March 2009 Volume 5 Issue 3

The Column

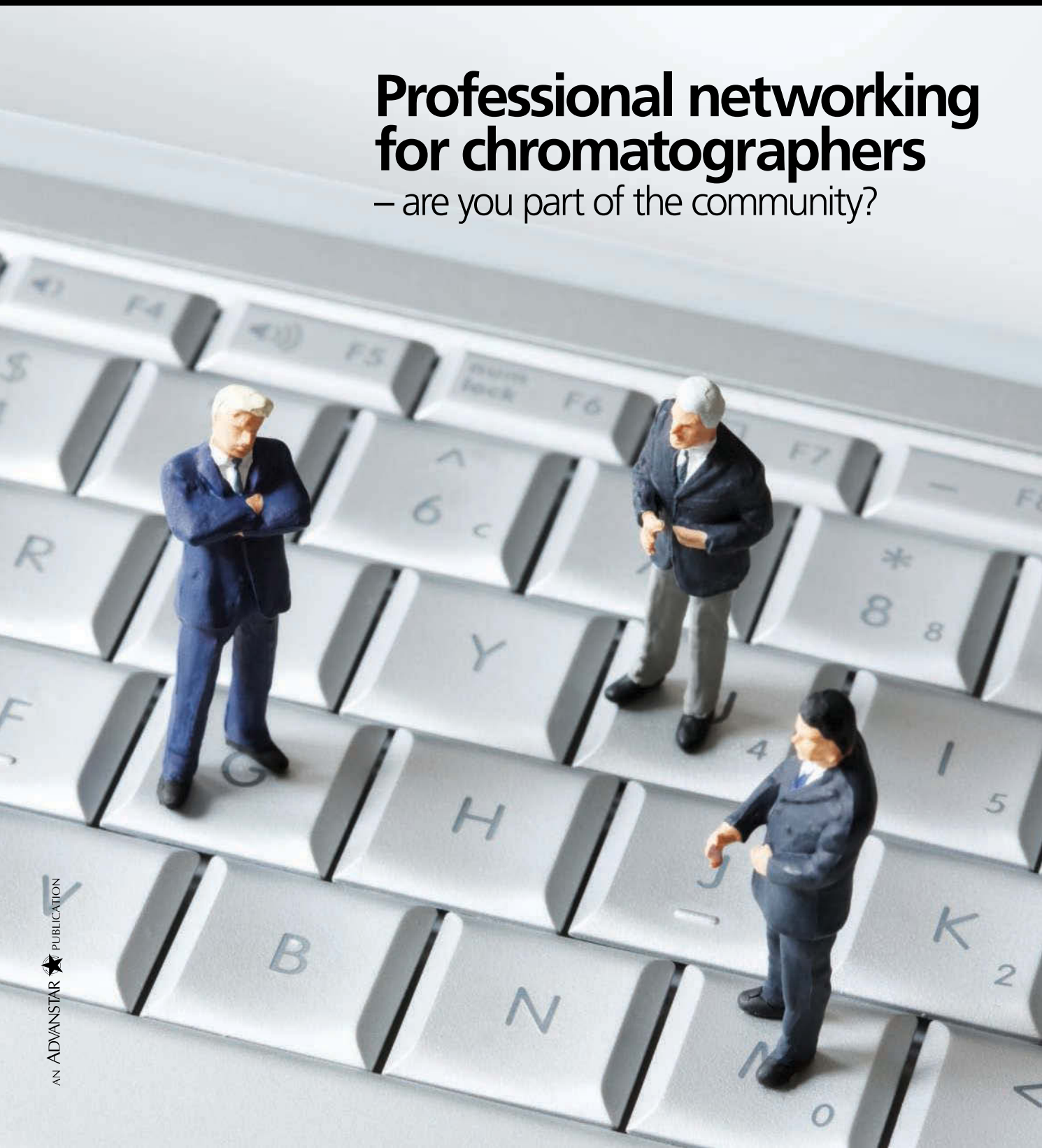
the interactive e-publication for the european separation science industry

Market Trends & Analysis
Molecular spectroscopy techniques

Pesticide screening
by GC–MS

Antidepressant bioanalysis
by SPE-LC–MS–MS

Professional networking for chromatographers
– are you part of the community?



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Mission Statement
The Column is the analytical chemist's companion within the dynamic world of chromatography. Interactive and accessible, it provides a broad understanding of technical applications and products while engaging, stimulating and challenging the global community with thought-provoking commentary that connects its members to each other and the industries they serve.

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The "Ultimate Chromatography Social and Professional Network" …Probably!

As the trend for using social and professional networking sites takes off, Incognito contributes to the hype by commenting on what's out there for chromatographers.

When you read this I'll be just back from Pittcon having spent some considerable time prior to the event lining up meetings for both business and pleasure, to make sure the trip is a successful one. Looking up who will be there, contacting them and arranging meeting logistics takes considerable time and effort.

In a (seemingly) unrelated conversation, my son asked me recently the difference between MSN Messenger and e-mail, to which I launched into a useless response involving postmen and synchronous communication — not really good enough for a nine year old!

These two events lead me to ponder what might be the ultimate way to communicate, network and share knowledge and experience within the chromatography community — perhaps the "Ultimate Chromatography Social and Professional Network".

Before I do this let me provide some information on the surprisingly large number of forums and networking groups that already exist. To keep the search manageable, I have concentrated only on chromatography interest groups.

Chromatography Forum (www.chromforum.org) — the most popular of all the chromatography "forums" with circa 7000 members worldwide and 50000+ posts on a diverse range of topics, arranged into LC, GC and LC–MS etc. This is an excellent professional resource for the chromatographer, with a high percentage of useful posts and replies, however, it's fairly "dry" with little "social interaction" other than a not very well populated "around the water cooler" section. The site is text based with very few chromatograms or other images included in the posts. It's also fairly daunting to newbies who are put off by the often highly technical language and I know of some who have dared to post only to be patronized or belittled by the responses offered.

Advantages — excellent for "many to many" communication with experts and peers.

Facebook (www.facebook.com) — the most populous social networking site that contains 10 "chromatography" related interest groups with around 159 members in total. Many of these are single interest groups that have not established a critical mass to stimulate any really useful debate however Facebook is much more suited to community facilitation — a way to share information in an easy and entertaining way. It's very interesting that some of the small groups have already been infiltrated with product specific advertising banners and posts touting new instruments or advertising staff vacancies. A famous study revealed an increased likelihood of securing a job via a (social) network contact with whom you have only a passing acquaintance rather than through employment agencies or via close personal friends (the so-called "strength of weak ties" phenomenon).[1]

Advantages — provides many possibilities for communication, excels at community facilitation using rich media and has a much more personal feel than forums.

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LinkedIn (www.linkedin.com) — is an on-line network of more than 35 million professionals from around the world. The site contains 5500 people with chromatography as a profile word and approximately six chromatography special interest groups with around 500 members. The largest group, however, is ChromForum with 222 members! Suffice to say that as we enter financially difficult times, "job jitter" has seen registrations at LinkedIn grow by more than 10% month on month.

Advantages — speed and efficiency with which you can "get to someone" to do business, form a partnership or get a job.

Flickr (www.flickr.com) — a site for publishing and sharing still images. Contains 456 images related to the search term "chromatography", most of which are paper or column chromatography of coloured substances.

Advantages — documenting a "series" of still images — imagine a step-by-step guide to removing and cleaning an ion source/flowcell etc. etc.

You Tube (www.youtube.com) — the most popular video sharing site. Returns 179 items from the search term "chromatography".

Advantages — excellent database with good video streaming technology for both serious and fun applications.

Skype (www.skype.com) — Largest voice over Internet protocol (VoIP) and synchronous video communication site. Not strictly social networking but a tool that I'm using more and more for audio and video conferencing and, interestingly, instant messaging also.

Advantages — superb (free) synchronous communication resource.

So, given what is already out there, my own specification of the "Ultimate Chromatography Networking Site" might look something like this.

General Features

- Allows broadcast to the global population (easily) when I require using various media types to those within pre-defined groups or individuals as I require.
- Offers a mechanism to rate content and should automatically influence the type, quality and frequency of content served up to me personally depending upon my affiliations and activity (if Amazon can do it with my shopping habits it's obviously possible!) It's also really important that the information is "pushed" to me (as in Blackberry technology) — frankly I'm not organized enough to go and "pull" all of the relevant stuff towards me — that would be almost self defeating!

More Specific Features

- Automatic creation of conference attendee lists with the ability to easily contact folks and book time in their conference calendar. Deliver conference highlights (social and professional, especially if I was not able to attend) within a week of the conference happening. I have one acquaintance who always blogs his last picture of the evening — amazing how many are of the sky or the ceiling! I also would like to see the plenary and other keynote lectures of the conference in video the next day — this might be automatically offered up to me if I belong to a particular interest group.
- Deliver on-line professional development (using media rich e-learning) and information on applications and techniques — so I can receive, learn and disseminate knowledge to peers and co-workers who would find such continuing professional development or day to day practical advice useful. This could also be used to develop learning routes for staff induction or more formal training etc.
- Post chromatograms/issues for troubleshooting information and know that I'll get a sound response during my working day ("forum" type activity but with better graphic capabilities and more synchronous on-line activity). I want to make a valid contribution to the forum to build trust with community members and I should also be able to promote my company and professional activities without it appearing as though all my replies had some business self interest at heart. My staff should be able to post to the forum without fear of patronizing responses.
- Ability to interface with users of new or emerging technologies to find out their experiences and honest opinions — is the technology living up to expectations, is it finding unsuspected applications, what is the reliability like, are there any design flaws etc.? It would be great to have an area for "Honest Instrument Demo" reports — posted by the client and the instrument demo chemist!

