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Review

Direct analysis of food samples by high-performance liquid chromatography

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Abstract

A short review on the sample analysis of food samples by high-performance liquid chromatography is presented. The paper is focused on direct injection of liquid samples, automated solid-phase extraction and column switching techniques, on-line dialysis and application of restricted-access media.

Keywords: Food analysis; Reviews; Column switching; Dialysis; Restricted-access media; Injection methods

Contents

1. Introduction

High-performance liquid chromatography (HPLC) is generally preferred in food analysis as a confirmatory method to immunological or microbial inhibition screening tests for the determination of additives, contaminants and natural compounds. Screening methods, except for a few immunoassays, are unable to determine individual components, and very often false-positive results are obtained.

The HPLC technique with UV, diode array, fluorescence or electrochemical detection, has an important place in the field of residual analysis. This technique gives one a real chance to separate simultaneously all analyzed compounds, together with their metabolites and degradation products. Very often this technique enables the determination of low

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concentration levels of one analyzed analyte in the presence of many other interfering and coeluting components which is valuable especially in combination with an effective sample preparation technique, mainly in the on-line mode. It is generally known that of the most difficult and time and labor consuming steps of the complete HPLC assay, there is clean-up and preconcentration of analytes from the biomatrix.

The commonly utilized preparation procedures as liquid–liquid extraction (LLE), solid-phase extraction (SPE), matrix solid-phase dispersion (MSPD) very often require many individual steps and they are applied in many cases off-line before the HPLC assay. The SPE technique is an exception, as it could be combined on-line with the HPLC equipment and in this configuration it represents one of the methods of direct analysis of samples.

In this article, the latest applications published in the literature from 1994 to 1999 are reviewed. All these contributions are dealing with direct analysis of food samples or their crude extracts. Authors of some articles published a comparison of direct analysis with other commonly applied preseparation, clean-up and preconcentration procedures. Special attention is devoted to column switching techniques. The paper focuses on the HPLC of all food matrices which can be injected directly into the column switching system without or with the simple preparation step.

The contribution is divided into the following parts: direct injection, automatic SPE and column switching, on-line dialysis, and application of RAM sorbents.

The preseparation and separation conditions are summarized in Tables 1–4.

The abbreviations used are listed at the end of this paper.

2. Direct injection

Alcoholic and non-alcoholic beverages can be in some cases analyzed directly after dilution and/or filtration. The ''cleanness'' of a chromatogram depends on the amount of colorants, preservatives and antioxidants, natural and artificial sweeteners and flavors, etc., present in samples as interferences.

In wine phenolic analysis, extraction induces many artefacts due to oxidation, isomerization or hydrolysis. Moreover, extraction is never exhaustive as wine is actually a partially colloidal solution; as a consequence, the recovery of many substances is low. Analysis by direct injection of wine into the chromatographic column avoided most of these difficulties, but required an efficient and thermostated column, a perfectly adapted gradient and the use of a diode-array detector. Up to 30 compounds might be identified and assayed [1].

Normal-phase HPLC on a CN column was developed for the quantitation of glucosides and isomers of a potential anti-fungal agent resveratrol in wine [2]. Free isomers were obtained by the enzymatic hydrolysis of glucosides with β-glucosidase. A 20-μl volume of untreated wine was injected into the HPLC system. Mean recoveries for all four analyzed compounds ranged from 100 to 106%. It was possible to detect the mentioned compounds above 0.11–0.3 μmol/l. The whole analysis took approximately 50 min.

The *cis*- and *trans*-isomers of resveratrol and their glucosides, catechin, epicatechin, quercetin and rutin were quantitated in wine using RP-HPLC with gradient elution and diode-array detection [3]. ODS served as the stationary phase; the gradient was formed by acetic acid, methanol and water. Wine samples were directly injected onto the column. Each analysis required an equilibration period of 10 min and a run time of 40 min for completion. The detection limits ranged from 30 μg/l to 1.5 mg/l. Recoveries approximated 100% (range 95.2–105.5%), and the method provided good precision with RSDs between 1.17 and 3.38%. The column could be used for at least 200 assays (20 μl sample injections).

A comparative evaluation of four methods to measure the concentrations of *cis*- and *trans*-resveratrol as well as total resveratrol in commercial wines was performed [4]. Two of these methods utilized GC–MS analysis and two utilized direct injection HPLC in normal-phase mode (isocratic elution) with absorbance detection at 306 nm, and RP-HPLC with gradient elution and diode-array detection. In the former HPLC method, wine sample was injected directly onto a CN column. Normal-phase chromatography was performed with a water–acetonitrile–methanol mobile phase. *cis*- and *trans*-resveratrol eluted at ca. 35 and 48 min, respectively.

Table 1
The preseparation and separation conditions for direct injection methods

Compound	Matrix	Preseparation	Column	Mobile phase	Detection	Ref.
Resveratrol	Wine	DI	LiChrospher 100 CN 250×4 mm, 5 μm	Water–MeCN–MeOH (90:5:5)	UV 306 nm	[2]
Phenolics	Wine	DI	ODS Hypersil 250×4 mm, 5 μm	Gradient acetic acid–MeOH–water	DAD 265–369 nm	[3]
Resveratrol	Wine	DI	LiChrospher 100 CN 250×4 mm, 5 μm	Water–MeCN–MeOH (90:5:5)	UV 306 nm	[4]
Resveratrol	Wine	DI	ODS Hypersil 250×4 mm, 5 μm	Gradient acetic acid–MeOH–water	DAD 265–369 nm	[4]
Phenolics	Wine	DI	Superspher RP18 250×4 mm, 22.5°C	Gradient acetic acid–MeCN–water (gradient phosphoric acid–water–MeCN)	DAD 254–450 nm (ED, FD)	[1,5,6]
Sorbic acid	Wine	Dilution, filtration, DI	Aminex HPX 87-H 300×7.9 mm, 65°C	0.005 *M* H_2SO_4–MeCN (75:25)	UV 258 nm	[8]
Catechins	Tea	Filtration, DI	Alltima C_{18} 250×4.6 mm, 5 μm	Gradient MeCN–acetate buffer, pH 4.5	UV 210 nm	[9]
Phenolics	Brandy	Filtration, DI	LiChrospher 250×4 mm, 5 μm	Gradient MeOH–acetic acid–water	UV 280 nm	[10]
Thiabendazole	Juice	Dilution, centrifugation, DI	Ultracarb 30 ODS 150×4.6 mm, 5 μm	MeCN–MeOH–water–ethanolamine (37:11:52:0.02)	FD ex 305 nm em 345 nm	[11]
4-Vinylguaiacol	Beer, worts	Sonication, filtration (beer), dilution, centrifugation, chill (worts), DI	Nucleosil C_{18} 250×4 mm, 10 μm	Water–MeOH–H_3PO_4 (54:45:1)	FD ex 259 nm em 341 nm	[13]
4-Vinylguaiacol, ferulic acid	Beer, worts	Sonication, filtration (beer), dilution, centrifugation, chill (worts), DI	Nucleosil C_{18} 250×4 mm, 10 μm	Water–MeOH–H_3PO_4 (64:35:1)	ED +0.9 V	[13]
Prenyl-flavonoids	Hops, beer	Sonication (beer), DI	Phenomenex RP18 250×4 mm, 5 μm	Gradient MeCN–1% formic acid	APCI-MS	[14]

Table 2
The preseparation and separation conditions for automatic SPE and column switching methods

