Multidimensional Chromatography

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Trace organic separations are often made difficult by the large number of substances present in various kind of samples, by the similarities among the analytes, and by the need to remove major components in the sample. The advantages of using hyphenated systems to tackle such problems have been demonstrated and sequential separation techniques are also well suited. A characteristic feature of these methods is the use of two or more columns for the separation.

Separation in two dimensions has a substantial history. While methods such as two-dimensional (2-D) electrophoresis and thin-layer chromatography are 2-D in space, coupled column techniques are 2-D in time. In *offline* techniques, the fractions of the first column are collected in vials and reinjected onto the second column later. Interest in these techniques has been revived by increased automation. In 1973 Huber *et al*. proposed a two-channel multicolumn system which allowed an imitation of 2-D chromatography with columns as they existed at that time. In *online* techniques, manual or automatic switching by a valve directs fractions between columns. Electrically controlled valves greatly facilitate the full automation of the chromatographic process, thus increasing the speed and the work capacity of the *high performance liquid chromatography* (HPLC) system. The terms column switching, coupled column chromatography and multidimensional chromatography are used interchangeably.

Column switching includes, in the widest sense, all techniques in which the flow path of the mobile phase is changed by valves, so the effluent from a primary column is passed to a secondary column for a defined period of time. An unlimited number of columns can theoretically be incorporated in a chromatographic network. However, in each successive step, the transferred fraction must be reconcentrated to reduce the dispersion of the analyte in the chromatographic system, and the dead volumes in the conections between columns and in any switching valves must be minimized to achieve maximum separation efficiency. The band spread problem can be approached by making a judicious choice of the order in which the dimensions are sequenced.

Multidimensional separations have been defined by Giddings as having only two criteria. The first is that sample components must be displaced by two or more separation techniques based on different separating mechanisms. The second is that components separated by any single separation dimension must not be recombined in any further separation dimension. Most coupled-column approaches proposed only subject a portion of the sample to full 2-D or *n*-D analysis and are useful for the resolution of a single fused peak from the first dimension. They do not permit a comprehensive 2-D or *n*-D separation of the entire sample. The term linear heart-cut approach, used by Deans, describes the use of on–off valves in order to isolate an effluent segment which is then injected into a subsequent column. Comprehensive automated systems are useful for the greater resolution of multiple fused peaks from the first dimension column and resolved in the second-dimension orthogonal separation system.

Basic Theory and Configurations

Giddings' treatment of the peak capacity for a single mode leads to the expression:

$$\phi = 1 + \frac{N^{1/2}}{m} \ln (1 + k_n) \qquad [1]$$

where $m = 4$ implies unit resolution (4σ separation), N is the plate number, and k_n is the retention factor for the last member of a series of peaks numbered from zero (nonretained) through to n (last peak out). A sequence of independent modes, each having a peak capacity ϕ_i, should exhibit a multiplicative separation effect with an overall result given by:

$$\phi = \phi_1 \times \phi_2 \times \ldots \phi_n \qquad [2]$$

With a conventional single-column approach the peak capacity increases with dwindling returns. To increase the peak capacity by a factor of 10, a 100-fold increase in column length would be necessary. In coupled chromatography however the effect is, theoretically, multiplicative and leads to an exponential increase with the number of columns within the configuration with different retention mechanisms.

Not all column types are compatible: typical examples of compatible systems with sufficiently different retention mechanisms are size exclusion and normal-phase chromatography, reversed-phase and ion

exchange chromatography, ion exchange and reversed-phase chromatography and polar bonded phase and normal-phase chromatography. The columns employed can have the same or different lengths: even a very short column, like a guard column, is used as the first column for some applications.

When one discrete zone is collected from the first-dimension column and reinjected into the second dimension, the resulting data are two individual one-dimension data sets. In linear coupled-column systems, the peak capacities of the individual columns can be summed, not multiplied. In so-called comprehensive automated system, the resulting data are a matrix, usually represented as a contour plot with each chromatographic separation along an axis.

The multidimensional resolution (R_s) for comprehensive multidimensional chromatography was suggested to be equal to the Euclidean norm of the resolution in each dimension. Schure *et al.* showed that this definition, under a certain set of assumptions, could be utilized to produce an experimentally simple method for 2-D resolution estimation:

$$R_s = \left[-\frac{1}{2} \ln \frac{1-P}{2} \right]^{1/2} \qquad [3]$$

where P is the ratio of the difference between the amplitude at the valley and average peak maximum (f) and the average maximum peak (g) of the resulting one-dimensional chromatogram at the peak maxima in the contour plot, as shown in **Figure 1** ($t_{1,x}$ and $t_{2,x}$ are the retention times of the peak maxima for peaks 1 and 2 in the x separation axis. The peak maxima in the y dimension are similarly denoted as $t_{1,y}$ and $t_{2,y}$).