- Open access to relevant application data in a form that is easily searchable, contains chromatograms and has sections to report on what happened when previous users attempted to reproduce the application. Clickable links to ordering sites for columns and chemicals would also be very useful.
- Quickly and effectively arrange synchronous meetings with peers and fellow members of interest groups to discuss topics of interest (i.e., it would have been opportune to arrange a meeting to discuss the MeCN shortage response from the MHRA recently and highlight efficient ways to deal with the issues raised by them and share good practice with colleagues who affiliated to the "Beat the Acetonitrile Crunch" interest group.)
- Ability to video conference with one (or a limited number) of network contacts and to synchronously share simple documents or my desktop without the need to use on-line meeting companies with all their attendant costs and admin. I'm doing quite a lot of this using Skype right now, however, the ability to share desktops is lacking.
- Instant messaging functionality for all of my peers/contacts with asynchronous message forwarding should they be unavailable at that time. I want to be able to find out quickly what my organization is doing and share key decisions with colleagues very quickly. My decision to service that LC equipment next Wednesday may have untold impact on other colleagues and not just those wanting to use the instrument on that day. I need, in fact, to develop a company "nervous system" that can quickly relay and gather input on all key decisions, big or small! I need my analytical chemistry network to be highly effective at delivering "ambient knowledge" about groups and topics close to me but also about emerging thought from the wider network community.
- Video and still images of how to perform essential maintenance on equipment — I don't really care if the manufacturer puts a lot of advertising around the page (no pop ups or passwords please — one of my pet hates is "closed groups" created by instrument manufacturers in Facebook!). If the advertising revenue supports the production of these valuable resources then more power to them all!
- Social/fun aspects — Darwin awards/IgNoble awards/Flash games/cool and crazy viral marketing pieces/crazy (but relevant) You Tube videos and all the other stuff that friends send to me that make the working day just a little more bearable — arranged so that these things don't interfere with work but augment the spirit of community.

Obviously my site specification is extensive and probably not possible in the short term, however, there are a few organizations out there who have the eye and ear of the community who could potentially begin the foray into this area. I'm suggesting that the folks behind the Chromatography Online (www.chromatographyonline.com) website (and the publishers of the magazine you are reading now), may be ideally placed to begin such an implementation. E-mail me at incognito@thecolumn.eu.com with your own proposals for the "Ultimate Chromatography Network Site" specifications and I'll levy the relevant people so that we can talk and network and share and learn more effectively…!

Reference

1. "The Strength of Weak Ties", *American Journal of Sociology*, **78**(6), 1360–1380, (May 1973).

News

Smells like ants

When people get lost in the desert they are well known for walking in circles in the featureless terrain. But how creatures indigenous to that part of the world can find their way has been something of a mystery to scientists. Research published in open access journal *Frontiers in Zoology* shows that desert ants from the inhospitable saltpans of Tunisia input both local smells and visual cues into their navigation systems to guide them home.[1] The process they use was identified after scientists from the Max Planck Institute for Chemical Ecology in Jena, Germany, used gas chromatography to verify that desert microhabitats have unique odour signatures that can guide the ants.

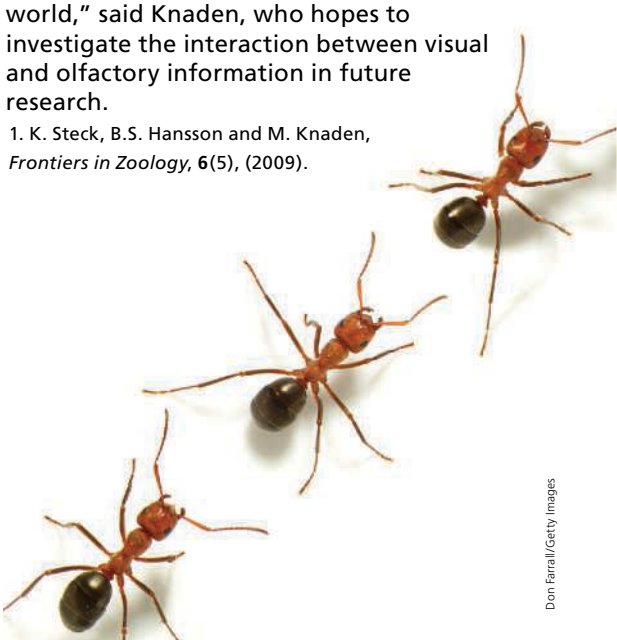
The use of environmentally derived olfactory landmarks has been shown for pigeons, while most ants rely rather on self-generated pheromone trails. However these ants roam for over 100 metres in search of food in a habitat where high temperatures and changeable food locations make pheromone trails ineffective.

Despite its homogenous appearance, the flat ground within the saltpan habitat differs slightly in its soil structure. Covered by a continuous salt crust, the surface is occasionally interrupted by clefts or by pieces of wood and halophytic plants and the signs of past periods of flooding. To check whether these structures result in different habitat odours, gas chromatography was used to analyse headspace samples of continuous salt crust, cleft salt crust, wood and halophytic plants. The emitted volatiles for each sample were relatively constant over two consecutive days, whereas the chromatograms differed among the samples, which mean they present potential olfactory landmarks.

After having identified some odours the researchers trained ants in field experiments to recognize ones pointing to a hidden nest entrance. Ants learned to associate their nest entrance with a single odour and discriminated the training odour against non-training odours. They even picked out the training odour from a four-odour blend.

"We are amazed to discover that while keeping track of the path integrator and learning visual landmarks, these ants can also collect information about the olfactory world," said Knaden, who hopes to investigate the interaction between visual and olfactory information in future research.

1. K. Steck, B.S. Hansson and M. Knaden, *Frontiers in Zoology*, **6**(5), (2009).



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[SFC]

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TharSFC introduces the new Method Station II Supercritical Fluid Chromatography (SFC) System. Designed for high throughput and high resolution of demanding separations encountered in drug research and development, this new analytical system features a faster, higher capacity autosampler, a larger Analytical-2-Prep™ column oven, and new and improved software. These new features provide unprecedented flexibility in an instrument that has a smaller footprint. For more information go to waters.com/SFC

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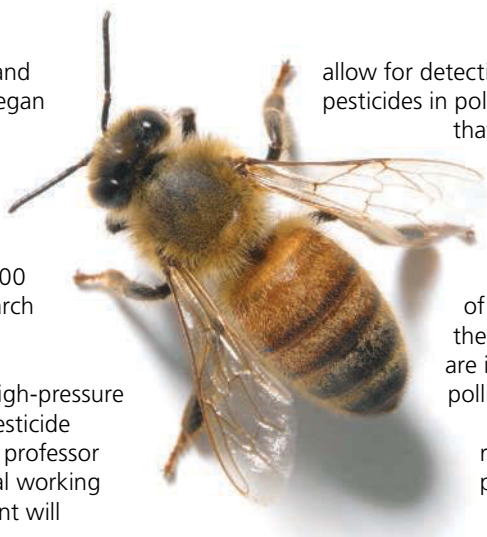


Don Farrall/Getty Images

Last year, Pennsylvania State University and ice cream manufacturer Häagen-Dazs began to work together to investigate Colony Collapse Disorder (CCD), a mysterious ailment that has decimated honeybee colonies around the world. This year, Häagen-Dazs has expanded that partnership with a second gift of $125000 to support ongoing and additional research and educational programmes related to honeybees.

This gift will include the purchase of high-pressure liquid chromatography equipment for pesticide analysis. According to Diana Cox-Foster, professor of entomology and co-chair of a national working group of CCD researchers, this equipment will allow for detection of chemicals including individual pesticides in pollen, wax and bee samples, chemicals that are potentially harmful to honeybees and other pollinators. Initial screenings can be performed using this equipment before more expensive analyses are undertaken.

"We anticipate that this piece of equipment will greatly facilitate the determination of how pesticides are impacting honeybees and other pollinators," said Cox-Foster.

For more information on honeybee research at Penn State, visit http://live.psu.edu/story/37918/nw4.

Separating and detecting toxins

A proposed European Community regulation sets a maximum level for fumonisins (FB1/FB2) in corn oil at 1000µg/kg, however traditional methods for detecting and quantifying low levels of mycotoxins such as fumonisins are specific to the individual toxins, which make it time-consuming, difficult and expensive to perform a comprehensive screen. The testing methods have had to be toxin specific because of the wide range of individual chemical and physical properties of the toxins as well as the large number of naturally occurring food constituents that may be extracted along with them and interfere with separation and detection.

However, researchers at the Munich Technical University and Bavarian Health and Food Safety Authority have developed a comprehensive mycotoxin screening method, based on the use of LCTech's GPC ULTRA gel permeation chromatography instrument. The new method relies on the fact that mycotoxins fall into the 200 to 600 molecular weight range and thus can be separated using GPC from food constituents that have much higher molecular weights.

In developing this comprehensive screen, researchers tried a range of different columns and found that the LCTech's MykoClean column successfully separated all 31 analytes from the oil. The recoveries obtained with the combined GPC-LC–MS–MS method were reported to range between 74–104%.

For additional detail, see the poster at http://www.pickeringlabs.com/pdf/080425-ua-Poster_30_Mykotoxin_Workshop1.pdf

Wataru Yanagida/Getty Images

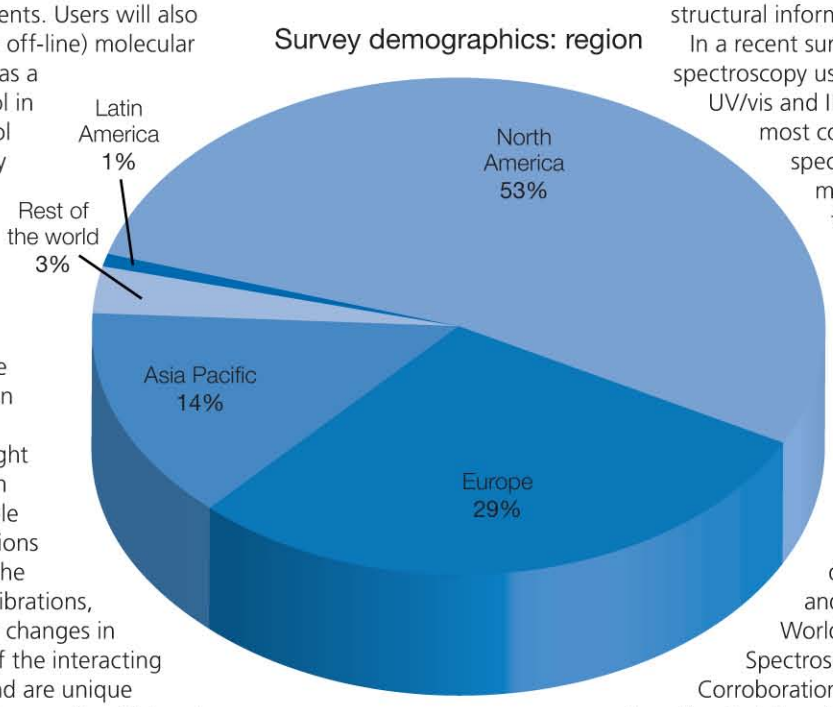
Links: **http://live.psu.edu/story/37918/nw4**
http://www.pickeringlabs.com/pdf/080425-ua-Poster_30_Mykotoxin_Workshop1.pdf

Market Profile: Molecular Spectroscopy Techniques

Similar to liquid and gas chromatography (LC and GC), molecular spectroscopy instruments are common tools in analytical and life science laboratories. Molecular spectroscopy technologies such as ultraviolet/visible (UV/vis) and infrared (IR) are often incorporated as detectors for LC and GC instruments. Users will also use stand-alone (or off-line) molecular spectroscopy units as a complementary tool in many quality control and R&D laboratory settings.

