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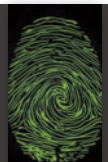
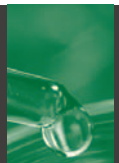


PEER-REVIEWED ARTICLE
Nontargeted soil analysis using DHS–TD–GC–MS



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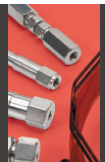

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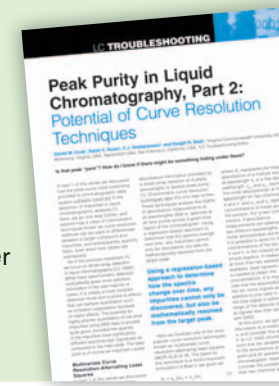

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Chemical Fingerprinting of Mobile Volatile Organic Compounds in Soil by **Dynamic Headspace–Thermal Desorption–Gas Chromatography–Mass Spectrometry**

Peter Christensen, Majbrit Dela Cruz, Giorgio Tomasi, Nikoline J. Nielsen, Ole K. Borggaard, and Jan H. Christensen,
University of Copenhagen, Copenhagen, Denmark

The chemical analysis of organic compounds in environmental samples is often targeted on predetermined analytes. A major shortcoming of this approach is that it invariably excludes a vast number of compounds of unknown relevance. Nontargeted chemical fingerprinting analysis addresses this problem by including all compounds that generate a relevant signal from a specific analytical platform and so more information about the samples can be obtained. A dynamic headspace–thermal desorption–gas chromatography–mass spectrometry (DHS–TD–GC–MS) method for the fingerprinting analysis of mobile volatile organic compounds (VOCs) in soil is described and tested in this article. The analysis parameters, sorbent tube, purge volume, trapping temperature, drying of sorbent tube, and oven temperature were optimized through qualitative and semiquantitative analysis. The DHS–TD–GC–MS fingerprints of soil samples from three sites with spruce, oak, or beech were investigated by pixel-based analysis, a nontargeted data analysis method.

Environmental samples contain thousands of organic compounds in complex mixtures (1), but the chemical analysis of organic compounds in environmental samples is typically targeted at a few chemical constituents that are already known and are expected to be present (2,3,4). In contrast, chemical fingerprinting aims to analyze all compounds from a complex mixture, which can be monitored with the selected analytical platform. The concept of chemical fingerprinting was first used in the 1970s for oil hydrocarbon fingerprinting to determine the source and weathering of crude oil and refined petroleum products (5). Since then, oil hydrocarbon fingerprinting has developed extensively and modern methods can now be used to monitor more than 1000 compounds in one single analysis (6). In the 1990s, fingerprinting methods were used for metabolomics and proteomics studies (7,8), and are now also used for plant and air matrices (9,10,11). Although the overall aim of chemical fingerprinting is to obtain a complete representation of a sample (for example, the whole metabolome of a cell), no single analytical technique exists that can fulfill this aim. Analytical techniques such as gas chromatography (GC) with mass spectrometry (MS) detection and liquid chromatography (LC) with MS detection are complementary methods that can be used with varying sensitivity to monitor compounds with different physical and chemical properties (for example, volatility and polarity). Each of these methods can be tuned to address different chemical windows by the choice of chromatographic mode or ionization source. Within soil science, substances in soil that can evaporate into the atmosphere, leach to surface and sub-surface water, or can be taken up by living organisms are of great interest for environmental, human health, and food perspectives (12). Several extraction techniques have been developed to transfer VOCs from various matrices

KEY POINTS

- The optimization of dynamic headspace, thermal desorption, and gas chromatographic parameters for analysis of mobile volatile organic compounds in soil slurry is investigated.
- DHS–TD–GC–MS chromatograms of mobile volatile organic compounds in soils were investigated by pixel-based chemometric data analysis.
- Terpenes in soils can be a potential biomarker for land use.

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Table 1: Monitored compounds used for the method optimization together with retention times, target and qualifier ion(s), and grouping of VOCs based on boiling points (bp). VOC group 1, bp < 35 °C; VOC group 2, 35 °C ≤ bp < 100 °C, and VOC group 3, 100 °C ≤ bp ≤ 218 °C (bp of naphthalene).

Compound	Retention Time (min)	Target Ion	Qualifier Ion(s)	VOC Group
Dichlorodifluoromethane	4.75	85	87/101	1
Chloromethane	5.98	50	52/15	1
Chloroethene	6.51	62	27/64	1
Bromomethane	7.33	94	96/79	1
Methanol	7.48	30	15/28	
Vinyl chloride	7.59	64	29/66	1
Trichloromonofluoromethane	8.00	101	103/105	1
1,1-Dichloroethene	8.61	61	96/98	1
Carbon disulfide	8.76	76	44/32	2
Acetonitrile	8.93	41	40/39	2
Allyl chloride	8.98	41	39/76	2
Dichloromethane	9.10	84	49/86	2
Water	9.26	16	19/20	
Acrylonitrile	9.29	53	52/26	2
(E)-1,2-Dichloroethene	9.32	61	96/98	2
1,1-Dichloroethane	9.66	63	65/27	2
Chloroform	9.68	83	85/47	2
Propionitrile	10.09	54	28/26	2
Methacrylonitrile	10.19	41	67/39	2
1,1,1-Trichloroethane	10.40	97	99/61	2
Carbon tetrachloride	10.51	117	119/82	2
2-Methyl-1-propanol	10.55	31	41/42	2
Benzene	10.63	78	77/52	2
1,2-Dichloroethane	10.67	62	27/49	2
Trichloroethylene	11.06	130	95/132	2
Methyl methacrylate	11.22	41	69/39	3
1,2-Dichloropropane	11.23	63	62/41	2
1,4-Dioxane	11.27	88	28/29	3
Dibromomethane	11.28	174	93/95	3
Bromodichloromethane	11.38	83	85/129	3
(Z)-1,3-Dichloro-1-propene	11.67	75	39/110	3
(E)-1,3-Dichloro-1-propene	11.75	75	39/49	3
Pyridine (from pyridine trifluoroacetate)	11.82	79	52/51	3
Toluene	11.90	91	92/65	3
Ethyl methacrylate	12.05	69	41/39	3
1,1,2-Trichloroethane,	12.17	97	83/61	3
Tetrachloroethylene	12.24	166	164/131	3
Dibromochloromethane	12.44	129	127/131	3
1,2-Dibromoethane	12.53	107	109/27	3
Chlorobenzene	12.86	112	77/114	3
1,1,1,2-Tetrachloroethane	12.92	131	133/117	3
Ethylbenzene	12.92	91	106/77	3
o-Xylene	13.01	91	106/77	3
p-Xylene	13.01	91	106/77	3
m-Xylene	13.29	91	106/77	3
Styrene	13.29	104	103/78	3

Table 1: (Continued) Monitored compounds used for the method optimization together with retention times, target and qualifier ion(s), and grouping of VOCs based on boiling points (bp). VOC group 1, bp < 35 °C; VOC group 2, 35 °C ≤ bp < 100 °C, and VOC group 3, 100 °C ≤ bp ≤ 218 °C (bp of naphthalene).

Compound	Retention Time (min)	Target Ion	Qualifier Ion(s)	VOC Group
Bromoform	13.44	173	171/175	3
1,1,2,2-Tetrachloroethane	13.74	83	85/95	3
(E)-1,4-Dichloro-2-butene	13.78	53	75/89	3
1,2,3-Trichloro-propane	13.79	110	75/77	3
Pentachloroethane	14.24	167	117/165	3
1,3-Dichlorobenzene	14.48	146	148/111	3
1,4-Dichlorobenzene	14.55	146	148/111	3
1,2-Dichlorobenzene	14.82	146	148/111	3
Hexachloroethane	15.04	201	117/119	3
1,2-Dibromo-3-chloropropane	15.40	157	75/155	3
Naphthalene	16.30	128	127/102	3

to a GC system (13,14). Most of these techniques can be grouped into solvent extraction, solid-phase extraction (SPE), gas extraction, and passive extraction (14). The U.S. Environmental Protection Agency Method 5035 for soil and waste samples recommends solvent extraction with methanol or polyethylene glycol for samples with high VOC concentration and gas extraction by purge-and-trap for VOC concentrations of less than 200 µg/kg (15). Purge-and-trap is able to automatically extract, concentrate, and transfer analytes to a GC system with little loss to the surroundings, and this is especially useful when working with trace amounts of VOCs (16,17). Dynamic headspace (DHS) is an alternative to purge-and-trap. In DHS the headspace above the sample, such as a soil slurry, is purged with inert gas during shaking or stirring and the VOCs are trapped on a sorbent tube. The sorbent tube is transferred to a thermal desorption unit (TDU), which is then heated for desorption (thermal desorption [TD]) of the VOCs and an inert gas carries the VOCs to the GC inlet. In this step, the direction of the gas flow thorough the desorption tube is reversed compared to the gas flow in the trapping phase. At the GC inlet the VOCs are focused, either cryogenically or by a sorbent before transfer to the GC column. By using DHS, the VOCs are dynamically removed from the sample, which mimics natural conditions better than batch extraction (18). The aim of this study was to develop and test a method for chemical fingerprinting of the mobile fraction of VOCs in soil using DHS–TD–GC–MS. Several parameters were optimized with a focus on optimal transfer of VOCs, while also reducing transfer of water. Following method optimization, soil samples representing three vegetation types were analyzed and a pixel-based chemometric approach was used to compare them to search for specific markers for land use.

Materials and Methods

Standards and Chemicals: EPA VOC Mix 6, EPA Appendix IX Volatiles Calibration Mix, and calcium chloride hexahydrate were supplied by Sigma Aldrich Denmark A/S. D_8-Naphthalene (Cambridge Isotope Labs., inc.) was obtained from VWR International A/S. Stock solutions and dilutions of mixtures were prepared in methanol (HPLC-grade, Rathburn Chemicals Ltd.) supplied by Mikrolab Aarhus A/S. Purified water was produced by a Millipore Milli Q Plus system.

Artificial Sample for Method Optimization: A test mix of EPA VOC Mix 6 and EPA Appendix IX Volatiles Calibration Mix was prepared by adding 10 µL of each mix to 180 µL of methanol to reach a final concentration of 100 ppm for each VOC. An artificial sample was then prepared in headspace vials (20 mL) containing 5 g of Ottawa sand and 10 mL of milli-Q water spiked with 1.0 µL of the test mix. Compounds, retention times, target and qualifier ions, and VOC group for the test mix are listed in Table 1.

Soil Samples: Soil samples were collected from three closely spaced forest sites in Vestskoven in Denmark during March 2017. According to the American Soil Taxonomy system, the soils at the three sites were classified as Typic Hapludalfs, which are important, productive, mainly temperate area soils (19). Each site represents a different vegetation type: beech (*Fagus sylvatica*), Norway spruce (*Picea abies*), and oak (*Quercus robur*), which were planted on former farmland in the early 1960s. At each site, the top 30 cm was removed from an area of 0.5 × 0.5 m and approximately 500 g of soil from the sides of the hole at a depth of 10–20 cm were transferred to 1 L blue cap bottles. Six samples were collected from each site and transferred to the laboratory. Each bottle was filled to the neck with 0.01 M $CaCl_2$ and shaken for 1 h in a bottom-over-end rotator at 10 rpm. From each sample, 10 mL of slurry were transferred to 20 mL amber headspace vials, avoiding plant debris floating on the top. Quality control (QC) samples were prepared by mixing 350 mL from one beech sample, 350 mL from one oak sample, and 450 mL from one spruce sample. The QC mix was shaken and 10 mL was transferred to each of six amber headspace vials. Six controls were also prepared in the same way as the soil samples but without adding soil.

Apparatus: The sample handling was performed by a MultiPurpose MPS2 autosampler equipped with a DHS station and agitator (Gerstel GmbH & Co. KG). The GC system was a 7890A with a 5973N MS (Agilent Technologies).

Analytical Method: One µL of deuterated internal standard solution (68 µg/mL d_8-naphthalene in methanol) was added to each sample and was then shaken at 1500 rpm for 3 min in the DHS station. The DHS extraction was performed with a N_2

Figure 1: Flow and temperature scheme for the analytical method.