Compound	Matrix	Preseparation	Precolumn	Column	Mobile phase	Detection	Ref.
Heterocyclic aromatic amines	Food	Dilution, homogenization, off-line automatic SPE		TSK ODS-80 250×4.6 mm, 5 μm TSK ODS-Super 100×4.6 mm, 2 μm	Gradient MeCN–10 m*M* ammonium acetate buffer, pH 3.2–10 m*M* ammonium acetate buffer, pH 4.0	UV, FD, EI-MS	[16]
Aflatoxins	Milk, corn, nut	Centrifugation (milk), extraction, filtration, dilution (corn, nut), off-line automatic SPE		ODS C_{18} 150×4.6 mm, 5 μm	MeOH–water (50:50)	FD ex 365 nm em 400 nm	[17]
PAHs	Oil, fat	Dilution, filtration, column switching	Chromspher PI 80×3 mm, 20°C	Two Chromspher 5 PAH 250×4.6 mm, 20°C	Gradient water–MeCN–ethyl acetate	FD	[21]
Penicillins, oxacillins	Pork meat	Extraction, column switching	LiChroCART LiChrospher RP-18 4×4 mm, 5 μm	LiChrospher 100 RP-18e 250×4 mm, 5 μm, 35°C	MeCN–0.2 *M* phosphate buffer, pH 3.0 (35:65) containing 2 m*M* EDTA	ED +0.65 V postcolumn derivatization	[22]
Streptomycin, dihydrostreptomycin	Animal tissue	Precipitation, extraction, centrifugation, off-line SPE, column switching	Inertsil C_8 40×4.6 mm, 5 μm	LC8-DB 250×4.6 mm, 5 μm	Water–MeCN (83:17) containing 10 m*M* HXSA and 0.4 m*M* NQS at pH 3.3	FD ex 347 nm em 418 nm postcolumn derivatization	[23]
Colistin	Milk, bovine tissues	Precipitation, off-line SPE, precolumn derivatization, column switching	LiChrospher 100 RP-18 30×4 mm, 5 μm, 35°C	LiChrospher 100 RP-18 125×4 mm, 5 μm, 35°C	MeCN–0.01 *M* phosphate buffer, pH 7.0 (68:32)	FD ex 340 nm em 440 nm	[24]
Heparin-binding proteins	Egg-white	Dilution, column switching	β-CD sulfate precolumn 10×6 mm	YMC-Pak ODS-AP 250×4.6 mm	Gradient MeCN–10 m*M* H_3PO_4 containing NaCl	UV 215 nm	[25]

Bensulfuron methyl	Rice, crayfish	Extraction, off-line SPE, column switching	Zorbax SB-Phenyl 150×4.6 mm, 5 μm	Zorbax Rx-C8 250×4.6 mm, 5 μm, 40°C	Gradient MeCN at pH 7.6	UV 254 nm	[26]
Proline	Beer	Precolumn derivatization, off-line SPE, filtration, column switching	C_{18} 250×4.6 mm, 5 μm	RN-β-CD 250×4.6 mm, 5 μm	MeCN–acetic acid (100:0.7)	FD ex 266 nm em 315 nm	[27]
Leucine, phenylalanine	Beer	Precolumn derivatization, off-line SPE, filtration, column switching	C_{18} 250×4.6 mm, 5 μm	β-CD 250×4.6 mm, 5 μm	MeOH–TEA–acetic acid (100:0.85:0.1)	FD ex 266 nm em 315 nm	[27]
Okadaic acid, dinophysisto-xin-1	Mussel	Homogenization, centrifugation, extraction, precolumn derivatization, column switching	Supelcosil LC-8-DB 250×4.6 mm, 5 μm (clean-up column), Nucleosil C_{18} 100×4 mm, 5 μm (trap column)	Supelcosil LC_{18} 250×4.6 mm, 5 μm	MeCN–water (90:10)	FD ex 365 nm em 415 nm	[28]
Glyphosate	Cereal sample	Extraction, off-line SPE, precolumn derivatization, column switching	Hypersil ODS 30×4.6 mm, 5 μm	Adsorbosphere NH_2 250×4.6 mm, 5 μm, 30°C	MeCN–0.05 *M* phosphate, pH 5.5 (35:65)	FD ex 263 nm em 317 nm	[29]
Tetracyclines	Animal tissues	Extraction, centrifugation, off-line SPE, column switching	Anagel-TSK-Chelate-SPW 10×6 mm, 10 μm	PLRP-S 150×4.6 mm, 5 μm	Gradient 0.1 *M* KH_2PO_4–0.01 *M* citric acid–0.01 *M* EDTA–MeOH–MeCN	UV 350 nm	[30]
Tetracyclines	Muscle, liver, eggs	Extraction, centrifugation, column switching	Anagel-TSK-Chelate-SPW 10×6 mm, 10 μm	PLRP-S 150×4.6 mm, 5 μm	Gradient 0.1 *M* KH_2PO_4–0.01 *M* citric acid–0.01 *M* EDTA–MeOH–MeCN	UV 350 nm	[31]

Table 3
The preseparation and separation conditions for on-line dialysis methods

Compound	Matrix	Preseparation	Precolumn	Column	Mobile phase	Detection	Ref.
Amoxicillin, cefadril	Muscle tissue	Homogenization, centrifugation, on-line dialysis, column switching	C_{18} 20×4.6 mm, 10 μm	ABZ Supelco 150×4.6 mm	MeCN–10 m*M* phosphate buffer, pH 7.0 (13:87)	UV 260 nm, postcolumn derivatization	[32]
Sugars	Beverages, yoghurt	On-line dialysis		NH_2-Zorbax 250×4.6 mm, 5 μm, 25°C	Water–MeCN (25:75)	RI	[33]
Sugars, organic acids	Beverages	On-line dialysis		ION-300 300×7.8 mm, 65°C	4.25 m*M* Sulphuric acid	RI	[33]
Amino acids	Food, beverages	Dilution, precolumn derivatization, on-line dialysis		Hypersil BDS C_{18} 150×4.6 mm, 3 μm, 40°C	Gradient MeOH–MeCN–10 m*M* phosphate buffer, pH 7.5 containing 0.8% THF	FD ex 335 nm em 440 nm	[34]

Table 4
The preseparation and separation conditions for RAM methods

Compound	Matrix	Preseparation	Precolumn	Column	Mobile phase	Detection	Ref.
Chloramphenicol	Animal tissue	Homogenization, extraction, centrifugation, off-line SPE, extraction, column switching (RAM)	(1) LC-HISEP 150×4.6 mm, 5 μm (clean-up column) (2) Supelcosil LC_{18} 150×4.6 mm, 5 μm (trap column)	Supelcosil LC_{18} 250×4.6 mm, 5 μm	MeCN–water–THF (80:18:2)	UV 278 nm	[37]
Domoic acid	Mussels, algae	Extraction, centrifugation, filtration, column switching (RAM)	LC-HISEP 150×4.6 mm, 5 μm	Supelcosil C_{18} DB 250×4.6 mm, 5 μm	MeOH–water formic acid (12:87:0.2)	UV 242 nm	[38]
Organochlorine pesticides	Milk	Dilution, column switching (RAM)	ISRP-C_{18} 30×4.6 mm	ODS-Hypersil 30×4.6 mm, 3 μm	100% MeCN	UV 254 nm	[39]
Carbofuran	Milk	Dilution, RAM		ISRP-C_8 100×4.6 mm	MeCN–phosphate buffer, pH 5.5 (20:80)	UV 220 nm	[40]

In the latter method, RP-HPLC was performed with a gradient comprising acetic acid, methanol and water. Retention times achieved were around 27 and 33 min. Detection limits and recoveries of both methods were similar (25 and 30 μg/l, and 97.5–105%, respectively). Results obtained by both methods were in good agreement. The only significant difference was an increase of approximately 15% in the mean *cis*-resveratrol concentrations provided by the latter method. This suggests that, since all reported peaks with the second method are subjected to purity and spectral identity analyses, the first method is not prone to overlapping contaminants that would give rise to falsely elevated values. The lower *cis*-resveratrol concentrations obtained in the first method may reflect the fact that absorbance was measured only at 306 nm, the maximum wavelength for *trans*-resveratrol, whereas that for the *cis*-isomer occurs at 280 nm. Since the second HPLC method allows the simultaneous determination of a wide range of biologically active phenols in red wine and has sophisticated software features to prevent false elevation by contaminating constituents eluting at or near the retention times of *cis*- and *trans*-resveratrol, it is the most flexible and robust of the current methods to quantitate these hydroxystilbenes in wine.