Molecular spectroscopy measures the interaction of light with a specific sample. The absorption, emission and/or reflection from a sample of light with wavelengths in the ultraviolet, visible and/or infrared regions is characteristic of the various molecular vibrations, rotations and other changes in the energy states of the interacting chemical species and are unique to differing molecular species. Molecular spectroscopy techniques are non-destructive and, except for nuclear magnetic resonance (NMR), they can analyse liquid, gaseous and solid samples.

Fluorescence, UV/vis and near infrared (NIR) spectroscopy techniques are quantitative techniques, while IR, NMR, colour and Raman spectroscopy provide qualitative measurements. NMR and IR both measure an absorbance spectrum, while colour and Raman spectroscopy measure scattered or reflected light. Of these techniques, NMR is the most powerful technique and can elucidate very specific structural information about a sample.



In a recent survey of 525 molecular spectroscopy users around the world, UV/vis and IR instruments were the most common type of molecular spectroscopy instruments mentioned, used by more than 70% and 50% of the respondents, respectively. Responses were obtained from a variety of industry sectors and over 40 different countries, albeit most respondents were from the US and Europe.

The foregoing data was extracted and adapted from SDi's Worldwide Molecular Spectroscopy Survey: Corroboration Market Trends and Revealing End-User Opinions. For more information, contact Glenn Cudiamat, vice president of Research Services, Strategic Directions International Inc., 6242 Westchester Parkway, Suite 100, Los Angeles, California 90045, USA, tel. +1 310 641 4982, fax +1 310 641 8851, e-mail: cudiamat@strategic-directions.com

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Rapid Large Volume Injection/GC–MS Analysis of Pesticides in Food Prepared by the QuEChERS Method

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[2]Shimadzu Europa GmbH, Duisburg, Germany.

In many analytical areas there is a tendency to save time in sample preparation. Regarding pesticide screening in food the well-known QuEChERS (Quick, Easy, Cheap, Efficient, Rugged and Safe)[1,2] method has been applied in many laboratories. This drastically reduces sample preparation effort when compared with the formerly used method with a final gel permeation chromatography (GPC) clean-up step. In contrast, when injecting the extracts prepared by QuEChERS many matrix signals can be observed in the GC–MS chromatogram. Full scan modes are, therefore, necessary to prevent false positive or false negative determination of target pesticides that could easily occur when running the gas chromatography–mass spectrometry (GC–MS) system in the more sensitive selected ion monitoring (SIM). To reach a high sensitivity for routine work in full scan mode, firstly, the GC–MS system should be a high-sensitivity instrument and secondly, a large volume injection further improves the limit of quantification (LOQ).

In this article a method called rapid large volume injection was used with a PTV injection port (Optic 3, ATAS GL International). Volumes up to 50 µL were injected with subsequent full scan GC–MS runs and the quantitative precision was checked by analysing round robin test samples. For the programmable temperature vaporization (PTV) insert special sintered glass liners were used. They do not have any filling material, preventing decomposition of fragile pesticides. Capacity for the large volume injection is achieved by the rough inner surface of the liners. This surface was SILTEK deactivated. To automatate the whole process after a liner was dirty (checked by a special degradation mixture) the LINEX automatic liner exchanger was installed and after about 80 injections the system performed the liner exchange automatically. The compound tables comprise of over 500 pesticides. Identification of the target compound was performed by checking full scan spectra and by using linear retention indices automatically checked as an additional filter.

Sample Preparation

The procedure involved the extraction of 10 g sample with 10 mL acetonitrile, followed by a liquid-liquid-partitioning step performed by adding 4 g anhydrous $MgSO_4$ plus 1 g NaCl, 1 g Na_3citrat and 0.5 g Na_2Hcitrat. The sample clean-up was performed using a rapid procedure called dispersive solid-phase extraction (SPE), in which 150 mg anhydrous $MgSO_4$ and 25 mg primary secondary amine (PSA) sorbent are mixed with 1 mL acetonitrile extract.

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After a second mixing and centrifugation step, the extract was transferred to autosampler vials for concurrent analysis by large volume GC–MS.

Experimental Conditions

The instrumentation was a GCMS-QP2010 Plus (Shimadzu Europa GmbH) with Optic 3 injector (ATAS GL International), AOC-5000 autoinjector (Shimadzu Europa GmbH) with an automatic glass liner exchanger option (LINEX, ATAS GL International). The chromatographic conditions were: VF-5-MS EZ guard column 30 m × 0.25 mm, 0.25 µm with an integrated retention gap of 10 m. The column temperature was set to 50 °C for 1 min (hold) then with 40 °C/min to 150 °C followed by 4.6 °C/min to 280 °C for 28.24 min, with a mean linear velocity of 30 cm/s (He). For the Optic 3 injector the temperature was set to 55 °C during the period of the solvent venting time and then ramped with 15 °C/s to 280 °C for the rest of the analytical run (59.75 min). The solvent venting at low temperature (55 °C) was optimized and finally set to 38 s at a split ratio of 50:1. The split was further programmed to transfer the analytes to the column.

This was achieved by closing the split after the venting time for 2 minutes (analyte transfer). The split was then reopened to 10:1 to purge residual solvent out of the liner. The injection volume was finally set to 30 µL. The Optic 3 is heated by direct ohmical heating. This leads to liner inner diameters of this PTV of about 3.4 mm, corresponding to typical hot split/splitless liner dimensions.

It is possible to ramp the Optic 3 up to a maximum of 30 °C/s even using these liners, which is in contrast to conventional PTVs that have indirect resistive heaters and correspondingly have typical inner diameters of about 1–2 mm. This has a strong influence on the method development in large volume injections. For the Optic 3 the injection speed up to about 100 µL is not as critical and, therefore, the liquid can be injected rather quickly [rapid large volume injection (RLVI)] while in the latter case a speed control of injection is important.

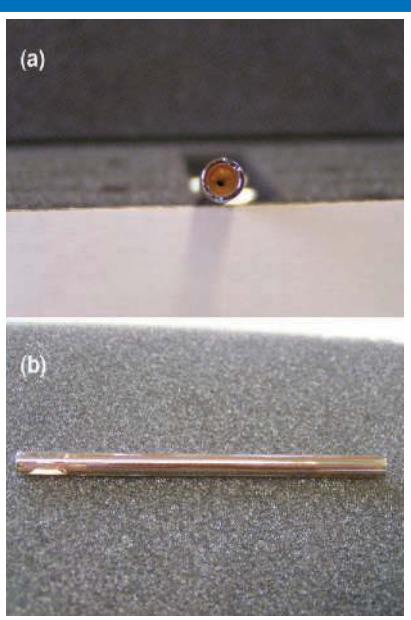
The mass spectrometer was operated in full scan mode to minimize false positive or false negative identification. The scan range was set to 50–550 *m/z*. The ion source temperature and the interface was set to 200 °C and 320 °C, respectively.

Results

The correct liner choice is critical to the success of any pesticide analysis using PTV injection. The liner must be thoroughly deactivated or many labile pesticides may decompose or adsorb in the inlet. For large volume injections the capacity of the glass insert is crucial. Any filling material such as glass wool or TENAX used in classical large volume injections that increases the injection volume capacity has to be avoided even if deactivated. For this reason, a glass insert with a rough surface (sintered glass liner, ATAS GL International) was chosen. With these glass liners the inner surface is covered by many small quartz beads to have a larger surface. When using a syringe with a side hole needle the liquid injected will be sprayed onto the wall surface of the liner. External experiments showed that even a 50 µL acetonitrile injection does not result in dropping any liquid out of the liner. These liners were deactivated by a double SILTEK (Restek) deactivation process.

In Figure 1 such a liner is shown. The inertness of the glass insert after subsequent injections of pesticide matrix

Figure 1: Sintered glass insert with glass beads on inner surface for Optic 3 with SILTEK deactivation.



was checked by a degradation of dichloro-diphenyl-trichloroethane (DDT) (also used in EPA 8270). The degradation of DDT must be below 20%. This was checked automatically in batch runs.

After optimization of the Optic 3 injector parameters the analytical performance of the repeatability was studied. Pesticide free extracts were spiked with a pesticides mixture (10–200 µg/L) and 10 subsequent 30 µL injections were made; quantification was fully automated. The result of the relative standard deviations (RSDs) was calculated from the concentration and was about 4.3% or better.

In the following steps the linearity of response was studied with standard solutions prepared in matrix extracts. The calibration curves generated from the matrix matched

Figure 2: Multiple pesticide-residue calibration run of a mixture of more than 50 pesticides on a VF-5-MS 30 m × 0.25 × 0.25 µm with an integrated retention gap of 10 m.

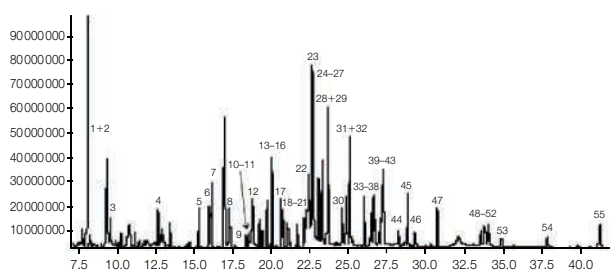


Figure 3: Strawberry sample (Germany) from the field. The pesticides cyprodinil 0.016 mg/kg, fludioxonil 0.022 mg/kg, trifloxystrobin 0.005 mg/kg, fenhexamid 1.078 mg/kg, boscalid 0.032 mg/kg and azoxystrobin 0.189 mg/kg.

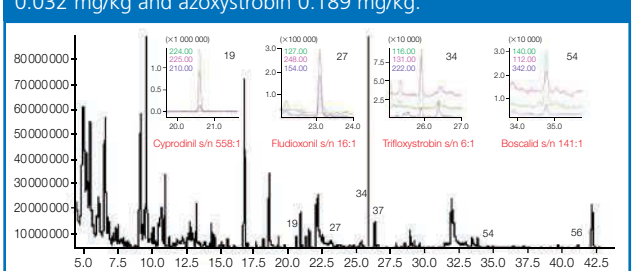


Table 1: Analytical data of a multi-residue pesticide mixture.