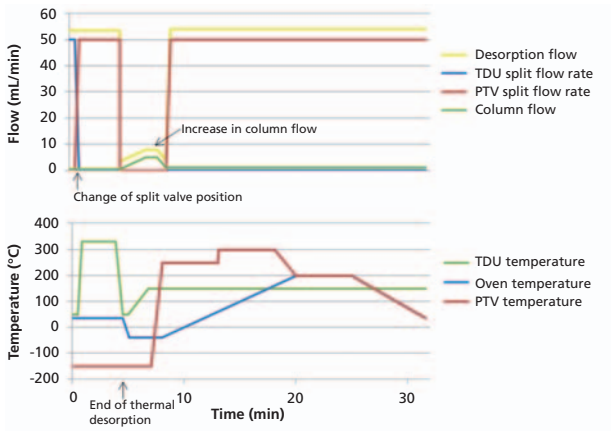


Figure 2: Area of VOCs and water for the five sorbent tubes (see Table 2 for further information, n = 1).

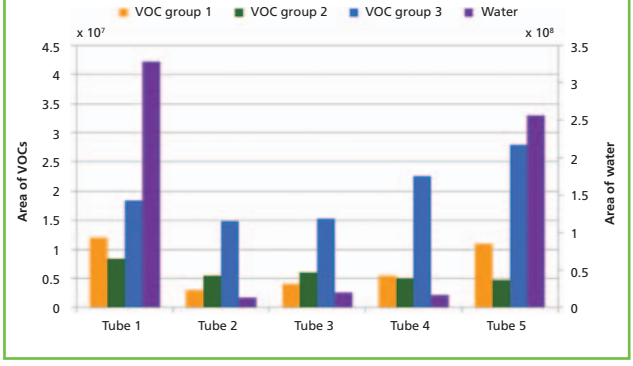


Figure 3: Transfer of VOCs and water for five purge volumes during the DHS extraction (n = 3). Error bars are ± 1 standard deviation.

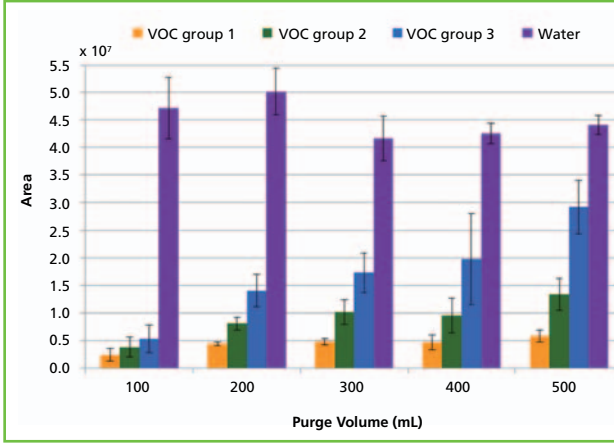
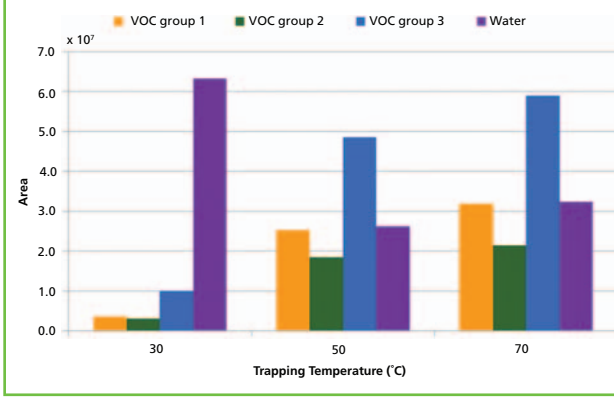


Figure 4: Area of VOCs and water for three trapping temperatures (n = 1).



purge flow of 50 mL/min for 10 min at 20 °C and analytes were trapped on sorbent tubes packed with Carbopack B + C and Carbosieve SIII (Gerstel GmbH & Co. KG) at 70 °C.

For transfer of analytes to the GC system, the sorbent tube was moved to the TDU, which was in solvent vent mode. Initially the total He flow rate was 53.5 mL/min, the septum purge flow rate was 0 mL/min (fixed), and the desorption flow rate was hence 53.5 mL/min. The TDU purge flow rate was 3 mL/min (fixed), the TDU split flow rate was 50 mL/min, and the column flow rate was 0.5 mL/min.

At 0.50 min (after the sorbent tube was moved to the TDU) the TDU split flow was changed to the PTV (Programmable Temperature Vaporizing) inlet split flow and kept at 50 mL/min (Figure 1). The pressure in the PTV inlet was 0.772 psi. The temperature of the TDU was held at 50 °C for 0.50 min, ramped to 330 °C at 720 °C/min, and held for 3 min (Figure 1). The analytes were cryo-focused in the liner in the PTV at -150 °C during the thermal desorption step. To avoid excessive use of liquid nitrogen, oven cooling was initiated after the thermal desorption step. The oven programme was hence started at 35 °C, decreased to - 40 °C at 120 °C/min, held for 2.875 min, increased to 200 °C at 20 °C/min, held for 5 min, and decreased to 35 °C at 25 °C/min (Figure 1). The oven reached - 40 °C but it was not possible to keep a rate of -120 °C/min. The hold time of 2.875 min was set to ensure that the -40 °C was reached. Transfer of analytes to the GC system can be improved by increasing the column flow rate before the PTV is heated. This was achieved with a column flow program starting at 0.5 mL/min, ramped to 5 mL/min at 1.95 mL/min per min, held for 1 min, and decreased to 1.1 mL/min at 5 mL/min per min (Figure 1). At the end of the flow program, the temperature program of the PTV was initiated. Here the temperature was increased by 12 °C/s to 250 °C, held for 5 min, increased by 10 °C/s to 300 °C, and held for 5 min.

The MS transfer line, ion source, and quadrupole temperatures were 230 °C, 230 °C, and 150 °C, respectively. Samples were analyzed in scan mode with a scan range of 10–300 mass-to-charge ratio (*m/z*). A 30 m × 0.25 mm, 1.4-µm VF-624ms column (Agilent J&W) was used.

Optimization Steps: Several parameters were optimized for the final method: type of sorbent tube, purge volumes, trapping temperature, drying of the sorbent tube, and initial oven temperature (Table 2).

The optimization steps for sorbent tube, trapping temperature, drying of the sorbent tube, and oven temperature were performed with a 30 m × 0.15 mm,

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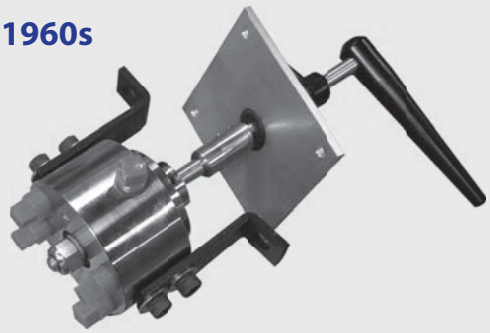

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Table 2: Optimization parameters and chosen settings for method optimization. Bold indicates setting chosen for the final method.

Sorbent Tube	Setting Evaluated				
	Carbopack C, Carbopack B, Carbosieve S-III	Tenax GR	Tenax TA	Carbopack B, Carbopack X	Carbopack B, Carbopack X, Carboxen-1000
Purge volume (mL)	100	200	300	400	**500**
Trapping temperature (°C)	30	50	**70**		
Drying of sorbent tube in DHS station (mL)	**0**	75	150	225	
Drying of sorbent tube in the TDU (mL)	**0**	75	150	225	
Oven temperature (°C)	**-40**	-20	0	35	

Figure 5: Extracted ion chromatogram of (a) bromomethane (*m/z* 94, VOC group 1), (b) dichloromethane (*m/z* 84, VOC group 2), (c) toluene (*m/z* 91, VOC group 3), and (d) pentachloroethane (*m/z* 167, VOC group 3) at initial oven temperatures of 35, 0, -20, and -40 °C.

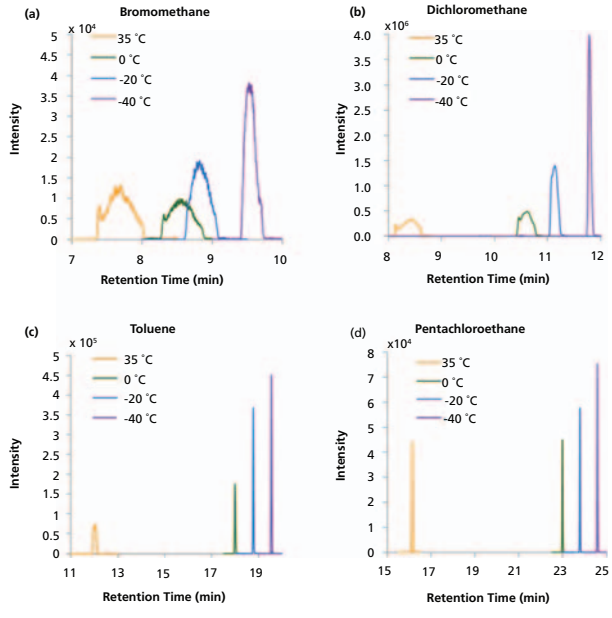
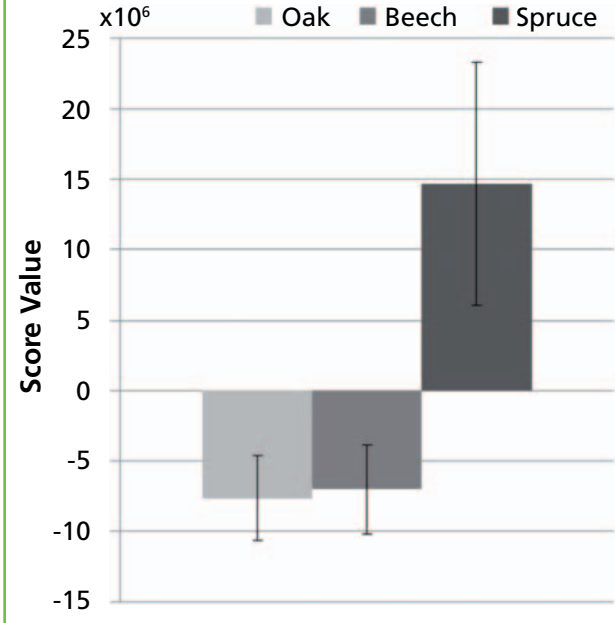


Figure 6: Average PC2 score values and standard deviations for samples representing oak, beech, and spruce (n = 6). Error bars are ± 1 standard deviation.



0.85-µm VF-624ms column (Varian) and modified methods compared to the final method described above were used.

For the optimization of the purge volume, the flow was kept constant at 50 mL/min and time was set to reach the designated purge volumes. To evaluate the sorbent tubes, the DHS extractions were performed with a purge flow of 25 mL/min for 8 min. The trapping temperature was 40 °C for the Tenax-based tubes (Table 2, tubes 2 and 3) and 50 °C for the Carbopack tubes (Table 2 – tubes 1, 4 and 5).

Data Analysis: For each optimization step, peaks were integrated and divided into their respective VOC group (Table 1). Evaluation of the parameters was based on the area of the VOCs and the area of the water peak (*m/z* 16). Overloading of the MS system occurred for *m/z* 17 and *m/z* 18 and therefore *m/z* 16 was the preferred choice for determination of the area of the water peak.

The total ion chromatograms (TICs) obtained from DHS–TD–GC–MS analysis of the soil extracts were investigated using a pixel-based chemometric approach where entire sections of chromatograms are analyzed without peak extraction (20). Mass-to-charge ratios below 35 as well as *m/z* 44 were removed from the TIC to exclude water, oxygen, nitrogen, and carbon dioxide. Baselines were removed by piece-wise linear subtraction of the lower part of a convex hull of each chromatogram (21) and samples were aligned using correlation optimized warping (COW) (22); the optimCOW procedure devised by Skov *et al.* (23) was used to find the optimal warping parameters. The scans before 9.25 min were excluded prior to alignment because the large irregular shifts in the early part of the chromatogram could not be satisfactorily aligned. The TICs were subsequently normalized

to Euclidean norm, thus removing information on analytical changes in signal intensity and concentration (21,24). The data were analyzed by principal component analysis (PCA), which was fitted according to a weighted least squares criterion using the inverse of the relative standard deviation of the QC samples as weights (25,26).

