Chromatography in Industry

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Key Words

industrial research, industrial/academic cooperation, robust methods, hyphenated systems, high-throughput experimentation

Abstract

This review focuses on the chromatography research that has been carried out within industry or in close cooperation with industry and that has been reported in the scientific literature between 2006 and mid-2008. Companies in the health care sector, such as pharmaceutical and biotechnology companies, are the largest contributors. Industrial research seems to take place in an open environment in cooperation with academia, peer companies, and institutions. Industry appears ready to embrace new technologies as they emerge, but they focus strongly on making chromatography work robustly, reliably, rapidly, and automatically. "Hyphenated" systems that incorporate on-line sample-preparation techniques and mass-spectrometric detection are the rule rather than the exception. Various multidimensional separation methods are finding numerous applications. Strategies aimed at speeding up the development of new chromatographic methods remain the focus of attention. Also, there is a clear trend toward exploring chromatographic methods for parallel processing along with other strategies for high-throughput analysis.

1. INTRODUCTION

LC: liquid chromatography

It is important to review chromatography in industry for several reasons. First, although the term chromatography commonly appears in the title, abstract, or key words of publications on the topic, the term industry does not. Therefore, it is not possible to search scientific databases and the literature using industry as a key word. Second, only a small fraction of all the chromatographic separations performed in industry are described in the scientific literature. There are no practical alternatives for computer-based library searching, and it is not possible for an academic observer to review research on chromatography in industry by any means other than through the published literature. Thus, this review exposes merely the tip of an iceberg. At best, the literature cited is representative of the chromatographic research performed in industry. As such, it may provide some indication of the directions in which chromatographic analyses are moving, both in industry and elsewhere.

What motivates industrial researchers and their employers to publish their work? There are several good reasons not to publish. For instance, the materials studied are often confidential, but even if they are not and if the chromatographic experiments concern products that are commercially available, companies may be reluctant to publish details about their products' chemical composition. If a company has made progress in analytical separation—essential for the publication of a paper in a journal devoted to analytical chemistry or chromatography—it may provide a company with a competitive edge. Also, the ability to perform a detailed analysis of a product or material may be of strategic importance.

Fortunately, there are also several good reasons to publish results. For a number of products, for example from the pharmaceutical industry, accepted analytical methods must be available prior to drugs' introduction into the market. In other cases, progress on analytical methodologies may be recognized as a means to enhance a company's scientific image. With talent being scarce, this factor may become increasingly relevant. Arguably, however, it is the individual industrial scientist who determines whether or not his or her work is published. It takes effort and motivation to clear the obstacles. In return, a scientist may gain little more than satisfaction and recognition, at least in the short run. In the long run, a good publication record may ensure a scientist's future employability.

1.1. Leading Companies

One way to search for scientific publications from industry is to sort all results according to the authors' affiliations. This does not allow one to rigorously divide all records by origin (e.g., academic, industrial), but it does permit one to establish which companies contribute most strongly to the scientific literature. Upon performing such an analysis, I obtained the results listed in **Table 1**. Unsurprisingly, the top five companies in the table (and 9 out of 15) are pharmaceutical companies. The pharmaceutical industry is known to be the strongest proponent of liquid chromatography (LC). Also, many pharmaceutical companies have merged in recent decades into a small number of very large conglomerates. Not only do these companies use chromatography to carry out their missions successfully, they also make significant contributions to the advancement of the field.

The sixth company in **Table 1**, Amgen, describes itself as a biotechnology company. Although the missions of pharmaceutical and biotechnology companies may overlap substantially (i.e., both aim to improve people's health), the main techniques they employ are different. Abbott Laboratories (ranked 11) defines itself neutrally as a health care company. There are two food (or nutrition) companies on the list, Nestlé (ranked 10) and Unilever (ranked 13), and a single chemical company, Dow Chemical (ranked 15). The latter's contributions to chromatography are especially laudable. In general, (very) large chemical companies contribute much less to the scientific literature than

Table 1 Companies with most scientific publications containing the word chromatography in the title (2006–2008)[1]

Rank	Company	Type of industry	Publications
1	Pfizer	Pharmaceutical	247
2	Merck[2]	Pharmaceutical	227
3	AstraZeneca	Pharmaceutical	204
4	Novartis	Pharmaceutical	162
5	GlaxoSmithKline	Pharmaceutical	160
6	Amgen	Biotechnology	135
7	Bristol-Meyers Squibb	Pharmaceutical	131
8	Eli Lilly	Pharmaceutical	92
9	Johnson & Johnson	Pharmaceutical	86
10	Nestlé	Nutrition/health care	82
11	Abbott Laboratories	Health care	68
12	Shering-Plough	Pharmaceutical	68
13	Unilever	Nutrition/personal care	48
14	Genentech	Biotechnology	46
15	Dow Chemical	Chemical	44

[1]Actual search word: chromatogr*. Data taken from Scopus™, **http://www.info.scopus.com**. Last accessed August 22, 2008.
[2]Manually screened to remove publications from Merck AG, Darmstadt, Germany.

do companies in the health care sector, at least on the subject of chromatography. Although a rapidly increasing fraction of the total scientific literature comes from Asia, the main industrial contributors are still based exclusively in Europe and North America.

1.2. Interindustry and Industry-Academic Cooperation

The total number of papers listed in the right-hand column of **Table 1** is 1800, but subtracting duplicate papers yields a revised total of 1622. This implies that up to 10% of the scientific contributions from these top-publishing companies reflect interindustry cooperation. However, it is not easy to distill the actual numbers from the raw data. A number of studies are conducted by large teams that include representatives from various companies (and possibly academia). Such projects are especially significant for subjects of general interest, such as the fundamentals of method validation (1). A larger number of papers reflect the results of cooperation projects between industry and academia. Such projects may be very useful for many reasons. Leading academic researchers, such as Pat Sandra and Jeremy Nicholson (see **Table 2**), enhance the visibility and the scientific image of a company, apart from their contributions in terms of knowledge and experience. The academic partners tend to bring with them an urge to publish, which restricts the types of projects that are amenable to cooperation. Shown in **Table 3** are the publication records for Pfizer and for AstraZeneca. These two companies may not be representative, but the exercise is certainly illustrative.

There is some ambiguity in these data. The number of papers for Pfizer is larger than that shown in **Table 1** because the search was performed on a later date. The data in **Table 3** suggest that the vast majority (some 75%) of the research published by these industries is performed in conjunction with an academic partner. Some papers are cowritten with companies active in the same (health care) field and some with other companies or institutions. The data seem to confirm

Table 2 Most prolific individual researchers from the 15 top-publishing companies listed in Table 1

Rank	Scientist	Affiliation	Number of papers
1	Ian Wilson	AstraZeneca	31
not ranked	Pat Sandra	University of Ghent[1]	19
2	Pavel Bondarenko	Amgen	18
2	Yunsheng Hsieh	Shering Plough	18
3	Tawakol El-Shourbagy	Abbott Laboratories	16
5	Mingshe Zhu	Bristol-Meyers Squibb	13
5	Christopher Welch	Merck	13
7	Mohamed Abdel-Rehim	AstraZeneca	12
7	Hans-Gerd Janssen	Unilever	12
8	Donglu Zhang	Bristol-Meyers Squibb	11
not ranked	Jeremy Nicholson	Imperial College London[2]	11
9	William Humphreys	Bristol-Meyers Squibb	10
9	Martin Kussman	Nestle	10
9	Jun Zhang	Abbott Laboratories	10
9	Peter Sajonz	Merck	10

[1]Publishes with Pfizer.
[2]Publishes with a variety of top-15 companies.

Table 3 Analysis of scientific output from two of the most prolific companies[1]

	Pfizer (254)			AstraZeneca (206)		
Research affiliation	Number of papers (%)	Examples	Reference	Number of papers (%)	Examples	Reference
Company sites	265 (104%)[2]	–	–	182 (88%)[3]	–	–
Health care companies	78 (31%)	Amgen	11	13 (6%)	GlaxoSmithKline	7
		Wyeth	9		Pfizer	4
Other companies	42 (17%)	–	–	28 (14%)	Waters	13
Noncommercial organizations	25 (10%)	Food and Drug Administration	5	29 (14%)	The Netherlands Cancer Institute	2
		National Institutes of Health, Bethesda	4		Scania Technical Center	2
					Lawrence Livermore National Laboratory	2
Universities	184 (72%)	Universiteit Ghent	19	158 (77%)	Karlstad University	16
		Vrije Universiteit Brussels	6		Imperial College London	11
					University of Manchester	8

[1]Data taken from Scopus™, **http://www.info.scopus.com**. Last accessed September 5, 2008.
[2]The percentage is greater than 100 because several company sites were involved in some of the same papers.
[3]The percentage is less than 100 because some references are nondescriptive.

the hypothesis that studies performed strictly within the confines of a particular industrial company are unlikely to be published. This finding leaves this reviewer with the uneasy feeling that however great the efforts are to discuss chromatography research in industry, much of the work ultimately proves to have been performed in academic laboratories.

MS/MS: tandem mass spectrometry

MS: mass spectrometry

RPLC: reversed-phase liquid chromatography

TOF: time of flight

2. SELECTED SUBJECTS

Below, I discuss a series of specific papers on a limited number of subjects. Because of length constraints, (much) more than 90% of the publications that might qualify for this review are not mentioned.

2.1. Enhanced Separation Performance

Ultraperformance liquid chromatography (UPLC) was conceived by the Waters Company, but the use of columns with smaller (<2 μm) particles to achieve better separation performance is much more widespread. Faster analyses and higher analytical throughput are more often the goal in industry than are highly efficient separations (high plate counts). Wren & Tchelitcheff (2) from AstraZeneca explored the use of UPLC for the analysis of compounds in pharmaceutical development and found it suitable for routine pharmaceutical analysis. De Villiers (from Pfizer) and colleagues (3) performed a practical evaluation of the possibilities and limitations of UPLC. They found high optimal velocities (3.7 mm s^{-1}) and low plate heights (4.4 μm). The authors compared the use of 1.7-μm particles at 1000 bar with conventional LC (3.5- and 5-μm particles at 400 bar) in terms of analysis speed and maximum efficiency. They concluded that the use of sub-2-μm particles is advantageous in terms of speed of analysis for required theoretical plate counts up to ~80,000. The same group (4) studied the effects of viscous heat dissipation on retention and efficiency using 2.1-mm–inner diameter (i.d.) columns at pressures up to 1000 bar using two different experimental setups. The use of a still-air column heater led to approximately adiabatic conditions. In this case, a longitudinal temperature gradient was formed across the length of the column, but no appreciable loss in efficiency occurred. When the authors used a water bath to control the temperature of the column, a radial temperature gradient occurred. In that case, a loss in efficiency was predicted and demonstrated experimentally.

