On-line Coupling of Solid-Phase Extraction to Liquid Chromatography—A Review

Ligang Chen, Hui Wang, Qinglei Zeng, Yang Xu, Lei Sun, Haoyan Xu, and **Lan Ding***
College of Chemistry, Jilin University, 2699 Qianjin Street, Changchun 130012, China

Abstract

Solid-phase extraction (SPE) is an effective sample preparation method for removal of interfering compound and enrichment of analyte. Liquid chromatography (LC) with various detectors is a main separation and detection technique used in the analytical field. This article reviews the literatures about the on-line coupling of SPE with LC. The advantages of on-line coupling are reduction of analysis time, sample contamination, and analyte losses, as well as improvement of precision and accuracy. The SPE sorbents including traditional materials, such as chemically bonded silica, ion-exchange and carbon-based materials, and some novel sorbents, such as restricted access material, molecularly imprinted polymer, immunosorbent, and monolithic material, used in the on-line analysis are discussed in detail. The on-line coupling of SPE-LC to other sample preparation techniques, such as supercritical fluid extraction, subcritical water extraction, microwave-assisted extraction, ultrasound-assisted extraction, and derivatization technique are also reviewed.

Introduction

The quality of sample preparation is a key factor in analytical chemistry. There is considerable interest in developing a new selective and sensitive method for extracting and isolating components from complex sample matrices (1). An ideal sample preparation methodology should be fast, accurate and precise, and consume little organic solvent. Other demands for modern sample preparation methods include sample integrity, high throughput, and compatibility with subsequent analysis (2).

Solid-phase extraction (SPE) is a widely used sample preparation technique (3). The analytes are transferred to solid sorbent where they are retained during the sampling process and then recovered by elution. The principal goals of SPE are trace analytes enrichment and sample clean-up. SPE has been used in many areas, including environmental, pharmaceutical, clinical, food, and industrial chemistry. Over time, various SPE formats and sorbents have been developed to facilitate the process of different sample types and to extend the scope of the method.

*Author to whom correspondence should be addressed: email dinglan@jlu.edu.cn.

The SPE technique not only can be performed in the off-line mode but also provides the possibility of on-line coupling to other analytical steps, such as chromatographic analysis. It enables partial or total automation of the analytical process, reduces analysis time, decreases analyte loss, increases sensitivity, and improves accuracy and precision. This review will discuss the on-line coupling of SPE to liquid chromatography (LC), which is a fast, modern, and reliable approach. The process and the mode of the on-line SPE-LC are similar. However, the sorbent used in the on-line SPE column is various, and developed quickly in recent years. The article reviews the sorbents used in on-line SPE detailedly, and the literatures about on-line coupling of SPE-LC to other sample preparation techniques are also summarized briefly.

A Survey of On-Line SPE-LC

Whether in the on-line or in the off-line mode, the SPE procedure consists of four steps: (*i*) Condition. An appropriate solvent is passed through the sorbent for activating the surface of the solid. Then the solvent is removed by a liquid, which is similar to the solvent used to dissolve sample. (*ii*) Sample loading. The sample is loaded onto the SPE column for retaining the analytes. (*iii*) Washing. The interfering compounds retained on the sorbent are removed with an appropriate solvent. (*iv*) Elution. The analytes are eluted from the sorbent with an appropriate solvent.

The most commonly method used for on-line coupling of SPE with LC is achieved through column switching. For this purpose, a small, typically 2–15 mm long and 1–4.6 mm i.d. precolumn used as SPE column is connected to a conventional LC analytical column via a switching valve. Column switching configurations can contain various numbers of precolumns, switching valves, and pumps. Figure 1 shows a simple column switching configuration. When the switching valve is in "position a," the sample is injected into the SPE column, which has been conditioned by appropriate solvents. Concurrently, the analytical column is equilibrated with the chromatographic mobile phase. The valve is switched to the "position b" after elution of the interfering compounds. The analytes are eluted from the

SPE column either in the back- or straight-flush mode using the chromatographic mobile phase and transferred into the analytical column. Afterwards, the valve is switched to its initial position (position a). The analytes are separated on the analytical column prior to detection. Simultaneously, the SPE column is reactivated and is ready to the next sample injection.

As compared with off-line SPE, on-line SPE offers a series of advantages and disadvantages (Table I). Firstly, on-line SPE technique reduces the sample preparation time and, thus, increases the sample throughput. Conditioning, washing, and elution steps can be performed automatically (4). Secondly, the technique can decrease the risk of sample contamination, eliminate the analyte loss by evaporation used in off-line mode, improve the accuracy, and reduce the risk for the operator (5). In addition, on-line SPE can decrease the solvent consumption and the costs for organic solvents waste disposal (6). In on-line mode, the whole extract is transferred to the LC analytical column, and in off-line mode, only a small aliquot is injected, so the sensitivity of the on-line method is increased. However, this easily leads to overloading of the analytical column, especially for the complex samples. In on-line mode, efficient cleaning of the extraction system is necessary in order to avoid memory effects.

On-line SPE Sorbents

In SPE, the analytes are partitioned between solid sorbent and liquid phase, and these analytes must have greater affinity for the solid sorbent than for the sample matrix. The choice of sorbent is a key point in SPE because it can control parameters such as selectivity, affinity, and capacity (8). This choice depends strongly on the analytes of interest and the interactions between chosen sorbent and the functional groups of the analytes. However, it also depends on the kind of sample matrix and its interactions with both the sorbent and the analytes (9). The traditional SPE sorbents include the chemically bonded silica, ion-exchange, and carbon-based materials. These kinds of sorbents have limited selectivity, in addition to the target analytes, many matrix constituents can also be enriched on them and disturb the chromatographic separation and detection. Some more selective sorbents have been developed, including restricted access material (RAM), molecularly imprinted polymer (MIP), and immunosorbent (IS). Moreover, monolithic material, as a new class of non-particulate sorbent, has attracted considerable attention in resent years.

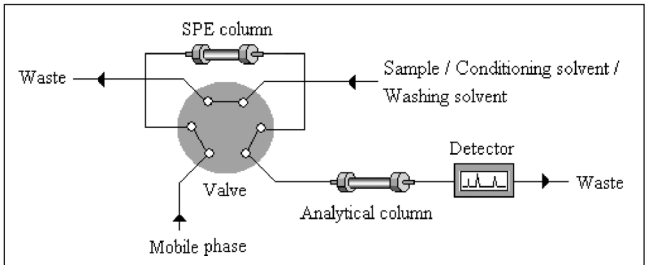

Figure 1. Typical configuration of on-line coupling of SPE to LC through column switching.

Table I. Advantages and Disadvantages of On-line SPE-LC

Advantages	Disadvantages
(a) Time savings and higher throughput	(a) Limited portability
(b) Improved precision and accuracy	(b) Expensive equipment.
(c) Safety.	One switching valve and one high pressure pump
The extraction and analytes take place in a	are needed at least
closed system, which decreased the exposure	(c) Matrix effects; ionic suppression or enhancement
to hazardous samples or solvent	in mass spectrometry detector
(d) Reusable SPE column	
(e) Minimal loss or degradation of analytes.	
No evaporation steps and direct elution of the analytes	
after preconcentration into LC column	
(f) Minimal consumption of organic solvent	

1. Silica-based, chemically bonded sorbents

Silica chemically bonded with various groups has been the most common material for SPE. These sorbents can be classified as reversed-phase sorbents with octadecyl (C18) or octacyl (C8) used for adsorbing non- or weak-polar compounds, or as normal-phase sorbents with cyanopropyl (CN) or aminopropyl (NH2) used for adsorbing polar compounds. Their interaction mechanisms are mainly based on Van der Waals forces between the analytes and the sorbents (9,10). These materials have been widely used as SPE sorbents in the on-line coupling with LC, especially for C18, which also is the most popular sorbent for LC analytical column. It is good that the sorbent used in the SPE column can be identical with the material packed in the analytical column. However, silica-based sorbents have several disadvantages, such as instability at extreme pH and the presence of some residual silanol groups. Some literature about applications of on-line coupling of SPE with silica-based sorbents to LC is summarized in Table II.