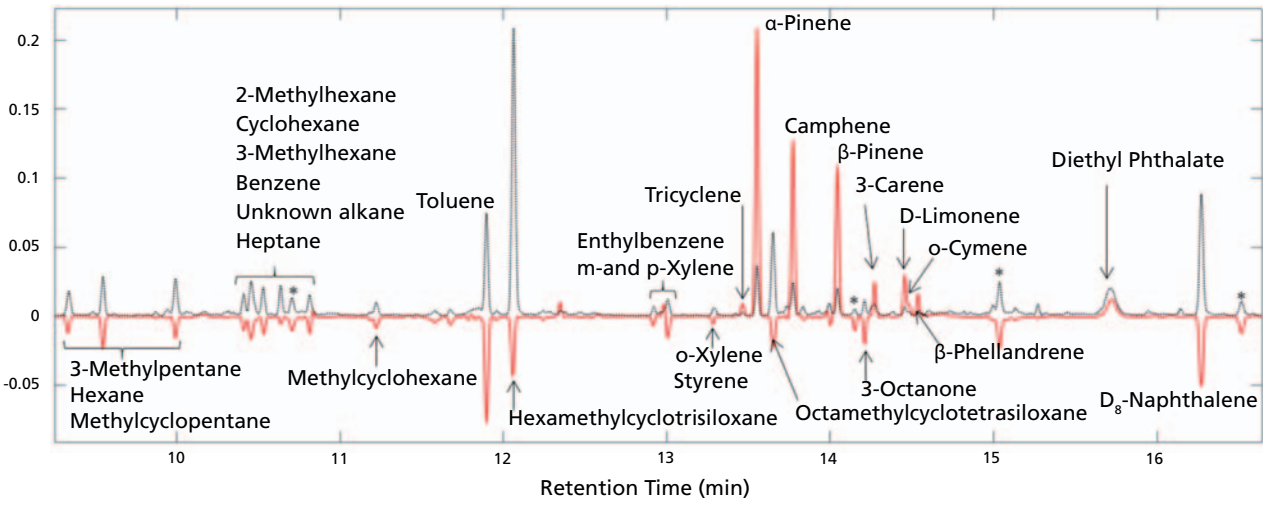
Results and Discussion

Optimization: One of the major challenges when analyzing VOCs in water samples and water suspensions on DHS–TD–GC–MS is to trap and isolate a large fraction of the VOCs and still eliminate water. Water can lead to chromatographic problems, such as poor peak shapes and split peaks, as well as retention time shifts as a result of solvent flooding (27). High amounts of water can also lead to carryover, higher detection limits, and poor reproducibility during the rapid heating of the inlet because of sample expansion beyond the capacity of the liner volume. Type of sorbent tube, purge volume, temperature during trapping, drying of the sorbent tube, and initial oven temperature were optimized to reduce the amount of water transferred from the sample while still obtaining high extraction efficiency and transfer of the VOCs from the sorbent tube to the GC column. The method targeted compounds with boiling points up to 218 °C. However, compounds with different boiling points were not necessarily affected the same way during extraction, trapping, transfer, and analysis. Therefore, the optimization parameters were evaluated based on a division of the VOCs into three groups. VOC group 1 included compounds with boiling points below 35 °C. These can easily volatilize at the sampling site and can be difficult to sample. VOC group 2 included compounds with boiling points between 35 °C and 100 °C. These are still very volatile, but are easier to sample compared to VOC group 1. VOC group 3 included compounds with boiling points between 100 °C and 218 °C. These are less likely to volatilize during sampling, but are also harder to extract with DHS than VOC groups 1 and 2 because they have a lower vapour pressure.

The most suitable sorbent tube traps all VOCs and is able to release them again during thermal desorption in the TDU, but does not trap any water and does not affect the VOC composition. Five sorbent tubes were tested for the trapping of VOCs. VOCs with boiling points below 100 °C (VOC groups 1 and 2) are likely be found at lower concentrations in soil samples than VOCs with boiling points above 100 °C as a result of volatilization in the field. Tube 1 was selected for the final analytical method because it provided the most efficient trapping of these low-boiling point VOCs and was the only sorbent tube that was able to trap the most volatile compound, dichlorodifluoromethane (Figure 2).

The purge volume for extraction should ensure highest possible transfer of VOCs, but not at the expense of also transferring a lot of water. Initial screening indicated that purge volumes of 30–400 mL during the DHS extraction were optimal and therefore purge volumes between 100–500 mL were tested in triplicates. The amount of water transferred to the sorption

Figure 7: PC2 loading plot. Red line indicates PC2 loading coefficients and dotted line indicates the average TIC. Terpenes have positive loading coefficients while most remaining peaks have negative coefficients. Compounds have been tentatively identified through a search in the NIST14 database. Asterisks indicate unknown compounds.



tube was relatively stable for the evaluated purge volumes (Figure 3). Transfer of VOCs largely increased with increasing purge volume, with VOC group 3 more affected than VOC groups 1 and 2. The optimal purge volume for all VOC groups was at 500 mL (Figure 3) and not at 300–400 mL as was found in the initial screening tests.

By increasing the trapping temperature, trapping of water can be limited. Trapping temperatures of 30 °C, 50 °C, and 70 °C were tested once. At trapping temperatures of 50 °C and 70 °C, trapping of water was reduced by approximately 50% compared to a trapping temperature of 30 °C (Figure 4). VOCs were trapped the least at 30 °C and slightly better at 70 °C than at 50 °C (Figure 4). The trapping temperature of 70 °C was therefore chosen.

Another way to remove water is by drying the sorption tubes in either the DHS station or in the TDU. Drying in the DHS station was performed with a N_2 flow through the tube (from the bottom and up), in the same way as the headspace was purged during the trapping. In the TDU, the drying was performed with a He flow from the top of the sorption tube to the bottom. The removal of water and VOCs was tested with a drying temperature of 70 °C, a flow of 35 mL/min in the TDU and DHS station, and with flow volumes in the range of 0–225 mL. Drying did not improve the VOC–water ratio and was therefore not implemented in the analytical method.

For the successful transfer of VOCs to the GC system, initial oven temperatures were also evaluated. The oven was cooled to initial temperatures of -40 °C, -20 °C, 0 °C, and 35 °C by the use of liquid nitrogen (except for 35 °C). The initial temperature of - 40 °C gave the highest and narrowest peaks (Figure 5); this was further improved for the final method using the same column as before with a larger inner diameter (0.25 mm instead of 0.15 mm) and film thickness (1.4 µm instead of 0.85 µm) leading to improved focusing on the column. The effect of the initial oven temperature was not seen for the very late-eluting compounds (Figure 5).

Soil Samples: The PCA of the preprocessed TICs showed a clear separation of spruce samples from the remaining samples along principal component (PC) 2. PC1 described variations in hexamethylcyclotrisiloxane,

Figure 8: Representative TICs of (a) spruce, (b) beech, and (c) oak where *m/z* 1–34 and 44 have been removed. Tentatively identified terpenes are marked with an asterisk (see Figure 9 for names).

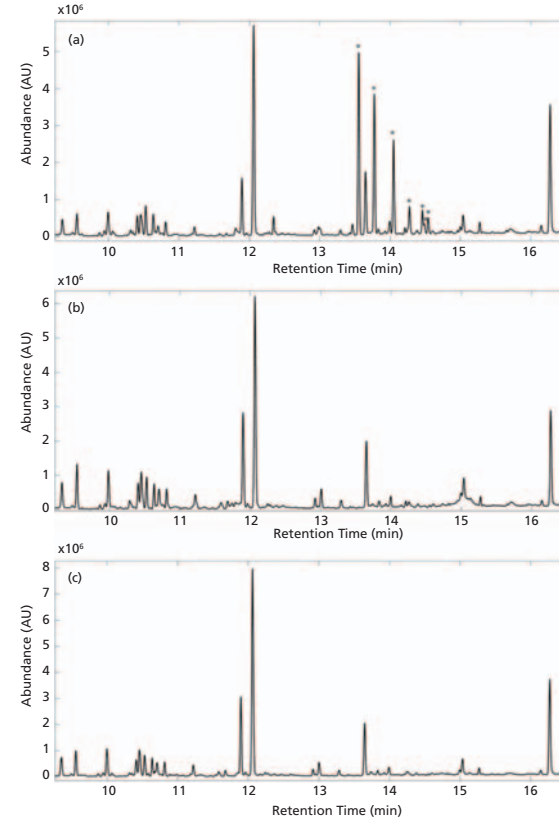
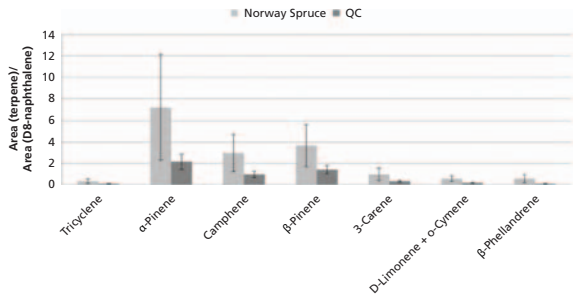


Figure 9: Precision of selected terpenes based on the area of the terpene divided by the area of d_8-naphthalene for QC samples (analytical precision) and samples representing spruce (combined sampling and analytical variation, n = 6). Error bars are ± 1 standard deviation.



octamethylcyclotrisiloxane, and diethyl phthalate. Spruce samples have positive PC2 score values while beech and oak samples have large negative PC2 scores (Figure 6). The separation in the PCA score plot can be explained from the corresponding loading plot (Figure 7). The positive scores indicate that the spruce samples contain relatively more (with respect to the average sample, which has score 0 by definition) of the compounds whose peaks have positive PC2 loading coefficients and relatively less of those with negative coefficients. For beech and oak samples the opposite is the case. Representative TICs of soil extracts from spruce, beech, and oak forest show that the TICs of soil extracts from spruce forest contain a number of peaks with positive PC2 loading coefficients that are not present in soil extracts from the beech and oak forests (Figure 8). The peaks with the largest PC2 loading coefficients were tentatively identified via a search in the NIST14 database. The majority of peaks with positive PC2 loading coefficients were terpenes, while peaks with negative PC2 loading coefficients were peaks that could also be found in the blank samples, such as d_8-naphthalene and hexamethylcyclotrisiloxane (Figure 7). The terpenes tentatively identified were α-pinene, β-pinene, camphene, 3-carene, D-limonene, *o*-cymene, and β-phellandrene.

In Figure 9 the precision of the terpenes is given based on the relative peak areas of the terpenes with respect to d_8-naphthalene for the quality control (QC) samples and the samples representing spruce. The samples representing beech and oak did not contain any of the terpenes. The precision of samples representing spruce was influenced by sample heterogeneity, as well as sampling and analytical variations. The QC samples were used to determine the analytical precision (repeatability) of the analytical method because these samples are analytical replicates. The repeatability calculated as relative standard deviations of the d_8-naphthalene standardized peak areas of terpenes in the QC samples was on average 27.5% (range 22.2–32.4%) and the sampling and analytical variation was on average 59.4% (range 46.1–68.1%) when calculated based on soil samples representing spruce. This means that the sampling variation can be estimated to an average value of 52.7%. These results demonstrate that the analytical uncertainty is acceptable and only contributes a little to the total uncertainty (59.4%).

With an unknown chemical profile of soil samples the benefit of calculating recoveries for the compounds in the test mixture is limited because these are not necessarily the compounds that are detected in the soil samples. All compounds in the test mix were detected at a level of 10 ng/mL in the artificial samples. The signal-to-noise ratio (*S/N*) was calculated for bromomethane, dichloromethane, toluene, pentachloroethane, and naphthalene as representatives of the three VOC groups. The *S/N* was in the range of 1300–6000 for the selected compounds in the test mix, which indicates that detection limits for these compounds are in the range of 5–23 ng/L.

The method was optimized to allow for nontargeted fingerprinting of soil samples. The method optimization was therefore based on peak areas and the chemometric analysis was performed on TICs. Thus only qualitative and semiquantitative data were presented. The nontargeted approach included all compounds that were detected compared to a targeted approach where only known constituents are analyzed. This provides improved information about the samples, and in this case, explains why soil samples from a spruce forest are different from soil samples from beech and oak forests. This could potentially lead to identification of new biomarkers for land use. For full quantitative analysis, it would be necessary to run standards and obtain better estimates of detection limits and limit of quantifications and recoveries specifically for the terpenes detected in the nontargeted fingerprinting to improve their applicability as a biomarker for land use.

Conclusion

A DHS–TD–GC–MS method was successfully optimized through qualitative and semiquantitative analysis and applied to soil samples representing spruce, oak, and beech. Nontargeted chemical fingerprinting analysis of the TICs of soil sample extracts showed that soil samples representing spruce differed from soil samples representing beech and oak because of the presence of terpenes. The optimized method was successfully used for the comparison of VOCs in soil samples from the three forest areas and for detection of terpenes as potential biomarkers for land use. The fingerprinting approach could be useful in other areas of research, such as metabolomics and petroleomics, and is not limited to environmental samples.