Boogers et al. (5) from DSM described a faster method for automatically derivatizing and analyzing amino acid mixtures resulting from the hydrolysis of casein and bovine serum albumin, reducing the run time by a factor of 2.5. Wang et al. (6) from Shering-Plough employed an LC–tandem mass spectrometry (MS/MS) method for determining testosterone and its metabolites in in vitro samples within 4 min. A group of GlaxoSmithKline scientists, Goodwin et al. (7), used LC with sub-2-μm particles combined with MS detection for the analysis of small-molecule drug candidates in plasma, reducing run times by a factor of three in comparison with conventional LC columns. Gika et al. (8) from AstraZeneca used complementary hydrophilic interaction chromatography (HILIC) and reversed-phase liquid chromatography (RPLC), both with sub-2-μm particles, for LC-MS-based metabonomic studies. Another AstraZeneca group, Wang & Zhang (9), used LC with sub-2-μm particles for rapidly quantifying levels of testosterone hydroxyl metabolites in rat liver microsomes.

Gika et al. (10) studied the applicability of RPLC with sub-2-μm particles in combination with time-of-flight (TOF)-MS for recording global metabolite profiles of human urine (**Figure 1**). The stability and repeatability of a gradient-elution method were determined by repeated analysis of a quality-control sample. The authors found that some system conditioning was necessary to obtain stable data. Thereafter, they obtained excellent repeatability in terms of retention time, signal

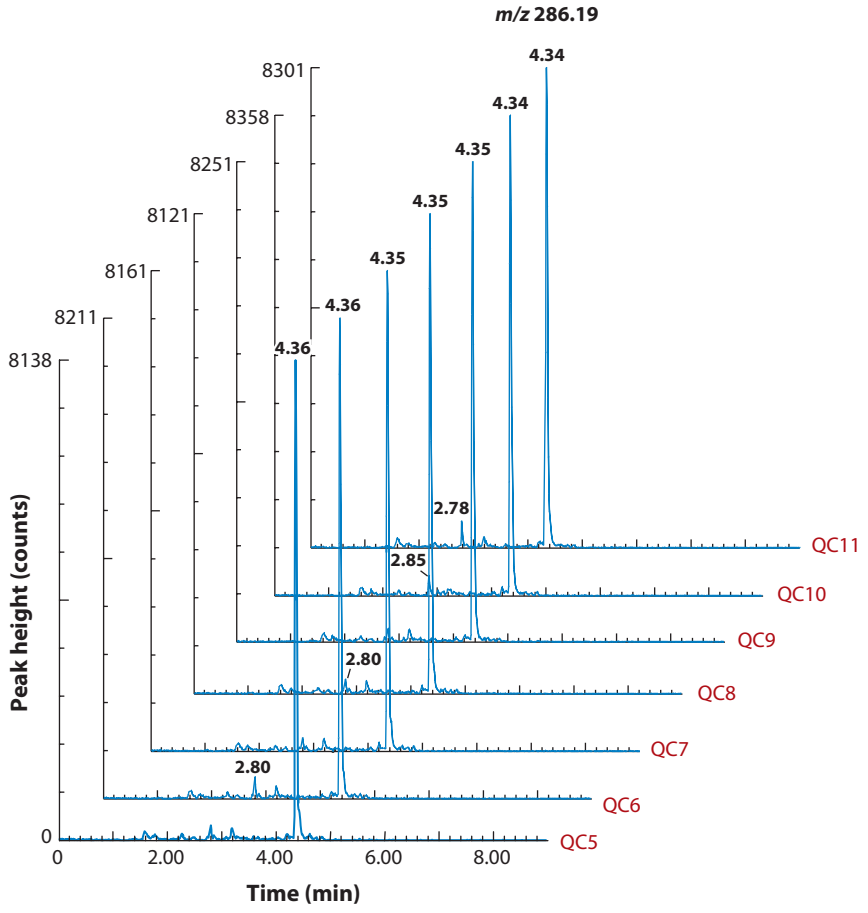

Figure 1

Fast, high-efficiency separations are of interest in industry, but repeatability and reliability are equally important. Repeatability is demonstrated in these extracted-ion chromatograms of an ion (*m/z* 286.19, retention time 4.3 min) in seven sequential quality-control (QC) samples run in between 67 test samples. Reproduced from Reference 10 with permission.

intensity, and mass accuracy, providing adequate within-day repeatability for the application. Gika's group (11) also investigated the use of elevated temperatures in combination with sub-2-μm particles. Elevated temperatures may lead to more efficient separations at lower pressures, and temperature programming may reduce the consumption of organic solvents. Isobaric high-temperature chromatography, where the temperature and flow rate follow a gradient program, was developed and evaluated against a conventional organic-solvent gradient. These studies showed that for urine (but not for plasma), chromatography at elevated temperatures provides better results than does conventional RPLC, resulting in higher peak capacities and better peak symmetry.

Dear et al. (12) from GlaxoSmithKline used a microplate imager as a radiodetection system for LC with sub-2-μm particles. The system exhibited robustness and sensitivity similar to those of a microplate scintillation counter typically used for off-line metabolite radiodetection. The same group utilized a microplate scintillation counter as a suitable radiodetection system for LC with sub-2-μm-particle columns (13). Licea-Perez et al. (14) from GlaxoSmithKline developed

semiautomated high-throughput LC-MS/MS methods for determining ethinyl estradiol and either 19-norethindrone or levonorgestrel in human plasma. Only 300 µl of plasma were required for sample preparation and analysis. LC-MS/MS also featured in the research of Wang et al. (15) from Shering-Plough, who developed an LC-MS/MS method using sub-2-µm particles for the determination of diastereomers in monkey plasma.

In comparison with sub-2-µm particles, monolithic columns seem to be used much less often. Huang et al. (16) from Bristol-Myers Squibb used a monolithic analytical column in combination with a high-flow direct-injection LC-MS/MS system to increase productivity for quantitative bioanalysis. They studied plasma samples containing a drug and its epimer metabolite. The same sample preparation, mobile phases, and MS conditions were used as with a conventional C_{18} column. Analysis times were reduced by a factor of two. In cooperation with GlaxoSmithKline, Costin et al. (17) designed an LC method using a monolithic column and chemiluminescence detection with tris(2,2′-bipyridyl)ruthenium(II) to determine the four predominant alkaloids—morphine, codeine, oripavine, and thebaine—during the extraction process from *Papaver somniferum* within 2 min. Altria et al. (18) from GlaxoSmithKline reported the separation of pharmaceutical substances using oil-in-water microemulsions as the eluent. Because of the high viscosity of the mobile phase, monolithic columns proved to be efficient in achieving rapid separations. The authors achieved separation of a test mix of paraben preservatives in both isocratic and gradient modes in less than 1 min.

Swinney et al. (19) from Johnson & Johnson employed an automated solvent-dispensing workstation capable of delivering volumes ranging from 10 ml to 4.5 liters for the preparation of solutions and mobile phases. The system was designed to address business, safety, and compliance needs while meeting or exceeding the precision and accuracy of current manual methods of preparation.

ESI: electrospray ionization

SPE: solid-phase extraction

2.2. Sample Preparation

Sample-preparation strategies are of great importance to industry. Automation may reduce demands on manpower from both a quantitative (time) perspective and a qualitative (training) perspective. If large numbers of samples are to be processed, automated systems are highly desirable. Integral systems in which different sample-preparation and/or analytical techniques are incorporated are commonly referred to as hyphenated systems.

Gika et al. (20) have investigated the effect of storage on samples for LC-MS-based metabonomics studies. They studied the stability of samples as a function of both storage time (up to six months at –20° C or –80° C) and the number of freeze-thaw cycles (up to nine) as well as the stability of samples in an autosampler tray (up to six days at 4° C). In the latter case, liquid chromatorgraphy–electrospray ionization (LC-ESI)-MS revealed clear differences after 48 h, whereas sample storage at either –20 or –80° C or up to nine freeze-thaw cycles did not affect the results.

Ridgway et al. (21) from Unilever have reviewed sample-preparation techniques for trace residues and contaminants in complex matrices, such as food. They discussed a wide variety of extraction techniques, classified as sample-preparation techniques, solid-phase extraction techniques, sorptive-extraction techniques, and techniques for the extraction of volatile analytes. Also, Chang et al. (22) from Abbott Laboratories have reviewed sample preparation in bioanalysis, with an emphasis on LC-MS. Their review covers pre-extraction and postextraction sample processing, protein precipitation, liquid-liquid extraction, off-line solid-phase extraction (SPE), and on-line SPE. Palandra et al. (23) from Pfizer have described a fully automated protein-precipitation technique for biological sample preparation aimed at the quantitation of drugs in various biological

matrices. Processing time took less than 30 s per sample, or approximately 45 min per 96-well plate. Ma et al. (24) from Amgen used a robotic system for the automated preparation of plasma samples by protein precipitation. The liquid-handling system featured an eight-channel liquid-handling arm, two robotic plate-handling arms, and two plate shakers. In addition, the system incorporated a robotic temperature-controlled centrifuge, a plate sealer, and a plate-seal-piercing station. Mohamed et al. (25) from Nestlé used a molecularly imprinted polymer for the selective extraction of the antibiotic chloramphenicol from milk. The enhanced selectivity of the sorbent resulted in a better recovery and a faster turnaround time. Ridgway et al. (26) evaluated solid-phase dynamic extraction for the extraction of nonpolar volatile aromatic analytes from aqueous solutions in both headspace and liquid-injection modes. This technique requires a gas-tight syringe equipped with a special needle that is coated on the inside with a nonpolar extraction phase. After absorption occurs inside the needle, the analytes are thermally desorbed directly into a gas chromatography (GC)-MS system. This technique was evaluated for the determination of furan, benzene, and toluene.

GC: gas chromatography

AstraZeneca scientists Abdel-Rehim et al. (27) evaluated 96-well pipette tips with a chemically bonded monolithic methacrylate sorbent plug (described in Reference 28) for SPE of pindolol and metoprolol from human plasma samples (**Figure 2**). Using this system, the authors prepared a 96-well plate in ~2 min. Xu et al. (29) from Abbott Laboratories employed a monolithic material as a support to extract a highly hydrophobic pharmaceutical compound and its hydroxylated metabolite from a urinary matrix prior to analysis by LC-MS/MS. The same group (30) performed an automated sample-preparation procedure using a monolithic phase based in an on-line extraction system for pharmaceutical components in plasma prior to LC-MS/MS analysis. The proteins were first precipitated with acetonitrile. The back pressure of the monolithic extraction cartridge remained the same after 450 samples were injected. The performance of this technique was compared with that of an automated 96-well liquid-liquid-extraction procedure, and the precision and accuracy were found to be similar.