Ye et al. developed a method using on-line SPE with C18 sorbent coupled to high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS–MS) to measure seven environmental phenols and five parabens in serum (11). This method simplified sample pretreatment process, and obtained the limits of detection (LODs) ranging from 0.1 to 0.5 ng/mL with a small amount of serum (0.1 mL). A sensitive and selective method was developed for quantifying cyclosporine A in the human peripheral blood mononuclear cells (PBMCs) by LC–ESI-MS (17). The on-line SPE was performed using a C8 column. The recovery (95.0–113.2%) and repeatability (5.1–9.9%) were found to be satisfactory. A fully automated method was developed for determination of a new antipsychotic drug amisulpride, which is a substituted benzamide (19). The serum or plasma sample was injected into the HPLC system and cleaned-up on a precolumn filled with Silica CN, and then the analyte was separated on Lichrospher CN column. The method can be applied to therapeutic drug monitoring and pharmacokinetic studies of amisulpride. An on-line SPE-LC method was

described for the determination of trovafloxacin in the human serum (22). The samples were deproteinized with acetonitrile and injected into an NH2 column for clean-up. Subsequently, a column switching system was used for quantitative transfer of the drug to a C18 analytical column. The recovery was 98.5% and the LOD was 0.1 µg/mL.

2. Ion-exchange sorbents

Ion exchange sorbents containing fixed ion-exchange sites are used to isolate ionic compounds from aqueous solutions. Both cation and anion functional groups can be bonded to the polymer, and then form different types of ion exchange sorbents, such as strong cation-exchange (SCX), weak cation-exchange (WCX), strong anion-exchange (SAX), and weak anion-exchange (WAX) sorbents. SCX sorbents contain ion-exchange sites consisting of sulfonic acid groups, and WCX sorbents contain ion-exchange sites consisting of carboxylic acid groups. On the other hand, quaternary amino groups are SAX sites, and primary, secondary and tertiary amine groups are WAX sites. These materials have attracted considerable attention due to their superior porosity, simple preparation procedure, and good permeability. Some examples about applications of on-line coupling of SPE with ion-exchange sorbents to LC are summarized in Table III.

Yamamoto et al. developed a novel method based on column-switching HPLC system using methylcellulose-immobilized strong cation-exchange (MC-SCX) precolumn for direct analysis of drugs in plasma (23). The precolumn was proved effective in the on-line enrichment of cationic drugs including pyridoxine, atenolol, and sulpiride that have different hydrophobicity, which are expressed as logarithm of octanol–water partition coefficient (log Pow, –0.80, –0.11, and 1.110) and pKa (8.67, 10.08, and 9.10) in plasma sample. The SCX precolumn also can be on-line coupled with HPLC–MS–MS for proteome analysis (25). This system exhibits good separation performance as well as good proteomic coverage in both one- and multidimensional separation, and represents a convenient, useful, and reliable approach for routine shotgun proteome analysis. Patsias et al. developed an automated method based on the on-line coupling of SAX-SPE and SCX-LC column followed by post-column derivatization and fluorescence detection for the trace determination of glyphosate and its primary conversion product aminomethyl phosphonic acid (AMPA) in water (27). By processing 100 mL samples, the LODs were 0.02 µg/L for glyphosate and 0.1 µg/L for AMPA, respectively.

3. Carbon based sorbents

Activated carbon has been used as sorbent for extracting low polarity organic compounds from water (28). However this sorbent was abandoned because irreversible adsorption and low recoveries were obtained for some analytes (29). The graphitized carbon black (GCB) obtained by heating carbon blacks at high temperature (2700–3000°C) has been achieved application as SPE sorbent (30). Some examples about applications of on-line coupling of SPE with carbons based sorbents to LC are summarized in Table IV. A GCB extraction cartridge has been evaluated for on-line coupling with a C8 analytical column to determine 11 carbamates and one carboximide pesticide in water (31). The analytes were not easily eluted from the GCB column, and this problem was partially solved by modifying the acetonitrile/water gradient to contain a front of 100% acetonitrile for a few seconds. In this way, resulting peaks are narrow and the dietofencarb/molinate pair is resolved.

The drawbacks of the GCB sorbents are that they have excessive retention (some analytes can even be irreversibly adsorbed)

Table II. Applications of On-line Coupling of SPE with Silica-based Sorbents to LC*

Analytes	Sample matrix	SPE material	Detection method	LOD (ng/mL)	Linearity range (ng/mL)	Recovery (%)	Precision (RSD, %)
Phenols and parabens (11)	Serum	C18	MS-MS	0.1–0.5	0–100	82–113	< 10
Steroids (12)	Yeast-mediated cell culture	C18	MS	0.12–0.36	0–50	> 94	< 8.8
Estrogens (13)	Waters	C18	UV	0.00098–0.0781	0.05–400	85–112	1.0–3.4
Tetracyclines (14)	Honey	C18	UV	5–12 (ng/g)	50–1000 (ng/g)	84.2–120.6	3.2–8.9
Zidovudine and its monophosphate (15)	Cell extracts	C18	UV	500; 100	500–100000	80.6–104.7	< 12
Andrographolide and dehydroandrographolide (16)	Plasma	C18	UV	19; 22	50–5000	92.0–102.1	1.2–6.5
Cyclosporine A (17)	Human peripheral blood mononuclear cells	C8	MS	5.0 (LOQ)	5–400	95.0–113.2	5.1–14.7
Methadone and its primary metabolite (18)	Human plasma	C8	MS	0.1 (LOQ)	0.1–25	> 95	< 10
Antipsychotic drug amisulpride (19)	Serum and plasma	CN	UV	5.0	10–600	91.9–99.4	2.8–11.3
Microcystins (20)	Water	CN	UV	0.02	0.05–2	94.2–99.7	3.1–7.8
Chlorthalidone (21)	Urine	CN	UV	20	100–20000	> 95	< 10
Trovafloxacin (22)	Serum	NH_2	UV	100	250–20000	98.5	2.6–11.3

* LOD = limit of detection; LOQ = limit of quantification.

Table III. Applications of On-line Coupling of SPE with Ion-exchange Sorbents to LC*

Analytes	Sample matrix	SPE material	Detection method	LOD (ng/mL)	Linearity range (ng/mL)	Recovery (%)	Precision (RSD, %)
Cationic drug (23)	Plasma	SCX	UV	1, 10, 50 (LOQ)	10–1000	91.6–100.0	0.03–14.48
Sotalol (24)	Plasma	SCX	UV	2.5	25–1000	60	5.8
Proteome analysis (25)	Yeast protein extract	SCX	MS-MS	n.r.	n.r.	n.r.	n.r.
Hydrochlorothiazide and losartan potassium (26)	Water	SCX	UV	70, 90	0.3–1.7; 0.4–1.8	95.6–118.0	2.7, 2.5
Glyphosate and aminomethyl phosphonic acid (27)	Water	SAX	Fluorescence	0.02, 0.1	1–1000 ng	84; 15	< 15

*LOD = limit of detection; LOQ = limit of quantification; n.r. = not reported.

Table IV. Applications of On-line Coupling of SPE with Carbon-based Sorbents to LC*

Analytes	Sample matrix	SPE material	Detection method	LOD (ng/mL)	Recovery (%)	Precision (RSD, %)
Carbamates and carboximide pesticide (31)	Water	GCB	UV	0.026–0.260	18–99	6–11
Polar pesticides (32)	Water	GCB	UV	0.05–0.16	63–75	4–8
Phenolic compounds (34)	Waters	PIGC	UV	0.1	55–105	< 15
Nitroaromatic explosives and related compounds (35)	Water	PIGC	MS	0.0025–0.563	74–96	2–7
Sulfonamides (36)	Eggs, pork	MWNT	UV	0.004.6–0.01	66.4–85.6	2.5–7.8

*The linearity range of the method was not reported in these applications; LOD: limit of detection.

and their mechanical stability is poor. Porous immobilized graphitic carbon (PIGC) is a new carbon based sorbent in which the graphite is immobilized on silica. It has a highly homogeneous crystalline structure made of large graphitic sheets (33). Crescenzi et al. developed an on-line system for the determination of nitroaromatic explosives and related compounds in water samples (35). In the system, an extraction column filled with different sorbents, such as restricted access material (RAM), C18, and PIGC were tested for on-line sample clean-up and analytes enrichment. The recoveries obtained using RAM and PIGC sorbents were in the range of 73–94% and 74–96%, respectively. However, C18 was not suitable in the work because the recoveries obtained were in the range of 13–91%. PIGC has better characteristics, especially in terms of durability and suppression of matrix effects. When analyzing spiked surface water with RAM, increased pressure was detected after ~ 20 samples, which was accompanied by higher variability in the analysis. When analyzing untreated surface water with the PIGC, it was only necessary to clean the extraction column every 50 samples in order to maintain satisfactory performance.