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Peak Purity in Liquid Chromatography, Part 2: Potential of Curve Resolution Techniques

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Is that peak "pure"? How do I know if there might be something hiding under there?

In part 1 of this series we discussed how the peak purity tools commonly provided in chromatographic data system software could aid in the detection of impurities in liquid chromatographic analysis (1). Here, we go one step further, and explore how a class of chemometric techniques known as *curve resolution methods* can be used to differentiate between a target compound and impurities, and subsequently quantify them, even when their peaks are overlapped.

As in the previous instalment (1), we focus on diode-array detection in liquid chromatography (LC–DAD). While mass spectrometric detection undoubtedly gives more selective information in the vast majority of cases, it is clearly a more complex detection mode and is prone to effects that can hamper quantitation such as ionization suppression because of matrix effects. The potential for highly precise quantitation of low-level impurities using DAD data is actually quite good, provided the spectra of the impurities have significantly different spectroscopic signatures as compared to the main peak. The latter point is of course an important caveat.

Multivariate Curve Resolution-Alternating Least Squares

In part 1 of this series we discussed the power of utilizing all of the absorbance information provided by a diode-array detector at multiple wavelengths to assess peak purity (1). Chemometric curve resolution techniques take this one step further. These techniques analyze the matrix of absorbance measurements at all wavelengths (that is, spectra) at all time points across a given time region of the chromatogram. Using a regression-based approach to determine how the spectra change over time, any impurities cannot only be discovered, but also be mathematically resolved from the target peak.

Using a regression-based approach to determine how the spectra change over time, any impurities cannot only be discovered, but also be mathematically resolved from the target peak.

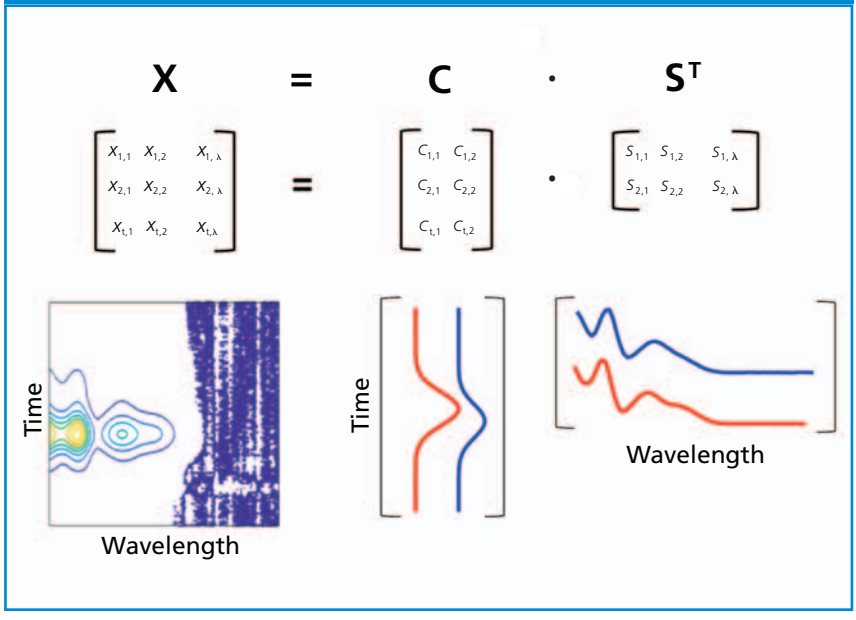
Here we illustrate one of the most popular curve resolution techniques, known as *multivariate curve resolution-alternating least squares* (*MCR-ALS*) (2–6). The basis for this technique is a multicomponent formulation of Beer's law given as:

$$A_\lambda = \varepsilon_{\lambda,X} b c_X + \varepsilon_{\lambda,Y} b c_Y \quad [1]$$

where A_λ represents the measured absorbance of a mixture solution at wavelength λ, b is the detection pathlength, $\varepsilon_{\lambda,X}$ and $\varepsilon_{\lambda,Y}$ represent the molar absorptivities at this wavelength for two chemical species X and Y, and c_X and c_Y represent the concentrations of these species in the solution. For a two-component mixture, if absorbance measurements are obtained at two different wavelengths, and the molar absorptivities are known, it is possible to solve for the concentrations of the two species, X and Y, in the mixture solution via simple algebra. If measurements at more than two wavelengths are available, least squares regression is needed to obtain the concentrations. It is important to note that the assumption that the two (or more) signals are linearly additive is only valid in cases where the total signal is within the linear range of the detector (for example, at signals less than about 1500 mAU with DAD).

At this point, we generalize the discussion to a measurement x, and consider this as a signal in an LC–DAD chromatogram, such that the variable $x_{i,j}$ refers to the absorbance at the ith time point and jth wavelength of the chromatogram. Additionally, we consider the possibility that more than two chemical species may be

Figure 1: Schematic for resolution of a spectrochromatogram represented by a matrix, **X**, into two component chromatograms and spectra contained by matrices **C** and **S**, respectively.



present in the sample within the chromatographic peak, which gives the following expression:

$$x_{i,j} = c_{i,1}s_{1,j} + c_{i,2}s_{2,j} + \ldots c_{i,N}s_{N,j} \quad [2]$$

Here, $c_{i,n}$ refers to the concentration of species *n* at the *i*th time point in the chromatogram, and $s_{n,j}$ refers to the molar absorptivity-pathlength product for species *n* at the *j*th wavelength. The full spectrochromatogram can be easily understood in terms of a matrix product. In matrix notation, equation 2 is commonly written as

$$\mathbf{X} = \mathbf{CS}^T \quad [3]$$

where the rows and columns of matrix **X** represent the absorbance at each wavelength and time point, respectively, and the superscript T refers to the matrix transpose. This concept is illustrated schematically in Figure 1. If the molar absorptivities are known at all measured wavelengths for all species present in the peak, then it is straightforward to solve for the resolved chromatograms, **C**, as follows:

$$\mathbf{C} = \mathbf{X}(\mathbf{S}^T)^\dagger \quad [4]$$

where the superscript † indicates the pseudo inverse operation. Equation 4 is simply a linear regression equation in matrix format. The columns of **C** are the individual component chromatograms (that is, each compound plus any background contributions), and the rows of $\mathbf{S}^T$ are the individual component spectra.

While in theory this approach could be a means of resolving overlapped chromatographic peaks, if there are unknown impurities present or uncharacterized mobile phase background components or species, then we do not have enough information to specify the **S** matrix. The MCR-ALS technique then becomes quite useful in this regard. Rather than exactly specifying **S**, an initial estimate for **S** is provided to the regression. This initial estimate can be obtained in a number of different ways. Pure variable methods are frequently used for this purpose. These methods seek to find the *N* most different spectra from the chromatographic data matrix, **X**, where *N* is the number of components needed to describe the measured data. The principle is that the most different spectra in the matrix are likely to be similar to the underlying pure component spectra. The caveat is that the number of components must be set by the user. Methods have been proposed for selecting the correct number of components such as scree plots; however, the only reliable method is evaluation of the results for multiple values of *N*. For a simple impurity screen, running MCR-ALS with two and three components to start should suffice, as one component would represent background, one would represent the target analyte, and if a third component is necessary, it is most likely because of an impurity peak.

A clear advantage to handling multiple chromatograms simultaneously is that calibration information and estimates of unknown concentrations can be obtained very efficiently.

Once this estimate for **S** is obtained, equation 4 is used to solve for the chromatographic profile matrix, **C**. Because the matrix **S** is only an approximation, **C** will only be an approximation as well. MCR-ALS can be considered an optimization method in which these **C** and **S** matrices are continuously improved with the goal of accurately representing the true underlying chromatographic and spectral profiles of each component. The power of MCR-ALS lies in the judicious implementation of constraints on the **C** matrix (and in subsequent steps, the **S** matrix as well) during this optimization. One frequently applied constraint is non-negativity, which allows the user to force the chromatographic profiles contained in **C** to have only positive values (6,7). Another constraint is unimodality, which forces each individual species chromatogram to exhibit a single peak (7). Many other constraints have been developed for MCR-ALS, but they are too numerous to describe here. Once **C** is constrained appropriately, the spectral matrix is updated via linear regression using equation 5:

$$\mathbf{S}^T = \mathbf{C}^\dagger\mathbf{X} \quad [5]$$

Now, constraints can be applied to this **S** matrix as well; non-negativity is frequently used in this case too.

By updating the **S** and **C** matrices in an alternating fashion (that is, equations 4 and 5), interspersed with the application of constraints, the final solutions for **C** and **S** will contain the pure component profiles of the individual chemical species within the chromatographic peak.

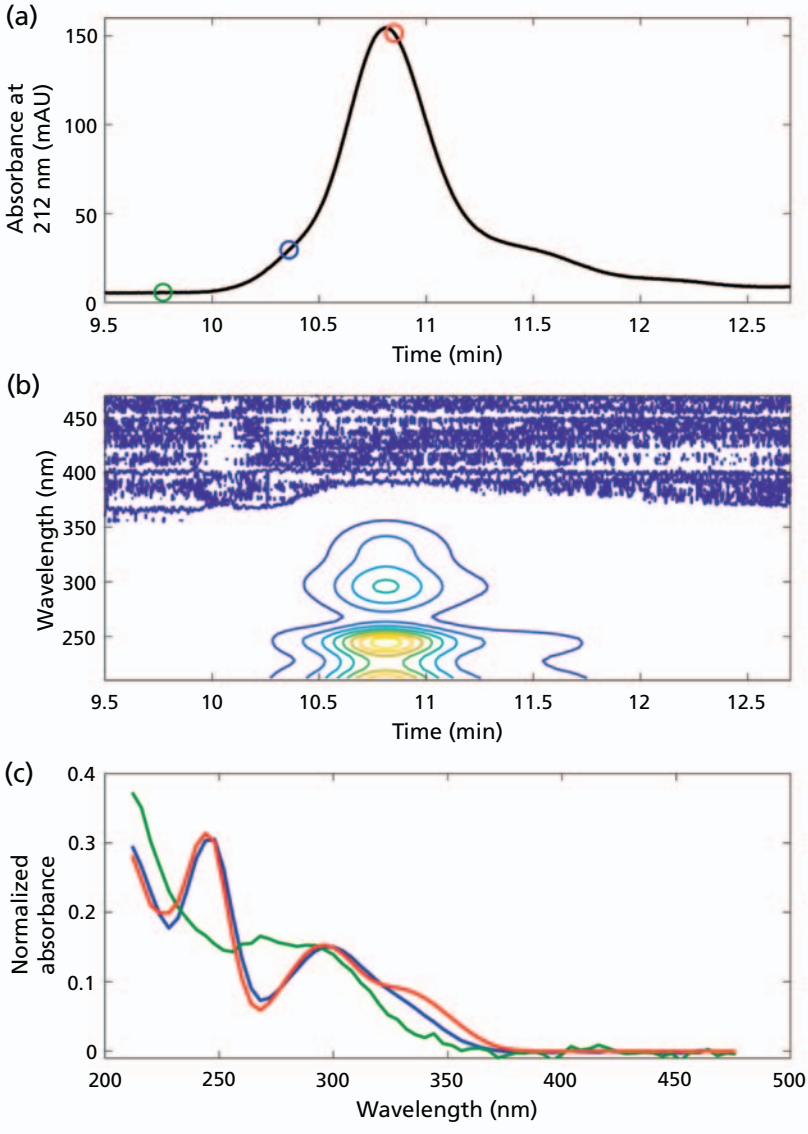
Application of MCR-ALS

We illustrate this approach using the chromatographic peak that was analyzed in part 1 of this series (1). Figure 2(a) shows the chromatographic peak, and Figure 2(b) shows the contour plot of the matrix **X**. We first applied a pure variable method (in this case the pure method in the Barcelona MCR-ALS toolbox, based on the SIMPLISMA algorithm [8–10]), and selected the three most different spectra within the spectrochromatogram. The corresponding time points are shown as circles in Figure 2(a), and the three spectra at these points are shown in Figure 2(c). It is likely that the spectrum shown in green represents a background spectrum, because it corresponds to a spectrum appearing in the baseline (green circle at 9.77 min in Figure 2[a]). After these initial estimate spectra are submitted to MCR-ALS, it should allow the algorithm to estimate the background contribution to the data, as well as the chromatographic peaks for each chemical species present within the profile.