Phenolic acids and esters, catechins and proanthocyanidins, flavonols and flavonol glycosides and other phenolic compounds were determined in wines using a C_{18} column thermostated at 22.5°C, diode-array detection and ternary gradient between 1% and 5(6)% acetic acid solutions and water–acetic acid–acetonitrile (65:5:30) [1,5,6]. The disadvantage of the used mobile phase was that acetic acid was present, which did not permit the detector to be used below 240 nm. In some cases, a binary gradient between phosphoric acid solution and acetonitrile was advantageous to record UV spectra at low wavelength and to distinguish two very closely related compounds (e.g., *trans*-resveratrol and *trans*-resveratrol-glycoside). Electrochemical and fluorescence detectors were sometimes useful. The injected volume was 50–70 μl and analysis required 150 min. The long time of analysis resulted in sensitivity to variations in temperature. Therefore, it was necessary to thermostate the analytical column. Thus, no variation >0.5 min was ever noted.

A direct injection RP-HPLC procedure was published for the separation and determination of the major carboxylic acids (tartaric acid, malic acid, lactic acid, citric acid) in grape must, red wine and white wine without interferences from sugars [7]. The acids were separated on C_{18} column, eluted with dilute acetic acid and monitored using UV detection at 254 nm.

Use of a hydrogensulfonated divinylbenzene–styrene copolymer HPLC column maintained at 65°C, isocratic elution with acetonitrile–0.005 *M* H_2SO_4 and UV detection at 258 nm permitted analysis by direct injection of white and red wines without significant interference between the peaks of sorbic acid and other sample components [8]. All samples were diluted 50 times with 0.005 *M* H_2SO_4 and filtered through a membrane filter (0.45 μm) before HPLC analysis. The limit of detection of the method was 0.01 mg/l with an average recovery of 99.6% and an average RSD of 0.87%. The whole analysis took approximately 20 min. HPLC results were compared with the results obtained using the official European Union spectrophotometric method, and a good correlation was shown, although the HPLC analyses were clearly more precise and accurate.

Four catechins were determined in tea [9]. Tea samples were prepared following the instructions provided on the package by dipping a tea bag in boiling water. After cooling, the sample was filtrated through the 0.2-μm porosity syringe filters. The experimental findings indicated losses of 4% or less of most analytes and 7% in the case of epicatechin gallate. All separations were conducted by RP-HPLC on a C_{18} column using gradient elution and DAD. Quantitative levels of tea catechins were determined using an acetonitrile–acetate buffer (pH 4.5) mobile phase. A second mobile phase of methanol–acetate buffer was used to confirm the identity of the catechins found. In addition, an acetonitrile–ascorbate buffer was used as the mobile phase in determining analyte losses occurring during sample preparation. Separations performed with ascorbic acid added to the mobile phase gave sharper peak shapes. This improvement, however, was partially offset by the somewhat higher background absorbance of the mobile phase at shorter wavelengths.

The same preparation method – filtration – was

employed for the treatment of nine polyphenols with low molecular mass occurring in brandy samples [10]. The relative simplicity of this wine industry product permitted the analysis by HPLC with direct injection. Membranes of 0.45 μm pore sizes were used for the sample filtration. The mobile phase consisted of methanol, acetic acid and water and separations were performed on the C_{18} column.

Dilution in methanol–water was used for the treatment of fruit juices and their concentrates in the determination of fungicide thiabendazole [11]. Any juice or concentrate that had pulp in it was centrifuged for 5 min at 10 000 *g*. Retention time was short, taking only 3.3 min. No interfering peaks occurred from juice and stored concentrate samples; however, with bulk concentrates, interferences can sometimes be a problem. To decrease the effects of interferences, the samples were diluted further, thus lowering the sensitivity, as the limit of quantitation increased from 5 ppb to 25 ppb. The evaluated method used a mobile phase consisting of acetonitrile, methanol, water and ethanolamine, and a C_{18} column. Detection was accomplished with a fluorescence detector. HPLC results were compared with ELISA analyses and very good agreement was shown.

Some phenolic compounds, catechin, epicatechin, chlorogenic acid, phloretin glucoside and phloretin xyloglucoside were also determined in several juice samples using RP-HPLC with DAD and direct sample injection [12]. The direct injection method gave good recovery, accuracy and precision with a detection limit of 1.6 mg/l.

A highly flavor-active phenolic compound, 4-vinylguaiacol, and its precursor, ferulic acid, were determined in beers and worts [13]. C_{18} stationary phase with the particles of 10 μm was used in the analytical column, and the mobile phase consisted of methanol, water and phosphoric acid. A run time of 45 min was sufficient for the simultaneous separation of both analytes. Detection was by either fluorescence monitoring (for 4-vinylguaiacol alone) or electrochemical detection (for simultaneous determination of both analytes). The methods were applied mainly to the analysis of production beers but were used also to monitor the decarboxylation of ferulic acid to 4-vinylguaiacol in wort during a fermentation. Pale beers were degassed, sonicated and filtered through 0.22 μm filters; stouts and wort samples were firstly diluted with methanol (1:1), centrifuged at 3000 *g* at 2°C and the supernatants were retained and chilled before direct injecting onto the HPLC system. Although only minimal sample clean-up was performed, there was no detectable deterioration in column performance after the analysis of approximately 1000 samples (the sample injection volume 25 μl). The detection limit for *trans*-ferulic acid by amperometric detection was 20 μg/l and that for 4-vinylguaiacol 10 μg/l, in comparison with 2 μg/l by fluorescence detection. RSDs were under 1% for both types of detection.

A method for the quantitation of six prenyl-flavonoids in beer and herb tea by HPLC–tandem mass spectrometry was developed [14]. Beer samples were degassed followed by sonication. Tea was prepared by steeping one bag in boiling water for 5 min. Separations were achieved on a C_{18} column with a linear solvent gradient of acetonitrile and aqueous formic acid. MS was operated using the atmospheric pressure chemical ionization source in the positive ion mode.

3. Automated solid-phase extraction and column switching

Solid-phase extraction is a technique that has a great potential for the automation. Other preseparation methods such as centrifugation, ultrafiltration, protein precipitation, liquid–liquid extraction are still difficult to be automated without using a rather expensive, and still not very versatile, robotic system.

There are two approaches to the automation of SPE: (i) off-line automated solid-phase extraction, (ii) on-line automated solid-phase extraction.