Carbon nanotubes (CNT) are novel carbon materials, which were first used in 1991 by S. Iijima (36). They can be divided into single-walled carbon nanotubes (SWNTs) and multiwalled carbon nanotubes (MWNTs) according to the carbon atom layers in the wall of the nanotubes. MWNTs have great analytical potential as effective sorbents for adsorbing metal ions, organic compounds and organometallic compounds (36). A method for the determination of residual sulfonamides in eggs and pork was established by on-line coupling of SPE with MWNTs to HPLC–UV (36). The developed method permitted the current HPLC separation and the next preconcentration proceeded in parallel, and thus one sample analysis can be finished within 35 min. For comparison, C18 was also tested for on-line clean-up. The MWNTs gave lower LODs (4.6–10.0 ng/L), higher enrichment factors (37.7–160.9), and lower RSDs (2.56–7.62%) than C18 with 4.8–16.4 ng/L of LODs, 46.8–129.8 of enrichment factors and 1.23–14.1% of RSDs.

4. Restricted access materials

Restricted access material (RAM) is designed specifically for the removal of macromolecules based on the size-exclusion mechanism (37). Only small molecules are able to penetrate into the pores of RAM and interact with a stationary phase bonded on their inner surface, while large molecules are excluded with the washing solvent (38). Several examples for the on-line coupling of RAM precolumns to LC are presented in Table V.

A fully automated method was developed for the determination of a new synthetic inhibitor of matrix metalloproteinases, Ro 28-2653, in ovine serum and plasma (41). Ro 28-2653 is very poorly soluble in water. The solubility is about 0.56 µg/mL at 25°C. The method was based on the coupling of a RAM precolumn for sample clean-up to a C8 analytical column. The method was validated over the analyte concentration ranging from 17.5 to 1950 ng/mL. The precision expressed as RSD was 4.2%.

The RAM precolumns were mainly used in the biological area for the removal of protein. However, they also can be used for analyzing the environmental samples, such as water or sediment (46,47,49,50). High molecular weight humic substances (fulvic and humic acids) existing in the environmental samples can be eliminated by the RAM column. A switching column system, which combines an RAM column and a C18 analytical column, was used for determination of tetracyclines in milk and environmental water samples (49). The RAM precolumns with C4, C8, and C18 bonded to the inner pore surfaces were tested, and C8 provided the best performance. The RAM column with C4 was not suitable because of the low affinity towards the polar tetracyclines [e.g. OTC breakthrough was observed with just 1 mL in a low organic content mobile phase, such as water–acetonitrile

(95:5)]. The C18 RAM column was not effective for remove matrix components.

The lifetime of the sorbent is one important factor for the SPE sorbent. Compared with common SPE sorbent, the lifetime of RAM sorbent is extremely long. The price of RAM columns is usually high and frequently exceeds the price of a common analytical column. However, most of the RAM precolumn has theoretical lifetime of injection of 100 mL of human plasma (38). If the sample injection volume is 50 µL, which means that the column can be used 2000 times without any changes in recovery, separation performance and back-pressure.

5. Molecularly imprinted polymers

In recent years, molecularly imprinted polymers (MIPs) have attracted much attention due to their outstanding advantages, such as predetermined recognition ability, stability, relative ease and low cost of preparation, and potential application to a wide range of target molecules (51). In the most common preparation process, monomers form complexes with template through covalent or non-covalent interactions and are then joined by using a cross-linking agent. After the removal of the template by chemical reaction or extraction, binding sites are exposed which are complementary to the template in size, shape, and position of the functional groups (52).

MIPs have been widely used as artificial receptors in separation, sensor, catalysis, and drug development and screening (53). Among them, the highly selective MIP solid-phase extraction (MISPE) technique used for extraction of analytes, which are present in low concentrations or in a complex matrix, has been widely investigated.

The MISPE has also been coupled on-line with LC, and some applications are presented in Table VI. The 4-nitrophenol (4-NP) MIPs were prepared using non-covalent molecular imprinting method (54). The river water samples spiked with eleven phenolic compounds at low levels were on-line pre-concentrated using this MISPE, and 4-NP can be selectively extracted. Compared with a commercially available highly cross-linked polymer (LiChrolut EN), the MIP yielded cleaner extracts. The similar system was also used for selective extraction and separation of triazine from water samples (57). The triazine enrichment factor up to 100 was obtained with good extraction efficiency (74–77%).

In the on-line SPE-LC, the compatibility between the elution solvent, which was used to desorb analytes from the SPE pre-

Table V. Applications of On-line Coupling of SPE with Restricted Access Materials to LC*

Analyte(s)	Sample matrix	Detection method	LOD (ng/mL)	Linearity range (ng/mL)	Recovery (%)	Precision (RSD, %)
Catecholamines (39)	Urine	Electrochemistry	0.52; 0.26; 23.98	15–500; 5–500; 50–500	95.3–97.1	1.9–4.3
2-Amino-1-methyl-6-phenylimidazo (4,5-b)pyridine (PhIP) (40)	Hemoglobin	MS–MS	0.03 fmol/mg	n.r.	> 95	< 15
Matrix metalloproteinase inhibitor (41)	Ovine plasma and serum	UV	5.0	17.5–1950	95	4.2
Cyproterone acetate (42)	Plasma	MS–MS	0.12	0.3–50	99.4	0.4–1.6
1- and 2-Naphthol (43)	Urine	Fluorescence	1.5; 0.5	0–360	93.3–107.9	2.0–7.2
Organophosphorus trimesters (44)	Plasma	MS–MS	0.2–1.8	1–100; 0.5–100 ng	60–92	0.9–5.2
(R)- and (S)-Propranolol (45)	Rat microdialysate	Fluorescence	10; 15	25–1000; 5–500	87.3–101.7	1.1–10.5
Macrolide antibiotics (46)	Water	MS–MS	0.002–0.006	0.02–5.0	86.5–98.3	2.9–8.9
Acidic herbicides (47)	Water	UV	n.r.	n.r.	86–102	3.0–7.0
Trimethoprim (48)	Milk	UV	5.0	n.r.	73	n.r.
Tetracyclines (49)	Milk and water	Fluorescence	0.015; 0.030	10–800	50–90	2–10
Alkylphenolic compounds and steroid sex hormones (50)	Sediment	MS	0.5–5 ng/g	n.r.	62–104	9.6–19

* LOD = limit of detection; n.r. = not reported.

Table VI. Applications of On-line Coupling of SPE with Molecularly Imprinted Polymers to LC*

Analyte(s)	Sample matrix	Template	Detection method	LOD (ng/mL)	Linearity range (ng/mL)	Recovery (%)	Precision (RSD, %)
4-Nitrophenol (54)	Water	4-nitrophenol	UV	n.r.	n.r.	38–58	< 8
4-Chlorophenols and 4-nitrophenol (55)	Water	4-chlorophenol	UV	n.r.	1–100	n.r.	< 11
Bisphenol A (56)	Water	P-tert.-butylphenol	Electrochemical	0.00036	0.0001–0.1	100.5	0.5–9.3
Triazines (57)	Water	Simazine	UV	0.07	n.r.	74–77	3.2–5.0
Ropivacaine and bupivacaine (58)	Plasma	Pentycaine	MS-MS	1.4; 1.9 nmol/L (LOQ)	n.r.	65–75	3–12
Basic drug alfuzosin (59)	Plasma	Alfuzosin	UV	15.0 (LOQ)	n.r.	99–101	2.0
Caffeine (60)	Human urine, coffee and beverages	Caffeine	UV	n.r.	180–1800	n.r.	1.7–11.1

* LOD = limit of detection; LOQ = limit of quantification; n.r. = not reported.

column, and the mobile phase, which was used to perform the separation on the analytical column, is very important. This problem has been successfully solved by introducing the eluent from the MIP precolumn to a sampling loop and subsequently injecting it into the analytical column. This method was used for the determination of caffeine in human urine, coffee, and beverages (60).