No.	RT	Substance	*m/z*	RI	Conc. mg/kg	Strawberry sample mg/kg
1	7.885	Dichloranilin-3.5	161	1340	0.1648	
2	8.059	Dichlobenil	171	1354	0.01715	
3	9.585	Nitrapyrin	194	1464	0.048	
4	12.707	Ethoprophos	97	1649	0.0408	
5	15.357	Terbuphos	57	1789	0.035	
7	16.028	Disulphoton	88	1823	0.0342	
6	16.176	Ethrimphos	153	1831	0.05958	
8	16.524	Flufenoxuron	126	1848	0.19	
9	17.241	Desmetryn	213	1885	0.0495	
10	17.605	Spiroxamin 1	100	1903	0.013	
11	18.522	Fenpropidin	98	1950	0.0103	
12	18.621	Spiroxamin 2	100	1955	0	
13	18.701	Fenitrothion	125	1959	0.0654	
14	19.217	Metolachlor	162	1985	0.025	
15	19.447	Fenpropimorph	128	1996	0.0105	
16	19.68	Tetraconazol	336	2008	0.053	
17	20.047	Pirimiphos-Ethyl	333	2027	0.0615	
18	20.616	Pendimethalin	252	2056	0.05	
19	20.685	Cyprodinil	224	2060	0.0245	0.016
20	21.014	Pyrifenox-z	92	2077	0.0624	
21	22.06	Pyriphenox-e	92	2131	0	
22	22.147	Chinomethionat	206	2136	0.0548	
23	22.554	Mepanipyrim	222	2158	0.051	
24	22.575	Endosulphan-a	159	2158	0.23	
25	22.964	Chlorfenson	111	2179	0.0505	
26	23.035	Fludioxonil	127	2184	0.1261	0.022
27	23.267	Oxadiazon	175	2195	0.0505	
28	23.524	Myclobutanil	179	2209	0.0565	
29	23.596	Buprofezin	105	2212	0.05265	
30	24.522	Endrin	81	2262	0.04905	
31	24.775	Fensulfothion	97	2276	0.05535	
32	24.96	Endosulphan-b	159	2286	0.0655	
33	26.021	Trifloxystrobin	116	2345	0.1875	0.005
34	26.332	Quinoxyfen	237	2362	0.02625	
35	26.481	Endosulphansulphate	272	2371	0.04545	
36	26.578	Fenhexamid	97	2376	0.097	1.078
37	26.825	Hexazinon	171	2390	0.0555	
38	27.06	Propagite 1	135	2403	0.0217	
39	27.106	Tebuconazol	125	2406	0.05275	
40	27.119	Propagite 2	135	2407	0	
41	27.238	Haloxyfop-ethoxyethylster	302	2414	0.067	
42	27.251	Triphenylphoshat (TPP - INSTD)	77	2414	0.05	
43	27.291	Piperonylbutoxid	176	2417	0.015	
44	28.129	Iprodion	314	2473	0.196	
45	28.769	Fenpropathrin	97	2502	0.0495	
46	29.209	Fenazaquin	145	2529	0.01035	
47	30.728	Acrinathrin	93	2620	0.0624	
48	33.547	Cyfluthrin 1	163	2787	0.0975	
49	33.624	Fenbuconazol	129	2792	0.029	
50	33.702	Cyfluthrin 2	163	2802	0	
51	33.868	Cyfluthrin 3	163	2810	0	
52	34.081	Cyfluthrin 4	163	2815	0	
53	34.764	Boscalid (Nicobifen)	140	2849	0.0594	0.032
54	37.773	Pyraclostrobin	132	2980	0.1275	
55	41.268	Azoxystrobin	344	3109	0.1584	0.189

standards were used for quantification, so that possible errors as a result of enhancement/suppression caused by the matrix effects could be minimized. All results were calculated using triphenylphosphate (TPP) as internal standard.

For the analysis of carrot extracts, for example, the correlation coefficients obtained for the calibration plots of all analytes were in the range 0.989–0.999 in the concentration range of 0.002–1.3 mg/L.

Applying this method, the lowest detection limit (LOD) for more than 500 analytes were in the range of 0.002–0.020 mg/kg depending on the substance.

Figure 2 shows the calibration run of a multiple pesticide-residue standard spiked with a blank strawberry sample. In Table 1 the retention times, linear retention indices (LRI) and concentrations are given.

Figure 3 shows the TIC chromatogram and the ion sets of cyprodinil, fludioxonil, trifloxystrobin and boscalid of a strawberry sample from the field (Germany). The target ions shown in this figure correspond to a range of the LOQ of 0.002–0.02 mg/kg. The LOD for this strawberry example is in the range of 0.0005–0.001 mg/kg.

Another critical point is the number of possible injections before the SILTEK deactivation is no longer stable enough to give reliable quantitative data. For this purpose, a degradation check standard was analysed to check the condition of the glass liner. A mixture of 5 ng pp'-DDT and endrin was periodically injected and analysed. The breakdown of pp'-DDT and endrin must be below 20%. pp'-DDD, pp'-DDE, endrin aldehyde and endrin-ketone are the metabolites from endrin and pp'-DDT. The check formula used was to calculate the total target areas of pp'-DDT + pp'-DDE + pp'-DDD/total target areas of pp'-DDE + pp'-DDD × 100%. In a batch series every fifth injection was done with the degradation check standard diluted in matrix extracts (for example apple matrix) and degradation check values were calculated.

Summary

The quantitative determination of multiresidue pesticides in food matrix according to the QuEChERS method can be successfully combined with a rapid large volume injection (RLVI) and a full scan GC–MS method.

The lowest determination limits (LOQ) were ⩽0.002 mg/kg. The observed RSDs of 4.3% and below indicate a high precision in routine work. Up to 80 injections of RLVI of 30 µL into a SILTEK deactivated sintered glass liner were possible.

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An SPE-LC–MS–MS Method for Analysing Antidepressants and their Metabolites in Plasma

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A rapid, sensitive and fully automated on-line solid-phase extraction-liquid chromatography-tandem mass spectrometry (SPE-LC–MS–MS) method was developed and validated for the direct analysis of 14 antidepressants and some of their metabolites in human plasma. Integration of the sample extraction and LC separation into a single system permitted direct injection of the plasma sample. The total process time was 20 minutes and only 50 µL of plasma was required. Extraction was reproducible with recoveries >99% for all the analytes. The method showed an excellent intra- and inter-assay precision for quality control (QC) samples spiked at a concentration of 40, 200 and 800 µg/L. The method was linear over the range 10–1000 µg/L ($r^2 > 0.99$). Limits of quantification (LOQ) were estimated to be 10 µg/L. Furthermore, the processed samples were demonstrated to be stable for at least 48 hours for most compounds. The method was subsequently applied to authentic clinical samples.

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Introduction

High-throughput analysis is becoming increasingly important in all areas of science; the forensic sciences being no exception. Moreover, because of the development of more potent drugs, drug concentrations in biological samples are often present at much lower levels than before. Therefore, fast analytical techniques with much higher sensitivity and selectivity are needed.

One of the bottlenecks in bioanalysis is often associated with the sample preparation requirements, especially if the method requires manual extraction techniques. For this reason, automation of off-line SPE is possible and the utility of "96-well SPE" was introduced some years ago. Nowadays, an even more attractive approach is on-line SPE; the entire process of conditioning, sample application, washing and elution takes place at constant flow-rates yielding better precision of quantitative methods in comparison with off-line vacuum driven extraction procedures. Another important advantage is that no manual transfers are made and that the whole of the eluate is loaded onto the LC column without the need for a pre-concentration step.

A very elegant system for rapid analysis of complex samples can be obtained by the on-line coupling of SPE. In this way, the sample is directly injected into the SPE-MS system and a rate-limiting step is eliminated. The Symbiosis Pharma system (Spark Holland, Emmen, The Netherlands), allows the simultaneous extraction of the second sample in one clamp and the elution of the first sample in the second clamp, thus leading to an optimal use of the extraction time. Several papers have been published concerning the analysis of drugs in biological samples with the Symbiosis system.[1–8]

The aim of this study was to develop a simple, rugged and high-throughput on-line SPE-LC–MS–MS method for rapid and simultaneous analysis of the main antidepressants prescribed in Belgium and some of their metabolites in plasma. The method involves a fully automated SPE system (Spark Symbiosis Pharma).

Materials and Methods

SPE–LC–MS–MS

Sample preparation: XLC (on-line SPE)

Sample extraction was performed using the on-line SPE Symbiosis Pharma system (Spark Holland). Nine hundred and fifty microlitres of 0.1% formic acid and 50 µL of the I.S. working solution (0.1 mg/L) were added to 50 µL of plasma. The following XLC programme was subsequently used; After conditioning with 1 mL of methanol, 1 mL of water and 1 mL of 0.1% formic acid, 100 µL of the diluted plasma sample was applied onto the SPE MCX cartridge (Oasis MCX Prospekt) using 1 mL of 0.1% of formic acid as transport solvent. Clean-up was accomplished with successive 1 mL washes of 0.1% formic acid and methanol. While the elution step was being performed, a new cartridge was conditioned, loaded and washed in the left clamp. The elution was performed with 300 µL of 5% ammonia in methanol (at 100 µL/min).

Chromatographic conditions

Focusing of the eluate was simultaneously performed as the compounds were eluted from the SPE cartridge by the use of a focusing column, Gemini C_{18} guard column (4 × 2.0 mm, 5 µm) (Phenomenex, Torrance, California, USA), and a gradient elution with 10 mM ammonium bicarbonate (pH 10) (a) and acetonitrile (b). A gradient was performed starting from 0% B and a flow-rate of 1 mL/min for 3 min, as the eluate was diverted to the waste using the MS–MS Rheodyne switching valve. At 3.01 min, a switch of the valve delivered the eluent to the analytical Gemini C_{18} column (150 × 2 mm, 5 µm) (Phenomenex) to start with the separation of the compounds at a flow-rate of 0.3 mL/min and 50% B over the next minute. From 4–5 min, B was subsequently increased to 70% and then kept for 6.5 min. At 11.5 min, B was increased to 95% in 1.5 min before returning to 50% within 0.5 min and equilibrating for 4.5 min. At 18 min a switch of the MS–MS valve diverted the eluent again to the waste, returning to the initial conditions to be ready for the analysis of the following sample.