3.1. Off-line automated solid-phase extraction

Nowadays there are many off-line SPE instruments on the market. Some of them perform all the SPE processes automatically but the transfer of the eluates from SPE to HPLC is manual (semi-automated systems). However, others are able to transfer

the eluates to the HPLC injector and perform the chromatographic analysis automatically so that the unattended sample preparation and analysis can be carried out (fully automated systems).

These automated off-line devices often use the same cartridges as in manual solid-phase extraction, placed in a rack [15]. Other parts of the device are: solvent dispensing system, eluate collector and drain for the waste liquids.

Polar and apolar heterocyclic aromatic amines were determined in heat-processing protein-rich foods (microwaved meat extract, vacuum dried meat extract, grilled beef, merguez sausage, chicken flavor pasta, peanut butter) using a robotic workstation [16]. Only simple dilution and homogenization of sample in sodium hydroxide solution were processed before loading into the automatic system. Robot performed elution of the analytes with dichloromethane from the Extrelut cartridges and loading them to the cation exchange ones. Apolar and polar components were eluted separately, treated on C_{18} cartridges, eluted with methanol–ammonia and collected in HPLC vials. The robotic procedure was completed in 4–5 h, including all conditioning steps. Separation was performed on the C_{18} analytical column using a gradient of the mobile phase consisting of two ammonium acetate buffers (pH 3.2 and 4.0) and acetonitrile. UV and fluorescence detectors were used to detect analyzed compounds. MS was used for qualitative confirming the identity of amines in complex samples.

Immunoaffinity column-based sample preparation procedure for rapid screening of aflatoxins in foods (milk, corn, nuts) was automated [17]. Milk samples were centrifuged, solid samples were ground and extracted with 60% aqueous methanol, filtered and diluted. After injecting into the immunoaffinity columns by the robot and washing, aflatoxins were eluted with 80% aqueous methanol. After immunoaffinity column clean-up, analytes were separated by RP-HPLC and determined using fluorescence detection without derivatization. Mean recoveries of aflatoxins B1, B2 and G1 added to corn and nuts at 9–36 ng/g were more than 85%, recoveries of aflatoxin G2 averaged 50%. The average recovery of aflatoxin M1 added to milk at 0.12–0.50 ng/ml was 78%. The above-mentioned automated system could assay samples at a rate of 15–30 min/sample.

3.2. On-line automated solid-phase extraction

On-line automated SPE devices were initially employed to preconcentrate the analytes present in a large volume of a sample. They form part of the so-called ‘‘column switching’’ or ‘‘coupled column’’ techniques (see below).

Solid phases in these systems are usually packed in stainless steel columns because SPE is carried out at high pressure. The first cartridges employed were the precolumns used in HPLC to protect the analytical columns so the cartridges for on-line SPE are normally referred to as precolumns [15].

3.3. Column switching

In HPLC, column switching (sometimes called multidimensional column chromatography) is a powerful technique for the separation and clean-up of multicomponent mixtures. In this approach, fractions from a precolumn (primary column) are transferred selectively to one or more analytical columns (secondary columns) for further separation. Column switching is used for: (i) trace enrichment of selected analytes; (ii) improving resolution of part of a complex sample (maximum resolution can be achieved by using different modes, stationary phases); and (iii) increasing sample throughput by using heart-cutting, backflushing, front- or end-cutting, and recycle chromatography [18].

The main advantages of column switching techniques for sample clean-up are: minimal sample handling, on-line sample processing, considerable time reduction, possibility of fully automation, greater precision and accuracy, improvement in the selectivity by combining different chromatographic modes, avoiding of an internal standard, protection of photo-labile analytes and minor consumption of organic solvents. On the other hand, the disadvantages of column switching are: requirement of switching valves and additional columns and/or pumping systems, requirement of compatible mobile phases and periodic replacement of pre-column [19].

In principle, any liquid biological sample free from suspended particles can be injected into a switching system. The type of a sample matrix and the amount of a sample injected determine the number of injections that can be processed before

replacing the precolumn. Centrifugation, dilution, filtration, prior off-line extraction and protein precipitation are used as sample treatment techniques before injection into switching system to increase precolumn lifetime [19].

In most cases, the precolumn is much shorter than the analytical column. The system consisting of two analytical columns combined through the switching valve (one analytical column is used instead of a precolumn) is employed to improve selectivity of the clean-up. The internal diameter of both precolumn and analytical column should be the same to minimize extra-column band broadening [19]. Particle size of precolumn packings used is mostly the same as for the analytical column, i.e., 5–10 μm. Reversed phases C_{18} and C_8 are very often employed.

On-line immunoaffinity extraction or antibody-mediated extraction is a variation of SPE where the ''bonded phase'' is specific for compounds of interest. This approach offers considerable advantages: the specificity of the antibody could be ''designed'' such that it is specific for only one molecule and/or its metabolites. Alternatively, if the compound is part of a series of homologues, the specificity of the antibody could be lowered so that all similarly structured compounds could cross-react and be extracted for the liquid chromatograph to separate and quantify [20].

3.3.1. Column switching techniques

The most commonly used column switching system is illustrated in Fig. 1. In the load position of the switching valve, the primary mobile phase flushes impurities and compounds with the weak retention from the precolumn. After rotating the switching valve into the inject position, the secondary mobile phase with a higher elution power than the primary mobile phase elutes the analytes from the precolumn onto the analytical column. After the transfer of the analytes is completed, the valve is rotated back. The analytes are separated with the secondary mobile phase at the analytical column. At the same time the primary column is reconditioned with the primary mobile phase.

The so-called ''back-flush'' technique is presented in Fig. 2. The difference between this method and

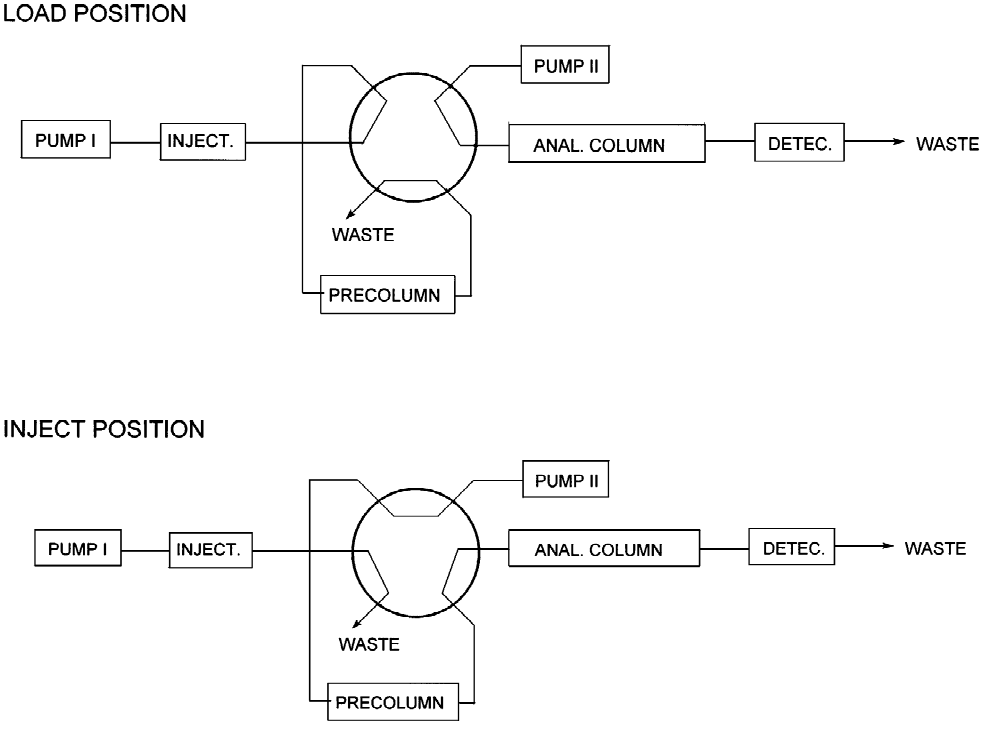

Fig. 1. Scheme of a column switching system.