6. Immunoaffinity extraction sorbents

The selectivity can be greatly enhanced by using materials involving antibodies chemically bonded onto silica-based sorbent to form so-called immunosorbent (IS) (61,62) which can be packed into precolumns for on-line extraction (63). Several examples of the on-line coupling of SPE with immunosorbents to LC are presented in Table VII. Bascarán et al. developed a new method for determination ochratoxin A in milk samples based on immunoaffinity column clean-up followed by on-line HPLC determination (64). Two different immunoaffinity columns were investigated. When compared with the OchraTest column, the Ochraprep provided higher recovery and lower RSD values.

Antibodies developed for low molecular weight molecules are not as selective as those developed for large proteins or virus, and usually they are able to recognize other molecules with similar structures. This so-called cross-reactivity of the antibodies was exploited and enhanced to obtain immunosorbents, which can isolate structurally related analytes. The characteristic of the antibodies allows the extraction of the mixture of seven phenylureas with recoveries > 75% (65). The method also can be used for the selective determination of a group of organic pollutants in groundwater and surface water (69). Compared to the US Environmental Protection Agency (EPA) reference method, the on-line method is an economical, time-saving, and user-friendly alternative.

7. Monolithic materials

Recently, a new type sorbent, monolithic material has attracted considerable attention. The monolith materials represent a relatively new class of stationary phases and sometimes they are also called continuous beds (phases) (70). The problems of large void volume between the packed particles and slow mass transfer, which exist in the conventional particulate material are overcome by this material (71). Monolithic materials can be explored as sorbents for on-line SPE. Several examples of the on-line coupling of SPE with monolithic materials to LC are presented in Table VIII.

Wei et al. developed an on-line method for determination of 1-adrenergic receptor antagonists including terazosin, alfuzosin, prazosin, and doxazosin in human plasma (74). The monolithic poly(glycidyl methacrylate-coethylene glycol dimethacrylate) [poly(GMA-EDMA)] WCX material used as SPE sorbent has good

Table VII. Applications of On-line Coupling of SPE with Immunosorbents to LC*

Analyte(s)	Sample matrix	Detection method	LOD (ng/mL)	Linearity range (ng/mL)	Recovery (%)	Precision (RSD, %)
Ochratoxin A (62)	Milk	Fluorescence	0.0005	0.005–0.1	72.3–97.5	2.2–6.5
Phenylureas (63)	River Seine water	UV	0.005–0.020	0.03–0.7	> 75	1.3–8.1
Flunitrazepam and metabolites (64)	Urine	UV	2.0	5–1000	> 92	< 9.9
β-Agonists (65)	Urine	MS–MS	0.01–0.05	0.05–10	n.r.	5.7–17.1
4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)-carbamoyl] benzoic acid (66)	Plasma	Radioimmunoassay	0.5 (LOQ)	0.5–10	88.9	4.0–11.9
Organic pollutants (67)	Water	MS	0.3	3–300	92	n.r.

* LOD = limit of detection; LOQ = limit of quantification; n.r. = not reported.

Table VIII. Applications of On-line Coupling of SPE with Monolithic Materials to LC*

Analyte(s)	Sample matrix	Detection method	LOD (ng/mL)	Linearity range (ng/mL)	Recovery (%)	Precision (RSD, %)
Pharmaceutical residues (70)	Water	MS	0.008–0.317	n.r.	9–107	5–48
Hydrophobic pharmaceutical compound and its metabolite (71)	Urine	MS-MS	n.r.	1.03–103	93–100	5.4–6.3
α1-Adrenergic receptor antagonists (72)	Plasma	UV	0.5	5–5000	> 80	< 15
Doxazosin (73)	Plasma	UV	n.r.	n.r.	> 90	0.8–2.6
Nicardipine; amlodipine (74)	Plasma	UV	0.2	0.5–50	75.5–83.0	2.9–20.5
DL-tetrahydropalmatine (75)	Corydalis yanhusuo	UV	1500	10000–300000	91	< 7
Phenolic compounds (76)	Water	UV	0.06	0.05–20	45.2–95.2	< 8
Bisphenol A (77)	Water	MS-MS	0.0003	0.001–0.16	53–113	1.5–6.3
Aflatoxin B1 (78)	Aqueous solutions	Fluorescence	50	100–1000	78.7–104.3	7.0
Drugs and metabolites (79)	Plasma	MS-MS	1.95 (LOQ)	1.95–1000	92.4–105.8	< 15

*LOD = limit of detection; LOQ = limit of quantification; n.r. = not reported.

permeability and biocompatibility and can be reused more than 300 times. The plasma samples obtained from hypertensive patients were analyzed, and the results were in agreement with those obtained by the conventional LLE-HPLC method. A method for determination of DL-tetrahydropalmatine (DL-THP) in Corydalis yanhusuo, a traditional Chinese herb, has been developed by Ou et al. (77). The L-THP imprinted monolithic column was prepared in stainless steel column by in situ polymerization method. Further, the monolith as the precolumn was on-line coupled with C18 column for determination of L-THP and its analogues. A rigid anti-bisphenol A (BPA) immunoaffinity column was successfully prepared by immobilizing BPA antibodies on the poly (ethylene dimethacrylate-glycidyl methacrylate) monolith via Schiff base reaction (79). The obtained monolithic column was on-line coupled to LC–MS–MS, and then applied to analyze BPA in real environmental water samples. The LOD is 0.3 ng/L when the sample volume was 100 mL.

On-line Coupling of SPE-LC to Other Sample Preparation Techniques

The on-line SPE-LC method is mainly used for the determination of liquid samples. If the samples are solid, the analytes in the sample matrix were usually extracted into solvent first. Then, the extract was introduced into the on-line system for further treatment and analysis. Alternatively, the SPE-LC method also can be further coupled on-line to other sample preparation techniques, such as supercritical fluid extraction (SFE), subcritical water extraction (SWE), microwave-assisted extraction (MAE), or ultrasound-assisted extraction (UAE) for direct analyzing solid samples (Table IX). In these coupling systems, the SPE column was used as interface for connecting SFE, SWE, MAE or UAE to LC.

Carbon dioxide is the typical extraction fluid in SFE. The SFE system can be easily on-line coupling with chromatography as CO_2 becomes gas upon depressurization and is easily eliminated from the analytical system. The solvating property of the fluid is also reduced substantially during the depressurization (82). The SFE was coupled with supercritical fluid chromatography (SFC) using a silica gel trap column as interface for the determination of capsaicinoids in the fruits of *Capsicum annuum L* (83). This method didn't need other pretreatment or much organic solvent. It is fast as one sample analysis can be completed within 20 min. An on-line method for determining nitroaromatic explosives in vapour phases is developed by combining SFE, SPE, and LC (84). The air samples were collected on the glass filters and polyurethane foam sorbents. The analytes extracted by SFE were transferred into the HPLC system via a PGC trap.

In pressurized liquid extraction (PLE), the extraction is performed with solvents at high temperature (up to 200°C) and pressure (up to 20,000 kPa) (85). A subclass of PLE is subcritical water extraction (SWE). The solubility of the analytes in water decreases dramatically when the water is cooled to ambient temperature. Trapping of the analytes to a solid-phase trap is thus relatively easy. The SWE has been coupled with SPE and chromatographic analysis for the determination of acid herbicides in different types of soil (86). A minicolumn containing C18 sorbent was proved as excellent material for the quantitative preconcentration of the 18 herbicides. The recoveries were in the range of 94.2–113.1%, and repeatabilities, expressed as RSDs, were in the range of 0.61–6.83%. The similar technique was also used for rapid determination of polar and medium polar contaminants in soil (87). Under the optimized conditions, recoveries of 11 out of 13 analytes ranged from 82% to 103% while those of the least hydrophilic pesticides, neburon and prochloraz, were 73% and 63%, respectively.