The results for MCR-ALS analysis of this peak using these spectra for initial estimates are shown in Figure 3. Two peak shape responses within the chromatogram are resolved as shown in Figure 3(a). These are two of the components contained in the matrix **C**, corresponding to two chemical species (peaks shown in blue and red), and a background contribution from the mobile-phase gradient shown in green. The normalized spectra contained in matrix **S**, which correspond to these species or contributions, are shown in Figure 3(b). Note that the non-negativity constraint has been applied to the components corresponding to the real chemical species (shown in red and blue), while the background component (green) was not constrained. This flexible application of constraints leads to a powerful algorithm for curve resolution.

Quantitation with MCR-ALS: A natural limitation of the MCR-ALS algorithm in this case is that there generally are multiple mathematical solutions that satisfy equation 3. Constraints are used to limit the possible solutions, but this generally does not provide a unique, chemically valid solution, especially when using MCR-ALS to analyze a single chromatogram, as described above. An extension of the MCR-ALS technique to analyze multiple chromatograms simultaneously is quite powerful in this regard, especially for quantitative analysis. In this approach, the analyst runs a series of calibration sample mixtures with varying concentrations of the target analytes, and obtains chromatograms for test samples

Figure 2: (a) Chromatogram of impure peak at 212 nm; (b) representation of this chromatogram as a contour plot where the *y*-axis is the UV-visible absorbance spectrum axis and the *x*-axis is the chromatographic time axis; (c) three most "pure" spectra within the spectrochromatogram found at the points circled in (a).



with unknown concentrations of the target analytes. Because MCR-ALS resolves signals resulting from individual chemical species, these calibration solutes are not required to be individual standards and can, in fact, be mixtures of the compounds of interest, minimizing the number of calibration samples that need to be analyzed. These measured spectrochromatograms are appended together along the time axis to form an augmented matrix **X** as follows:

$$\mathbf{X} = \begin{bmatrix} \mathbf{X}_{c,1} \\ \mathbf{X}_{c,2} \\ \vdots \\ \mathbf{X}_{c,L} \\ \mathbf{X}_{u,1} \\ \mathbf{X}_{u,2} \\ \vdots \\ \mathbf{X}_{u,M} \end{bmatrix} \quad [6]$$

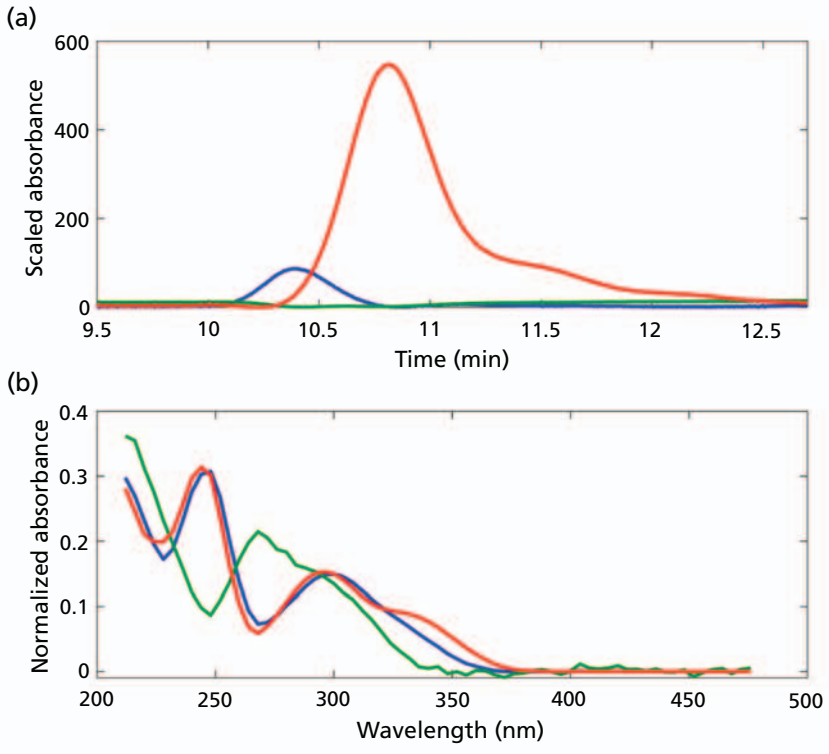
where the $\mathbf{X}_c$ are the L calibration chromatograms and the $\mathbf{X}_u$ are the M unknown chromatograms. MCR-ALS is carried out similarly to the approach described above. The resulting **S** matrix still consists of the N spectra of the pure component species, but the resulting **C** matrix now consists of $L + M$ resolved chromatograms for each of the N species, appended together similarly as shown in equation 6. The resolved chromatograms and spectra for a dataset of five calibration standards, C1–C5, and one unknown, U1, are shown in Figure 4 (that is, $L = 5$; $M = 1$). The table above the figure shows the known concentrations of the standard mixtures, and it can be seen that the scaled peak intensities in the chromatograms (Figure 4[a]) are proportional to these concentrations. By integrating these resolved chromatographic peaks, calibration curves can be constructed, as shown in Figure 5.

MCR-ALS is able to distinguish compounds with even small differences in spectra given a large enough S/N.

A clear advantage to handling multiple chromatograms simultaneously is that calibration information and estimates of unknown concentrations can be obtained very efficiently. Another advantage is the potential to add additional constraints to the analysis, which further limits the possible solutions for **C** and **S**. For example, if a blank chromatogram is included in the data set, the contributions of the chemical species for this chromatogram can be set to zero forcing the blank to be modelled using only the background components. Additionally, calibration constraints can be added to the analysis, which constrain the peak areas for the calibrated samples to follow an expected relationship between detector signal and concentration (11–13).

Of particular note here is the fact that two compounds present in the unknown sample have been reliably quantified, despite the resolution between the two peaks being

Figure 3: MCR-ALS results from the chromatogram shown in Figure 1. (a) Resolved pure component chromatograms; (b) resolved pure component spectra. The red and blue curves represent chemical species and the green curves represent background contributions.



significantly less than 1, and a high degree of similarity between their spectra. Here the chromatographic resolution of the two peaks is approximately 0.6.

The performance of the MCR-ALS algorithm is highly dependent on the similarity of the spectra of the species contributing to the overlapped peak, as well as the signal-to-noise ratio (S/N) of the peaks.

Peak Capacity Enhancements via MCR-ALS

The performance of the MCR-ALS algorithm is highly dependent on the similarity of the spectra of the species contributing to the overlapped peak, as well as the signal-to-noise ratio (S/N) of the peaks. Here the similarity of the spectra for the two analytes psoralen and angelicin can be expressed by the correlation coefficient, which is 0.98 (see part 1 for further discussion).

The improvement of effective chromatographic performance can be quantified in terms of the peak capacity of the separation. The peak capacity of a gradient separation, n_c, can be estimated as follows:

$$n_c = \frac{t_{grad}}{w_b R_s'} \quad [7]$$

where t_{grad} is the time of the gradient, and w_b is the average width of the peaks at the base. The R_s' term is the resolution required for effective quantitative analysis (14). Typically, chromatographers use an R_s' value of 1 when calculating peak capacity. Clearly, if peaks can be quantified at a resolution of less than 1 using curve resolution as discussed above, then the effective peak capacity has been increased. In recent work, we have developed a quantitative relationship between peak capacity and the signal-to-noise ratio of neighbouring peaks and spectral similarity as measured by correlation coefficient. As an example, if the correlation coefficient between the overlapped spectra is 0.89 and S/N is 50, the chromatographic resolution required for quantitation is $R_s' = 0.3$. This results in a roughly threefold improvement in peak capacity relative to conventional use of DAD where the only means of separation is that provided by the column itself. Clearly, MCR-ALS can provide a significant enhancement in chromatographic method performance.

Availability of MCR-ALS in Software Packages

One hurdle to widespread usage of MCR-ALS is the lack of implementation of curve resolution options in commercial chromatographic data systems. Although commercial data systems for spectroscopy instruments (for example, infrared) frequently provide MCR-ALS or related curve resolution tools within their software, this situation is as not common for chromatographic data systems. To the best of our knowledge, only Shimadzu has recently added this capability to its data system software (15). The other option for chromatographers wishing to apply these methods to their data is to use one of the many available MCR-ALS toolboxes available for use in the Matlab programming environment. Eigenvector Research, Inc. sells its PLS Toolbox package, which includes MCR-ALS (16). Matlab toolboxes are freely available from the Barcelona MCR-ALS group (10,17) and the Olivieri group (18), with the latter toolbox specifically focused on calibration applications. The Olivieri and Barcelona MCR-ALS toolboxes are also available for users without access to Matlab through a stand-alone graphical user interface (17,18). There is also an ALS package available for the open-source R statistical software environment (19).

Because of the lack of integration with instrumental software, an extra step is required to export the raw spectrochromatogram and read it into the third-party software packages listed above. Unfortunately, this approach is not always straightforward,

Figure 4: (a) Resolved chromatograms for five calibration mixtures (C1 through C5) containing psoralen and angelicin; table shows the corresponding concentrations; (b) Resolved spectra for psoralen (red), angelicin (blue), and a gradient background contribution (green).

Concentrations (ppm)

	C1	C2	C3	C4	C5	U1
Psoralen	50	5	10	20	1	?
Angelicin	5	1	50	20	10	?

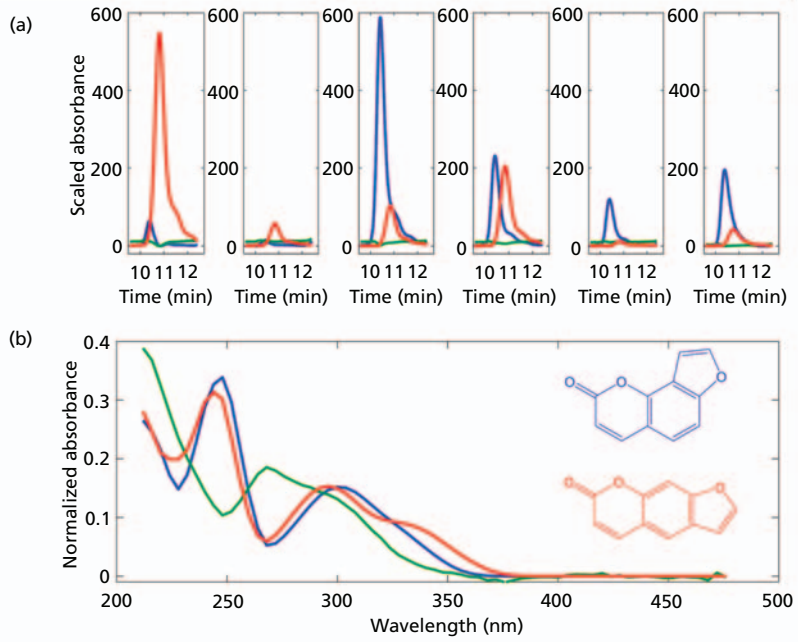
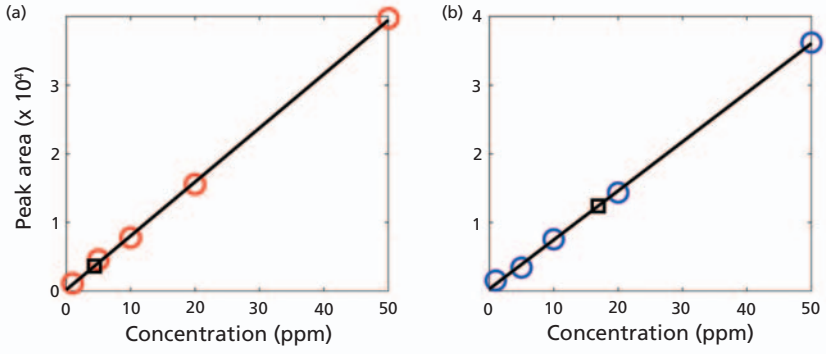


Figure 5: Calibration curves for (a) psoralen and (b) angelicin from MCR-ALS results. Coloured circles indicate calibration points; black squares denote unknown sample points.



depending on the instrument software. Although a few extra minutes may be required to move the data and to analyze with the third-party software, it will often require less time than it would take to analyze samples using different chromatographic columns or to vary other method parameters to resolve impurity peaks and increase confidence that none are present.

Concluding Remarks

To those of us who have utilized MCR-ALS for chromatographic analyses, it is clear that this technique adds a powerful tool to the chromatographer's arsenal. While the peak purity approaches described in part 1 of this series can identify whether impurities are present, MCR-ALS can resolve the pure chromatographic profile, allowing quantitation of the target analyte and the impurity if standards are available for the compound. As mentioned earlier, MCR-ALS does require that compound spectra be at least slightly different; however, MCR-ALS is able to distinguish compounds with even small differences in spectra given a large enough S/N as shown in Figure 3.