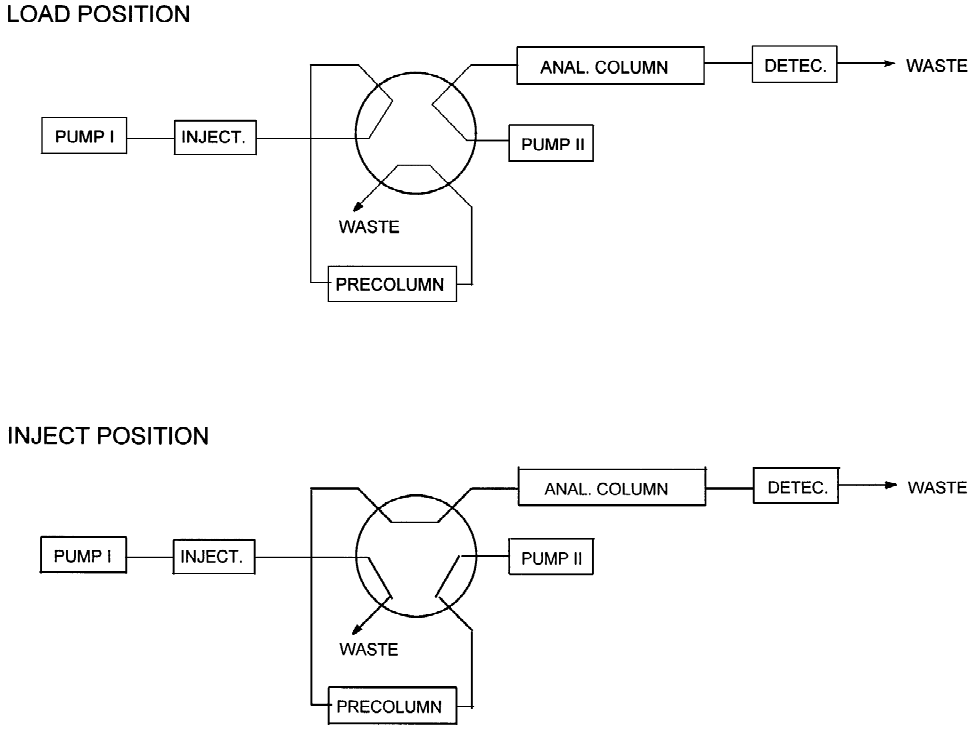

Fig. 2. Scheme of a column switching system in back-flush mode.

the method mentioned above, is in the flow direction of the secondary mobile phase through the precolumn in the injection position. In this position, the analytes are transferred from the precolumn onto the analytical column in the reversed flow. In this case, band broadening of the transferred zone on the precolumn is minimized.

Fig. 3 presents the connection of a precolumn as a loop. It is the simplest setup of the column switching system that does not require additional chromatographic equipment, especially pumps.

If wide or narrow cut of the chromatographic effluent from the precolumn is transferred to the analytical column by flow switching, switching techniques named according to the transfer fraction are front-, heart- and end-cuttings (the analyte zone elutes at the front or in the middle or at the end of the chromatographic effluent of the precolumn).

An automated on-line method for the determination of polycyclic aromatic hydrocarbons in edible oils and fats was developed using an LC–LC coupling of a clean-up donor–acceptor complex chromatography (DACC) column to two analytical columns [21]. Propanol was added to the samples and they were filtered prior to the automated treatment. After washing the DACC column with propanol, the backflush mode and the HPLC gradient (water, acetonitrile and ethyl acetate) were initialized and the analytes were eluted to the analytical columns and detected using fluorescence detection. Compared to the traditional methods this technique significantly reduced the amount of solvent waste and considerably saved time. The total analysis time of one sample was approximately 80 min. Quantification limits less than of 0.1 μg/kg of the individual PAHs were achieved.

Much more complicated manual sample treatment followed by automated SPE was necessary in the method for the determination of penicillins and oxacillins in pork meat [22]. Samples were extracted several times with acetonitrile, dichloromethane, light petroleum and phosphate buffer (pH 7). SPE and elution onto the analytical column were performed by the on-line sample preparator. A short C_{18}

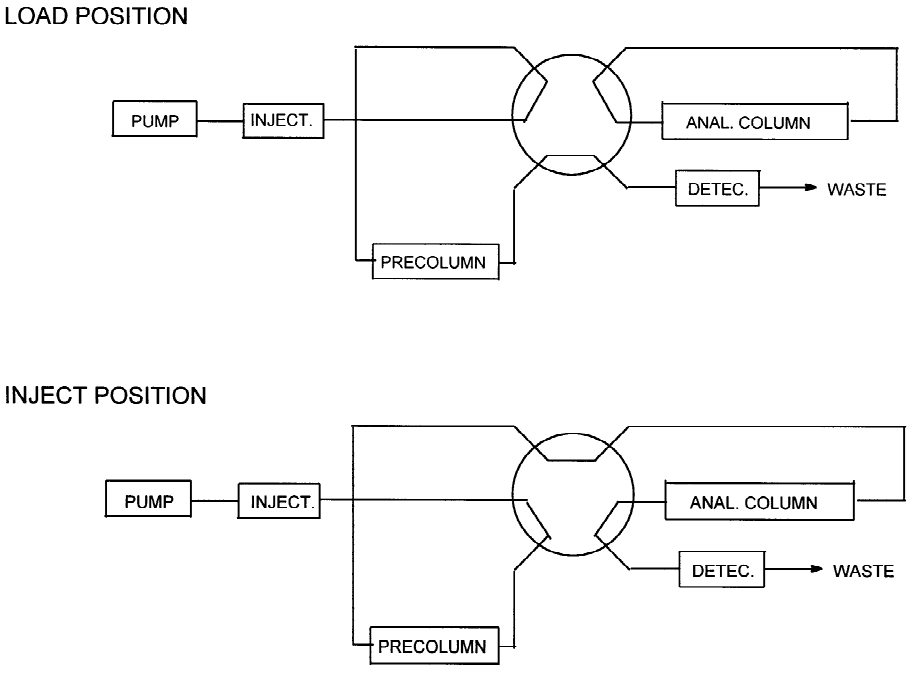

Fig. 3. Connection of a precolumn as a loop.

precolumn was used and drugs were eluted with the mobile phase containing acetonitrile, EDTA and phosphate buffer (pH 3). The recoveries were found to be nearly 100%. It was possible to use one cartridge for at least 25 analyses with identical analytical performance. Analytes were detected electrochemically on glassy carbon electrode after the photochemical degradation. The reaction products formed could be detected with detection limits 1–5 ng (using 200 μl injections).