The MAE process involves disruption of hydrogen bonds, as a result of microwave-induced dipole rotation of molecules, and migration of ions, which enhance the penetration of solvent into matrix (89). Chen et al. developed a rapid technique based on dynamic MAE coupled on-line with SPE–HPLC for the determination of organochlorine pesticides (OCPs) in grain samples (90). The reliability and repeatability of the method are

Table IX. On-line Coupling of SPE-LC to other Sample Preparation Techniques*

Analyte(s)	Sample matrix	SPE material	Coupling technique	LOD (ng/g)	Linearity range (ng/g)	Recovery (%)	Precision (RSD, %)
Capsaicinoids (81)	*Capsicum annuum* L	Silica gel	SFE-SPE-LC–UV	500 ng	10000–10180000	92.1	n.r.
Explosives (82)	Vapour phase	PGC	SFE-SPE-LC–UV	9.5–56.8 ng	n.r.	87–103	2.6–10.9
Chlorophenoxy acid herbicides (84)	Soil	C18	SWE-SPE-LC–UV	n.r.	n.r.	91.2–113.1	0.6–6.8
Polar and medium polar contaminants (85)	Soil	C18	SWE-SPE-LC–MS	n.r.	n.r.	63–103	8–15
Chlorophenols, chloroanilines, (86) methylanilines, caffeine, nitrotoluenes and polychlorinated biphenyls (86)	Sand or paper	C18	SWE-SPE-LC–UV	n.r.	n.r.	87–108	3–24
Organochlorine pesticides (88)	Grain samples	C18	MAE-SPE-LC–UV	19–37	80–4000	86–105	1.2–8.7
polycyclic aromatic hydrocarbons (89)	Soil	C18	MAE-SPE-LC–FD	30	n.r.	98–99	2.5–5.6
N-methylcarbamates (90)	Soil and food	C18	UAE-SPE-LC–FD	3-12	0.5–10 µg/mL	77–101	3.1–7.5
Phenolic acids (91)	Lamiaceae herbs	SAX	UAE-SPE-LC–UV	2-5 ng	n.r.	90–106	0.4–11
Formaldehyde (92)	Textile	SCX	UAE-SPD-LC–UV	60	500–200000	97.1–98.2	3.2
Malondialdehyde (93)	Mouse tissue	Resin	SPD-LC–FD	17 ng/mL	190–5500 ng/mL	106	< 5
Estrogens (94)	Wastewater treatment plants	HLB	SPD-LC–MS	0.4-0.7 ng/L	1–400 ng/L	79.7–95.3	4.6–10.8

*LOD = limit of detection; n.r. = not reported; HLB = Hydrophile lipophile balance.

improved, and the risks of sample loss and contamination are decreased as well. Criado et al. used on-line MAE-SPE-LC system for determination of polycyclic aromatic hydrocarbons (PAHs) in soils (91). The LOD was 0.03 µg/g and the precision was 3%.

In the UAE, the sample was extracted with solvent under the help of acoustic vibration. The UAE has been coupled on-line with SPE, LC, post column derivatisation and fluorescence detection for the determination of *N*-methylcarbamates in soil and food (92). This method allows extraction of the carbamates from soil and food, with recoveries similar to those provided by the EPA method. A fast and efficient on-line coupled dynamic UAE to LC method was developed via a SPE trap filled with SAX material for the determination of phenolic acids in Lamiaceae herbs (93). The whole analysis process including extraction, clean-up and analysis can be completed within 30 min, which was about half of the time required for conventional off-line analysis.

The derivatization technique is important for increasing the sensitivity of some analytes. In the conventional procedures, the derivatization reaction occurs in the liquid phase. Afterward, a new derivatization method, solid phase derivatization (SPD), was developed, in which the reaction was carried out on solid support. The SPD technique combines the two steps of extraction and derivatization, thus minimizes the number of steps for sample purification and concentration, and offers the possibility of automation (94).

In the SPD, the derivatization may occur simultaneously with extraction, or subsequent to extraction. In the first case, a derivatization reagent was adsorbed on the SPE sorbent prior to introducing the analytes. An example is the analysis of malondialdehyde with dansyl hydrazine as derivatization reagent followed by the on-line HPLC determination (95). Compared with the conventional method, the proposed method has advantages in automation, selectivity, precision (RSD < 5) and sensitivity (LOD, 0.02 µg/mL). In the second case, the derivatization reagent was introduced into the SPE column after loading the analytes. An on-line method by coupling of SPD to LC–MS was developed for determination of estrogens in water (96). The optimized on-line protocol has been demonstrated to be an improvement over existing methods due to its greater sensitivity (LOQ, 1.0 ng/L) with low sample volume (1.0 mL) and the total analysis time (extraction, derivatization and determination) is less than 17 min.

Conclusions

The on-line coupling of SPE to LC has been widely used in recent years. In this technique, the inherent advantages of SPE and LC are put together in a single methodology with the following features: sensitivity, selectivity, accuracy, reliability, automation, high throughput, and minimal sample manipulation. Most sorbents used in the off-line SPE also can be used in the on-line SPE. The selectivity of classic SPE sorbents including the chemically bonded silica, ion-exchange and carbon materials is limited. In addition to the target analytes, many matrix constituents can also be enriched by these sorbents, and thus disturb the subsequent chromatographic separation and detection. Nowadays, there is a considerable interest in developing selective sorbents including RAM, MIP, and IS for extracting and isolating components from complex sample matrices. The monolithic materials also have attracted great interests in recent years due to their high permeability. The SPE-LC technique can be further coupled on-line with other sample preparation techniques, such as SFE, SWE, MAE, and UAE for directly analyzing solid samples, in which all analysis and sample extraction procedures take place in a closed, automated system. The SPD technique combines the two steps of extraction and derivatization, and also can be used in the on-line analysis for increasing the analytical efficiency.

Acknowledgements

This work was supported by the Natural Science Foundation of China. (Grant number: 20875037).