Tandem mass spectrometry

A Quattro Premier tandem mass spectrometer (Waters, Milford, Massachusetts, USA) was used. Ionization was achieved using electrospray in positive ionization mode (ESI+). Nitrogen was used as nebulization and desolvation gas at a flow-rate of 800 L/h and heated to 350 °C. Capillary voltage and source block temperature were 1 kV and 120 °C, respectively. The selected MRM transitions for the antidepressants are shown in Table 1.

Table 1: MRM transitions and conditions for all the compounds and their deuterated analogues. Underlined transitions were used for quantification.

Compound	Precursor ion (*m/z*)	Product ion (*m/z*)	Cone voltage (V)	Collision energy (eV)
Trazodone	372.1	147.9	40	35
		176.0		25
Fluvoxamine	319.1	70.8	25	15
		86.8		15
Norfluoxetine	296.1	30.0	15	8
		133.9		6
Paroxetine	330.1	69.8	40	28
		192.1		20
Citalopram	325.1	108.8	30	25
		115.8		25
Venlafaxine	278.1	57.8	20	18
		260.1		12
Fluoxetine	310.1	43.9	20	12
		147.9		8
Desipramine	267.2	44.0	25	30
		71.8		15
Nortriptyline	264.1	90.8	25	20
		233.2		15
Norclomipramine	301.1	43.9	25	35
		71.8		20
Imipramine	281.2	57.7	25	35
		85.7		10
Sertraline	306.1	158.8	15	30
		275.0		12
Amitriptyline	278.1	90.8	30	25
		104.8		20
Clomipramine	315.1	57.9	30	30
		85.8		18
Paroxetine-d_6	336.1	75.8	35	32
Fluoxetine-d_6	316.1	43.9	20	12
Desipramine-d_3	270.2	74.8	25	15
Imipramine-d_3	284.2	88.8	25	18
Clomipramine-d_3	318.1	88.8	30	20

On-line SPE–LC–MS–MS assay validation

Linearity, limit of quantification (LOQ), limit of detection (LOD), precision and accuracy

Linearity was checked over their therapeutic range and weighting factors were determined according to least square regression analysis.

QCs were prepared for every run in blank plasma at a concentration of 40, 200 and 800 µg/L. Intra-assay precision was evaluated by replicate (n = 5) analysis of the three QC samples in one run. Inter-assay precision was evaluated by replicate analysis of the QC samples in several experiments performed on six different days.

Selectivity and specificity

The selectivity of the method against endogenous interferences was verified by examination of the chromatograms obtained after the extraction of six different blank plasma samples.

Stability of samples

The stability of the drugs in plasma was monitored in diluted plasma samples as follows; 50 µL of blank plasma spiked at the initial concentrations of 40, 200 and 800 µg/L (n = 9, at each concentration) were diluted with 950 µL of 0.1% formic acid. The I.S. was added to the control samples (n = 3) and the concentrations were determined immediately. Another pool of samples were kept in the autosampler at 6 ± 2 °C and analysed after the addition of the I.S., after 24 hours (n = 3) and 48 hours (n = 3). For an evaluation of freeze/thaw stability, samples at concentrations of 40 and 200 µg/L (n = 3) were spiked with the I.S. after three freeze-thaw cycles and analysed. Stability was tested against a lower percentage limit corresponding to 90% of the mean value of control samples by one-sided t-test ($P < 0.05$).

Assessment of matrix effects

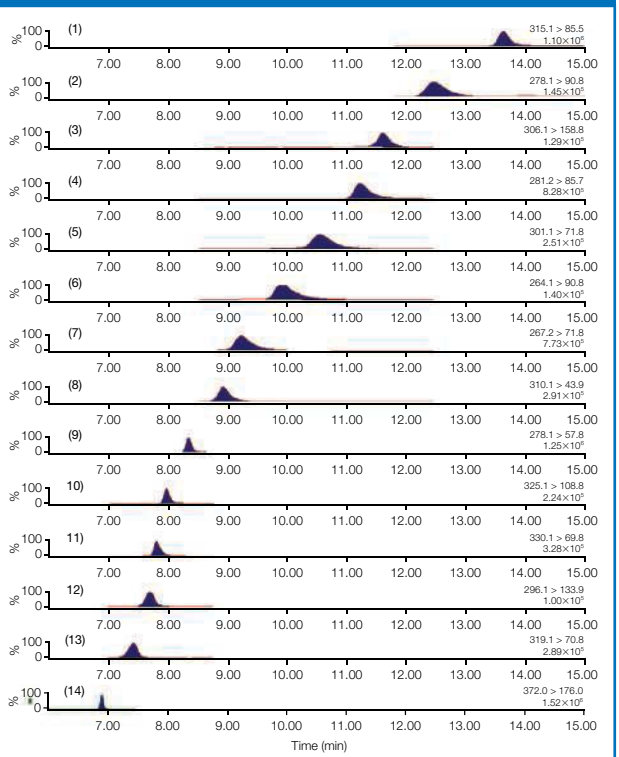
Two different analyses were performed. The first one involved a post-column infusion experiment. The study was based on a continuous post-column infusion of a mixture of the drugs and their internal standards (10 µg/L at a flow-rate of 10 µL/min) to produce a constant elevated response in the MRM channels. The interference of this constant response was monitored following the injection of plasma samples [in two different anticoagulants: sodium citrate (n = 3) and sodium fluoride (n = 3)] and compared with the response following the injection of mobile phase only. A second

type of experiment comprises a comparison of the peak responses of the analysis of a blank plasma sample spiked at 1000 µg/L calibrator (n = 3) with those obtained from water spiked at the same concentration level.

Recovery

A 1000 µg/L calibrator (n = 3) was loaded and washed in a first SPE cartridge while a second cartridge was placed in series to determine the breakthrough of the first one. Both cartridges were subsequently eluted independently.

Figure 1: MRM chromatograms obtained following the analysis of a spiked plasma sample with 10 µg/L of (1) clomipramine, (2) amitriptyline, (3) sertraline, (4) imipramine, (5) norclomipramine, (6) nortriptyliline, (7) desipramine, (8) fluoxetine, (9) venlafaxine, (10) citalopram, (11) paroxetine, (12) norfluoxetine, (13) fluvoxamine and (14) trazodone. Peak intensity is shown on the right-hand corner of each trace.



Recovery was considered to be the ratio between the response obtained after elution of the first cartridge and the total response (sum of both the first and the second SPE cartridge).

Results and Discussion

In each instance, a weighted (1/x) linear regression line was applied, except for trazodone, nortriptyline and norclomipramine, for which a quadratic response was found to be more suitable to obtain the best fit across the calibration range. Correlation coefficient of $r^2 > 0.99$ was achieved in the range investigated: from 10–1000 µg/L. Figure 1 shows the MRM chromatograms obtained following the analysis of the lowest calibrator (10 µg/L). At this concentration a signal-to-noise (S:N) > 10:1 was observed for the qualifier and RSD > 20% so the criteria for LOQ were satisfied.

Selectivity of the method was achieved by a combination of retention time, precursor and product ions.

The most prominent precursor–product transitions were used for the quantification of the non-deuterated compounds and the next most abundant, used as qualifiers.

Table 2: Reported therapeutic range and linearity data for 14 antidepressants and their metabolites.[9]

Compound	Therapeutic range (µg/L)	r^2	LOQ (µg/L)	LOD (µg/L)
Trazodone	500–2500	0.99995	10	0.5
Fluvoxamine	50–250	0.99848	10	1
Norfluoxetine	100–500	0.99890	10	1
Paroxetine	10–75	0.99939	10	0.5
Citalopram	20–200	0.99793	10	0.5
Venlafaxine	250–750	0.99727	10	0.5
Fluoxetine	150–500	0.99720	10	1
Desipramine	75–250	0.99945	10	1
Nortriptyline	50–250	0.99922	10	1
Norclomipramine	150–550	0.99702	10	1
Imipramine	45–150	0.99951	10	0.5
Sertraline	50–250	0.99900	10	1
Amitriptyline	50–300	0.99845	10	0.5
Clomipramine	20–250	0.99947	10	0.5

Table 3: Intra-assay and inter-assay precision and bias of the QC samples prepared in plasma at a concentration of 40, 200 and 800 µg/L

Compound	Concentration of QC	Intra-assay precision (n = 5) Mean concentration found (µg/L)	RSD (%)	Bias (%)	Inter-assay precision (n = 6) Mean concentration found (µg/L)	RSD (%)	Bias (%)
Trazodone	40	43.6	6.1	9.0	41.0	11.5	2.5
	200	233.2	1.5	16.6	199.6	7.0	−0.2
	800	692.6	4.7	−13.4	673.4	13.2	−15.8
Fluvoxamine	40	41.9	7.2	4.8	36.5	10.9	−8.8
	200	216.5	3.3	8.3	189.4	7.6	−5.3
	800	843.8	3.8	5.5	807.0	8.7	0.9
Norfluoxetine	40	45.6	7.5	14.0	41.1	14.3	2.8
	200	236.1	3.8	18.1	193.2	15.0	−3.4
	800	880.9	7.5	10.1	801.5	8.6	0.2
Paroxetine	40	40.8	6.4	2.0	38.5	6.5	−3.8
	200	212.8	2.2	6.4	193.6	7.2	−3.2
	800	807.3	4.3	0.9	805.9	8.8	0.7
Citalopram	40	44.1	4.4	10.3	43.1	7.8	7.8
	200	222.0	2.0	11.0	201.3	7.8	0.7
	800	796.7	2.8	−0.4	780.7	10.1	−2.4
Venlafaxine	40	44.0	5.6	10.0	43.1	12.5	7.8
	200	227.4	1.7	13.7	198.3	10.4	−0.8
	800	780.8	5.5	−2.4	785.4	13.0	−1.8
Fluoxetine	40	43.8	7.3	9.5	40.0	10.7	0.0
	200	220.0	3.0	10.0	196.3	9.8	−1.8
	800	817.0	8.3	2.1	814.3	9.9	1.8
Desipramine	40	43.3	5.4	8.2	40.5	5.4	1.3
	200	213.7	3.1	6.9	194.4	6.2	−2.8
	800	830.6	3.2	3.8	791.0	11.1	−1.1
Nortriptyline	40	41.7	6.4	4.3	38.1	8.4	−4.8
	200	211.0	3.6	5.5	192.1	8.3	−4.0
	800	813.4	5.1	1.7	800.0	13.6	0.0
Norclomipramine	40	40.0	6.5	0.0	36.6	15.4	−8.5
	200	226.9	15.5	13.5	197.0	7.0	−1.5
	800	798.6	3.4	−0.2	808.9	5.5	1.1
Imipramine	40	43.4	2.3	8.5	40.1	6.4	0.3
	200	226.1	1.3	13.1	198.1	7.4	−1.0
	800	827.1	3.8	3.4	806.5	11.4	0.8
Sertraline	40	42.2	5.2	5.5	37.2	14.1	−7.0
	200	221.9	3.5	11.0	193.9	9.2	−3.1
	800	850.2	5.5	6.3	824.6	7.6	3.1
Amitriptyline	40	38.3	5.9	−4.3	35.2	10.1	−12.0
	200	203.1	3.1	1.6	181.8	9.2	−9.1
	800	805.6	4.2	0.7	780.1	9.7	−2.5
Clomipramine	40	43.9	6.5	9.8	41.5	9.9	3.8
	200	219.1	1.7	9.6	207.1	7.5	3.6
	800	764.4	5.1	−4.5	808.2	10.3	1.0

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Injection of single analyte solutions did not produce interference in the other MRM channels. Linearity data, LOQ and LOD are shown in Table 2.