Xu et al. (31) from Merck and BioMarin designed on-line extraction assays for use in combination with "high-turbulence" LC-MS/MS analysis of MK-0974 in human plasma and urine. They advocate a four-step strategy to deal with extraction recovery, carry-over, and analyte loss to urine

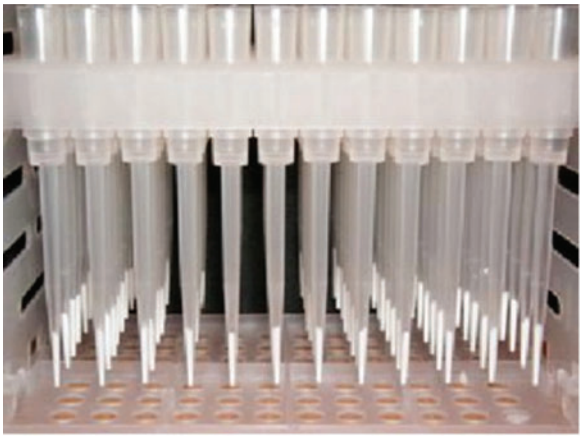

Figure 2

Parallel sample processing may be an important opportunity for industry. This battery consists of 96 pipette tips that contain a monolithic adsorbent created by the ultraviolet polymerization of methacrylates. Reproduced from Reference 27 with permission.

containers. Rezzi et al. (32) from Nestlé used an automated SPE-RPLC system that can be combined with nuclear magnetic resonance (NMR) spectroscopy. Their system allowed the identification of 72 metabolites of various molecular classes in human urine. Matthews & Woolf (33) from Merck used a method for determining a γ-secretase inhibitor (a potential treatment for Alzheimer's disease) in human plasma and cerebrospinal fluid. Their method involves performing liquid chromatography–atmospheric-pressure chemical ionization (LC-APCI)-MS/MS after SPE in the 96-well format. Amgen researchers Chelius et al. (34) utilized an automated proteolytic digestion to assist in pharmaceutical stability studies of antibody drugs and drug candidates. The performance of the 96-well-plate digestion procedure was comparable to the currently used manual approach; however, it was faster and less labor intensive, and the tryptic digests were more repeatable.

APCI: atmospheric-pressure chemical ionization

2.3. Chiral Separations

The separation of enantiomeric drugs or metabolites will probably remain an active area of research for the pharmaceutical industry. In their article, Akin et al. (35) stressed the importance of chirality in the biological activity and commercial viability of drug candidates, thereby underscoring the importance of enantioselective separations. The authors tested and compared a number of commercial screening tools for chiral separations, using a number of new chiral compounds as a challenging test. McConnell et al. (36) have extensively reviewed the contributions of chiral separations and other analytical techniques for determining the absolute stereochemistry of target molecules in drug discovery. They stress that analytical potential has been critical to the successful discovery and development of chiral drugs in the pharmaceutical industry.

Wetli & Francotte (37) from Novartis used an automated platform for screening chiral separations, which they claim allows many tests to be performed in a relatively short time, leading to increased productivity in preparative separations. A strategy employed by Johnson & Johnson, described by Huybrechts et al. (38), uses four commercially available polysaccharide-based chiral stationary phases and two different types of mobile phases. The system was initially based on solvent- and column-switching devices, which were subsequently replaced by a system that allowed eight high-performance liquid chromatography (HPLC) runs to be conducted in parallel. Sajonz et al. (39) from Merck attempted to use a commercial (Eksigent Express 800) microfluidic eight-channel HPLC instrument for multiparallel normal-phase chiral analysis (**Figure 3**). The enantiopurity of test mixtures containing the two enantiomers of benzoin and the closely related (R,S)-dihydrobenzoin prepared on a 96-well microplate was asserted within 2 h. The authors claimed that this technology is highly beneficial for use in a high-throughput environment.

New chiral selectors are unlikely to be developed in industry, but they are likely to be tested in academic-industrial cooperation projects. In cooperation with Eli Lilly, Dan Armstrong's group (40) successfully tested its boron-containing selector for separating primary amine–containing racemates. Zhou et al. (41) at Merck compared the enantioselectivity of two chiral stationary phases based on immobilized proteins such as α1-acid glycoprotein and ovomucoid protein. Boesten et al. (42) from DSM reported the enantiomeric resolution of a series of unsaturated N-methyloxycarbonyl-α-H-α-amino acids on macrocyclic glycopeptide stationary phases. They applied this method for monitoring the conversion and product enantiomeric excess of an enzymatic hydrolysis reaction.

The separation of chiral metabolites is a greater challenge than are chiral formulations. De Meulder (from Johnson & Johnson) and colleagues (43) employed two different LC-MS/MS methods using a cellulose-based column. One method was used for the quantitative determination of risperidone and the enantiomers of its active metabolite 9-hydroxyrisperidone (paliperidone) in human plasma, and the other method was applied to the determination of the enantiomers

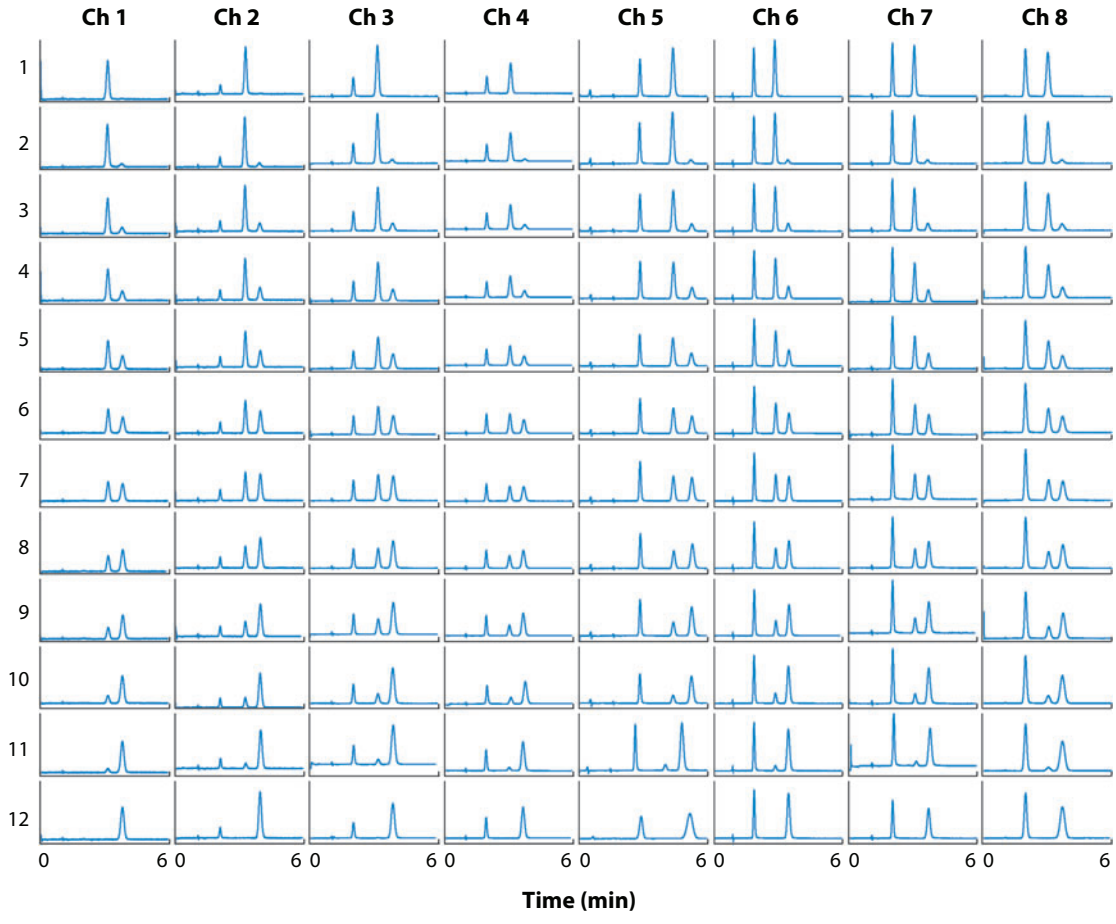

Figure 3

Examination of the suitability of a microfluidic multiparallel high-performance liquid chromatography system for high-throughput chiral separations. Parallel channels are indicated as Ch 1 through Ch 8. Reproduced from Reference 39 with permission.

of 9-hydroxyrisperidone in human urine. A particularly difficult separation was achieved by Wickremsinhe et al. (44), who used LC-MS to determine the four stereoisomers of the active metabolite of prasugrel in human plasma. The authors used statistical analyses of the individual chromatographic peak areas to allow quantitative measurements.

Chen et al. (45) used an APCI interface to hyphenate packed-column supercritical-fluid chromatography (SFC) with MS/MS for the analysis of propranolol enantiomers in mouse blood. Zhang et al. (46) used the "simulated moving bed" concept to efficiently perform chiral SFC separations. Ease of solvent removal may be a strong aspect of SFC for preparative separations. Gahm et al. (47) reported a particular case of optimizing a chiral SFC purification method. Leonard et al. (48) used semipreparative SFC to produce gram amounts of enantiopure intermediate for initial studies. They also described how this method was scaled up to preparative chiral HPLC on a 300-mm-i.d. column to produce sufficient amounts of two enantiopure intermediates, which allowed them to ultimately obtain 170 g of a preclinical candidate. Mukherjee & Cook from AstraZeneca (49) addressed one of the weaknesses of carbon dioxide–based SFC, namely its ability to analyze aqueous samples.

A Pfizer scientist, Ramstad (50), used capillary electrokinetic chromatography (CEC) to determine the enantiomeric purity of three pharmaceutical compounds. In this study, highly sulfated γ-cyclodextrin was the chiral recognition agent present in the elution buffer at 5%. CEC allows high-resolution separations and the use of fairly expensive buffer additives. However, it does not readily allow upscaling. The same is true for capillary electrophoresis (CE) (51) and for micellar electrokinetic chromatography (MEKC) (52). In another MEKC-related study, Hou et al. (53) describe what may become another rare application of this technique in industry: separating and detecting ephedrine alkaloids with MEKC-ESI-MS. In their search for a "generalized detector for enantio-enrichment," Welch et al. (54) from Merck compared two fast chromatographic methods: chiral SFC and achiral chromatography with circular dichroism detection.

CEC: capillary electrokinetic chromatography

CE: capillary electrophoresis

Mommers et al. (55) from DSM introduced a GC method for analyzing both the conversion and the enantiomeric excess of samples arising from alcohol-dehydrogenase reactions. The chiral compounds were a series of saturated, straight-chain alcohols ranging from 2-butanol to 2-heptanol. They were converted on-line to the corresponding trifluoroacetylated derivatives by injecting trifluoroacetic anhydride into the column shortly after injection of the aqueous samples. This procedure was much simpler and faster than conventional off-line protocols. Begnaud et al. (56) from Firmenich performed a chiral analysis of 3-methyl-3-sulfanylhexan-1-ol by multidimensional GC and chiral GC–olfactometry. The latter technique allowed the on-line evaluation of the sensory character of both enantiomers via a sniffing port. The authors reported a double-cool-strand interface that makes it possible to significantly delay the olfactometric detection of the second-eluting enantiomer, thereby lowering the risk of sensory saturation.

2.4. Method Development and Validation

Methods are at the heart of chromatographic activity in industry. Huge numbers of people (analysts, technicians, operators, etc.) apply analytical methods. Only a few scientists develop them, and often several others help to validate them. The quality of methods determines the quality of measurements, and in many industries, the quality of measurements determines the quality of the product and ultimately the success of the operation.

