical industry. A lot of great work has already been done in supporting chemical synthesis of drug substances, particularly small molecules. In the areas of formulation development, PK determination, vaccines, and biologics, LC is heavily employed. Green chromatography, however, is not so common. The authors believe that there are good opportunities in these areas to implement green chromatography.

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Adam Socia is an Associate Principle Scientist in the Analytical Sciences group at Merck & Co., Inc., West Point, Pennsylvania, USA. He completed his Ph.D. at Drexel University and his MS at Sacred Heart University, focusing on the development of novel separations for amino acids and other charged analytes. Adam’s current role is in providing analytical support for both solid-dosage and sterile-liquid early-stage pharmaceuticals. Adam has written about his research in several peer-reviewed journals and presented both oral and poster presentations at numerous conferences.

Yong Liu is a Principal Scientist in the Department of Analytical Sciences at Merck & Co., Inc., West Point, Pennsylvania, USA. He has been in Merck for 17 years in the field of analytical chemistry and quality control for small molecule pharmaceuticals, siRNAs, lipids, peptides, and proteins both in drug substance and drug product. Currently, he is leading a group in providing analytical support for drug product development covering phase I through phase III and drug product degradation impurity structure elucidation by mass spectrometry. He has 40 publications with research interests in separation science, structural elucidation of degradation impurities of drug molecules, and studying of drug–drug and drug–excipient interactions via MS. He obtained bachelor’s degree from Wuhan University, a master’s degree from University of Central Florida, and a Ph.D. degree from Rutgers University, both in organic chemistry.

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www.vicidbs.com

VICI AG International, Schenkon, Switzerland.

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www.peakscientific.com/genius

Peak Scientific, Scotland, UK.

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http://bit.ly/2w0w0dC

Gilson, Middleton, Wisconsin, USA.

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www.gerstel.com

Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany.

Solving Recovery Problems in Solid-Phase Extraction

In this excerpt from *LCGC*'s e-learning platform, CHROMacademy.com, recovery problems in solid-phase extraction (SPE) are discussed.

Problems with quantitation in solid-phase extraction (SPE) may be associated with an inability to recover target analytes or internal standards from the extraction sorbent. These problems may be the result of poor choices in the chemistry of the protocol, or issues with the processing of the method.

The choice of sorbent chemistry is very important, and, to achieve the optimum extraction selectivity, one needs to choose a sorbent based on the analyte and matrix component chemistry. It is very useful to know the LogP/LogD values and pK_a values wherever possible. In general terms, the specificity of the sorbent–analyte interaction increases in the following order:

- non-functionalized polymer sorbents
- hydrophobic sorbents (such as C18, C8, CN; more polar sorbents are more selective)
- polar functionalized polymeric sorbents
- strong or weak ion-exchange sorbents
- mixed mode sorbents (silica or polymer hydrophobic materials with ion-exchange functional groups)

The strength of electrostatic interactions between analytes and sorbent, some 15 times stronger than simple hydrophobic interactions, means that sorbents containing ion-exchange moieties are likely to give the most selective extraction under most circumstances.

Of course, although the ability of the sorbent to retain the analyte is of great importance, one also needs to consider the requirement to selectively elute the analyte, which again requires knowledge of the analyte chemistry and the interactions with the sorbent. For the avoidance of doubt: in solid-phase extraction, the ability to be selective invariably equates to the extent of analyte recovery.

To ensure optimum analyte retention, one should consider both the sample diluent and the sorbent equilibration solvent. If an analyte is loaded in a solvent that is too highly eluting, then analyte breakthrough may occur or final elution volumes may be larger than necessary. For hydrophobic sorbents (and analytes), use a sample diluent with as little organic solvent as possible to ensure sample solubility. If the sorbent requires a conditioning (wetting) step, which is typically 100% organic solvent, ensure that an equilibration step is used to lower the elutropic strength of the solvent within the sorbent bed prior to the sample loading step.

If ion-exchange sorbents are used, the pH of the sample diluent should be adjusted to ensure target analytes are in their ionized form before loading. Similarly, the pH of the equilibration solvent should be adjusted to ensure that any weak ion-exchange ligands are in the charged form (the pK_a of weak ion-exchange sorbents can be obtained from the manufacturer's literature), to promote efficient retention of the target analytes. Further, one should consider loading the sample at a lower flow rate than is usual, because of the point-to-point nature of the electrostatic interactions in ion exchange, which require some degree of molecular orientation to occur, and therefore are inherently slower than other retention mechanisms. It may be necessary to include a "soak" time, in which the flow is stopped and the sample remains stationary within the sorbent bed to maximize analyte retention and prevent poor analyte recovery. Soak times of 30 s to several minutes can be necessary to ensure optimum recovery, and can be used with all types of sorbents.

Wash solvents, used to selectively elute interferences from the sorbent while retaining the analyte, should be strong enough to obtain the cleanest extract possible. However, care needs to be taken to avoid analyte breakthrough. Optimization of wash solvent strength should be performed by titrating the elution strength in discrete steps over several tubes or wells containing the same nominal analyte amount, plotting the solvent strength against analyte recovered, choosing the highest solvent strength that does not contain any analyte response. Remember here also that the organic solvent type and composition can be used in tandem with solution pH and ionic strength in order to achieve a "strong" wash solvent that removes a large amount of sample matrix components but does not cause analyte breakthrough. The "mixed-mode" phases are particularly useful in this respect because the dominant retention mechanism can be "flipped" between hydrophobic and hydrophilic/electrostatic using pH and solvent strength, resulting in improved sample cleanliness and recovery.

Similarly, the strength of the elution solvent should be optimized by evaluating successively stronger solvents, until one observes an unacceptable amount of interferences or background within the final eluate. Again, solvent strength, pH, and ionic strength should all be considered for optimization of elution strength, and soak steps may also be required to optimize recovery.

One further, more esoteric cause of poor recovery is the phenomenon of dewetting. If a less miscible solvent is loaded onto the sorbent bed (hexane following an aqueous solvent, for example), it may be necessary to fully dry the sorbent bed under high vacuum (or positive pressure) for several minutes, to avoid exclusion of the second solvent from the sorbent pore structure by the first solvent.

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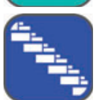
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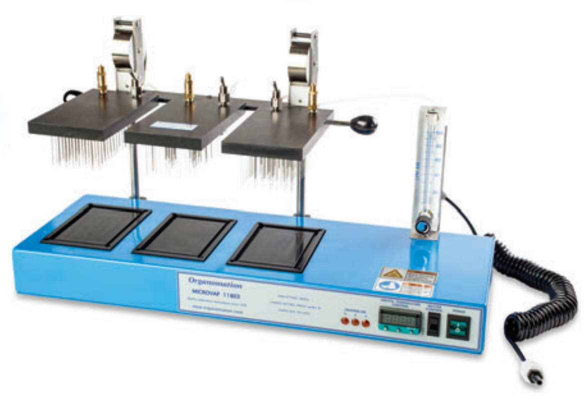

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