The intra-assay precision (repeatability) and inter-assay precision were satisfactory, with all relative standard deviations less than 20% (Table 3). Results indicated that the accuracy of the assay was >81%.

The stability of spiked samples (40, 200, 800 µg/L) was monitored at 24 hours and 48 hours while kept in the autosampler at 6 ± 2 °C. No statistically significant difference could be observed for the three different concentrations, except for clomipramine and norclomipramine where a slightly negative trend was observed ($P > 0.05$), but did not compromise quantification.

Post-column infusion experiments were performed to provide information of the effect of the matrix throughout the course of the elution time for the analytes. No changes in response were observed. A second experiment was performed and we compared peak responses obtained when the antidepressants were spiked with a blank plasma sample, with the responses obtained when the antidepressants were added to a sample where the plasma was substituted with water. No statistically significant differences in peak areas were observed.

Table 4: Extraction recovery and matrix effect. Data represents the mean of three experiments with a 1000 µg/L calibrator.

Compound	Recovery (%) (n = 3)	Estimated matrix effect after SPE (%) (n = 3)
Trazodone	99.9	–10.9
Venlafaxine	99.8	–10.6
Citalopram	99.9	–5.9
Desipramine	99.8	–9.1
Imipramine	99.9	–7.1
Nortriptyline	99.6	–11.8
Amitriptyline	99.9	–12.8
Paroxetine	99.8	–6.7
Fluvoxamine	99.7	–16.8
Norfluoxetine	99.8	0.5
Fluoxetine	99.9	–12.1
Sertraline	99.9	–15.2
Clomipramine	99.7	–17.7
Norclomipramine	99.9	–10.4

Very high and reproducible recoveries were obtained with this SPE procedure for all analytes and all compounds were totally eluted from the SPE cartridge at the elution step conditions (Table 4).

The validated SPE-LC–MS–MS method was applied to the analysis of 11 authentic samples from clinical cases and previously analysed by liquid chromatography coupled to diode array detector (DAD) using a routine screening method. They were positive for citalopram (n = 1), fluoxetine (n = 6), norfluoxetine (n = 6), trazodone (n = 3), venlafaxine (n = 1), amitriptyline (n = 2) and nortriptyline (n = 2). The citalopram positive sample could not be detected by the LC–DAD because of the low concentration (14 ng/mL). The measured concentrations were sub or in the therapeutic range and several samples had to be re-analysed after 1:10 dilution with blank plasma, which demonstrated the high sensitivity of the method.

Conclusions

The combination of on-line SPE with MS–MS allowed the development of a high-throughput, fast and sensitive method using just 50 µL of sample and with a 20 min total analysis time without compromising the method validation criteria. The method was successfully applied to 11 authentic plasma samples.

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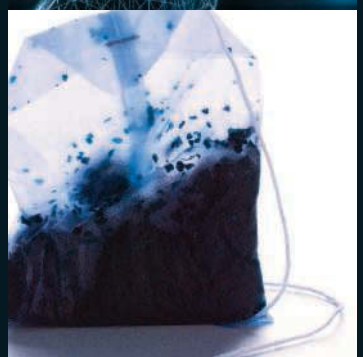

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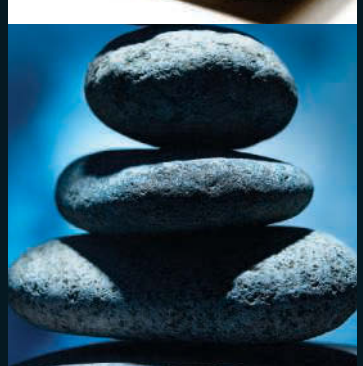

March 2009

www.chromatographyonline.com

Analysis of Food Samples with Ion Chromatography after In-line Dialysis

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Run a difficult food sample on your IC and you stand a big chance that you will wreck the column. Of course, you can waste a lot of time on tedious sample preparation steps to eliminate undesired matrix components. Or you can go for Metrohm's automated compact stopped-flow dialysis providing optimum separation while protecting your column from detrimental compounds.

Introduction

Ion chromatography (IC) as an analytical technique has experienced an impressive surge in popularity because of the simplicity and robustness of the method, the improved reliability and the great choice of columns, detectors and applications. For a sample in a homogeneous matrix, very little sample preparation is required and results can be obtained within a matter of minutes. In complex matrices carrying high organic loads such as wastewater, soil eluates or dairy products, a more extensive sample preparation is mandatory to prevent destruction of the column.

Although numerous sample preparation techniques have been developed, such as the Carrez precipitation for protein-containing samples, most of them are tedious and error-prone. To overcome these shortcomings, Metrohm launched the first coupling of IC with dialysis in 1997. Since then the procedure has been further improved and allows for an efficient in-line elimination of undesired matrix components in a variety of demanding sample types.

Using, as examples, an ultra-high temperature (UHT) processed milk and a baby milk powder sample, this work presents a fully automated sample preparation set-up coupled to the new ion chromatograph 881 Compact IC pro (Figure 1). Calibration parameters, carryover and recovery rates were tested with multi-anion standards.

Figure 1: The 881 Compact IC pro with the 858 Professional IC Sample Processor with dialysis cell and 800 Dosino. Instrument control, data acquisition and processing were performed by MagIC Net software.

Materials and Methods

Instrumentation

- 881 Compact IC pro
- 858 Professional IC Sample Processor
- 800 Dosino
- Dialysis equipment

Reagents and eluents

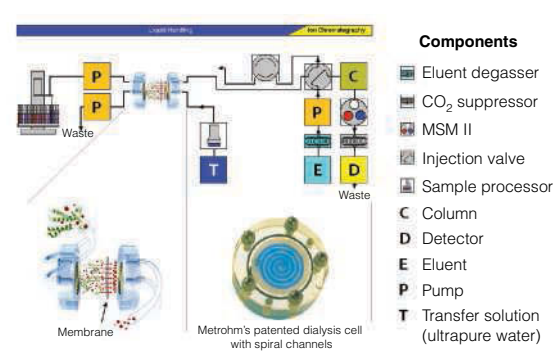
All standard solutions and eluents were prepared with deionized water having a specific resistance higher than 18 MΩ·cm. Two standard solutions covering the concentration ranges 1.0…3.6 mg/L and 10…36 mg/L served to determine the system characteristics.

The ultra-high temperature (UHT) processed milk and the baby milk powder were purchased from Migros, Switzerland.

Compact Stopped-Flow Dialysis

Dialysis is based on the selective diffusion of molecules or ions from one liquid (donor or sample solution) to another (acceptor solution) through a membrane. The driving force for the transfer is the concentration gradient across the membrane.

Figure 2: Schematic diagram showing both the in-line dialysis cell and its coupling to the compact IC. The diagram and photograph in the lower part of the figure show Metrohm's patented spiral-flow dialysis cell.



Contrary to dynamic dialysis, where two solutions continuously pass through the dialysis module, at least one solution is temporarily stopped until the concentration in the acceptor solution is the same as that in the donor solution. This patented stopped-flow procedure takes between 10 and 14 minutes and can be directly coupled to an IC set-up. As the dialysis is performed during the recording of the previous sample's chromatogram, the overall analysis time is not prolonged.

Whereas in the conventional set-up two double-channel peristaltic pumps transport the sample and the acceptor solution to and from the dialysis cell, in compact dialysis a Dosino doses ultrapure water through the acceptor compartment of the cell. The stopped-flow status is achieved by stopping the Dosino and blocking the outlet capillary of the cell by feeding it through the valve of the sample processor. The latter, depending on its valve position, allows or blocks the acceptor solution flow (Figure 2).

System Characteristics

Calibration

Five concentration levels (0.5, 1, 5, 10 and 20 mg/L) prepared from a multi-ion standard were used for external calibration (Table 1).

Carryover

Carryover was evaluated by injection of an ultrapure water blank immediately after injection of a standard (Table 2).

Recovery rates

To determine recovery rates, results obtained by direct injection were compared to those obtained by injection of the dialysate (Table 3).

Dairy Samples

UHT processed milk

Prior to analysis, the UHT processed milk sample was diluted 1:100 with ultrapure water and placed in the sample vials upon the rack of the sample processor. The subsequent dialysis of the milk sample and the injection of the dialysate onto the separation column was fully automated. The calculation was performed automatically using integration software MagIC Net 1.1 against the previously prepared calibration plots (see section "Calibration").

Under the conditions described in the caption of Figure 3, excellent baseline separation of chloride, phosphate and sulphate is achieved within 12 min. Repetitive analyses showed no trending in peak areas or retention times, which suggests that sample proteins did not pass the membrane.

Baby food milk powder

Following the manufacturer's instructions, the baby food milk powder was replenished with water. Prior to analysis, the prepared milk sample was diluted 1:100.

As with the UHT milk sample, the chromatographic conditions applied provide an excellent baseline separation for chloride, phosphate and sulphate (Figure 4).

Conclusion

The analytical challenge treated in the present work consists in the determination of chloride, phosphate and sulphate in the presence of difficult sample matrices that interact with the stationary column phase or even render

Table 1: Correlation coefficients and relative standard deviations of the five-point anion calibration.