The system based upon the indirect transfer technique was applied to the determination of antibiotics streptomycin and dihydrostreptomycin in pork and bovine muscle and kidney [23]. Diluted perchloric acid solution was used to precipitate proteins and extract the analytes from the tissue. After centrifugation, the extract was further cleaned up by off-line solid-phase extraction on a cation-exchange SPE column. The analytes were eluted with phosphate buffer (pH 8) and chromatographed using on-line column enrichment liquid chromatographic system with the postcolumn derivatization and fluorescence detection. Both columns (enrichment and analytical) were C_8 stationary phases with 5 μm particles. The primary mobile phase was aqueous solution of 1-hexanesulfonic acid, the secondary mobile phase consisted of aqueous solutions of 1-hexanesulfonic acid, acetonitrile and a derivatizing agent 1,2-naphthoquinone-4-sulfonic acid (pH 3.3). The sample was loaded onto the enrichment column, and this column was flushed with the primary mobile phase for 5 min to elute coextracted materials to waste while retaining the analytes on the column. The column selection valve was then switched to the injection position and the secondary mobile phase eluted the analytes from the precolumn to the analytical column. Whole analysis time of 25 min was achieved. The retention times of streptomycin and dihydrostreptomycin were 22 and 23 min. The baseline resolution of the two drugs was not achieved at these chromatographic conditions. Because it was considered unlikely that both drugs would be encountered in the same sample, a mobile phase was chosen that allowed the identification of each drug while minimizing analysis time. Recoveries achieved were 61.1% for streptomycin and 55.3% for dihydrostreptomycin at the concentration levels of 40–80, and 200–400 ppb, respectively. The

detection limits were 10 ppb for streptomycin and 20 ppb for dihydrostreptomycin.The sample enrichment column began to deteriorate after about 150 injections (injection volume 2 ml).

A similar system was used for the determination of antibiotic colistin in milk and four bovine tissues (muscle, liver, kidney and fat) [24]. Sample treatment consisted of the protein precipitation using 10% trichloroacetic acid, solid-phase purification on C_{18} SPE cartridges, and precolumn derivatization with *o*-phtalaldehyde and 2-mercaptoethanol in borate buffer (pH 10.5). The resulting reaction mixture was injected into the switching HPLC system including a precolumn and an analytical column, all packed with RP-18. Washing the precolumn and final elution onto the analytical column were conducted using acetonitrile–phosphate buffer (pH 7) mixtures. Recoveries were higher than 60%. The duration of the washing and elution steps and the precolumn reequilibration before the next analysis was 26 min. The precolumn was changed after every 30 injections (200 μl sample loop).

A very simple preseparation technique was developed for the determination of lysozyme and other proteins binding on heparin in egg white [25]. Sample was only 10-fold diluted with water and injected into the column switching system. After sample loading, the precolumn (β-CD sulfate-immobilized hydrophilic vinyl-polymer gel) was washed with water, then with Tris–HCl buffer (pH 7.5) containing various concentrations of NaCl, and finally again with water. The analytes were backflushed with the mobile phase consisting of acetonitrile, phosphoric acid, sodium chloride and water onto the analytical column (C_{18}) and detected at 215 nm. The non-specific binding of proteins on the precolumn was eliminated completely by the washing with the buffer containing NaCl over 60 m*M*. On the other hand, almost quantitative retention of the proteins was obtained at least up to 180 m*M*. The β-CD sulfate precolumn was compared with the heparin precolumn. The heparin-binding proteins were retained almost quantitatively on both precolumns, and other proteins were completely removed. Better resolution was obtained with the β-CD sulfate precolumn. Retentions of heparin-bonding proteins achieved at this type of the precolumn were in the range 97–102% and no decrease in retention was shown after about 400 cycles operation during 2 years (500 μl sample volume).

Herbicid bensulfuron methyl was determined in rice and crayfish [26]. After the analyte was extracted from the sample with methylene chloride, the extract was concentrated and cleaned up using off-line normal-phase SPE. The analyte was eluted from the cartridge with the mixture of isopropanol, methanol and hexane, evaporated and reconstituted in acetonitrile–water. RP-HPLC with column switching and UV detection was applied to the further sample clean-up and the determination of bensulfuron methyl. Only one pump, but two switching valves were used. Chromatography started by injecting prepared sample onto the primary column (phenyl) with the primary mobile phase containing 35% acetonitrile at pH 3.2. At this pH, bensulfuron methyl is uncharged and relatively nonpolar. It migrated through the phenyl column slowly relative to most matrix compounds. As the analyte was eluting from the phenyl column, the second valve was switched, thereby transferring the analyte to the secondary column (Rx-C8) within a 2–3 min time window. Under these conditions (lower pH), bensulfuron methyl concentrated onto the head of the C_8 column to minimize peak broadening due to a longer retention time. After 2–3 min, the first valve was switched, and the analyte was eluted from the C_8 column using a gradient of 15–25% acetonitrile at pH 7.6. At this higher pH, bensulfuron methyl is negatively charged and consequently more polar. It eluted rapidly from the second column. The primary column was cleaned with 60% aqueous acetonitrile buffered at pH 7.6 after the analysis. Even after more than 50 sample injections (injection volume 50 μl), no column deterioration was observed. Time of the analysis including whole column switching preseparation step was 60 min. Bensulfuron methyl was recovered at 70–116% (at concentration levels of 0.008–1.0 ppm).

A method for the determination of three amino acids (leucine, phenylalanine and proline) enantiomeric composition in beers was presented using a column switching system [27]. Columns used were C_{18} and RN-β-CD (for the determination of proline) or β-CD (determination of leucine, phenylalanine). Samples, in which leucine and phenylalanine were determined, were firstly cleaned up using strong

cationic cartridges. All samples were precolumn-derivatized with 9-fluorenylmethyl chloroformate at pH 8.0. After derivatization and acidification to pH 4.0, the postreaction mixture was purified using off-line SPE. The analytes were eluted with pure diethyl ether and, after reconstitution in the mobile phase, they were injected onto the C_{18} column. A small portion of the eluting peak of interest was introduced from the C_{18} column onto the chiral column. The mobile phase for the reversed-phase system consisted of acetonitrile, acetic acid and water. Chiral columns were used in the polar organic modes, mobile phases consisted of acetonitrile and acetic acid, and when using β-CD column, there was also triethylamine in the mobile phase. The effluent from the C_{18} column was detected using a UV detector, the effluent from the chiral column was monitored using a fluorescence detector.

A complex column switching system consisting of three columns, two switching valves and two pumps was developed for the determination of diarrhetic shellfish poisons (okadaic acid and dinophysistoxin-1) in mussels and mussel products [28]. Samples were homogenized in methanol–water and centrifuged followed by extraction with dichloromethane and derivatization with 9-anthryldiazomethane in acetone at 25°C. The column switching system consisted of a C_8 clean-up column, a C_{18} trap column and a C_{18} analytical column. Both mobile phases used were mixtures of acetonitrile and water. The technique called heart-cut was used to transfer narrow zones of the analytes from the clean-up column to the trap column. The analytes were washed from the trap column in back-flush mode to the analytical column and detected using a fluorescence detector. The detection limit for both compounds was 0.5 ng (20 μl injection) and the recoveries were higher than 90%.

The combination of extraction, off-line SPE, precolumn derivatisation and column switching was reported for the determination of the herbicide glyphosate in cereal samples [29]. They were extracted with water, centrifuged and passed through the C_{18} SPE cartridge. Derivatisation was performed at pH 9.0 using 9-fluorenylmethyl chloroformate in acetonitrile. The columns used in the column switching system were a short C_{18} precolumn and a NH_2 analytical column. The mobile phase consisted of acetonitrile and phosphate buffer (pH 5.5). Using this mobile phase, the glyphosate derivative is ionized which results in little retention on C_{18} and adequate retention on the amino column. All interferences with more C_{18} retention were retained on the C_{18} column and sent to waste by rinsing with the mobile phase during the separation of the analyte on the NH_2 column. Off-line SPE was necessary to remove a large amount of interferences. The clean-up approach is similar to that in LC–LC analysis. The detection limit of 0.5 mg/kg and recoveries of 61–99% were achieved. The analytical procedure provided a throughput of at least 25 samples per day.