Xiao et al. (57) from Shering-Plough designed a strategy for developing LC separations of challenging pharmaceutical molecules. They used a commercial computer-assisted chromatographic method–development tool and an automated column-switching system. Jimidar et al. (58) from Johnson & Johnson applied CE to separating low-molecular-weight compounds in the late phase of pharmaceutical development. Late-phase methods are developed for transference to quality-control labs and are supposed to last throughout the product lifetime. Coffman et al. (59) from Wyeth and Genentech developed purification processes for protein biopharmaceuticals, where several dozen chromatographic columns are typically evaluated. The authors describe a batch-binding method of screening chromatographic purification conditions in a 96-well format with a robotic liquid-handling system. Wiendahl et al. (60), in cooperation with Novo Nordisk and Bayer, carried out high-throughput screening of conditions for designing and optimizing chromatographic processes. The authors performed breakthrough and elution experiments for two and three proteins, respectively, on several different miniaturized columns. Xu (61) from Shering-Plough reported the development of rapid and reliable LC-MS/MS methods for pharmacokinetics studies.

Larcinese et al. (62) from Firmenich modeled peak migration in fast LC separations. The authors claim that their method allows experimental conditions for optimal resolution to be established more rapidly, and their approach has been applied to the separation of a vanilla-aroma formulation. Kozlowski & Dalterio (63) from Bristol-Myers Squibb examined the role

SEC: size-exclusion chromatography; equivalent to gel-permeation chromatography

of the sample solvent and the injection volume during method development for semipreparative RPLC. Turnpenny et al. (64) from Pfizer developed a turbulent-flow chromatography method, and Smalley et al. (65) from Bristol-Myers Squibb developed a turbulent-flow LC-MS/MS method for the analysis of digoxin and its internal standard digitoxin. Another group of Bristol-Myers Squibb scientists, Xiao et al. (66), designed LC methods for potential extractables from commercial elastomeric stoppers in a complex surfactant matrix. Sajonz et al. (67) from Merck created a method to extract the unstable active pharmaceutical ingredient ertapenem, an antibiotic. Several impurities or degradation products were synthesized or prepared by controlled degradation and the relative response factors of these were determined. Loeser et al. (68) from Novartis developed a system that allows automation of pH-optimization measurements during LC method development. They used a quaternary pump to deliver two different aqueous buffer components in a consistent proportion to simulate a single, premixed buffer component while simultaneously producing a solvent gradient by increasing the concentration of organic modifier.

Validating analytical methods is much more important for industry than it is for academia. Extensive validation is usually required by regulatory authorities, but it is desirable to perform a thorough validation of a given method before it is put to routine use. Between 2006 and 2008, more than 50 papers whose titles contained the word validation were published by one of the top 15 publishers. Most of these papers described the validation of a specific method. In some cases, the authors performed cross-validation of two different methods. For example, De Beer et al. (69) in cooperation with Pfizer compared a Raman method for determining medroxyprogesterone acetate in a pharmaceutical suspension with a reference HPLC method. A software solution for automating the chromatographic method validation process, reported by Lukulay & Morgado from Pfizer (70), has also been employed.

Despite the vast body of work on the subject, discussions on how to perform method validation are still ongoing, and researchers have not yet reached a consensus on all aspects (71). Kaltenbrunner (from Amgen) and colleagues (72) have attempted to apply theoretical concepts of ion-exchange chromatography to establish a more structured approach to the validation of preparative separation methods for proteins. Rozet et al. (1) have described an objective means of establishing whether a method is expected to yield results with sufficient accuracy. They proposed to use the β-expectation tolerance interval (or accuracy profile) for this purpose and introduced a "fit-for-purpose" concept to select the most appropriate function for a calibration curve. Additionally, Nowatzke & Woolf (73) have reported good practices for monitoring (*a*) the stability of analytes in biological samples collected during clinical studies and (*b*) the quality of stock solutions.

2.5. Quality Control and Process Analytical Technology

Process analytical technology (PAT) is a system for rapidly and frequently performing analyses at many stages of the manufacturing process of a given substance (i.e., a synthetic drug). There are many advantages associated with closely monitoring the process, rather than simply verifying the quality of a product after the entire manufacturing process is completed.

Industry's departure from off-line batch analysis and its trend toward on-line process analysis are reflected in the contemporary literature. For example, Burdick et al. (74) computed tolerance intervals and used these to define validation acceptance criteria for performance parameters for the chromatographic purification of a recombinant protein. Mollerup et al. (75), in cooperation with Novo Nordisk, attempted to transform the purification of proteins by size-exclusion chromatography (SEC) into a well-articulated process within a PAT framework. In their report, the authors discussed the general thermodynamic principles of ligand binding and various models

of multicomponent adsorption in ion-exchange and hydrophobic interaction chromatography. They determined the model parameters from experimental data and compared the simulated chromatograms to experimental data for validation.

Another report (76) discussed the screening techniques used to identify suitable ion-exchange resins and they described the optimization of a purification step based on models of the adsorption isotherm. Gavin & Olsen (77) from Eli Lilly took a "quality-by-design" approach to developing an ion-pair LC method for the analysis of atomoxetine hydrochloride. Rathore (from Amgen) and colleagues (78) undertook a case study of PAT, wherein they evaluated the usefulness of a commercial on-line LC system for real-time pooling of process chromatography columns based on product quality attributes.

Squibb et al. (79) from Pfizer have reported that most major pharmaceutical companies maintain large collections of liquid samples—often containing more than 1,000,000 stock solutions—that are typically dissolved in dimethyl sulfoxide. Due to the inherent inaccuracies of high-throughput gravimetric analysis and automated dilution, stock-solution concentrations can vary significantly from the nominal values. The authors present an LC method for measuring the purity, identity, and concentration of these stock solutions using four different columns and parallel ultraviolet (UV), ESI-MS and evaporative light-scattering detectors.

MALDI: matrix-assisted laser desorption/ionization

LC × LC: comprehensive two-dimensional liquid chromatography

2.6. Proteins

Proteomics does not feature in many of the papers that originate from industry. The field remains dominated by academia and research institutes, and chromatography plays a minor role in this area in comparison with MS. However, industry tends to participate in large cooperation projects and consortia. For example, Bayer took part in a study on peptidomic analysis of rat urine using CE-MS (80). Mueller et al. (81) from Novartis coupled LC with matrix-assisted laser desorption/ionization (MALDI)-MS and MALDI-MS/MS techniques for proteome analysis. The authors explored both on-line and off-line interfaces and determined that the off-line mode was preferable. Li (from Pfizer) and colleagues (82) demonstrated that quantitation at the MS and MS/MS level can be accomplished by a variety of labeling techniques. Using LC-MS, Li et al. performed quantitative analyses of the MRP2/ABCC2 protein, which plays an important role in the excretion of drugs through the liver. The actual compound analyzed was a unique hexadecameric tryptic peptide. LC-MS has also been used by Zamani et al. (83), in cooperation with AstraZeneca, to study the conformation of a monoclonal antibody. Kajdan et al. (84) from Dow Chemical employed a comprehensive two-dimensional liquid chromatography (LC × LC) method to characterize recombinant proteins. The authors used cation-exchange chromatography in the first dimension and RPLC in the second dimension, and their system was coupled on-line via ESI to a TOF-MS for detailed protein characterization.

The Staby group (85, 86) from Novo Nordisk have performed a great deal of work in comparing different anion-exchange resins for protein purification. Similarly, Pabst (from Pfizer) and colleagues (87) compared strong anion exchangers for the purification of a polyethylene glycol (PEG)ylated protein. They studied the effect of PEGylation on ion-exchange adsorption using bovine serum albumin as a model system. Fisher (from Amgen) and associates (88) performed high-throughput purification of Fc-fusion proteins. They reported that screening protein molecules for a particular biological activity sometimes requires the purification of hundreds of proteins before the lead candidate is found. In cooperation with Genentech and Bayer, Bajaj et al. (89) used a new flow-cell that allowed simultaneous measurement of protein concentration and intensity of scattered light in combination with SEC. This technique allowed the authors to study protein self-association and measure second virial coefficients to quantify

solution nonideality. They used a modified Debye light-scattering equation to obtain association-equilibrium constants for β-lactoglobulin Finally, Karnoup et al. (90) from Dow Chemical utilized an LC method to determine protein glycosylation in monoclonal antibody samples derived from transgenic plants, then compared the results with those obtained from glycopeptide profiling by MALDI-MS.

GC × GC: comprehensive two-dimensional gas chromatography

2.7. Food

Many typical methods for food analysis have been described in recent literature, but few of them originated from industry. However, two scientists from Firmenich, Impellizzeri & Lin (91), used an LC method to perform a quantitative analysis of oleocanthal in extra-virgin olive oils. A compound known as deacetoxy ligstroside aglycone is known to be responsible for the throat irritation caused by olive oils. In a similar experiment, Cooper (from Nestlé) and colleagues (92) applied LC to separate and quantify six of the major chocolate polyphenols in 3 min. They also performed chiral analyses of epicatechin and catechin.

Published research suggests that food industries are embracing increasingly complex analytical tools. For instance, the Rochat group (93) from Firmenich used a comprehensive two-dimensional GC technique (GC × GC) to detect sulfur compounds in the aroma of roast beef. The authors also employed (*a*) a TOF-MS detector to pinpoint ~70 sulfur compounds in the GC × GC chromatogram, of which 50 were identified, and (*b*) GC-olfactometry, a common technique in food analysis, to establish the most important sulfur compounds. Van Platerink et al. (94) from Unilever used LC-MS to quantify angiotensin I–converting enzyme (ACE)-inhibiting peptides in plasma samples collected from human volunteers following consumption of a peptide-enriched drink designed to lower blood pressure. The authors tested and evaluated four different sample-preparation methods. After removing proteins by acidification and heating, they injected at least 200 samples in large volumes before the system had to be cleaned.

Guillotin et al. (95) worked with Degussa to perform a detailed characterization of commercial pectins. The authors used CE with very small amounts of sample to determine the degree of amidation, the degree of methyl-esterification, and consequently the degree of substitution of the pectins. Also, the authors successfully employed CE to determine the distribution of methyl-esters over the galacturonan backbone and the extent to which they are bunched together, known as the degree of blockiness. In addition to using CE, the Guillotin group (96) utilized anion-exchange chromatography to study the intermolecular distributions of amide groups within two pectins. After removal of the methyl esters, the different populations showed almost equal degrees of amidation, but the values of the degrees of blockiness were not the same. The authors concluded that the amidation process was heterogeneous.

Nilsson et al. (97) worked with AkzoNobel to characterize mixtures of substituted cellulose and starch. The authors used (*a*) selective hydrolysis with specific enzymes to achieve separation of the two polymers in the mixture, (*b*) SEC for various hydrolyzed fractions, (*c*) anion-exchange chromatography to determine the amounts of unmodified glucose units in the fractions, and (*d*) ESI-MS to determine the substituents.

Van Platerink et al. (98) from Unilever describe a two-dimensional LC-MS method for the identification of small, hydrophilic ACE–inhibiting peptides in enzymatically hydrolyzed milk proteins. Hydrophilic fractions that were poorly separated in RPLC were resolved more clearly by HILIC. The authors analyzed narrow fractions collected from the HILIC column for their ACE-inhibiting potential in an at-line assay. They further analyzed fractions showing significant inhibition of ACE through the use of LC-MS for structure elucidation.