	Fluoride	Chloride	Nitrite	Bromide	Nitrate	Phosphate	Sulphate
Correlation coefficient	0.99995	0.99996	0.99999	0.99996	0.99994	0.99990	0.99997
RSD (%)	1.516	1.242	0.834	1.169	1.479	2.491	1.176

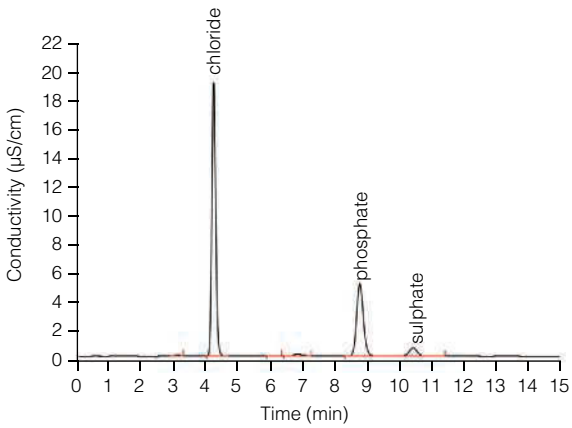
Table 2: Carryover in percent determined for the concentration ranges 1.0…3.6 mg/L and 10…36 mg/L.

	Fluoride	Chloride	Nitrite	Bromide	Nitrate	Phosphate	Sulphate
Low standard conc.	0.24	0.15	0.17	0.20	0.18	0.11	0.28
High standard conc.	0.49	0.12	0.13	0.22	0.11	0.00	0.38

Table 3: Anion recovery rates.

	Low standard concentration (1.0...3.6 mg/L)					High standard concentration (10...36 mg/L)				
	Direct injection		With dialysis		Recovery	Direct injection		With dialysis		Recovery
	Mean (mg/L)	RSD (%)	Mean (mg/L)	RSD (%)	rate (%)	Mean (mg/L)	RSD (%)	Mean (mg/L)	RSD (%)	rate (%)
Fluoride	1.06	0.12	1.03	0.24	97.2	10.81	0.09	10.57	0.06	97.8
Chloride	3.01	0.04	2.97	0.03	98.7	31.58	0.03	31.22	0.06	98.9
Nitrite	2.94	0.32	2.91	0.15	99.0	30.01	0.30	29.81	0.04	99.3
Bromide	1.02	0.08	1.01	0.00	99.0	10.50	0.04	10.38	0.17	98.9
Nitrate	3.02	0.07	2.97	0.00	98.3	30.80	0.03	30.40	0.03	98.7
Phosphate	3.81	0.17	3.47	0.10	91.1	33.74	0.02	31.83	0.03	94.3
Sulphate	3.52	0.09	3.35	0.07	95.2	35.57	0.04	34.17	0.07	96.1

Figure 3: Anion chromatogram of a UHT dialysate containing 9.88 mg/L chloride, 17.40 mg/L phosphate and 1.09 mg/L sulphate (after 1:100 dilution of the sample). Column: Metrosep A Supp 5 – 100, eluent: 3.2 mmol/L sodium carbonate and 1.0 mmol/L sodium hydrogen carbonate, flow: 0.7 mL/min, column temperature: 30 °C, injection volume: 20 µL, acceptor solution: ultrapure water, dialysis time: 14 min.

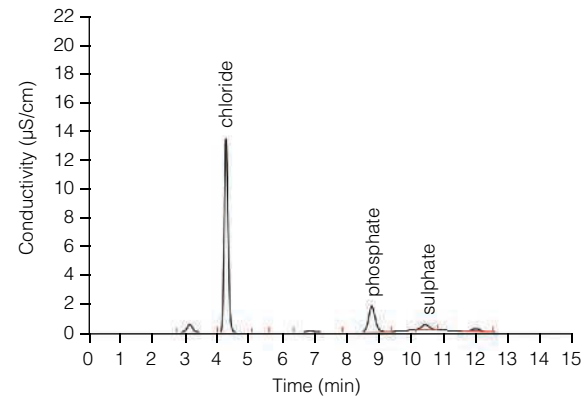


it unusable. Metrohm's patented stopped-flow dialysis coupled to the new 881 Compact IC pro ion chromatograph overcomes these drawbacks.

Two standard solutions covering the concentration ranges 1.0…3.6 mg/L and 10…36 mg/L as well as two samples, an ultra-high temperature (UHT) processed milk and a baby milk powder, were characterized in terms of analyte concentration, relative standard deviation, calibration quality, carryover and recovery rates. While the five-point calibration curves yielded correlation coefficients (R) better than 0.9999, carryover between two subsequent injections of a concentrated sample and a blank was less than 0.49%. Recoveries for the low (1.0…3.6 mg/L) and high standard concentrations (10…36 mg/L) were within 91…99% and 94…100%, respectively.

Automated compact stopped-flow dialysis is a highly efficient sample preparation technique that ensures optimum separation performance by protecting the column from detrimental matrix constituents.

Figure 4: Anion chromatogram of a baby food milk sample containing 7.37 mg/L chloride, 7.41 mg/L phosphate and 0.76 mg/L sulphate (after 1:100 dilution of the sample). Chromatographic conditions correspond to those given in Figure 3.



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Efficient Validation of GPC/SEC Instruments with EasyValid

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Validation is a time consuming process, consisting of many steps. OQ/PV for GPC/SEC systems can be efficiently done with PSS EasyValid, a tool that ensures fast and accurate validation and provides typical GPC/SEC results. It is suitable for all GPC/SEC systems, independent on instrument model, detector type and system manufacturer.

Introduction

Validation consists of several phases that verify pre-defined performance specifications:

- Design qualification (DQ): helps to answer the question: Is the system doing the right job?
- Installation qualification (IQ): qualifies the analytical system for correct installation in the users laboratory.
- Operational qualification (OQ, a.k.a. performance verification, PV): qualifies the analytical system for correct operation.
- Performance qualification (PQ): helps to keep laboratory productivity high.

In general instrument manufacturers supply tools for IQ, that can be used for validation independent on the analytical task. However, DQ and OQ/PV require validation with respect to the analytical technique. Because GPC/SEC is used to measure molar masses and molar mass distributions, the OQ/PV step should document that precise and accurate molar masses are obtained. In addition GPC/SEC relevant parameters, such as repeatability and detector drift, should be investigated and documented within this phase. This can be done using a dedicated GPC/SEC validation tool.

PSS EasyValid validation kit is used for comprehensive and fast OQ/PV of GPC/SEC systems, independent on instrument model and manufacturer. All concentration detectors such as refractive index (RI), ultraviolet (UV), infrared (IR) and/or evaporating light scattering (ELS) can be validated at the same time saving time and solvent. Molar mass sensitive detectors (on-line viscometers and on-line MALLS/LALLS/RALLS detectors) can be part of the validation, but should be validated additionally using dedicated light scattering/viscometer validation kits (e.g., PSS Visco/LS validation kit).

Instrumentation/Equipment

An example OQ/PV was performed on a PSS SECcurity GPC/SEC system consisting of

- an isocratic pump
- an autosampler with variable injection volume

Figure 1: Example printout with molar masses and molar mass averages for the primary poly(styrene) standard with the narrow molar mass distribution. This documents that the overall system can measure correct GPC/SEC results.

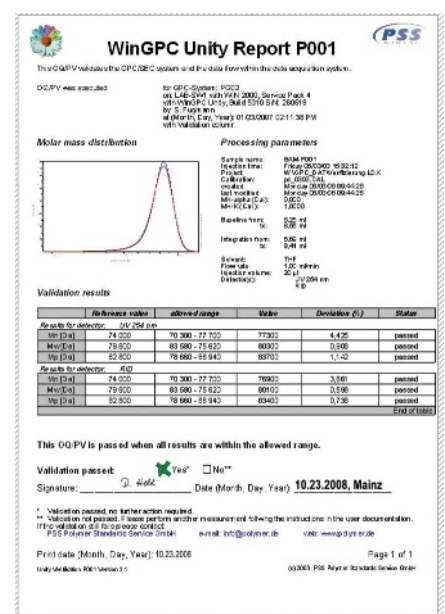
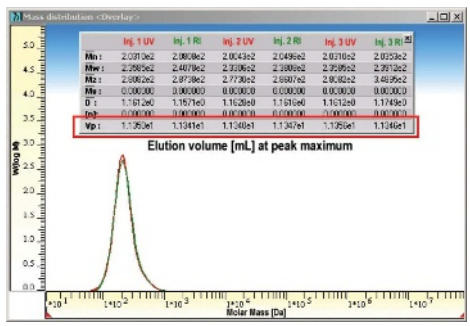


Table 1: Overview validation results EasyValid OQ/PV: all results are compared to the expected values and maximum deviations stated in the EasyValid user documentation (in agreement with ISO/EN 13885 and ASTM D5296-05).

Parameter	UV detector result	RI detector result
Plate count (1/m)	50 300; passed	48 100; passed
Molar mass results validation sample 1	Passed for all 3 injections	Passed for all 3 injections
Molar mass results validation sample 2	Passed for all 3 injections	Passed for all 3 injections
Repeatability (%)	0.14; passed	0.05; passed
Detector S/N (%)	0.13; passed	0.25; passed
Detector drift (%)	−0.19; passed	−1.56; passed

Figure 2: Repeatability for three injections of the primary poly(styrene) standard with the narrow molar mass distribution documented by the measured elution volume at the peak maximum for the UV and RI detector.



- an ultraviolet spectrophotomer (UV) working at 254 nm
- a differential refractometer (RI)

Data acquisition, calibration and evaluation was done using PSS WinGPC Unity with ReportDesigner option.

The PSS EasyValid validation kit used for OQ/PV consists of

- a SDV validation column
- a kit of 6 different reference and validation substances in colour coded glass vials
- column and test certificates
- a detailed user documentation with examples and layouts for consistent documentation.

The validation samples in the kit are primary standards and characterized through an extensive and comprehensive independent round robin test organized by the German Institute for Material Research (BAM).