A method for the determination of tetracyclines in sheep liver and cattle kidney using metal chelate affinity chromatography (MCAC)–RP-HPLC was developed [30]. Drugs were extracted with succinate buffer and centrifuged. Simple one-step deproteinization procedures were examined first as a preseparation step before column switching HPLC analysis. Recoveries of drugs were low and in some cases there were chromatographic interferences. Therefore the use of a variety of off-line SPE-based procedures was investigated using methanol for elution. Eluates were injected into the column switching system. A metal chelate affinity (iminodiacetic-bonded hydrophilic polymeric support) precolumn was preloaded with copper(II). In the absence of copper, the tetracyclines passed through the chelate precolumn. Analytes were back-flushed with an phosphate–citrate buffer containing EDTA from the precolumn onto the analytical column (PLRP-S styrene–divinylbenzene copolymer) and separated via a gradient of the mobile phase containing mentioned buffer with EDTA, methanol and acetonitrile. Recoveries were in the range 36–89% at 10–300 μg/kg levels. The limits of detection were 10–50 μg/kg.

An improvement on previously reported on-line MCAC–HPLC method for tetracyclines was published [31]. The throughput was increased by the use of an organic solvent (ethyl acetate) extraction, eliminating the need for off-line SPE clean-up prior to the on-line MCAC treatment. This method was applied to the surveillance of animal products (muscle, liver, eggs) for the presence of tetracycline residues. Approximately 150 sample extracts (1.5 ml injections) could be processed before the MCAC column performance started to deteriorate. Re-

coveries were generally higher than those obtained using earlier procedure, and ranged from 42 to 101%. Detection limits were estimated to be 3–6 μg/kg. These represented an improvement on those obtained in [30], particularly for chlortetracycline, which was not detectable below 50 μg/kg.

4. On-line dialysis

Automated column liquid chromatographic determination of amoxicillin and cefadroxil in bovine serum and muscle tissue using on-line dialysis could also be used for the direct sample analysis [32]. The method is based on the on-line combination of dialysis and solid-phase extraction for the sample preparation and HPLC with the UV detection for the separation of analytes. The authors used a Gilson ASTED system on-line combined with an HPLC instrument. The ASTED system was slightly modified by the authors. Instead of a dilutor used to transport the dialysate from the dialysis block to the precolumn, a conventional HPLC pump, which can handle much higher back pressures, was installed in order to optimize the concentration step and to enable using, e.g., longer precolumns, smaller particles and/or higher flow-rate. The muscle tissue samples were homogenized with a buffer using a blender and after the centrifugation the supernatant was injected into an ASTED system. The complete optimized time schedule for the preseparation step was worked-out. In order to enhance the UV detectability of the analytes, postcolumn addition of sodium hydroxide was recommended, giving the possibility to shift detection to 260 nm instead of 230 nm. The method shows a good linearity and repeatability for both analytes. The limit of detection was 0.2 μg/g tissue. The method has a sample throughput of 30 samples per 24 h.The authors discussed the factors limiting the applicability of dialysis, e.g.,dilution of the sample and subsequent preconcentration of the analytes on SPE precolumns is therefore necessary. They confirmed the low selectivity of dialysis because the separation is only based on differences in molecular size. The trace level determination of analytes in complex biological and food samples is therefore difficult. Recoveries were 32% (2% RSD) and 34% (2% RSD) for amoxicillin and cefadroxil. The authors have also discussed in detail the selectivity and the performance of the total on-line system. They recommended to improve the selectivity of the assay in the case of kidney and liver samples by postcolumn labeling with fluorescamine (for many interferences in these matrices).

The ASTED system incorporated on-line with the HPLC instrument has also been used for the analysis of sugars and organic acids in food and beverages [33]. Raw liquid food samples containing complex matrices such as dairy products or soft drinks were injected directly into the HPLC system. Until recently, the clean-up techniques prior to the HPLC determination of sugars and organic acids were manual and off-line, including liquid–liquid extraction, solid-phase extraction, precipitation and centrifugation. The on-line clean-up of a variety of raw foods permitted the determination of colorants in desserts and confectionery, nitrofurans in eggs and meat homogenates, aflatoxins in milk. The authors used two methods for the analyte separation. The first one was used to determine only sugars from soft drinks and dairy products (orange syrup, cola drinks, reconstituted baby milk and liquid chocolate yoghurt). In this method the amino silica column was applied. In the second method, fruit juices and fermented beverages (grape juice, red wine, white wine, apple juice and cider) were analyzed on an ion-exchange column for the simultaneous determination of sugars and some related compounds such as glycerol and ethanol. All basic chromatographic conditions were optimized (mobile phase, column temperature, injection volume). In the first method five sugars (i.e., fructose, glucose, sucrose, maltose, lactose) were separated. The mobile phase was a mixture of acetonitrile–water (7:3). In the second method, the objective was to separate simultaneously six organic acids (i.e., citric, tartaric, malic, succinic, lactic and acetic acids). In this work the sample on-line dialysis was performed without the need for a trace enrichment step. The LOD was 0.16 g/l for fructose and glucose and varied from 0.28 g/l to 0.41 g/l for disaccharides. Recoveries of sucrose, maltose and lactose (4.5–4.9%) were lower than those of the hexoses (6.3–7.1%). In the second method, the LOD varied from 6.7 to 9.5%. The authors used internal standards to minimize any

possible fluctuations during dialysis caused by the composition of the matrix. This assay was well suited for the removal of macromolecular matrix interferents such as proteins, polysaccharides and condensed phenolic compounds.

Fully automated derivatization, on-line dialysis using the ASTED and the HPLC analysis of amino acids in food, beverages and feedstuff has also been published [34]. A fully automated sample processor performed precolumn derivatization of primary and secondary amino acids with *o*-phthalaldehyde–3-mercaptopropionic acid and 9-fluorenylmethyl chloroformate (FMOC), respectively. Twenty-five amino acids (including cysteine) present in food products were separated in 24 min using a one-step binary gradient. This method is specially dedicated to food applications as feedstuff protein hydrolysate, an orange juice and red wine. All samples determined in this work had to be automatically diluted before analysis, what means that no sensitivity limitation occurs.

5. Application of restricted access media sorbents

New possibilities are also in the application of RAM sorbents (restricted access media), not only in packing analytical columns for the direct application of sample extracts (one column system) but also for the column switching mode (two column system). In this case the precolumn packed with the RAM sorbent is connected with the analytical column for the separation and determination of all analytes in food sample. The column switching system could be automated using the autosampler incorporated into the applied HPLC system [35]. The RAM sorbents have a hydrophobic interior and a hydrophilic barrier, which allows the passage of small molecules and restricts the access of large molecules. A hydrophobic interior retains small molecules. These sorbents combine both reversed-phase and size-exclusion principles. The RAM sorbents are divided into four groups: physical diffusion barrier with monofunctional and difunctional phases and chemical diffusion barrier with monofunctional and difunctional phases [36].