A host of methods exist for coupling the various separation systems. In order to set up a switching network, the separation problem must be analysed and a valve configuration selected according to the solution. A review of commonly used switching networks is given by Ramsteiner (see Further Reading). These techniques only require minor modifications to existing equipment and, of equal importance, enable the sample preparation procedures to be completely automated.

For multidimensional chromatography a standard high pressure liquid chromatograph is used with the addition of one or more switching valves. These valves may be simple, manually operated six-, eight- or 10-port valves or may be automatically controlled. An eight-port valve with matching sample loops is typically used when the comprehensive mode of operation is utilized. When coupling two or more separation techniques online, not only has an interface to be constructed but attention must be given to the effects of the injection volume and flow rates on the performance of the coupled system.

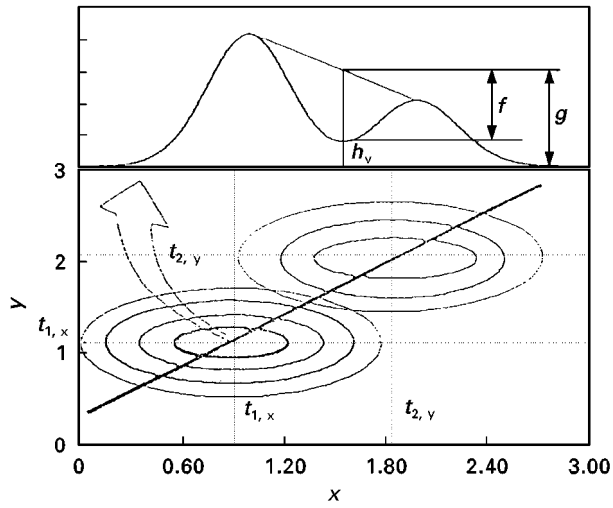

Figure 1 Schematic diagram of the two-dimensional resolution measurement using a 2-D contour (bottom) and the corresponding slice for resolution determination. (Reproduced with permission from Murphy RE, Schure MR and Foley JP (1998) *Analytical Chemistry* 70: 1585–1594. Copyright 1998 American Chemical Society.)

Scope of Multidimensional Liquid Chromatography

Multidimensional liquid chromatography (LC) is a powerful approach for demands such as improved analyte detectability and separating power, or the generally recognized need to increase sample throughput. In this sense, the main applications of multidimensional systems are analyte purification and enrichment, and improvement of the separation process (for instance, by increasing column length or by reducing the time of analysis), as will be illustrated in some detail below. In addition, because of their inherent flexibility, systems can be designed to achieve various objectives within a chromatographic network.

Sample clean-up

The main drawback of LC for the analysis of complex samples is the time-consuming and laborious nature of the sample pretreatment. Classic methods of clean-up often involve one or more liquid–liquid extractions, which have disadvantages such as the use of large volumes of organic solvents, the risk of contamination or the loss of analytes during evaporation and the final introduction of an aliquot of the (concentrated) extract into the LC column. Whereas

a chromatographic run often requires a few minutes, sample preparation time can be 1–2 h, and typically accounts for at least one-third of the error generated during the performance of the analytical method. Solid-phase extraction, although most commonly used as an offline technique, enables more rapid sample processing. Moreover, since a variety of stationary phases are available, better selectivity is generally achieved. However, the risk of contamination or loss of analytes, and the final introduction of an aliquot of the extract are ever-present drawbacks. In this respect, the employment of coupled columns is very well suited for the purification of complex samples before chromatography and today, coupling LC-LC is a well-established technique for sample clean-up, especially in the analysis of environmental, biomedical, pharmaceutical and food(stuff) samples.