Experimental conditions

Column:	PSS SDV EasyValid (8 × 300 mm)
Solvent:	THF
Flow-rate:	1 mL/min
Temperature:	25 °C
Samples:	1 column test sample 2 PSS round robin certified reference materials in colour coded autosampler vials [Poly(styrene)s with a narrow and with a broad molar mass distribution]
Calibration:	PSS Poly(styrene) ReadyCal Kit, 3 colour coded autosampler vials with 4 different molar masses each.
Concentration:	Sample dependent pre-weight in
Injection volume:	20 µL

Results

Figure 1 shows the UV and RI results obtained for one injection of the primary poly(styrene) standard with the narrow molar mass distribution. The WinGPC ReportDesigner has automatically set the passed/failed flags for the molar mass averages. All molar masses are within the expected range (compare also EasyValid user documentation), therefore, this part of the OQ/PV is passed. The reports for the repeat injections and for the sample with the broad molar mass distribution show similar results.

In addition to the molar masses the repeatability of the system, detector noise and drift were analysed. Figure 2 shows an overlay of three injections for the sample with the narrow molar mass distribution. The measured elution volumes at the peak maximum are highlighted. The deviations for the elution volume is in the expected range (compare EasyValid user documentation, expected results in agreement with ISO/EN 13885/ASTM D5296-05), so that this part of the validation is also successfully passed.

Table 1 summarizes all results for this validation step. The overall OQ/PV is passed, because all results are within the expected range (compare EasyValid user documentation).

Required time for OQ/PV:

- work time including sample preparation, system preparation, calibration, data evaluation: 85 minutes
- total time including sample runs and documentation: 385 minutes (approx. 6.5 hours).

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Alternate Selectivity for Polar Compounds in Hydrophilic Interaction Liquid Chromatography (HILIC) Using a New Amino Type HILIC Column

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Hydrophilic interaction liquid chromatography (HILIC) offers unique advantages for the separation of very polar compounds when compared to reversed-phase chromatography. A new silica based HILIC phase was developed to provide additional selectivity options in HILIC separations. The separation of water soluble vitamins on the new TSKgel NH2-100 HILIC column and on the well known TSKgel Amide-80 HILIC column demonstrates the differences in selectivity.

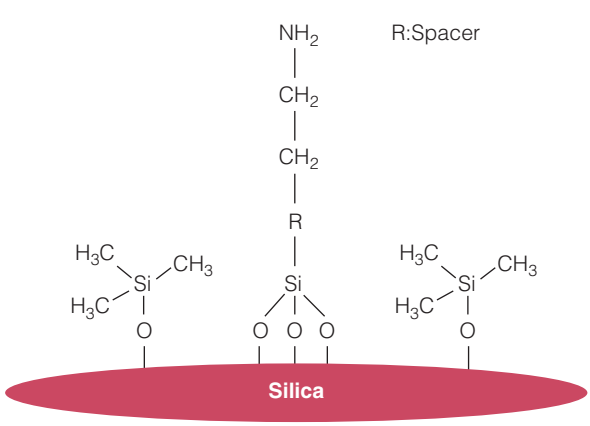
Introduction

HILIC is used primarily to separate polar and hydrophilic compounds.[1,2] Target applications for HILIC include the analysis of saccharides, glycosides, oligosaccharides, peptides and hydrophilic drugs. Altering selectivity plays a major role in maximizing resolution. The availability of an additional TSK-GEL HILIC phase provides a powerful tool for rapid method development.

Hydrophilic interaction chromatography

HILIC has similarities to normal phase chromatography with regard to the nature of the stationary phase. However, the eluents used for HILIC are similar to those known from reversed-phase chromatography. Typical mobile phases are mixtures of acetonitrile and water or aqueous buffers, applied in isocratic or gradient mode. It is commonly believed that in HILIC the aqueous content of the mobile phase creates a water rich layer on the surface of the stationary phase. This allows for partitioning of solutes between the more organic mobile phase and the aqueous layer. Hydrogen bonding and dipole–dipole interactions are the dominating retention mechanisms in HILIC mode. The number of polar groups, as well as the conformation and solubility of the sample in the mobile phase determine the elution order.

Figure 1: Schematic diagram of the new HILIC phase.



HILIC phases

Typical HILIC stationary phases are silica or polymer particles carrying polar functional groups (e.g., amino, amide or zwitterionic groups). TSKgel Amide-80 HILIC columns are well established for the analysis of glycans by HPLC or HPLC–MS.[3,4,5] Packed with spherical silica particles that are covalently bonded with non-ionic carbamoyl groups they offer expanded stability. Conventional amino type HILIC columns have limited stability in aqueous solutions but sometimes the selectivity of an amino ligand might better suit the target HILIC application. Therefore, we developed a new HILIC phase, which combines the amino ligand functionality with high durability.

The new amino type HILIC phase is based on a 3 µm silica particle with 100 Å pores, which is treated with a special endcapping procedure. Amino groups are introduced step wisely after endcapping (Figure 1). The amino groups act as HILIC functional groups without any peak splits. Because of a high ligand density and large

Figure 2: H-u-plots of TSKgel Amide-80 and NH2-100 HILIC. Conditions: TSKgel NH2-100 3 µm and TSKgel Amide-80 3 µm (4.6 mm i.d. × 15 cm L each); sample: uracil eluent: H_2O/acetonitrile = 10/90; flow-rates: 0.1~2.4 mL/min; temp.: 40 °C; UV @ 254 nm.

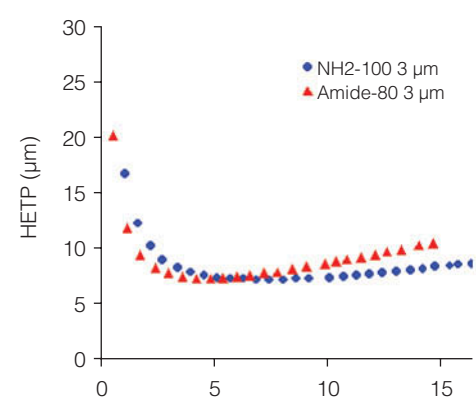
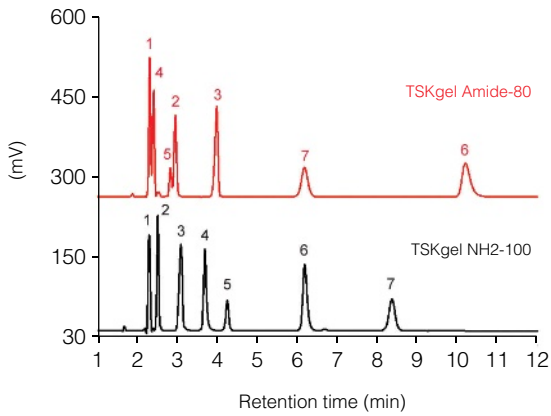


Figure 3: Comparison of selectivity. Separation of water soluble vitamins on a TSKgel Amide-80 and TSKgel NH2-100 column. Peaks: 1 = nicotinamide; 2 = vitamin B2; 3 = pyridoxine; 4 = nicotinic acid; 5 = vitamin C; 6 = vitamin B1; 7 = vitamin B12.



surface area TSKgel NH2-100 3 µm columns show the strongest retention for very polar compounds among the commercially available HILIC columns.

Figure 2 shows a comparison of the H-u plots of the TSKgel Amide-80 and NH2-100 HILIC phases. The optimum HETP for the amino type column is reached at a flow-rate of 1.2 mL/min at a pressure of 6 MPa. At increased flow-rates the H-u-curve is relatively flat. This allows using this column for fast separations at elevated flow-rates without impairing separation efficiency.

The availability of two TSK-GEL HILIC phases offering different selectivity features allows for adopting both, the stationary as well as the mobile phase to optimize the separation of a given sample. Available in a particle size of 3 µm, both columns are ideally suited for high efficiency HPLC as well as HPLC–MS analysis

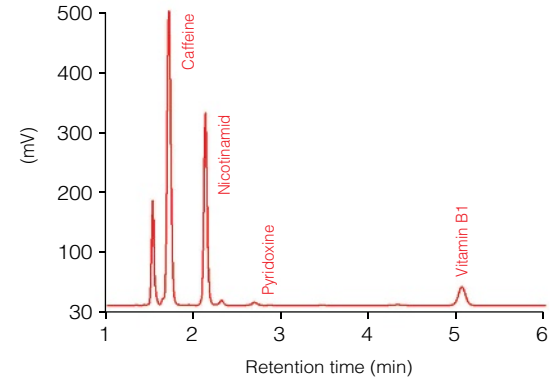
HILIC Analysis of Water Soluble Vitamins

HILIC separations are performed either in isocratic mode with a high percentage of organic solvent or with gradients starting with high percentage of organic solvent and ending with a high portion of aqueous solvent, which is opposite to reversed phase. The elution order of compounds is usually inversed as well. As a result in HILIC mode polar compounds are very well separated according to increased polarity. We present the analysis of water soluble vitamins (Nicotinamide, Nicotinic acid, Pyridoxine and Vitamins B1, B2, B12 and C) as an example for the separation of polar compounds on the two available TSK-GEL HILIC phases. For difficult separations of the very polar vitamins of the B complex HILIC separation is advantageous over reversed-phase analysis, especially when combined with highly sensitive mass spectrometric detection.

Material and Methods:

Columns:	TSKgel NH2-100 3 µm, 4.6 mm i.d. × 15 cm L
	TSKgel Amide-80 3 µm, 4.6 mm i.d. × 15 cm L
Mobile phase:	25 mM phosphate buffer (pH 2.5)/ACN = 30/70
Flow-rate:	1 mL/min
Temperature:	40 °C
Detection:	UV @ 254 nm

Figure 4: Analysis of vitamins in an energy drink.



Sample:	Figure 3: vitamin standard mixture
	Figure 4: energy drink (filtrated, diluted in ACN (1:1))
Injection:	5 µL

Figure 3 shows the separation of a standard solution of water soluble vitamins on a TSKgel NH2-100 column compared to the separation on a TSKgel Amide-80 column. Both columns have the same dimension (4.6 mm i.d. × 15 cm L) and particle size (3 µm). Flow-rate and mobile phase were identical as well. The elution order of the compounds varies when applying the same mobile phase to both columns: The new amino type column shows a stronger retention for nicotinic acid, vitamin C and vitamin B12 while retention of vitamin B1, B2 and pyridoxine is reduced. Figure 4 shows the analysis of a commercially available energy drink on the new amino-type HILIC column, after filtration and addition of the same volume of acetonitrile.

Conclusion

TSKgel Amide-80 HILIC columns have been used for years for a broad range of HILIC applications. The new 3 µm TSKgel NH2-100 columns provide an additional selectivity option, when increased retention or alternate selectivity is needed. This new bonded phase provides a powerful tool for robust method development in hydrophilic interaction liquid chromatography.

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