2.8. Oil and Petrochemicals

Very few papers have been published in the area of oil and petrochemicals. Kleinert (from BASF) & Lunze (99) studied the control of chromatographic simulated moving bed processes, using on-line product-purity measurements and reconstructed wave fronts for control purposes. McEwen (100) from DuPont reported using an LC-MS instrument to perform GC-MS. An atmospheric pressure photoionization (APPI) interface was used for GC-MS; the results thus obtained were compared with those from APCI-GC-MS and from conventional electron-ionization GC-MS. APPI ionization proved to be similar to low-energy electron ionization. Luong et al. (101) from Dow Chemical contributed to progress in the field of GC by developing a solventless column test. In this technique, a special plunger-in-needle syringe and GC conditions allow one to inject minute amounts of probes without the need for a solvent. Thus, it may be possible to achieve a better (i.e., more sensitive) characterization of GC columns in a shorter time, given that there would be no need to remove solvent. Luong et al. (102) reviewed low-thermal-mass GC, which allows fast temperature programming—including a rapid cool-down cycle. Winniford et al. (103) from Dow Chemical evaluated a miniaturized pulsed discharge detector in conjunction with GC × GC. In combination with a pyrolysis unit, the authors applied this technique to the characterization of a polyethylene copolymer.

An intriguing application of chromatography was described in detail by Lustig et al. (104) from Dupont. In this report, single-stranded DNA was wrapped helically around individual single-walled carbon nanotubes (CNTs) to form DNA/CNT hybrids, which were then separated by ion-exchange chromatography. Each hybrid was found to elute at an ionic strength that depended upon the electronic band structure of the core nanotube. Thus, a method was obtained for separating CNTs based on chirality. Typical industrial applications were described by Dow Chemical scientists Luong et al. (105), who presented a multidimensional GC method for determining low amounts of oxygenates in hydrocarbon matrices, and Gras et al. (106), who designed a GC method using sulfur-chemiluminescence detection to measure individual sulfur compounds and total sulfur content in hydrocarbon matrices.

An industrial activity that is well known to the public is environmental research. An example of an experiment in this area is that of Wortberg et al. (107) from BASF, who developed an on-line GC-MS system for automated monitoring of crude wastewater at a complex chemical–production site. Direct aqueous injection was made possible by a special, two-stage injector consisting of a splitless vaporization chamber on top of a programmed-temperature vaporizing injector equipped with a Tenax-packed line. In the implementation, two instruments operated alternately, each performing one analysis every 40 min. When one of approximately 140 monitored compounds exceeded a specified threshold, either an alarm was set off or the wastewater stream was diverted to an "off-spec tank." The GC-MS system operated quasi-continuously, with a system availability exceeding 98%.

The environmental area of research lends itself well to cooperation among industries and between industry and academia; such cooperation may be particularly beneficial to industry because involvement in such studies is thought to enhance the corporate image. For example, results published by Sanderson et al. (108) demonstrated industrial interest in the monitoring of alkyl sulfates and alkyl ethoxysulfates in river sediments. Also, crop-protection companies have reported effective methods for residue analysis. For example, Zimmer (from Bayer) and associates (109) reported on an LC-MS/MS method for determining deltamethrin residues in plant materials. Sagawa et al. (110), also from Bayer, used LC-MS/MS to determine the six trichothecene mycotoxins in rice. Brudin (from Syngenta) and colleagues (111) used one- and two-dimensional separations for the characterization of sulfonated lignins, which are used as natural detergents in agricultural formulations.

Although fewer in number than environmental research studies, toxicology reports represent another field in which publication of research results may be used to enhance a corporation's image. Zhang et al. (112) from Dow Chemical used LC-ESI-MS to study hemoglobin-methylation effects induced by exposure to methyl methane sulfonate. The same group used an LC-MS/MS system employing positive APPI to quantify 8-hydroxydeoxyguanosine in DNA samples (113) and the DNA adduct of thymidylyl(3′-5′)thymidine methyl phosphotriester (114).

2.9. Polymers

SEC (also known as gel-permeation chromatography) is frequently applied in industry to characterize all types of polymers. In the area of polymer research, progress is highly dependent on the willingness of industrial researchers to share their knowledge with others. Wallace Yau has been exemplary in this respect. Now at Dow Chemical, Yau built a system that combines SEC with temperature-rising elution fractionation and three on-line detectors for infrared spectrometry, viscometry, and light scattering (115). Barth & Saunders (from Dupont and Polymer Laboratories, respectively) (116) have reviewed column technology for SEC.

Unique new separation methods may yet emerge from industry, provided that the need is great enough to justify the efforts. Recently, Meunier et al. (117) from Dow Chemical proposed molecular topology fractionation (MTF) in response to the industrial need for improved characterization methods for long-chain-branched polymers. The authors applied this technique to the separation of polystyrene stars and long-chain-branched polyethylene fractions. Edam et al. (118), in cooperation with Dow Chemical, have realized separations between very high-molecular-weight linear and branched polymers by combining MTF with SEC in a comprehensive two-dimensional LC system. This approach in principle allows one to unravel the degree-of-branching and molecular-weight distributions.

Kaal & Janssen (from Atas GL and Unilever, respectively) (119) have published an excellent review of methods that extend GC's range of applicability. Although the applicability of GC can be enhanced by exploiting better equipment and columns, improvement occurs mainly through the reduction of the analytes' molecular weight and polarity. The authors describe four generic routes for extending the applicability of GC: high-temperature GC, derivatization, pyrolysis, and thermochemolysis.

Analytical ultracentrifugation and hydrodynamic chromatography, as demonstrated by Wohlleben (from BASF) & Lechner (120), can be used to obtain complementary information about heterogeneous polymer dispersions. Erdner et al. (121) from DuPont studied the effects of deuterated solvents in SEC of water-soluble polymers. For aqueous SEC, the use of deuterium oxide slightly increased the SEC elution volume. A moderate increase in bandbroadening (and a corresponding moderate decrease in column efficiency) was observed when using D_2O. Also, the authors evaluated the use of deuterated chloroform as eluent instead of chloroform, and they observed no hydrodynamic volume changes. Another application of SEC was described by Normand et al. (122) from Firmenich, who discretized SEC chromatograms of corn maltodextrins. They used anion-exchange chromatography with pulsed amperometric detection to characterize commercially available oligomers and subsequently extrapolated the discretization to the entire SEC profile. The authors claim that this procedure eliminated the apparent, chemically irrelevant, continuous molar-weight distribution obtained by SEC.

Rittig (from BASF) and colleagues (123) studied the characterization of polyacetals by LC and LC $\times$ LC in combination with various other techniques (e.g., MALDI-TOF-MS and infrared and

NMR spectroscopy). They identified cyclic oligomers and polymers with various end groups. De Geus et al. (124), working with DSM, also used LC at the critical conditions to gain insight into the initiation process of enzymatic ring-opening polymerization reactions of monofunctional alcohols.

Peters (from DSM) and coworkers (125) studied UV-cured networks prepared from mixtures of difunctional (PEG diacrylate) and monofunctional (2-ethylhexyl acrylate) acrylates. This particular type of polymer allowed complete hydrolysis, and the authors analyzed the resulting fragments by aqueous SEC coupled to on-line RPLC. Materials with different mean network densities and fractions of dangling chain ends were prepared by varying the concentration of monofunctional acrylate in the polymerization mixture. Peters et al. also calculated various network parameters, such as the number of cross-linked PAA units, the degree of cross-linking, and the network density, which is the molar concentration of effective network chains between cross-links per volume of polymer. The authors compared the mean molar mass of chains between chemical network junctions with results obtained from solid-state NMR and dynamic mechanical analysis and found them to be similar.

In a cooperation projection between the German Institute for Polymers and Basell, Albrecht et al. (126) studied the separation of ethylene-propylene copolymers based on chemical-composition high-temperature-gradient LC. The authors obtained information about the separated fractions by coupling the LC system with Fourier transform infrared spectroscopy through a solvent-elimination interface. Another cooperation was reported by Knecht et al. (127): The German Institute for Polymers and BASF jointly studied multidimensional chromatographic separations of hydrophilic polymers such as PEG–poly(vinyl alcohol) graft copolymers, which are used as coatings for pharmaceutical tablet formulations. LC $\times$ LC allowed separation of these polymers by molar mass and chemical composition.

Peters et al. (128, 129) used low-molecular-weight model compounds to mimic cross-linking reactions in peroxide-cured EPDM (terpolymerized ethylene, propylene, and diene monomer) rubber. The authors studied the complex mixtures arising from the model reactions with GC-MS and GC $\times$ GC–MS. The approach allowed them to gain new insights into the cross-linking mechanisms at work. Belov et al. (130) from DuPont used inverse GC to investigate the thermodynamics of sorption of organic vapors in perfluorinated polymers. In another cooperation, Adamska et al. (131) along with Bayer developed an inverse GC method for determining the Hansen solubility parameters of pharmaceutical excipients. Lastly, Kaal et al. (132), in cooperation with Unilever, successfully hyphenated SEC with pyrolysis-GC-MS for the analysis of copolymers. Their technique provides accurate chemical-composition information as a function of the molecular-weight information provided by SEC. The authors extended this technique to water-soluble polymers (133) by coupling aqueous LC directly to pyrolysis-GC-MS.

3. CONCLUSIONS

In this review, I have attempted to provide a reasonable perspective on the types of chromatographic research undertaken in industry today. As I explained at the outset of this review, this account is neither exhaustive nor comprehensive. Moreover, because I have restricted my data to published research, only a small fraction of the chromatographic activities in industry has been described. Due to length constraints, I have deliberately omitted many good papers. More importantly, as discussed above (**Table 3**), most of the research published by industry results from cooperation projects. This is a fair reflection of the world we live in: Scientists frequently compete, but they also cooperate.

4. PERSPECTIVES

Industry is still striving for improvements in its chromatographic operations, but it is reluctant to take on this task alone. Industry-academic cooperation is increasingly abundant, as is evident from the scientific literature. Industry is quick to embrace new developments from equipment manufacturers, provided that these are tuned to existing needs. Also, the advantages of new technology need to be convincingly demonstrated, and the source(s) must be trustworthy.

Industry still needs speed and efficiency. However, in most cases saving analyst time is much more important than saving analysis time. The demand for automated, hyphenated systems for sample preparation will increase, provided that these systems are robust, reliable, and easy to use. The same criteria apply to hyphenated detection systems. The immense success of GC-MS and LC-MS, LC-MS/MS, and GC × GC is due to highly reliable hardware and high-quality, easy to use software. Despite the complexity of these techniques, they have become robust and reasonably affordable. Other complex (hyphenated) systems can find their way into industry if the above criteria are met. In some cases, when a great many samples need to be analyzed in a short time, high-throughput experimentation is desirable. Fast chromatographic separations are of interest, as is more rapid sample preparation. Because the latter is often more time consuming than the former, parallel sample processing is an important area for development.