A column switching system using a RAM sorbent precolumn and UV detection was published for the HPLC determination of chloramphenicol in animal tissue [37]. Chloramphenicol (CAP), D-*threo*-1-*p*-nitrophenyl-2-dichloracetylamino-1,3-propanediol is a bacteriostatic with a broad spectrum of activity, often applied in veterinary medicine. The EU established the MRL (maximal residual limit) for CAP at 10.0 μg/kg. The preseparation and clean-up procedures published before, were rather complicated and varied for the different tissues. In the presented paper the applicability of the method was tested for different tissues of pig (liver, kidney, muscle, fat with skin), turkey (liver, kidney, muscle, fat, skin) and trout (muscle, skin). After cutting tissues in small pieces and the homogenization, the samples were mixed with sand and dried before mixing with acetonitrile (3 ml) and centrifuging for 5 min at 2980 *g*. The clear acetonitrile solution was decanted. The procedure was repeated and fraction evaporated to dryness. The clean-up and preconcentration steps were realized using SPE C_{18} with acetonitrile and silica gel cartridges with ethyl acetate as solvents. Solvents were evaporated to dryness, hexane was added to the residue and an aliquot (50 μl) of the lower aqueous phase was injected directly into the HPLC column switching system as it was found out that the extract purification with C_{18} and silica SPE cartridges was not sufficient. A HISEP column was developed for removing proteins and the other macromolecules (RAM sorbent) which are eluted near the eluent front and CAP is effectively retained. For the HPLC analysis two C_{18} columns were applied, a short one, as a trap column, and the second one as the analytical column. After a complete amount of CAP has been trapped on the trap column, it was eluted to the analytical column and analyzed isocratically (acetonitrile–water–THF) at 278 nm. The authors evaluated the linearity and repeatability of the whole method. During the validation, 1400 samples were analyzed over a period of 12 weeks. Quantitation was performed by the external standard calibration technique. Recoveries for different tissue samples were in the range 82–113%.

A rapid and sensitive automatic method was presented for the determination of domoic acid (DA) in mussels and algae using HPLC with a column switching system and UV detection [38]. Interfering peaks resulting from matrix protein components are

excluded by use of the RAM column in the preseparation step. Sample material was extracted with pure water and the crude extract was directly injected. DA is a toxin, which belongs to a group of naturally occurring neurotoxic amino acids and its monitoring in seafood and algae is required by institutions responsible for public food in the USA and Europe. In this paper, the HISEP RAM precolumn was combined with the analytical RP-18 column. Two mobile phases were used, 0.2% formic acid in acetonitrile for the preparation (RAM precolumn) and acetonitrile–water–formic acid for the separation on the analytical column. The same so-called internal-surface RP-18 precolumn has also been used in the previous paper [37]. Mussel material was mixed with water and sonicated for 5 min. After boiling for 5 min, the mixture was centrifuged at 5000 *g* and the supernatant was filtered. The extract was injected directly into the column switching system. Lyophilized algae material was also mixed with water and sonicated for 5 min. After the filtration, the supernatant was injected into the HPLC system. The detection limit for DA determination was 1 ng (0.125 mg DA/kg mussel tissue or 2.0 mg DA/kg algae tissue). This assay allows to monitor the preliminary tolerable amount for DA in seafood in Europe (20.0 mg DA/kg mussel).

The direct injection on an ISRP (RAM) column with column switching was described for the analysis of organochlorine pesticides in plain milk [39]. A simple and rapid procedure for the extraction and separation of aldrin, DDT, endrin, heptachloro- and methoxychloro-organochlorine pesticides in raw milk has been developed without the special pretreatment of the sample. The raw milk was diluted with the mobile phase (water–acetonitrile) to 50% and 25% milk solutions. The milk was fortified with known amounts of organochlorine pesticides. The extraction of milk proteins was realized on the ISRP C_{18} column and the HPLC separation on an ODS Hypersil column. Both columns were combined in a switching system. The UV detection was carried out at 254 nm. The used HSA ISRP column provided good results in the extraction of milk proteins due to two factors. First, the milk proteins are not adsorbed by human serum albumin immobilized on the surface of the silica gel and second, the milk proteins are not able to get into the small silica pores. Recoveries of the HSA ISRP column for organochlorine pesticides in milk was 99.3% ($n=5$).

The carbamates have been available since the 1960s as an alternative to organochlorine pesticides and have been used as contact insecticides for the control of external parasites. HPLC analyses of pesticides require very often sample preseparation procedures involving protein precipitation, LLE and analyte preconcentration. HSA ISRP C_{18} column has also been applied on-line for the determination of carbofuran in raw milk by direct injection into an HPLC system [40]. The mobile phase was phosphate buffer (pH 5.5)–acetonitrile and the UV detection was performed at 220 nm. The best results (rapid on-line extraction and minimum clogging of the HPLC system or column) were obtained with the 5% milk sample. Milk proteins were eluted rapidly from HSA RAM column (retention time 3.3 min), retention time of carbofuran was 5.9 min. Recoveries were 98.4–102.7% for the extraction of 0.062–1.00 μg/l carbofuran in milk. The reproducibility of the method was statistically evaluated. The detection limit was 0.025 μg/l.

6. Conclusion

In conclusion, it seems to be obvious that the application of direct analysis in the HPLC assay of food samples is not so simple in comparison to clinical samples. In many cases, especially for semi-solid and solid food matrices, e.g., tissue, fat, eggs, milk products, plants and herbs, the additional preseparation or cleaning steps have to be involved before the direct injection of sample into the HPLC system (homogenization, deproteinization, ultrafiltration, LLE, SPE, etc.). But automated SPE and column switching techniques including RAM sorbent applications give new possibilities to reduce the preseparation and preconcentration steps to a minimum. Very often only crude extracts could be applied. New approaches to the extraction procedures, e.g., microwave extraction, pressurized liquid extraction could reduce time and solvent consumption remarkably and the resulting extracts are clean

enough for the direct injection into the HPLC column switching system.

7. Nomenclature

HPLC	High-performance liquid chromatography
UV	Ultraviolet
DAD	Diode-array detection
LLE	Liquid–liquid extraction
SPE	Solid-phase extraction
MSPD	Matrix solid-phase dispersion
DSA	Direct sample analysis
RAM	Restricted-access media
RP-HPLC	Reversed-phase high-performance liquid chromatography
ODS	Octadecylsilane
GC–MS	Gas chromatography–mass spectrometry
RSD	Relative standard deviation
ELISA	Enzyme-linked immunosorbent assay
MS	Mass spectrometry
LC	Liquid chromatography
DACC	Donor–acceptor complex chromatography
PAHs	Polyaromatic hydrocarbons
EDTA	Ethylene diamine tetraacetic acid
CD	Cyclodextrin
MCAC	Metal chelate affinity chromatography
ASTED	Automated sequential trace enrichment dialysis
LOD	Limit of detection
FMOC	9-Fluorenylmethyl chloroformate
CAP	Chloramphenicol
EU	European Union
MRL	Maximal residual limit
THF	Tetrahydrofuran
DA	Domoic acid
ISRP	Internal surface reversed-phase
HSA	Human serum albumin
ASE	Accelerated solvent extraction
DI	Direct injection
MeCN	Acetonitrile
MeOH	Methanol
HXSA	Hexanesulfonic acid
NQS	1,2-Naphthoquinone-4-sulfonic acid
TEA	Triethylamine
ED	Electrochemical detection
FD	Fluorescence detection
ex	Excitation
em	Emission
APCI	Atmospheric pressure chemical ionization
EI	Electrospray ionization
RI	Refractive index
Tris	tris(Hydroxymethyl)aminomethane
DDT	1,1,-Trichloro-2,2-bis(*p*-chlorophenyl)ethane

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