Reference

1. M. Gilar, E.S.P. Bouvier, and B.J. Compton. Advances in sample preparation in electromigration, chromatographic and mass spectrometric separation methods. *J. Chromatogr. A* **909:** 111–135 (2001).
2. S. Rodriguez-Mozaz, M.J. Lopez de Alda, and D. Barceló. Advantages and limitations of on-line solid phase extraction coupled to liquid chromatography–mass spectrometry technologies versus biosensors for monitoring of emerging contaminants in water. *J. Chromatogr. A* **1152:** 97–115 (2007).
3. C.F. Poole. New trends in solid-phase extraction. *Trends Anal. Chem*. **22:** 362–373 (2003).
4. B.Á. Sánchez, F.P. Capote, J.R. Jiménez, and M.D. Luque de Castro. Automated solid-phase extraction for concentration and clean-up of female steroid hormones prior to liquid chromatography–electrospray ionization–tandem mass spectrometry: an approach to lipidomics. *J. Chromatogr. A* **1207:** 46–54 (2008).
5. Y. Watabe, T. Kondo, T. Nishikawa, T. Fujita, K. Kaya, and K. Hosoya. Fully automated liquid chromatography–mass spectrometry determination of 17β-estradiol in river water. *J. Chromatogr. A* **1120:** 252–259 (2006).
6. J.B. Quintana, M. Miró, J.M. Estela, and V. Cerdà. Automated on-line renewable solid-phase extraction-liquid chromatography exploiting multisyringe flow injection-bead injection lab-on-valve analysis. *Anal. Chem.* **78:** 2832–2840 (2006).
7. T. Hyötyläinen. Principles, developments and applications of on-line coupling of extraction with chromatography. *J. Chromatogr. A* **1153:** 14–28 (2007).
8. N. Masqué, R.M. Marcé, and F. Borrull. New polymeric and other types of sorbents for solid-phase extraction of polar organic micropollutants from environmental water. *Trends Anal. Chem*. **17:** 384–394 (1998).
9. N. Fontanals, R.M. Marcé, and F. Borrull. New hydrophilic materials for solid-phase extraction. *Trends Anal. Chem.* **24:** 394–406 (2005).
10. L.A. Berrueta, B. Gallo, and F. Vicente. A review of solid phase extraction: basic principles and new developments. *Chromatographia* **40:** 474–483 (1995).
11. X. Ye, L.J. Tao, L.L. Needham, and A.M. Calafat. Automated on-line column-switching HPLC–MS/MS method for measuring environmental phenols and parabens in serum. *Talanta* **76:** 865–871 (2008).
12. C. Cheng and H.-R.Tsai. Analysis of steroids in yeast-mediated cell culture by on-line solid-phase extraction coupled high-performance liquid chromatography electrospray- ionization/mass spectrometry and novel continuous postcolumn infusion of internal standard technique. *Anal. Chim. Acta.* **623**: 168–177 (2008).
13. S. Wang, W. Huang, G. Fang, J. He, and Y. Zhang. On-line coupling of solid-phase extraction to high-performance liquid chromatography for determination of estrogens in environment. *Anal. Chim. Acta* **606:** 194–201 (2008).
14. J. Li, L. Chen, X. Wang, H. Jin, L. Ding, K. Zhang, and H. Zhang. Determination of tetracyclines residues in honey by on-line solid-phase extraction high-performance liquid chromatography. *Talanta* **75:** 1245–1252 (2008).
15. I. Lefebvre, J.-Y. Puy, C. Perrin, and C. Périgaud. Quantification of zidovudine and its monophosphate in cell extracts by on-line solid-phase extraction coupled to liquid chromatography. *J. Chromatogr. B* **858:** 2–7 (2007).
16. L. Chen, A.Yu, X. Zhuan, K. Zhang, X. Wang, L. Ding, and H. Zhang. Determination of andrographolide and dehydroandrographolide in rabbit plasma by on-line solid phase extraction of high-performance liquid chromatography. *Talanta* **74**: 146–152 (2007).
17. N. Ansermot, M. Fathi, J.-L. Veuthey, J. Desmeules, D. Hochstrasser, and S. Ru. Quantification of cyclosporine A in peripheral blood mononuclear cells by liquid chromatography-electrospray mass spectrometry using a column-switching approach. *J. Chromatogr. B* **857:** 92–99 (2007).