The principle of multidimensional chromatography for sample clean-up is to transfer an effluent cut containing the analytes from a primary to a secondary column, whereas the remaining effluent containing unwanted compounds is vented to waste. In particular, the online coupling of a primary column, for the pre-separation of the analytes, with the analytical column via a switching valve greatly facilitates the analysis, as shown in **Table 1.** This table compares sample preparation steps typically involved in liquid–liquid extraction, and solid-phase extraction on to disposable cartridges, with those required by the multidimensional approach. As can be deduced from this table, a multidimensional approach drastically reduces, or even eliminates, manual sample preparation steps, and the entire analysis can be fully automated. In addition, the total time required for analysis is greatly shortened and the selectivity that can be reached is comparable to (and sometimes better than) that obtained with traditional liquid–liquid or solid-phase extraction. Additional advantages over classical procedures are avoidance of an internal standard (and thus of sample dilution) and protection of the analytes during analysis (for example, light-sensitive or oxidation-sensitive compounds, as they can be kept away from light and air).

The success of the clean-up process mainly depends on the type of sample, the working conditions used in the first chromatographic dimension and the configuration of the system.

Type of sample In principle, any liquid sample can be processed by multidimensional LC (solid samples require prior dissolution and homogenization). However, the stability of the chromatographic system is often limited when processing complex matrices. For example, up to several hundred samples can be analysed with satisfactory stability when analysing water samples or biofluids with a low protein content (such as urine). However, regeneration or replacement of the primary column after a few injections may be required for fluids with a large amount of proteins (for instance, plasma or blood) due to the irreversible adsorption of the proteins to the stationary phase, or to the clogging of the pre-column. In most instances, this problem can be overcome simply by centrifugation or filtration of the sample. A prior treatment such as acidification, or the addition of an organic solvent or a selective displacer, aimed to increase the free concentration of analytes tightly bound to matrix constituents may also be required.

Primary column The effectiveness of clean-up mainly depends on the ability of the primary column to

Table 1 Procedures for sample clean-up by liquid–liquid and solid-phase extraction on to a disposable cartridge and multidimensional approach

Liquid–liquid extraction	*Solid-phase extraction*	*Multidimensional LC*
Pipetting out the sample	Pipetting out the sample	Primary column conditioning and sample injection
Addition of an internal standard	Addition of an internal standard	
Addition of an organic solvent	Cartridge conditioning	
Agitation		
Centrifugation		
Separation	Elution of the sample	Switching-valve rotation
Possibly re-extraction	Matrix elimination by washing the cartidge	Elution of the analytes and insertion into the secondary column
Agitation		
Centrifugation		
Separation	Elution of the analytes	
Solvent evaporation	Solvent evaporation	
Redissolution	Redissolution	
Filtration	Filtration	
Injection	Injection	

retain selectively the compounds of interest. In general, small columns (often called pre-columns), typically 2–10 × 1–4.6 mm i.d., are preferred. Small size reduces cost, allows fast sampling and prevents band broadening during the analyte transfer to the analytical column. Longer columns provide higher resolution, which might be required for some applications. On the other hand, particle sizes in the 10–40 μm range are used to prevent pre-column clogging.

The stationary phase must be chosen according to the type of sample and the characteristics of the compounds to be analysed. For example, common reversed-phase materials, which are used in most published procedures, allow the retention of a wide variety of compounds of low to medium polarity. This makes the multidimensional approach very useful in the biomedical field, since proteins, salts and other highly polar matrix constituents are flushed to waste in the first fraction of eluent, while retaining the compounds of interest. Next, the analytes are desorbed and transferred to the analytical column for complete resolution. Obviously, pre-columns with a stationary phase similar to that of the analytical column provide little extra selectivity, but flushing of the pre-column with the correct solvent gives the required selectivity for most applications. This is illustrated in **Figure 2**, which shows the elution time profile obtained for untreated urine (50 μL) in a 20 × 2.1 mm i.d. pre-column packed with a C_{18} stationary phase. When using water as the mobile phase (washing solvent), the vast majority of matrix components are eliminated from the pre-column within the first 1–1.5 mL of eluent. Therefore, satisfactory preseparation can be achieved by incorporating a flushing step, provided that the analytes are not eluted from the first column during the washing stage. As an example, **Figure 3** shows the chromatograms obtained for a urine sample on a C_{18} analytical column, after flushing the pre-column with 1 mL of water. This figure also shows the chromatogram obtained for the same sample when a C_{18} solid-phase extraction cartridge was used offline for sample clean-up. As can be seen from this figure,