With the advent of PAT, the use of chromatography in quality-control situations may decline, unless very rapid and reliable methods are developed. In contrast, the quality control of complex products, such as food and natural materials, will become more important, and in this area chromatography is indispensable. Related to the latter development is a much increased interest in chromatography-performance relationships. Predicting product properties from (complex) chromatograms is a great challenge. Although statistical (multivariate) methods may be used for this purpose, ideally a better understanding of products and processes should go hand in hand with a better understanding of chemical structure and chromatography.

SUMMARY POINTS

1. Health care companies seem more inclined to publish their chromatographic results than does the chemical industry. Arguably, health care companies are also more inclined to cooperate with one another and with academia.
2. Enhanced throughput, automation, on-line sample preparation, and—as a summarizing entity—hyphenation are abundantly used in studies originating in industry, suggesting that industry is still searching for complete turnkey solutions to replace complex manual protocols.
3. Two-dimensional separations are used frequently in industry and elsewhere. Industry will continue to put such techniques to good use, provided that they contribute to the solutions of real problems.
4. SFC seems to be enjoying a third wave of interest. This technique is making significant inroads into the field of chiral separations.
5. Whereas proteomics research is performed mainly at academic research centers, industry is actively investigating protein-purification techniques.

DISCLOSURE STATEMENT

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LITERATURE CITED

1. Rozet E, Hubert C, Ceccato A, Dewé W, Ziemons E, et al. 2007. Using tolerance intervals in pre-study validation of analytical methods to predict in-study results. The fit-for-future-purpose concept. *J. Chromatogr. A* 1158:126–37
2. Wren SAC, Tchelitcheff P. 2006. At-line coupling of UPLC to chip-electrospray FTICR-MS. *J. Chromatogr. A* 1119:140–46
3. De Villiers A, Lestremau F, Szucs R, Gélébart S, David F, Sandra P. 2006. Evaluation of ultra performance liquid chromatography. Part I: Possibilities and limitations. *J. Chromatogr. A* 1127:60–69
4. De Villiers A, Lauer H, Szucs R, Goodall S, Sandra P. 2006. The influence of frictional heating on temperature gradients in ultra-high pressure liquid chromatography on 2.1 mm i.d. columns. *J. Chromatogr. A* 1113:84–91
5. Boogers I, Plugge W, Stokkermans YQ, Duchateau ALL. 2008. Ultra-performance liquid chromatographic analysis of amino acids in protein hydrolysates using an automated pre-column derivatisation method. *J. Chromatogr. A* 1189:406–9
6. Wang G, Hsieh Y, Cui X, Cheng KC, Korfmacher WA. 2006. Ultra-performance liquid chromatography/tandem mass spectrometric determination of testosterone and its metabolites in in vitro samples. *Rapid Commum. Mass Spectrom.* 20:2215–21
7. Goodwin L, White SA, Spooner N. 2007. Evaluation of ultra-performance liquid chromatography in the bioanalysis of small molecule drug candidates in plasma. *J. Chromatogr. Sci.* 45:298–304
8. Gika HG, Theodoridis GA, Wilson ID. 2008. Hydrophilic interaction and reversed-phase ultra-performance liquid chromatography TOF-MS for metabonomic analysis of Zucker rat urine. *J. Sep. Sci.* 31:1598–608
9. Wang D, Zhang M. 2007. Rapid quantitation of testosterone hydroxyl metabolites by ultra-performance liquid chromatography and mass spectrometry. *J. Chromatogr. B* 855:290–94
10. Gika HG, Macpherson E, Theodoridis GA, Wilson ID. 2008. Evaluation of the repeatability of ultra-performance liquid chromatography-TOF-MS for global metabolic profiling of human urine samples. *J. Chromatogr. B* 871:299–305
11. Gika HG, Theodoridis G, Extance J, Edge AM, Wilson ID. 2008. High temperature–ultra performance liquid chromatography-mass spectrometry for the metabonomic analysis of Zucker rat urine. *J. Chromatogr. B* 871:279–87
12. Dear GJ, Patel N, Weightman A, Pirard H, Talvitie M. 2008. Utilizing a -100 degrees C microplate CCD imager, yttrium silicate–coated 384 microplates and ultra-performance liquid chromatography for improved profiling of radiolabeled drug metabolites in complex biological samples. *J. Chromatogr. B* 868:49–57
13. Dear GJ, Patel N, Kelly PJ, Webber L, Yung M. 2006. TopCount coupled to ultra-performance liquid chromatography for the profiling of radiolabeled drug metabolites in complex biological samples. *J. Chromatogr. B* 844:96–103
14. Licea-Perez H, Wang S, Bowen CL, Yang E. 2007. A semi-automated 96-well plate method for the simultaneous determination of oral contraceptives concentrations in human plasma using ultra performance liquid chromatography coupled with tandem mass spectrometry. *J. Chromatogr. B* 852:69–76
15. Wang G, Hsieh Y, Cheng K-C, Morrison RA, Venkatraman S, et al. 2007. Ultra-performance liquid chromatography/tandem mass spectrometric determination of diastereomers of SCH 503034 in monkey plasma. *J. Chromatogr. B* 852:92–100
16. Huang MQ, Mao Y, Jemal M, Arnold M. 2006. Increased productivity in quantitative bioanalysis using a monolithic column coupled with high-flow direct-injection liquid chromatography/tandem mass spectrometry. *Rapid Commum. Mass Spectrom.* 20:1709–14

17. Costin JW, Lewis SW, Purcell SD, Waddell LR, Francis PS, Barnett NW. 2007. Rapid determination of Papaver somniferum alkaloids in process streams using monolithic column high-performance liquid chromatography with chemiluminescence detection. *Anal. Chim. Acta* 597:19–23
18. Altria KD, Marsh A, Clark BJ. 2006. High performance liquid chromatographic analysis of pharmaceuticals using oil-in-water microemulsion eluent and monolithic column. *Chromatographia* 63:309–14
19. Swinney K, Young B, Jakubik ME, Clark H, Troisi J, Fermier AM. 2007. An automated mobile phase preparation workstation. *J. Chromatogr. A* 1141:182–90
20. Gika HG, Theodoridis GA, Wilson ID. 2008. Liquid chromatography and ultra-performance liquid chromatography–mass spectrometry fingerprinting of human urine: sample stability under different handling and storage conditions for metabonomics studies. *J. Chromatogr. A* 1189:314–22
21. Ridgway K, Lalljie SPD, Smith RM. 2007. Sample preparation techniques for the determination of trace residues and contaminants in foods. *J. Chromatogr. A* 1153:36–53
22. Chang MS, Ji Q, Zhang J, El-Shourbagy TA. 2007. Historical review of sample preparation for chromatographic bioanalysis: pros and cons. *Drug Dev. Res.* 68:107–33
23. Palandra J, Weller D, Hudson G, Li J, Osgood S, et al. 2007. Flexible automated approach for quantitative liquid handling of complex biological samples. *Anal. Chem.* 79:8010–15
24. Ma J, Shi J, Le H, Cho R, Huang JC, et al. 2008. A fully automated plasma protein precipitation sample preparation method for LC-MS/MS bioanalysis. *J. Chromatogr. B* 862:219–26
25. Mohamed R, Richoz-Payot J, Gremaud E, Mottier P, Yilmaz E, et al. 2007. Advantages of molecularly imprinted polymers LC-ESI-MS/MS for the selective extraction and quantification of chloramphenicol in milk-based matrixes. Comparison with a classical sample preparation. *Anal. Chem.* 79:9557–65
26. Ridgway K, Lalljie SPD, Smith RM. 2006. Comparison of in-tube sorptive extraction techniques for non-polar volatile organic compounds by gas chromatography with mass spectrometric detection. *J. Chromatogr. A* 1124:181–86
27. Abdel-Rehim M, Persson C, Altun Z, Blomberg L. 2008. Evaluation of monolithic packed 96-tips and liquid chromatography–tandem mass spectrometry for extraction and quantification of pindolol and metoprolol in human plasma samples. *J. Chromatogr. A* 1196/1197:23–27
28. Altun Z, Hjelmström MA, Abdel-Rehim M, Blomberg LG. 2007. Surface modified polypropylene pipette tips packed with a monolithic plug of adsorbent for high-throughput sample preparation. *J. Sep. Sci.* 30:1964–72
29. Xu RN, Boyd B, Rieser MJ, El-Shourbagy TA. 2007. Simultaneous LC-MS/MS quantitation of a highly hydrophobic pharmaceutical compound and its metabolite in urine using online monolithic phase-based extraction. *J. Sep. Sci.* 30:2943–49
30. Xu RN, Fan L, Kim GE, El-Shourbagy TA. 2006. A monolithic phase based on-line extraction approach for determination of pharmaceutical components in human plasma by HPLC-MS/MS and a comparison with liquid-liquid extraction. *J. Pharm. Biomed. Anal.* 40:728–36
31. Xu Y, Willson KJ, Musson DG. 2008. Strategies on efficient method development of on-line extraction assays for determination of MK-0974 in human plasma and urine using turbulent-flow chromatography and tandem mass spectrometry. *J. Chromatogr. B* 863:64–73
32. Rezzi S, Vera FA, Martin FP, Wang S, Lawler D, Kochhar S. 2008. Automated SPE-RP-HPLC fractionation of biofluids combined to off-line NMR spectroscopy for biomarker identification in metabonomics. *J. Chromatogr. B* 871:271–78
33. Matthews CZ, Woolf EJ. 2008. Determination of a novel γ-secretase inhibitor in human plasma and cerebrospinal fluid using automated 96-well solid phase extraction and liquid chromatography/tandem mass spectrometry. *J. Chromatogr. B* 863:36–45
34. Chelius D, Xiao G, Nichols AC, Vizel A, He B, et al. 2008. Automated tryptic digestion procedure for HPLC/MS/MS peptide mapping of immunoglobulin γ antibodies in pharmaceutics. *J. Pharm. Biomed. Anal.* 47:285–94
35. Akin A, Antosz FJ, Ausec JL, Greve KF, Johnson RL. 2007. An orthogonal approach to chiral method development screening. *Curr. Pharm. Anal.* 3:53–70
36. McConnell O, Bach A II, Balibar C, Byrne N, Cai Y, et al. 2007. Enantiomeric separation and determination of absolute stereochemistry of asymmetric molecules in drug discovery: building chiral technology toolboxes. *Chirality* 19:658–82