18. D. Whittington, P. Sheffels, and E.D. Kharasch. Stereo selective determination of methadone and the primary metabolite EDDP in human plasma by automated on-line extraction and liquid chromatography mass spectrometry. *J. Chromatogr. B* **809:** 313–321 (2004).
19. J. Sachse, S. Härtter, H. Weigmann, and C. Hiemke. Automated determination of amisulpride by liquid chromatograph with column switching and spectrophotometric detection. *J. Chromatogr. B* **784:** 405–410 (2003).
20. H.S. Lee, C.K. Jeong, H.M. Lee, S.J. Choi, K. S. Do, K. Kim, and Y.H. Kim. On-line trace enrichment for the simultaneous determination of microcystins in aqueous samples using high-performance liquid chromatography with diode-array detection. *J. Chromatogr. A* **848:** 179–184 (1999).
21. S.C. Salado and L.E. Vera-Avila. On-line solid-phase extraction and high-performance liquid chromatographic determination of chlorthalidone in urine. *J. Chromatogr. B* **690:** 195–202 (1997).
22. S. Bompadre, L. Ferrante, L. Leone, and S. Ripa. Quantification of trovafloxacin in serum by high-performance liquid chromatography with on-line solid-phase extraction. *Chromatographia* **49:** 185–187 (1999).
23. E. Yamamoto, T. Sakaguchi, T. Kajim, N. Mano, and N. Asakawa. Novel methylcellulose- immobilized cation-exchange precolumn for on-line enrichment of cationic drugs in plasma. *J. Chromatogr. B* **807:** 327–334 (2004).
24. P. Chiap, A. Ceccato, B.M. Buraglia, B. Boulanger, and P. Hubert. Development and validation of an automated method for the liquid chromatographic determination of sotalol in plasma using dialysis and trace enrichment on a cation-exchange precolumn as on-line sample preparation. *J. Pharm. Biomed. Anal.* **24:** 801–814 (2001).
25. F. Wang, X. Jiang, S. Feng, R. Tian, X. Jiang, G. Han, H. Liu, M. Ye, and H. Zou. Automated injection of uncleaned samples using a ten-port switching valve and a strong cation-exchange trap column for proteome analysis. *J. Chromatogr. A* **1171:** 56–62 (2007).
26. M.A. Obandoa, J.M. Estela, and V.Cerdá. Multi-syringe chromatography (MSC) system for the on-line solid-phase extraction and determination of hydrochlorothiazide and losartan potassium in superficial water, groundwater and wastewater outlet samples. *J. Pharm. Biomed. Anal.* **48:** 212–217 (2008).
27. J. Patsias, A. Papadopoulou, and E. Papadopoulou-Mourkidou. Automated trace level determination of glyphosate and aminomethyl phosphonic acid in water by on-line anion-exchange solid-phase extraction followed by cation-exchange liquid chromatography and post-column derivatization. *J. Chromatogr. A* **932:** 83–90 (2001).
28. E. Matisová and S. Krabáková. Carbon sorbents and their utilization for the preconcentration of organic pollutants in environmental samples. *J. Chromatogr. A* **707:** 145–179 (1995).
29. C. Crescenzi, A. Di Corcia, A. Marcomini, and R. Samperi. Determination of nonionic polyethoxylate surfactants in environmental waters by liquid chromatography/electrospray mass spectrometry. *Anal. Chem.* **67:** 1797–1804 (1995).
30. M.-C. Hennion. Graphitized carbons for solid-phase extraction. *J. Chromatogr. A* **885:** 73–95 (2000).
31. J. Slobodník, Ö. Öztezkizan, H. Lingeman, and U.A.Th. Brinkman. Solid-phase extraction of polar pesticides from environmental water samples on graphitized carbon and Empore-activated carbon disks and on-line coupling to octadecyl-bonded silica analytical columns. *J. Chromatogr. A* **750:** 227–238 (1996).
32. J. M. Soriano, B. Jiménez, J. C. Moltó, and G. Font. Behaviour of graphitized carbon black in the extraction of polar nonionic nitrogen-containing pesticides a checking of hypothese. *J. Liq. Chromatogr. Relat. Technol.* **23:** 2829–2838 (2000).
33. V. Coquart and M.-C. Hennion. Trace-level determination of polar phenolic compounds in aqueous samples by high-performance liquid chromatography and on-line preconcentration on porous graphitic carbon. *J. Chromatogr. A* **600:** 195–201 (1992).
34. D. Puig and D. Barcelö. Comparison of different sorbent materials for on-line liquid solid extraction followed by liquid chromatographic determination of priority phenolic compounds in environmental waters. *J. Chromatogr. A* **733:** 371–381 (1996).
35. C. Crescenzi, J. Albiñana, H. Carlsson, E. Holmgren, and R. Batlle. On-line strategies for determining trace levels of nitroaromatic explosives and related compounds in water. *J. Chromatogr. A* **1153:** 186–193 (2007).
36. G.-Z. Fang, J.-X. He, and S. Wang. Multiwalled carbon nanotubes as sorbent for on-line coupling of solid-phase extraction to high-performance liquid chromatography for simultaneous determination of 10 sulfonamides in eggs and pork. *J. Chromatogr. A* **1127:** 12–17 (2006).
37. S. Souverain, S. Rudaz, and J.-L. Veuthey. Restricted access materials and large particle supports for on-line sample preparation: an attractive approach for biological fluids analysis. *J. Chromatogr. B* **801:** 141–156 (2004).
38. P. Sadílek, D. Satínsky, and P. Solich. Using restricted-access materials and column switching in high-performance liquid chromatography for direct analysis of biologically-active compounds in complex matrices. *Trends Anal. Chem.* **26:** 375–384 (2007).
39. E. Rozet, R. Morello, F. Lecomte, G.B. Martin, P. Chiap, J. Crommenc, K.S. Boos, and Ph. Hubert. Performances of a multidimensional on-line SPE-LC-ECD method for the determination of three major catecholamines in native human urine: validation, risk and uncertainty assessments. *J. Chromatogr. B* **844**: 251–260 (2006).
40. R. Busquets, L. Puignou, and M.T. Galceran. Determination of 2-amino-1-methyl-6- phenylimidazo(4,5-b)pyridine (PhIP) in hemoglobin using on-line coupling of restricted access material to liquid chromatography–mass spectrometry. *Anal. Chim. Acta* **559:** 45–53 (2006).
41. P. Chiap, M. Piette, B. Evrard, F. Frankenne, B. Christiaens, G. Piel, D. Cataldo, J.-M. Foidart, L. Delattre, J. Crommen, and Ph. Hubert. Automated method for the determination of a new matrix metalloproteinase inhibitor in ovine plasma and serum by coupling of restricted access material for on-line sample clean-up to liquid chromatography. *J. Chromatogr. B* **817:** 109–117 (2005).
42. B. Christiaens, M. Fillet, P. Chiap, O. Rbeida, A. Ceccato, B. Streel, J. De Graeve, J. Crommen, and Ph. Hubert. Fully automated method for the liquid chromatographic–tandem mass spectrometric determination of cyproterone acetate in human plasma using restricted access material for on-line sample clean-up. *J. Chromatogr. A* **1056:** 105–110 (2004).
43. R. Preuss and J. Angerer. Simultaneous determination of 1- and 2-naphthol in human urine using on-line clean-up column-switching liquid chromatography–fluorescence detection. *J. Chromatogr. B* **801:** 307–316 (2004).
44. N. Amini and C. Crescenzi. Feasibility of an on-line restricted access material/liquid chromatography/tandem mass spectrometry method in the rapid and sensitive determination of organophosphorus triesters in human blood plasma. *J. Chromatogr. B* **795:** 245–256 (2003).
45. C. Mišlanová, A. Stefancová, J. Oravcová, J. Horecky, T. Trnovec, and W. Lindner. Direct high-performance liquid chromatographic determination of (R)- and (S)-propranolol in rat microdialysate using on-line column switching procedures. *J. Chromatogr. B* **739:** 151–161 (2000).
46. J. Ding, N. Ren, L. Chen, and L. Ding, On-line coupling of solid-phase extraction to liquid chromatography–tandem mass spectrometry for the determination of macrolide antibiotics in environmental water. *Anal. Chim. Acta* **634:** 215–221 (2009).
47. E.A. Hogendoorn, E. Dijkman, and B. Baumann. Strategies in using analytical restricted access media columns for the removal of humic acid interferences in the trace analysis of acidic herbicides in water samples by coupled column liquid chromatography with UV detection. *Anal. Chem.* **71:** 1111–1118 (1999).
48. E. Blahová, L. Bovanová, and E. Brandšteterová. Direct HPLC analysis of trimethoprim in milk. *J. Liq. Chromatogr. Relat. Technol.* **24:** 3027–3035 (2001).
49. J. Chico, S. Meca, R. Companyó, M.D. Prat, and M. Granados. Restricted access materials for sample clean-up in the analysis of trace levels of tetracyclines by liquid chromatography Application to food and environmental analysis. *J. Chromatogr. A* **1181:** 1–8 (2008).
50. M. Petrovica, S. Tavazzib, and D. Barceloa. Column-switching system with restricted access precolumn packing for an integrated sample cleanup and liquid chromatographic–mass spectrometric analysis of alkylphenolic compounds and steroid sex hormones in sediment. *J. Chromatogr. A* **971:** 37–45 (2002).
51. H. Yang, S. Zhang, F. Tan, Z. Zhuang, and X. Wang. Surface molecularly imprinted nanowires for biorecognition. *J. Am. Chem. Soc.* **127:**1378–1379 (2005).
52. C. He, Y. Long, J. Pan, K. Li, and F. Liu. Application of molecularly imprinted polymers to solid-phase extraction of analytes from real samples. *J. Biochem. Biophy. Meth.* **70:** 133–150 (2007).
53. F.G. Tamayo, E. Turiel, and A. Martín-Esteban. Molecularly imprinted polymers for solid-phase extraction and solid-phase microextraction: Recent developments and future trends. *J. Chromatogr. A* **1152:** 32–40 (2007).
54. N. Masqué, R.M. Marcé, F. Borrull, P.A.G. Cormack, and D.C. Sherrington. Synthesis and evaluation of a molecularly imprinted polymer for selective on-Line solid-phase extraction of 4-nitrophenol from environmental water. *Anal. Chem.* **72:** 4122-4126 (2000).
55. E. Caro, R.M. Marcé, P.A.G. Cormack, D.C. Sherrington, and F. Borrulla. On-line solid-phase extraction with molecularly imprinted polymersto selectively extract substituted 4-chlorophenols and 4-nitrophenol from water. *J. Chromatogr. A* **995:** 233–238 (2003).
56. Y. Watabe, T. Kondo, M. Morita, N. Tanaka, J. Haginaka, and K. Hosoy. Determination of bisphenol A in environmental water at ultra-low level by high-performance liquid chromatography with an effective on-line pretreatment device. *J. Chromatogr. A* **1032:** 45–49 (2004).
57. B. Bjarnason, L. Chimuka, and O. Ramstrom. On-Line solid-phase extraction of triazine herbicides using a molecularly imprinted polymer for selective sample enrichment. *Anal. Chem.* **71:** 2152–2156 (1999).
58. L.I. Andersson, M. AbdeI-Rehim, L. Nicklasson, L. Schweitz, and S. Nilsson. Towards molecular-imprint based SPE of local anaesthetics. *Chromatographia* Suppl. **55:** S65–S69 (2002).
59. F. Hugon-Chapuis, J.U. Mullot, G. Tuffalb, M.-C. Henniona, and V. Pichona. Selective and automated sample pretreatment by molecularly imprinted polymer for the analysis of the basic drug alfuzosin from plasma. *J. Chromatogr. A* **1196:** 73–80 (2008).
60. G. Theodoridis, C.K. Zacharis, P.D. Tzanavaras, D.G. Themelis, and A. Economou. Automated sample preparation based on the sequential injection principle Solid-phase extraction on a molecularly imprinted polymer coupled on-line to high-performance liquid chromatography. *J. Chromatogr. A* **1030:** 69–76 (2004).
61. F. Becher, E. Duriez, H. Volland, J. C. Tabet, and E. Ezan. Detection of functional ricin by immunoaffinity and liquid chromatography-tandem mass spectrometry. *Anal. Chem.* **79:** 659–665 (2007).
62. D. Sircar and P.V. Subbaiah. Isoprostane measurement in plasma and urine by liquid chromatography–mass spectrometry with one-step sample preparation. Clin. Chem. **53:** 251–258 (2007).
63. D. Stevenson. Immuno-affinity solid-phase extraction. *J. Chromatogr. B* **745:** 39–48 (2000).
64. V. Bascarán, A. H. de Rojas, P. Chouciño, and T. Delgado. Analysis of ochratoxin A in milk after direct immunoaffinity column clean-up by high-performance liquid chromatography with fluorescence detection. *J. Chromatogr. A* **1167:** 95–101 (2007).
65. E. Schoenzetter, V. Pichon, D. Thiebaut, A. Fernandez-Alba, and M.-C. Hennion. Rapid sample handling in microcolumn-liquid chromatography using selective