Here we have limited our discussion to impurity analysis in LC–DAD; however, it is worth noting that MCR-ALS finds use in many other analyses such as metabolomics and environmental analyses as well as other instrumental techniques from hyperspectral imaging to LC with mass spectrometric detection to two-dimensional liquid chromatography (3,4,20,21). The latter will be the focus of the next instalment in this series where we will look at how the additional separation dimension can help in the quest to determine peak purity particularly when spectrally indistinguishable impurities are present.

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New Sample Preparation Products and Accessories

Douglas E. Raynie, Sample Preparation Perspectives Editor

This yearly report on new products introduced at Pittcon (or in the preceding year) covers sample preparation instrumentation, supplies, and accessories.

New sample preparation technologies introduced in the past year, while not necessarily disruptive, take giant leaps in that direction. These technologies will eliminate solvent use and operating costs without sacrificing efficiency or selectivity. Thus, two new product introductions in the past year, CEM's Energized Dispersive Guided Extraction (EDGE) system and Entech's Pulsed Evaporative Concentration Extraction (PECE) system lead the way in terms of product innovations.

Our annual review of sample preparation products covers the previous year. The primary focus is new product introductions at Pittcon, although this is not the exclusive focus. In late 2017, the *LCGC* editorial staff submitted a survey to vendors of sample preparation products. Responses to this survey are compiled in this review. Additionally, a keyword search using the terms "sample preparation" and "extraction" was conducted for exhibitors at Pittcon 2018; then each of these vendors was visited. Even a conversation on the shuttle bus from the hotel to the convention center yielded knowledge of new product technology. Although attempts were made to be as inclusive as possible, we apologize for any oversight.

This review is presented in three sections. First, we discuss two innovative directions in extraction technology. Next, new solid-phase sorbents and sorbent-based products are presented. Sorbent technologies are seeing increased development for both general and selective purposes. Finally, we turn to other sample preparation instrumentation, accessories, and supporting technologies. To provide readers 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, and the text highlights particularly worthwhile products.

New sample preparation technologies introduced in the past year, while not necessarily disruptive, take giant leaps in that direction. These technologies will eliminate solvent use and operating costs without sacrificing efficiency or selectivity.

New Extraction Modes

Energized Dispersive Guided Extraction: Foregoing the application of microwaves upon which the company was founded, CEM introduced the EDGE system as its entry into the world of pressurized solvent extraction (PSE). (For full disclosure, I evaluated early iterations of the equipment during CEM product development.) A schematic of the system's extraction process is shown in Figure 1. This approach is somewhat different than that used by other vendors. In this approach, extracting solvent is initially added through the bottom of the sample vessel. Then the sample is fully wetted by a solvent spray coming from the top. As the solvent-coated vessel walls are heated, a thermal gradient is created, which results in a pressure gradient. The increased pressure forces solvent down through the sample, which CEM calls the energized dispersive effect. Extraction temperatures up to 200 °C can be used, though 100 °C is generally sufficient. Data for the extraction of several semivolatile organic compounds (SVOCs) in environmental solids show quantitative recoveries. The system is reportedly capable of running 12 samples/h. The company received a bronze Pittcon Today Excellence Award for the system among vendors with similar reported annual sales.

Pulsed Evaporative Concentration Extraction: Previously, Entech Instruments developed vacuum assisted sorbent extraction (VASE) and sorbent pens for headspace sampling of volatile compounds. They have expanded this approach to SVOCs and less volatile materials, with up to six-ring aromatics, using what they term pulsed evaporative concentration extraction (PECE). PECE operates by taking advantage of solute volatilization at reduced pressure, with cycles consisting of periods of increased pressure with

Table 1: Sample preparation sorbent products

Supplier	Product Name	Product Type	Mode	Base Material	Functional Group	Dimensions	Comments
Agilent Technologies	Captiva EMR-Lipid	Cartridges, 96-well plates	Pass-through sample cleanup	Proprietary	Proprietary	96-well plates and 1-, 3-, and 6-mL cartridges	Effective removal of phospholipids and other lipids from foods and biological matrices
Biotage	Isolute Filter+	96-well plate	Filtration	High purity polypropylene, polyethylene, PVDF	NA	0.2-µm frits	High-performance filtration of aqueous biological samples
	Evolute Hydro	96-well plate	SPE with in-well hydrolysis	PS-DVB	Acid, base, neutral (ABN) or cation exchange	10, 30 mg/well	Hydrolysis of urine samples for LC–MS
Eprep Pty Ltd	µSPEed cartridges	Micro SPE cartridge	Reversed phase, normal phase, HILIC	Silica and divinylbenzene	C18, C8, phenyl, strong anion and cation exchange	3-µm sorbents	MicroSPE with one-way check valve
	Customizable Micro Separation/ Reactor cartridges	Bioreactor cartridges	Customizable chemistry (*in situ* immobilization of ligand and antibody)	NA	NA	3-µm sorbents	Trypsin digests, targets sample cleanup
Hilicon AB	iSPE-HILIC Spin	Cartridge SPE	Hydrophilic interaction	Silica	Hydroxyethyl amide, sulphate, quaternary ammonium	1 mL with 10–50 mg HILIC material; 50-µm, 60-Å silica	Tailor-made for SPE of hydrophilic compounds including glycopeptides, glycans, and neurotransmitters
	iSPE-HILIC 96-well	96-well plate	Hydrophilic interaction	Silica	Hydroxyethyl amide, sulphate, quaternary ammonium	96 wells with 25–100 mg HILIC material per well; 50-µm, 60-Å silica	Tailor-made for SPE of hydrophilic compounds including glycopeptides, glycans, and neurotransmitters
Macherey-Nagel	Chromabond Flash RS Sphere SiOH	Flash chromatography cartridge	Normal phase	Silica	Unmodified	Columns with 4–330 g, 15–25 µm particles	Lower backpressure due to efficient packing of spherical particles
	Chromabond HLB	SPE columns, cartridges, 96-well plates	Hydrophilic-lipophilic balance	Silica	*N*-vinylpyrrolidone-divinylbenzene copolymer	30–60 µm particles, 65-Å pores, 750 m^2/g surface area, 1–14 pH stability	Used for extraction of polar organic molecules from polar matrices
Millipore Sigma	Supel Genie Online SPE cartridges	Online cartridge SPE	Sample cleanup	Silica	C18, RP-amide, or HybridSPE (zirconia for phospholipid removal)	20 mm × 4.0 mm	Robust cartridges for bioanalysis, food and beverage, environmental analysis
National Chromatography	Hi-purity SPE	Cartridge SPE	Reversed phase	Silica	C8, C18, C30	Varies	Environmental, other applications

Table 1: (Continued) Sample preparation sorbent products

Supplier	Product Name	Product Type	Mode	Base Material	Functional Group	Dimensions	Comments
Optimize Technologies	Opti-Lynx 2	Hand-tight trap column	Sample cleanup, desalting, detergent removal	NA	NA	2.1 mm × 5 mm, 3.0 × 5 mm, 4.6 × 5 mm	Zero dead volume connection, rated to over 6000 psi with quarter-turn connection
Orochem Technologies, Inc.	Panthera Deluxe	Cartridge, 96-well plate SPE	Reversed phase	Polydivinyl-benzene	Hydrophilic and hydrophobic interactions	1-, 3-, 6-, and 10-mL cartridges, 1- and 2-mL/well plates	Biological sample extraction for a wide range of small molecule analytes
	QuEChERS Specialty for Hemp and Stevia	Dispersive SPE	Adsorption	NA	Various	2- and 15-mL centrifuge tubes	Extraction of pesticides from hemp and stevia leaves
Phenomenex	Strata DE SLE	Supported liquid extraction	Sample cleanup	Diatomaceous earth	None	200-µL plate, 400-µL plate, 2-mL tube, 20-mL tube	Liquid–liquid and supported liquid extractions
SiliCycle	SiliaFast FaPEx	Cartridge SPE	Varies	Silica	Primary secondary amine, C18, carbon black	Varies	Fast pesticide extraction
UCT, Inc.	Abalonase Ultra	Beta-glucuronidase abalone enzyme	NA	NA	Liquid enzyme activity ≥ 150,000 units/mL	NA	Enzymatic hydrolysis
	Enviro-Clean WAX columns	Cartridge SPE	Weak anion exchange	Divinylbenzene	Polyamino	NA	Analysis of per- and polyfluoroalkyl substances in drinking water
	Refine 96-well ultrafiltration plate	96-well plate	Precipitation and filtration	Proprietary submicrometer frit combination	NA	96-well plate	Precipitation of plasma and serum, on-plate urinary hydrolysis and filtration
Waters Corporation	Oasis PRiME MCX	Cartridge, 96-well plate SPE	Mixed mode (reversed phase and cation exchange)	Polymeric	NA	1-, 3-, 5-, 20-, 35-mL and 10–500 mg cartridges; 10-, 30-, 60-mg/well plates	Phospholipid removal from biological matrices

lower temperature, and application of temperature differentials across their extraction vessel. This process results in the evaporation of analytes followed by condensation onto the sorbent pen; by cycling through the process, analytes build up on the sorbent material. When combined with splitless injection for gas chromatography (GC), significant sensitivities are achieved.

Solid-Phase Sorbents and Products

The use of solid-phase sorbents in techniques like solid-phase extraction (SPE), solid-phase microextraction (SPME), stir-bar sorptive extraction (SBSE), and others is becoming well-established for analytical extraction and sample cleanup, though development of new, often selective, phases continues. Products and accessories specifically for these sorbent-based extractions will be discussed in the following section, but here we look specifically at new (bulk) sorbent phases. These sorbents are summarized in Tables 1 and 2. Note that this year there did not appear to be any emerging theme in new sorbents. One modality (for example, reversed phase versus mixed mode versus ion exchange, and so on) or technique (cartridge-based SPE, dispersive SPE, SPME, and so forth) did not predominate the product offerings. Notable sorbent offerings are discussed below.

One problem associated with QuEChERS (quick, easy, cheap, effective, rugged, and safe) and other forms of dispersive SPE is the coextraction of lipids from

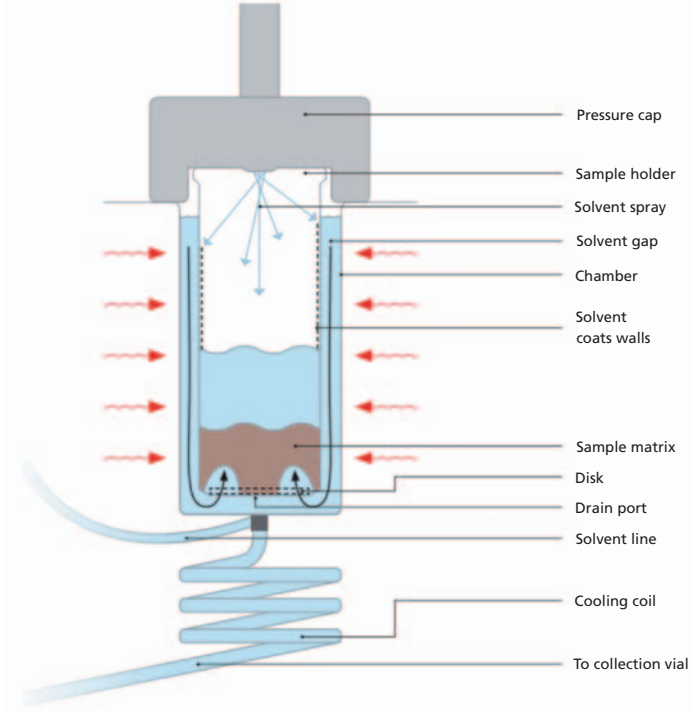
Table 2: Sample preparation instrumentation