37. Wetli HA, Francotte E. 2007. Automated screening platform with isochronal-parallel analysis and conditioning for rapid method development of chiral separations. *J. Sep. Sci.* 30:1255–61
38. Huybrechts T, Török G, Vennekens T, Sneyers R, Vrielynck S, Somers I. 2007. Multimodal HPLC screening of polysaccharide-based chiral stationary phases. *LC-GC Eur.* 20:320–35
39. Sajonz P, Schafer W, Gong X, Shultz S, Rosner T, Welch CJ. 2007. Multiparallel microfluidic high-performance liquid chromatography for high-throughput normal-phase chiral analysis. *J. Chromatogr. A* 1145:149–54
40. Wang C, Armstrong DW, Risley DS. 2007. Empirical observations and mechanistic insights on the first boron-containing chiral selector for LC and supercritical fluid chromatography. *Anal. Chem.* 79:8125–35
41. Zhou L, Mao B, Ge Z. 2008. Comparative study of immobilized alpha 1 acid glycoprotein and ovomucoid protein stationary phases for the enantiomeric separation of pharmaceutical compounds. *J. Pharm. Biomed. Anal.* 46:898–906
42. Boesten JMM, Berkheij M, Schoemaker HE, Hiemstra H, Duchateau ALL. 2006. Enantioselective high-performance liquid chromatographic separation of N-methyloxycarbonyl unsaturated amino acids on macrocyclic glycopeptide stationary phases. *J. Chromatogr. A* 1108:26–30
43. De Meulder M, Remmerie BMM, de Vries R, Sips LLA, Boom S, et al. 2008. Validated LC-MS/MS methods for the determination of risperidone and the enantiomers of 9-hydroxyrisperidone in human plasma and urine. *J. Chromatogr. B* 870:8–16
44. Wickremsinhe ER, Tian Y, Ruterbories KJ, Verburg EM, Weerakkody GJ, et al. 2007. Stereoselective metabolism of prasugrel in humans using a novel chiral liquid chromatography-tandem mass spectrometry method. *Drug Metab. Dispos.* 35:917–21
45. Chen J, Hsieh Y, Cook J, Morrison R, Korfmacher WA. 2006. Supercritical fluid chromatography-tandem mass spectrometry for the enantioselective determination of propranolol and pindolol in mouse blood by serial sampling. *Anal. Chem.* 78:1212–17
46. Zhang Y, Dai J, Wang-Iverson DB, Tymiak AA. 2007. Simulated moving columns technique for enantioselective supercritical fluid chromatography. *Chirality* 19:683–92
47. Gahm KH, Tan H, Liu J, Barnhart W, Eschelbach J, et al. 2008. Purification method development for chiral separation in supercritical fluid chromatography with the solubilities in supercritical fluid chromatographic mobile phases. *J. Pharm. Biomed. Anal.* 46:831–38
48. Leonard WR Jr, Henderson DW, Miller RA, Spencer GA, Sudah OS, et al. 2007. Strategic use of preparative chiral chromatography for the synthesis of a preclinical pharmaceutical candidate. *Chirality* 19:693–700
49. Mukherjee PS, Cook SE. 2006. A feasibility study on direct assay of an aqueous formulation by chiral supercritical fluid chromatography (SFC). *J. Pharm. Biomed. Anal.* 41:1287–92
50. Ramstad T. 2006. Enantiomeric purity methods for three pharmaceutical compounds by electrokinetic capillary chromatography utilizing highly sulfated-gamma-cyclodextrin as the chiral selector. *J. Chromatogr. A* 1127:286–94
51. Ramstad T, Johnson RL. 2006. Method for enantiomeric purity of a quinuclidine candidate drug by capillary electrophoresis. *J. Sep. Sci.* 29:2056–64
52. Antczak A, Ramstad T, Johnson R. 2006. A CD-MEKC method utilizing a neutral surfactant for enantiomeric purity determination of an oxazolidinone drug candidate. *Chromatographia* 64:57–64
53. Hou J, Zheng J, Rizvi SAA, Shamsi SA. 2007. Simultaneous chiral separation and determination of ephedrine alkaloids by MEKC-ESI-MS using polymeric surfactant I: Method development. *Electrophoresis* 28:1352–63
54. Welch CJ, Biba M, Sajonz P. 2006. Fast methods of enantiopurity determination for the Soai reaction: Towards a general enantioenrichment detector. *Chirality* 19:34–43
55. Mommers JHM, de Wildeman SMA, Koolen WAF, Duchateau ALL. 2008. Enantioselective gas chromatographic analysis of aqueous samples by on-line derivatisation. *J. Chromatogr. A* 1182:215–18
56. Begnaud F, Starkenmann C, Van de Waal M, Chaintreau A. 2006. Chiral multidimensional gas chromatography (MDGC) and chiral GC-olfactometry with a double-cool-strand interface: application to malodors. *Chem. Biodivers.* 3:150–60

57. Xiao KP, Xiong Y, Liu FZ, Rustum AM. 2007. Efficient method development strategy for challenging separation of pharmaceutical molecules using advanced chromatographic technologies. *J. Chromatogr. A* 1163:145–56
58. Jimidar MI, Nyen PV, Ael WV, Smet MD. 2008. Method development for pharmaceutical analysis. *Sep. Sci. Technol.* 9:63–94
59. Coffman JL, Kramarczyk JF, Kelley BD. 2008. High-throughput screening of chromatographic separations: I. Method development and column modeling. *Biotechnol. Bioeng.* 100:605–18
60. Wiendahl M, Wierling PS, Nielsen J, Fomsgaard Christensen D, Krarup J, et al. 2008. High throughput screening for the design and optimization of chromatographic processes: Miniaturization, automation and parallelization of breakthrough and elution studies. *Chem. Eng. Technol.* 31:893–903
61. Xu X. 2007. HPLC method development for drug discovery LC-MS assays in rapid PK applications. *Sep. Sci. Technol.* 8:317–51
62. Larcinese JP, Avaltroni F, Normand V. 2007. Non-intuitive separation of vanilla compounds using rapid resolution high-performance liquid chromatography. *J. Chromatogr. Sci.* 45:629–34
63. Kozlowski ES, Dalterio RA. 2007. Analyte solvent and injection volume as variables affecting method development in semipreparative reversed-phase liquid chromatography. *J. Sep. Sci.* 30:2286–92
64. Turnpenny P, Fraier D, Chassaing C, Duckworth J. 2007. Development of a micro-turbulent flow chromatography focus mode method for drug quantitation in discovery bioanalysis. *J. Chromatogr. B* 856:131–40
65. Smalley J, Marino AM, Xin B, Olah T, Balimane PV. 2007. Development of a quantitative LC-MS/MS analytical method coupled with turbulent flow chromatography for digoxin for the in vitro P-gp inhibition assay. *J. Chromatogr. B* 854:260–67
66. Xiao B, Gozo SK, Herz L. 2007. Development and validation of HPLC methods for the determination of potential extractables from elastomeric stoppers in the presence of a complex surfactant vehicle used in the preparation of parenteral drug products. *J. Pharm. Biomed. Anal.* 43:558–65
67. Sajonz P, Wu Y, Natishan TK, McGachy NT, DeTora D. 2006. Challenges in the analytical method development and validation for an unstable active pharmaceutical ingredient. *J. Chromatogr. Sci.* 44:132–40
68. Loeser E, Babiak S, Zhu P, Yowell G, Konigsberger M, Drumm P. 2006. Automation of pH optimization experiments during LC development. *Chromatographia* 63:345–51
69. De Beer TRM, Baeyens WRG, Vermeire A, Broes D, Remon JP, Vervaet C. 2007. Raman spectroscopic method for the determination of medroxyprogesterone acetate in a pharmaceutical suspension: validation of quantifying abilities, uncertainty assessment and comparison with the high performance liquid chromatography reference method. *Anal. Chim. Acta* 589:192–99
70. Lukulay P, Morgado J. 2006. Automation of the chromatographic analytical method validation process. *LC-GC N. Am.* 24:150–56
71. Viswanathan CT, Bansal S, Booth B, DeStefano AJ, Rose MJ, et al. 2007. Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. *Pharm. Res.* 24:1962–73
72. Kaltenbrunner O, Giaverini O, Woehle D, Asenjo JA. 2007. Application of chromatographic theory for process characterization towards validation of an ion-exchange operation. *Biotechnol. Bioeng.* 98:201–10
73. Nowatzke W, Woolf E. 2007. Best practices during bioanalytical method validation for the characterization of assay reagents and the evaluation of analyte stability in assay standards, quality controls, and study samples. *AAPS J.* 9:E117–22
74. Burdick R, Gleason T, Rausch S, Seely J. 2007. Using tolerance intervals for setting process validation acceptance criteria. *BioPharm. Int.* 20:40–46
75. Mollerup JM, Hansen TB, Kidal S, Staby A. 2008. Quality by design—thermodynamic modelling of chromatographic separation of proteins. *J. Chromatogr. A* 1177:200–6
76. Mollerup JM, Hansen TB, Kidal S, Sejergaard L, Staby A. 2007. Development, modelling, optimisation and scale-up of chromatographic purification of a therapeutic protein. *Fluid Phase Equilib.* 261:133–39
77. Gavin PF, Olsen BA. 2008. A quality by design approach to impurity method development for atomoxetine hydrochloride (LY139603). *J. Pharm. Biomed. Anal.* 46:431–41