on-line immunoaffinity extraction. *J. Microcolumn Separations* **12:** 316–322 (2000).
66. I. Deinl, L. Angermaier, C. Franzelius, and G. Machbert. Simple high-performance liquid chromatographic column-switching technique for the on-line immunoaffinity extraction and analysis of flunitrazepam and its main metabolites in urine. *J. Chromatogr. B* **704:** 251–258 (1997).
67. J. Cai and Jack Henion. Quantitative multi-residue determination of 3-agonists in bovine urine using on-line immunoaffinity extraction-coupled column packed capillary liquid chromatography-tandem mass spectrometry. *J. Chromatogr. B* **691:** 357–370 (1997).
68. M. Itoh and G. Kominami. On-line immunoaffinity extraction followed by high-performance liquid chromatography and radioimmunoassay for a novel retinobenzoic acid, AM-80, inhuman plasma. *J. Immunoass. Immunoch.* **22:** 213–223 (2001).
69. S. Ouyang, Y. Xu, and Y. H. Chen. Selective determination of a group of organic compounds in complex sample matrixes by LC/MIMS with on-Line immunoaffinity extraction. *Anal. Chem.* **70:** 931–935 (1998).
70 A. Maruška and O. Kornyšova. Application of monolithic (continuous bed) chromatographic columns in phytochemical analysis. *J. Chromatogr. A* **1112:** 319–330 (2006).
71. E.G. Vlakh and T.B. Tennikova. Preparation of methacrylate monoliths. *J. Sep. Sci.* **30:** 2801–2813 (2007).
72. J. Bones, K. Thomas, P.N. Nesterenko, and B. Paull. On-line preconcentration of pharmaceutical residues from large volume water samples using short reversed-phase monolithic cartridges coupled to LC-UV-ESI-MS. *Talanta* **70:** 1117–1128 (2006).
73. R.N. Xu, B. Boyd, M.J. Rieser, and T.A. El-Shourbagy. 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:** 2943–2949 (2007).
74. X. Wei, J. Yin, G. Yang, C. He, and Y. Chen. On-line solid-phase extraction with a monolithic weak cation-exchange column and simultaneous screening of a1-adrenergic receptor antagonists in human plasma. *J. Sep. Sci.* **30:** 2851–285 (2007).
75. G. Yang, H. Liu, Y. Zhang, S. Wang, J. Yi, and B. Yin, Y. Chen. On-line simultaneous removal of human serum albumin and enrichment of doxazosin using a weak cation-exchange monolithic column. *J. Chromatogr. A* **1129:** 231–235 (2006).
76. X. Wei, G. Yang, L. Qi, and Y. Chen. Determination of nicardipine and amlodipine in human plasma using on-line solid-phase extraction with a monolithic weak cation-exchange column. *Talanta* **77:** 1197–1202 (2009).
77. J. Ou, L. Kong, C. Pan, X. Su, X. Lei, and H. Zou. Determination of dl-tetrahydropalmatine in Corydalis yanhusuo by l-tetrahydropalmatine imprinted monolithic column coupling with reversed-phase high performance liquid chromatography. *J. Chromatogr. A* **1117:** 163–169 (2006).
78. J. Ou, L. Hu, L. Hu, X. Li, and H. Zou. Determination of phenolic compounds in river water with on-line coupling bisphenol A imprinted monolithic precolumn with high performance liquid chromatography. *Talanta* **69:** 1001–1006 (2006).
79. L. Li, J. Wang, S. Zhou, and M. Zhao. Development and characterization of an immunoaffinity monolith for selective on-line extraction of bisphenol A from environmental water samples. *Anal. Chim. Acta.* **620:** 1–7 (2008).
80. E. Calleri, G. Marrubini, G. Brusotti, G. Massolini, and G. Caccialanza. Development and integration of an immunoaffinity monolithic disk for the on-line solid-phase extraction and HPLC determination with fluorescence detection of aflatoxin B1 in aqueous solutions. *J. Pharm. Biomed. Anal.* **44:** 396–403 (2007).
81. X. Zang, R. Luo, N. Song, T. K. Chen, and H. Bozigian. A novel on-line solid-phase extraction approach integrated with a monolithic column and tandem mass spectrometry for direct plasma analysis of multiple drugs and metabolites. *Rapid Commun. Mass Spectrom.* **19:** 3259–3268 (2005).
82. T. Hyötyläinen. Principles, developments and applications of on-line coupling of extraction with chromatography. *J. Chromatogr. A* **1153:** 14–28 (2007).
83. K. Sato, S.S. Sasaki, Y. Goda, T. Yamada, O. Nunomura, K. Ishikawa, and T. Maitani. Direct connection of supercritical fluid extraction and supercritical fluid chromatography as a rapid quantitative method for capsaicinoids in placentas of capsicum. *J. Agric. Food Chem.* **47:** 4665–4668 (1999).
84. R. Batlle, H. Carlsson, E. Holmgren, A. Colmsjö, and C. Crescenzi. On-line coupling of supercritical fluid extraction with high-performance liquid chromatography for the determination of explosives in vapour phases. *J. Chromatogr. A* **963:** 73–82 (2002).
85. E. Björklund and T. Nilsson. Pressurised liquid extraction of persistent organic pollutants in environmental analysis. *Trends Anal. Chem.* **19:** 434–445 (2000).
86. J.L. Luque-García and M.D. Luque de Castro. Coupling continuous subcritical water extraction, filtration, preconcentration, chromatographic separation and UV detection for the determination of chlorophenoxy acid herbicides in soils. *J. Chromatogr. A* **959:** 25–35 (2002).
87. C. Crescenzi, A. D. Corcia, M. Nazzari, and R. Samperi. Hot phosphate-buffered water extraction coupled on-line with liquid chromatography/mass spectrometry for analyzing contaminants in soil. *Anal. Chem.* **72:** 3050–3055 (2000).
88. B. Li, Y. Yang, Y. Gan, C.D. Eaton, P. He, and A.D. Jones. On-line coupling of subcritical water extraction with high-performance liquid chromatography via solid-phase trapping. *J. Chromatogr. A* **873:** 175–184 (2000).
89. L. Chen, H. Jin, L. Ding, H. Zhang, X.Wang, Z. Wang, J. Li, C. Qu, Y. Wang, and H. Zhang. On-line coupling of dynamic microwave-assisted extraction with high-performance liquid chromatography for determination of andrographolide and dehydroandrographolide in Andrographis paniculata Nees. *J. Chromatogr. A* **1140:** 71–77 (2007).
90. L. Chen, L. Ding, H. Jin, D. Song, H. Zhang, J. Li, K. Zhang, Y. Wang, and H. Zhang. The determination of organochlorine pesticides based on dynamic microwave-assisted extraction coupled with on-line solid-phase extraction of high-performance liquid chromatography. *Anal. Chim. Acta* **589:** 239–246 (2007).
91. A. Criado, S. Cárdenas, M. Gallego, and M. Valcárcel. Direct automatic screening of soils for polycyclic aromatic hydrocarbons based on microwave-assisted extraction/fluorescence detection and on-line liquid chromatographic confirmation. *J. Chromatogr. A* **1050:** 111–118 (2004).
92. A. Caballo-López and M.D. Luque de Castro. Continuous ultrasound-assisted extraction coupled to on line filtration–solid-phase extraction–column liquid chromatography– post column derivatisation–fluorescence detection for the determination of N-methylcarbamates in soil and food. *J. Chromatogr. A* **998:** 51–59 (2003).
93. M. Kivilompolo and T. Hyötyläinen. On-line coupled dynamic sonication-assisted extraction– liquid chromatography for the determination of phenolic acids in Lamiaceae herbs. *J. Chromatogr. A* **1216:** 892–896 (2009).
94. L. Chen, H. Jin, L. Wang, L. Sun, H. Xu, L. Dinga, A. Yu, and H. Zhang. Dynamic ultrasound-assisted extraction coupled on-line with solid support derivatization and high-performance liquid chromatography for the determination of formaldehyde in textiles. *J. Chromatogr. A* **1192:** 89–94 (2008).
95. H.L. Lord, J. Rosenfeld, S. Raha, and M.J. Hamadeh. Automated derivatization and analysis of malondialdehyde using column switching sample preparation HPLC with fluorescence detection. *J. Sep. Sci.* **31:** 387–401 (2008).
96. A. Salvador, C. Moretton, A. Piram, and R. Faure. On-line solid-phase extraction with on-support derivatization for high-sensitivity liquid chromatography tandem mass spectrometry of estrogens in influent/effluent of wastewater treatment plants. *J. Chromatogr. A* **1145:** 102–109 (2007).

Manuscript received December 11, 2008;
revision received February 25, 2008.