Supplier	Product Name	Application	Main Use	Important Feature	Comments
Biotage	TurboVap LV	Solvent evaporation of tubes and vials	Fast sample dry-down	Easily exchangeable manifolds extend functionality, flow gradients for programmable evaporation	Uses gas vortex shearing technology
	TurboVap II	Solvent evaporation of 50–200 mL volumes	Polymer identification, forensics, evolved gas analysis	Trap and collect analytes from a few degrees above ambient to 1400 °C, with capability to add reactant gas	Newly designed valve oven, resistive heating coil
CDS Analytical	CDS 6200 Pyroprobe and modular autosampler	Thermal extraction device for thermal desorption and extraction, dynamic headspace, and pyrolysis sampling for GC	NA	0.2-µm frits	High-performance filtration of aqueous biological samples
	CDS 7450 purge-and-trap system with 7550 Thermal Desorption Tower	Collecting and introducing VOCs and SVOCs in water, soil, and thermal desorption tubes to GC	Purge and trap dynamic headspace sampling for EPA methods 524, 624, and 8260	Chemically inert sample pathway. Moisture control and foam sensor	Available as stand-alone or coupled units
Entech Instruments	7650HS-CTS large-volume headspace system	Food, flavour, and fragrant analysis	Static headspace analysis	New multicapillary column trapping system	Traps all compounds boiling from -50 to >400 °C
Eprep Pty Ltd.	ePrep sample preparation workstation	Standalone chromatography sample preparation workstation	Calibration standard preparation, dilution, aliquots, filtering, reagent addition, mixing, micro SPE	Robotic sample preparation for 5–1000 samples	USB or Bluetooth connectivity
Fluid Management Systems	EZSpe	Semiautomated SPE system	Drinking and wastewater analysis	Runs up to six samples simultaneously	Uses all SPE cartridge sizes
Fritsch GmbH	Variable Speed Rotor Pulverisette 14	Silica	*N*-Vinylpyrrolidone–divinylbenzene copolymer	30–60 µm particles, 65-Å pores, 750-m^2/g surface area, 1–14 pH stability	Used for extraction of polar organic molecules from polar matrices
	Planetary Mill Pulverisette 5	Wet and dry grinding of hard, medium-hard, soft, brittle, and moist samples	Mechanical alloying, mixing, and homogenizing	Also reliably homogenizes down to the nano range	Built-in safety features with clamping of grinding bowl
	Knife Mill Pulverisette 11	Homogenizing samples with up to four cutting edges	Foodstuffs, feed testing, pharmaceuticals	Fast and gentle comminution of moist, oily, and fatty samples	Difficult to grind samples may be embrittled with liquid nitrogen. Sample material maintains coldness in grinding vessel
Gerstel	Sample ID barcode reader for sequence generator	Routine analysis laboratory operation	Automated analysis sequence generation based on reading barcodes of a series of samples placed in the autosampler	Automated sample logging, method activation, and sequence set-up	Full traceability
	Thermal Desorber TD 3.5+	Direct thermal extraction of analytes from liquids or solids	Thermal desorption of analytes concentrated on sorbent tube, SBSE, SPME fibre, or directly from sample and transfer to GC	Integrated with PTV sample inlet for analyte cryofocusing	May also be used for dynamic headspace analyte concentration
LCTech GmbH	DEXTech Pure	Sample cleanup for PCB and dioxin analysis	Acidic silica gel, aluminum oxide, and carbon columns for fractionation of PCBs and dioxins	Compliant with EPA and European regulatory agencies	Measurement of PCBs and dioxins separately within one GC–MS run each
Leap Technologies	MFx Collector	Used with 96-well plates	Automated fraction collection	Unique dynamic flow reservoir	Flowpath allows minimal peak diffusion
	HDX	Automated hydrogen-deuterium exchange	Protein binding studies in pharmaceutical development	Automated, with pH control and quenching before LC–MS injection	Inline digestion with accurate temperature control

Table 3: Sample preparation accessories

Supplier	Product Name	Application Area	Product Type	Suggested Application	Comments
Argos Technologies	Omega Zen pipette controller	Liquid dispensing	Ergonomic and precise control of liquid transfer	For repeated transfers; housing is ideal for use in cleanrooms and fume hoods	Easy-to-read LCD indicates aspirate–dispense speed, gravity mode, and battery charge
Eprep Pty Ltd	µXact^3 handheld digital syringe	Liquid dispensing	Programmed micro SPE and filtering, sequenced methods, aqueous and organic liquid dispensing	Precise step programmable operation at pressure	Quick change syringe, constant or variable flow rate, touch-screen programming, calibration standards module
MicroSolv Technology Corporation	U-2D glass-lined 96-well plates	Bioanalysis	96-well plate	Compatible with Fast Sample Prep and glass inserts	Inserts can be separated from base for visual or thermal control; 350, 500, and 1000-µL inserts
Polymer Char	Solvent Handling Trolley	Transportation and transfer of solvents	Solvent storage and dispensing	Transfer of large solvent volumes	Improved laboratory safety; capacity large enough to refill several instruments
Thermo Fisher Scientific	Guardcap vial caps	Inline matrix elimination	Functionalized autosampler vial caps for sample pretreatment	Cation exchange hydronium and sodium form sulfonate and hydrophilic divinylbenzene. Removal of alkaline earth and transition metals, pH adjustment of basic samples, and removal of particulates and hydrophobic substances	Removes up to 1000 mg/L of divalent cations from 800-µL sample; neutralizes up to 500 µL of 50 mM NaOH. Filters over 80% of particles greater than 20 µm

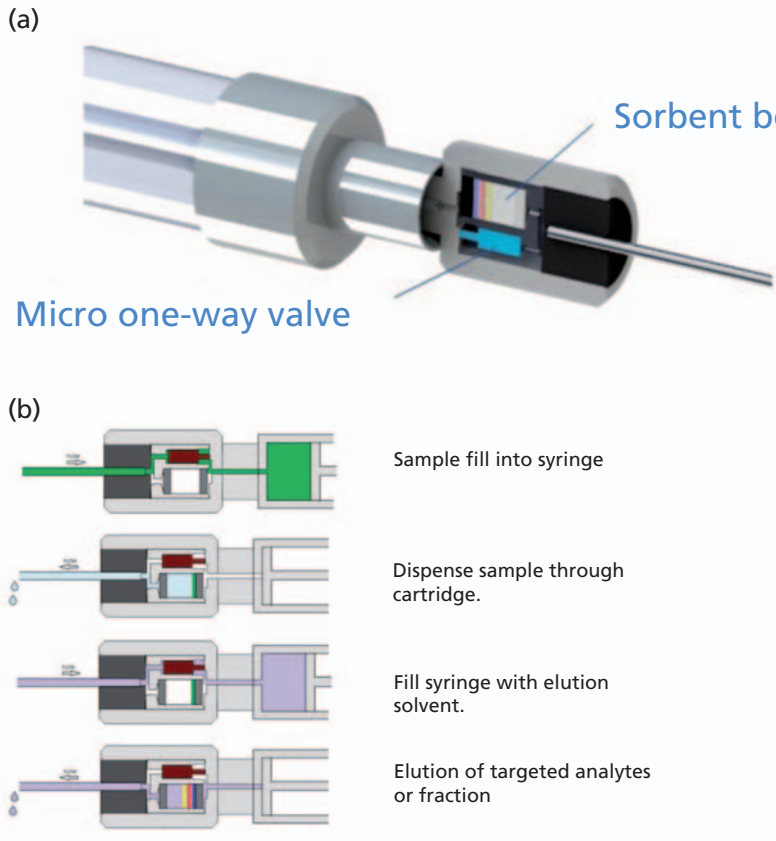
Figure 1: Schematic diagram of the extraction vessel and solvent flow during energized dispersive guided extraction. (Courtesy of CEM Corp.)



fatty samples. This problem includes interferences associated with phospholipids after protein precipitation of biological fluids. Agilent Technologies addresses this problem with the development of the Captiva EMR-Lipid sorbent. The Captiva product appears to be a restricted access material along the lines of Agilent's Bond Elut EMR-Lipid sorbent, introduced last year, in a format amenable to cartridges and 96-well plates. Company literature shows superior results to other sorbent-based materials, such as zirconia. (For full disclosure, I am involved in an Agilent-funded project investigating the physical chemistry of dispersive SPE sorbents.)

Eprep, an Australian company, developed a series of syringe-based microseparation cartridges for SPE. Although other syringe-based forms of SPE, including Micro Extraction by Packed Sorbent (MEPS, by SGE), dispersive

Figure 2: µSPEed cartridges from Eprep, showing (a) the integrated one-way valve and (b) the sample loading and dispersion, solvent loading, and elution steps. (Courtesy of Eprep Pty Ltd.)



pipette extraction, and others are well-established, what distinguishes the Eprep µSPEed product is the use of a miniature, one-way valve, depicted in Figure 2, which allows greater control over solvent flow. Combined with the increased surface area of the 3-µm particles used, significant savings in time and solvent volume are achieved, along with very high analyte concentration factors.

Sample Preparation Instrumentation, Accessories, and Related Products

Sample preparation instrumentation and other products, including those previously discussed, are summarized in Tables 2 and 3.

Sample Preparation Instrumentation: Sample concentration via solvent evaporation is important in many sample preparation processes. Biotage expanded its popular TurboVap line with two new offerings. This equipment has smaller footprints and an improved nozzle design and sensors for extended use. After solvent evaporation, the next step typically involves solvent transfer, such as internal standard or reagent addition or aliquot generation. This array of steps can be robotically handled with Eprep's sample preparation workstation, with capability to handle up to 1000 samples.

Sample particle size reduction and sample homogenization are vital in many instances. Fritsch emphasized its expanded product line with new cutting, impacting, knife, and planetary mills. With proper selection of the suitable mill, brittle, hard, fibrous, moist, or oily samples can be accommodated with mills with smaller footprints and increased capacities.

Accessories: Every once in a while, a new product addresses a need so obvious that we were all blind to it. Polymer Char introduced a solvent handling trolley, a cart with associated connections and valving to transfer fresh and waste solvents to chromatographic systems. Not only will this system prevent the necessity of frequently lifting solvent bottles, but safety advantages associated with reduced spillage, minimal exposure to solvent vapours, and less direct solvent contact should make this worth considering in all laboratories.

Not tabulated, the Xylem model 4100 VOC analyzer and 4760 purge-and-trap concentrator from OI Analytical were upgraded with new gaskets, automated standard addition, and related features for improved performance. A new company, Biotix Fluid Innovation, developed a locking microcentrifuge tube to alleviate spillage and errors in the laboratory.

Conclusion and Future Directions

New sample preparation products in the past year seem very interesting. On one hand, significant developments in extraction, headspace and thermal desorption, sample grinding, liquid dispensing, and selective sorbents were highly prevalent. Yet there was no clear-cut focus, no long-standing industry problem being addressed. Whether the trend in coming years is aimed at tool-building or problem-solving for food, environmental, and related analysis is the question. Nonetheless, highly significant offerings were made this year that should excite all analysts.

"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 via e-mail to LCGCedit@ubm.com

HPLC system

The Agilent 1260 Infinity II Prime LC system provides the highest convenience standard in the 1260 Infinity II LC portfolio, as well as an extended pressure range (up to 800 bar), superior quaternary mixing, and specifically designed columns, according to the company. The automated instrument features reportedly increases analytical laboratories' efficiency and the intelligent system emulation technology (ISET) offers method transfers from many Agilent and third-party legacy instruments. The improved convenience level of the 1260 Infinity II Prime LC is highlighted with a local user interface—the Agilent InfinityLab LC Companion.
www.agilent.com
Agilent Technologies, Inc., California, USA.

Chromatography software

Clarity enables the control of hundreds of different instruments from one environment. Its wide range of data acquisition interfaces allows connection to virtually any chromatograph. Clarity is multilingual; users can switch between six languages. With easy operation, outstanding user' support, and optional extensions for various applications, such as, PDA, MS, GPC, NGA, and many more. A free demo is available from DataApex's website.
www.dataapex.com
DataApex, Prague, The Czech Republic.

MALS detector

The new Postnova PN3621 Maximum Angle MALS detector sets a high standard for precise multi-angle light scattering detection for size-exclusion chromatography (SEC) and field-flow fractionation (FFF), according to the company.
The detector simultaneously measures the scattering intensity at a maximum of 21 angles, which enables determination of absolute molecular weight and size of proteins, polymers, and nanoparticles.
www.postnova.com
Postnova Analytics GmbH, Landsberg, Germany.

Solid-phase extraction

The hydrophilic-lipophilic balanced polymeric SPE adsorbent Chromabond HLB is specially designed for the enrichment of hydrophilic analytes, including pesticides and pharmaceuticals, from polar matrices such as water, blood serum, or food. Linked hydrophilic groups of the HLB copolymer interact with polar functional groups of the analytes while the lipophilic backbone interacts with nonpolar hydrocarbon residues to provide enhanced retention. Macherey-Nagel offers a broad range of columns, cartridges, and 96-well plates packed with Chromabond HLB adsorbent.
www.mn-net.com
Macherey-Nagel GmbH & Co. KG, Düren, Germany.

Purification columns

Centrifugal partition chromatography columns offer high-injection capacities of milligrams to multi-kilograms in the natural product purification process, resulting in 95% recoveries with 99% purity.
www.gilson.com/en/AI/Products/80.320/Default.aspx#.Wh19sVWnFaQ
Gilson, Middleton, Wisconsin, USA.