78. Rathore AS, Yu M, Yeboah S, Sharma A. 2008. Case study and application of process analytical technology (PAT) towards bioprocessing: use of on-line high-performance liquid chromatography (HPLC) for making real-time pooling decisions for process chromatography. *Biotechnol. Bioeng.* 100:306–16
79. Squibb AW, Taylor MR, Parnas BL, Williams G, Girdler R, et al. 2008. Application of parallel gradient high performance liquid chromatography with ultra-violet, evaporative light scattering and electrospray mass spectrometric detection for the quantitative quality control of the compound file to support pharmaceutical discovery. *J. Chromatogr. A* 1189:101–8
80. Frommberger M, Zürbig P, Jantos J, Krahn T, Mischak H, et al. 2007. Peptidomic analysis of rat urine using capillary electrophoresis coupled to mass spectrometry. *Proteomics–Clin. Appl.* 1:650–60
81. Mueller DR, Voshol H, Waldt A, Wiedmann B, Van Oostrum J. 2007. LC-MALDI MS and MS/MS–an efficient tool in proteome analysis. *Sub-cell. Biochem.* 43:355–80
82. Li N, Nemirovskiy OV, Zhang Y, Yuan H, Mo J, et al. 2008. Absolute quantification of multidrug resistance-associated protein 2 (MRP2/ABCC2) using liquid chromatography tandem mass spectrometry. *Anal. Biochem.* 380:211–22
83. Zamani L, Andersson FO, Edebrink P, Yang Y, Jacobsson SP. 2008. Conformational studies of a monoclonal antibody, IgG1, by chemical oxidation: structural analysis by ultrahigh-pressure LC-electrospray ionization time-of-flight MS and multivariate data analysis. *Anal. Biochem.* 380:155–63
84. Kajdan T, Cortes H, Kuppannan K, Young SA. 2008. Development of a comprehensive multidimensional liquid chromatography system with tandem mass spectrometry detection for detailed characterization of recombinant proteins. *J. Chromatogr. A* 1189:183–95
85. Staby A, Jensen RH, Bensch M, Hubbuch J, Dünweber DL, et al. 2008. Comparison of chromatographic ion-exchange resins VI. Weak anion-exchange resins. *J. Chromatogr. A* 1164:82–94
86. Staby A, Jacobsen JH, Hansen RG, Bruus UK, Jensen IH. 2006. Comparison of chromatographic ion-exchange resins V. Strong and weak cation-exchange resins. *J. Chromatogr. A* 1118:168–79
87. Pabst TM, Buckley JJ, Ramasubramanyan N, Hunter AK. 2007. Comparison of strong anion-exchangers for the purification of a PEGylated protein. *J. Chromatogr. A* 1147:172–82
88. Fisher SM, Trail D, Hecht RI. 2006. The high throughput purification of Fc-fusion proteins. *Proc. Biochem.* 41:2473–76
89. Bajaj H, Sharma VK, Kalonia DS. 2007. A high-throughput method for detection of protein self-association and second virial coefficient using size-exclusion chromatography through simultaneous measurement of concentration and scattered light intensity. *Pharm. Res.* 24:2071–83
90. Karnoup AS, Kuppannan K, Young SA. 2007. A novel HPLC-UV-MS method for quantitative analysis of protein glycosylation. *J. Chromatogr. B* 859:178–91
91. Impellizzeri J, Lin J. 2006. A simple high-performance liquid chromatography method for the determination of throat-burning oleocanthal with probated anti-inflammatory activity in extra virgin olive oils. *J. Agric. Food Chem.* 54:3204–8
92. Cooper KA, Campos-Giménez E, Jimenez Alvarez D, Nagy K, Donovan JL, Williamson G. 2007. Rapid reversed phase ultra-performance liquid chromatography analysis of the major cocoa polyphenols and inter-relationships of their concentrations in chocolate. *J. Agric. Food Chem.* 55:2841–47
93. Rochat S, de Saint Laumer J-Y, Chaintreau A. 2007. Analysis of sulfur compounds from the in-oven roast beef aroma by comprehensive two-dimensional gas chromatography. *J. Chromatogr. A* 1147:85–94
94. Van Platerink CJ, Janssen H-G, Horsten R, Haverkamp J. 2006. Quantification of ACE inhibiting peptides in human plasma using high performance liquid chromatography-mass spectrometry. *J. Chromatogr. B* 830:151–57
95. Guillotin SE, Bakx EJ, Boulenguer P, Schols HA, Voragen AGJ. 2007. Determination of the degree of substitution, degree of amidation and degree of blockiness of commercial pectins by using capillary electrophoresis. *Food Hydrocolloids* 21:444–51
96. Guillotin SE, Mey N, Ananta E, Boulenguer P, Schols HA, Voragen AGJ. 2006. Chromatographic and enzymatic strategies to reveal differences between amidated pectins on a molecular level. *Biomacromolecules* 7:2032–37
97. Nilsson C, Asplund A, Schagerlö FH, Melander C, Andersen A, et al. 2007. Studies of the separation and characterisation of mixtures of starch and cellulose derivatives by use of chromatography and mass spectrometry. *Anal. Bioanal. Chem.* 387:2045–55

98. Van Platerink CJ, Janssen H-G, Haverkamp J. 2008. Application of at-line two-dimensional liquid chromatography-mass spectrometry for identification of small hydrophilic angiotensin I-inhibiting peptides in milk hydrolysates. *Anal. Bioanal. Chem.* 391:299–307
99. Kleinert T, Lunze J. 2008. Decentralised control of chromatographic simulated moving bed processes based on wave front reconstruction. *J. Proc. Contr.* 18:780–96
100. McEwen CN. 2007. GC/MS on an LC/MS instrument using atmospheric pressure photoionization. *Int. J. Mass Spectrom.* 259:57–64
101. Luong J, Gras R, Jennings W. 2007. An advanced solventless column test for capillary GC columns. *J. Sep. Sci.* 30:2480–92
102. Luong J, Gras R, Mustacich R, Cortes H. 2006. Low thermal mass gas chromatography: principles and applications. *J. Chromatogr. Sci.* 44:253–61
103. Winniford BL, Sun K, Griffith JF, Luong JC. 2006. Universal and discriminative detection using a miniaturized pulsed discharge detector in comprehensive two-dimensional GC. *J. Sep. Sci.* 29:2664–70
104. Lustig SR, Jagota A, Khripin C, Zheng M. 2006. Universal and discriminative detection using a miniaturized pulsed discharge detector in comprehensive two-dimensional GC. *Mater. Res. Soc. Symp. Proc.* 923:1–6
105. Luong J, Gras R, Yang G, Sieben L, Cortes H. 2007. Capillary flow technology with multi-dimensional gas chromatography for trace analysis of oxygenated compounds in complex hydrocarbon matrices. *J. Chromatogr. Sci.* 45:664–70
106. Gras R, Luong J, Shearer R. 2007. A unified approach for the measurement of individual or total volatile organic sulfur compounds in hydrocarbon matrices by dual-plasma chemiluminescence detector and low thermal mass gas chromatography. *J. Chromatogr. Sci.* 45:671–76
107. Wortberg M, Ziemer W, Kugel M, Müller H, Neu H-J. 2006. Monitoring industrial wastewater by online GC-MS with direct aqueous injection. *Anal. Bioanal. Chem.* 384:1113–22
108. Sanderson H, Price BB, Dyer SD, DeCarvalho AJ, Robaugh D, et al. 2006. Occurrence and hazard screening of alkyl sulfates and alkyl ethoxysulfates in river sediments. *Sci. Total Environ.* 367:312–23
109. Zimmer D, Philipowski C, Posner B, Gnielka A, Dirr E, Dorff M. 2006. Determination of deltamethrin residues in plant materials by liquid chromatography/tandem mass spectrometry with electrospray ionization. *J. AOAC Int.* 89:786–96
110. Sagawa N, Takino T, Kurogochi S. 2006. A simple method with liquid chromatography/tandem mass spectrometry for the determination of the six trichothecene mycotoxins in rice medium. *Biosci. Biotechnol. Biochem.* 70:230–36
111. Brudin S, Berwick J, Duffin M, Schoenmakers P. 2008. One-dimensional and two-dimensional liquid chromatography of sulphonated lignins. *J. Chromatogr. A* 1201:196–201
112. Zhang F, Bartels MJ, Pottenger LH, Schisler MR, Grundy JJ, Gollapudi BB. 2008. Quantitation of methylated hemoglobin adducts in a signature peptide from rat blood by liquid chromatography/negative electrospray ionization tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* 22:1455–60
113. Zhang F, Stott WT, Clark AJ, Schisler MR, Grundy JJ, et al. 2007. Quantitation of 8-hydroxydeoxyguanosine in DNA by liquid chromatography/positive atmospheric pressure photoionization tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* 21:3949–55
114. Zhang F, Bartels MJ, Pottenger LH, Gollapudi BB, Schisler MR. 2007. Quantitation of lower levels of the DNA adduct of thymidylyl(3′-5′)thymidine methyl phosphotriester by liquid chromatography/negative atmospheric pressure chemical ionization tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* 21:1043–48
115. Yau WW. 2007. Examples of using 3D-GPC-TREF for polyolefin characterization. *Macromol. Symp.* 257:29–45
116. Barth HG, Saunders GD. 2006. High-performance SEC column technology. *LC-GC N. Am.* 24(Suppl. 4):38–43
117. Meunier DM, Stokich TM Jr, Gillespie D, Smith PB. 2007. Molecular topology fractionation of polystyrene stars and long chain branched polyethylene fractions. *Macromol. Symp.* 257:56–70
118. Edam R, Meunier DM, Mes EPC, Van Damme FA, Schoenmakers PJ. 2008. Branched-polymer separations using comprehensive two-dimensional molecular-topology fractionation × size-exclusion chromatography. *J. Chromatogr. A* 1201:208–14

119. Kaal E, Janssen JGM. 2008. Extending the molecular application range of gas chromatography. *J. Chromatogr. A* 1184:43–60
120. Wohlleben W, Lechner MD. 2008. AUC and HDC characterization of heterogeneous polymer dispersions. *Colloid Polym. Sci.* 286:149–57
121. Erdner JM, Barth HG, Foley JP, Payne WG. 2006. Size-exclusion chromatography using deuterated mobile phases. *J. Chromatogr. A* 1129:41–46
122. Normand V, Avaltroni F, Bouquerand P. 2006. Mathematical discretization of size-exclusion chromatograms applied to commercial corn maltodextrins. *J. Chromatogr. Sci.* 44:91–95
123. Rittig F, Fandrich N, Uriel M, Schrepp W, Just U, Weidner SM. 2006. Structure determination of polyacetals by liquid chromatography and hyphenated techniques. *Macromol. Chem. Phys.* 207:1026–37
124. de Geus M, Peters R, Koning CE, Heise A. 2008. Insights into the initiation process of enzymatic ring-opening polymerization from monofunctional alcohols using liquid chromatography under critical conditions. *Biomacromolecules* 9:752–57
125. Peters R, Litvinov VM, Steeman P, Dias AA, Mengerink Y, et al. 2007. Characterisation of UV-cured acrylate networks by means of hydrolysis followed by aqueous size-exclusion combined with reversed-phase chromatography. *J. Chromatogr. A* 1156:111–23
126. Albrecht A, Heinz L, Lilge D, Pasch H. 2007. Separation and characterization of ethylene-propylene copolymers by high-temperature gradient HPLC coupled to FTIR spectroscopy. *Macromol. Symp.* 257:46–55
127. Knecht D, Rittig F, Lange RFM, Pasch H. 2006. Multidimensional chromatographic techniques for hydrophilic copolymers. II. Analysis of poly(ethylene glycol)-poly(vinyl acetate) graft copolymers. *J. Chromatogr. A* 1130:43–53
128. Peters R, Tonoli D, van Duin M, Mommers J, Mengerink Y, et al. 2008. Low-molecular-weight model study of peroxide cross-linking of ethylene-propylene (-diene) rubber using gas chromatography and mass spectrometry I. Combination reactions of alkanes. *J. Chromatogr. A* 1201:141–50
129. Peters R, van Duin M, Tonoli D, Kwakkenbos G, Mengerink Y, et al. 2008. Low-molecular-weight model study of peroxide cross-linking of ethylene-propylene-diene rubber using gas chromatography and mass spectrometry II. Addition and combination reactions. *J. Chromatogr. A* 1201:151–60
130. Belov N, Yampolskii Y, Coughlin MC. 2006. Thermodynamics of sorption in an amorphous perfluorinated rubber studied by inverse gas chromatography. *Macromolecules* 39:1797–804
131. Adamska K, Bellinghausen R, Voelkel A. 2008. New procedure for the determination of Hansen solubility parameters by means of inverse gas chromatography. *J. Chromatogr. A* 1195:146–49
132. Kaal ER, Alkema G, Kurano M, Geißler M, Janssen JGM. 2007. On-line size exclusion chromatography-pyrolysis-gas chromatography for copolymer characterization and additive analysis. *J. Chromatogr. A* 1143:182–89
133. Kaal ER, Kurano M, Geißler M, Janssen JGM. 2008. Hyphenation of aqueous liquid chromatography to pyrolysis–gas chromatography and mass spectrometry for the comprehensive characterization of water-soluble polymers. *J. Chromatogr. A* 1186:222–27

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