FID gas station

VICI's DBS range of FID gas stations with software control and alarm capability allows GC users to reap the benefits offered by hydrogen carrier gas, whilst overcoming the safety concerns, according to the company. The company reports that this system combines the reliability of the VICI DBS hydrogen and zero air generators into one compact and convenient FID package.
www.VICIDBS.com
VICI AG International, Schenkon, Switzerland.

Microchip column

µPAC is PharmaFluidics' chip-based chromatography column for nano-liquid chromatography. Perfect order in the separation bed is achieved by etching a regular pattern of pillars into a silicon wafer using micromachining technology. The column allows high-resolution separation of tiny, complex biological samples, with an unprecedented robustness. µPAC is suitable for lipidomic, metabolomic, and peptide profiling, according to the company.

www.pharmafluidics.com
PharmaFluidics, Ghent, Belgium.

MALS detector

The µDAWN is, according to the company, the world's first multi-angle light scattering (MALS) detector that can be coupled to any UHPLC system to determine absolute molecular weights and sizes of polymers, peptides, and proteins or other biopolymers directly, without resorting to column calibration or reference standards. The WyattQELS Dynamic Light Scattering (DLS) module, which measures hydrodynamic radii "on-the-fly", reportedly expands the versatility of the µDAWN.

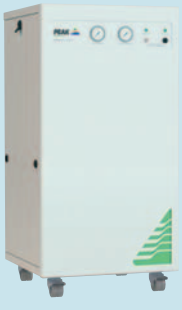
www.wyatt.com
Wyatt Technology, Santa Barbara, California, USA.

Thermal desorption tubes

Designed for material emissions-, flavour-, and air analysis, the TD 3.5+ processes 3.5" tubes or Gerstel plus tubes and offers enhanced recovery and sensitivity, according to the company. The liner-in-liner design without transfer line reduces analyte loss and memory effects. Up to 240 samples are processed automatically. In combination with DHS 3.5+, dynamic headspace from 10 mL up to 1 L volume is performed.

www.gerstel.com
Gerstel GmbH & Co. KG, Mülheim an de Ruhr, Germany.

Nitrogen generator



Peak Scientific's new Infinity XE 50 Series nitrogen generator system is designed to cater for numerous applications across a typical laboratory. Delivering a variable flow of nitrogen gas ranging from 11 L/min to 432 L/min at purities up to 99.5%, the system is ideally suited for a wide range of applications including LC–MS, ELSD, sample evaporation, NMR, FTMS, and gloveboxes.

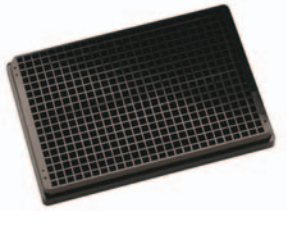
www.peakscientific.com/infinityxe50
Peak Scientific Instruments Ltd, Glasgow, Scotland, UK.

Preparative system

Quattro countercurrent and centrifugal partition chromatographs and extractors are designed to work with, and complement, standard flash and HPLC laboratory and process instrumentation. When appropriate, replacing the solid–liquid columns with unique liquid–liquid instrumentation allows preparations from milligram to tonnes every year. No on-column adsorption or degradation will occur, according to the company. A mass-balance is the norm for CCC/CPC. Typically a 50–80% solvent saving occurs. Standard biphasic solvents, ionic liquids, liquid chiral selectors, and ion exchangers may all be used. According to the company, crude material that would poison standard columns can be injected without causing contamination.

www.quattroprep.com
AECS-QuikPrep Ltd., London, UK.

Circular dichroism microplate



Porvair Sciences has introduced a new black Krystal UV Quartz bottomed microplate that offers optical transmission in the wavelength range of 185 nm to 1100 nm making them ideal for laboratories looking to make circular dichroism (CD) measurements. The microplates allow these measurements to be made in a convenient ANSI/SLAS compliant 96- or 384-well microplate footprint using next-generation CD spectrometers. The proprietary black polystyrene polymer mix ensures low cross-talk whilst the top-quality quartz bottom gives reduced birefringence, essential for good CD measurements.

www.porvair-sciences.com/krystal-uv-transparent
Porvair Sciences Ltd., Wrexham, UK.

HPLC Troubleshooting: **Autosampler Contamination**

An excerpt from *LCGC*'s e-learning tutorial on troubleshooting autosampler contamination at CHROMacademy.com

Although modern autosamplers may differ in their sampling principle (push to fill, pull to fill, integral loop, and so forth), they contain many similar features, including a needle for aspirating sample from the vial, a port to introduce the sample into a loop, and a valve that allows the sample plug to be automatically introduced into the mobile-phase flow. Although contamination may arise from several of the system components, the autosampler is arguably the most susceptible because it handles each sample in its nondiluted form and, in most cases, contains large surface areas and narrow-diameter tubing within the hydraulic pathways of the device. We need to be extra careful to avoid contamination issues, which can give rise to poor quantitative reproducibility (poor relative standard deviation between repeat injections of the same sample) and carryover from sample to sample that may lead to extraneous peaks within the chromatogram.

The sample needle is used to pierce the vial septum and withdraw the sample liquid, usually as a result of negative pressure created by the backstroke of a filling or metering pump. The needle is typically manufactured from passivated steel and contacts the sample on both its inside and outside surface. For this reason, it is important that both the inside and outside surfaces are properly washed between sample injections and care should be taken to program the wash cycle of the autosampler to effectively remove any contamination. You should consider the volume of wash solution with which the inside of the needle is flushed (at least 10× injection volume is recommended as a minimum) and the number of flushes (with washes in progressively cleaner solvents being optimal). Some instruments will allow wash solutions to be aspirated from different bottles, which may improve the efficiency of the wash. The wash solutions should be very carefully chosen and their eluotropic strength matched to that of the most highly retained analytes; for example, if the analyte is eluted at a composition of 60% organic, consider that concentration for the wash solution strength. Don't make the mistake of choosing a solvent that is highly solubilizing for the matrix, it is the analyte solubility that concerns us more here.

Wash bottle caps and injection ports (which typically contain a rubberized or polymeric seal), are also sources of contamination because the needle will pass through them after having recently been immersed in the sample. If contamination is suspected, it is good practice to change these components or thoroughly clean them in strong solvent before retesting. If it is feasible, it may be more beneficial to remove any seals or caps from wash bottles or vials to assess the impact on reducing contamination. Injection port seals should be well maintained and regularly replaced, and the nature of the injection port wash solvents (if fitted) should be considered in line with analyte solubility characteristics.

Perhaps the most insidious component for contamination is the injection valve itself, which contains a rotary seal that moves under pressure to "join" different hydraulic pathways within the autosampler, most notably to achieve injection of the loop contents into the mobile phase flow. The rotor seal, which is the moving component and through which all sample material passes, is constructed from a plastic material, typically Vespel or Tefzel, and several external ports are connected to the various lines within the instrument. With use, the channels on the surface of the rotor seal may become scratched and roughened, which will promote the adsorption of analyte materials—this condition is especially problematic when the analyte has a greater affinity for the rotor seal material. One should ensure the correct rotor seal material is used (least adsorptive) and that the seal is inspected and maintained on a regular basis (as part of the preventative maintenance routine). Ensure that all connections into the valve are properly made and that there are no unswept volumes in any of the connections.

It is sometimes necessary to passivate the autosampler when dealing with analyte components that are known to be highly adsorptive on the metal surfaces (especially tubing) within the sampler, although this step has become increasingly unnecessary as manufacturers continue to improve their own passivation techniques. There are several chemical recipes to achieve passivation, and some involve flushing with various aqueous and organic solvents before treatment with solutions of strong acid. Your instrument manufacturer should be able to guide you through this process.

More Online:

Get the full tutorial at
www.CHROMacademy.com/Essentials
(free until 20 July).

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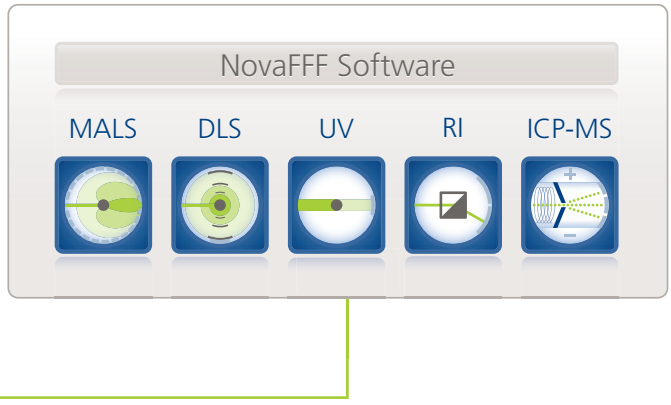
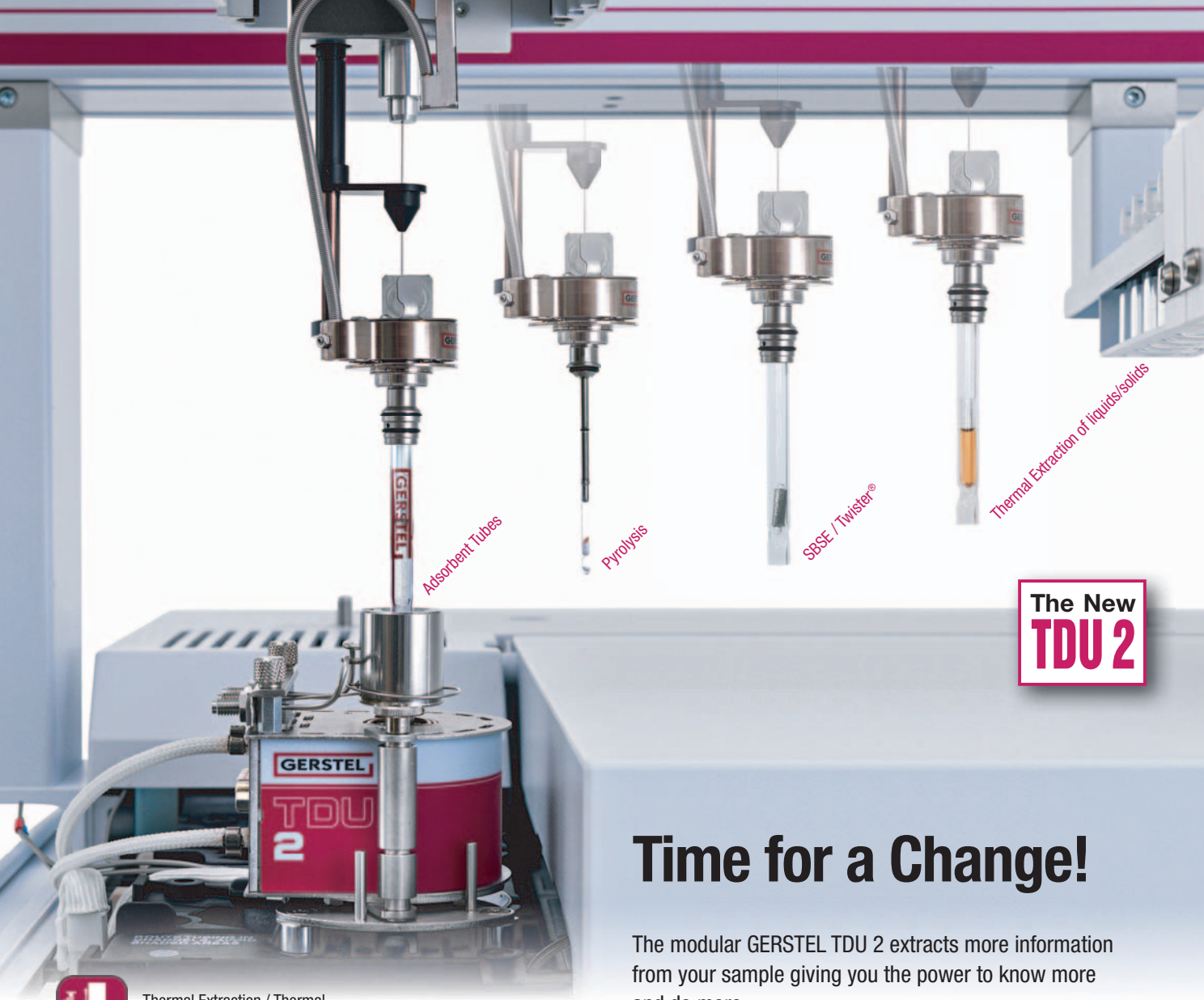


Contact us for more information: **www.postnova.com**



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