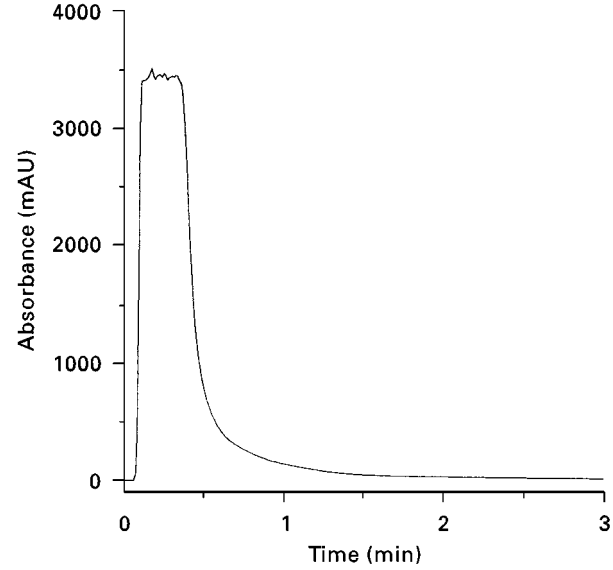

Figure 2 Elution time profile obtained for untreated urine in a pre-column packed with C_{18} stationary phase.

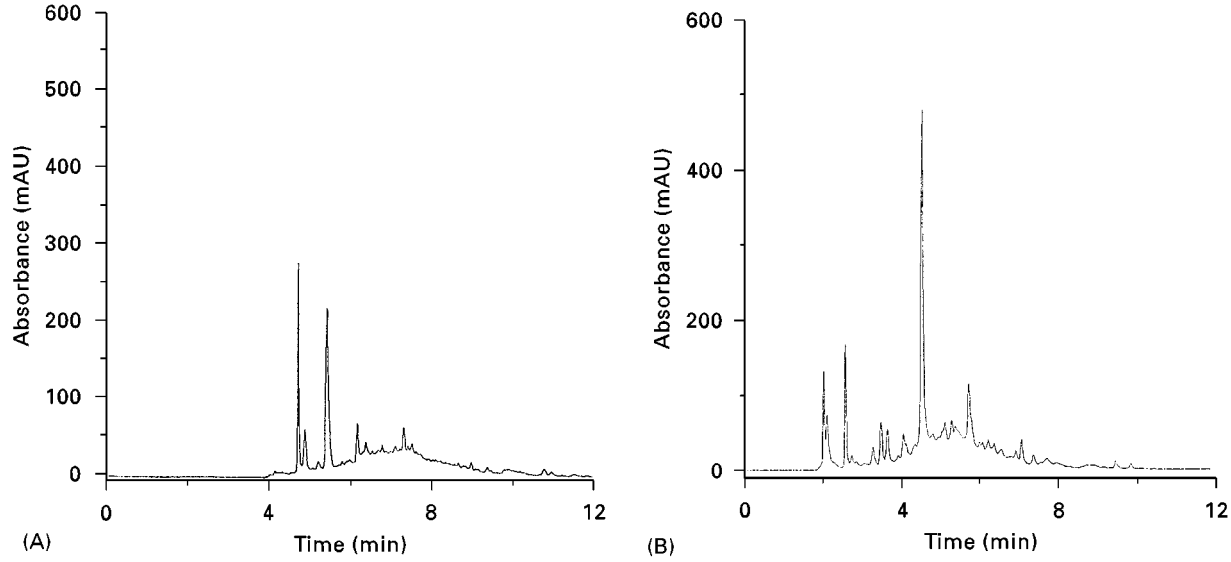

Figure 3 Typical chromatograms obtained from a urine sample by (A) online and (B) offline sample clean-up. Conditions: detection wavelength 230 nm; analytical column, Hypersil ODS C_{18} (5 μm, 250 × 4 mm i.d.); mobile phase, acetonitrile–0.05 mol L^{-1} phosphate buffer (pH 3).

the selectivity that can be reached is comparable to or even better than that obtained with solid-phase extraction cartridges. In some instances, selectivity can be improved simply by using a buffer or a specific modifier (an organic solvent or a surfactant) in the washing eluent.

Alternatively, a different chromatographic mode can be used to prepare the analytes. For example, gel permeation materials are effective for the elimination of high molecular mass matrix components before reversed-phase chromatography, which is particularly useful in the analysis of biofluids. A packing material specially designed for this field is the internal surface reversed-phase silica support (ISPR). ISPR materials confine the partitioning phase exclusively to the internal regions of the particles. Therefore, only interaction with small molecules is possible while the macromolecules are unretained since the external surface is nonadsorptive to them. The analysis of polar compounds requires different stationary phases. For example, ion exchangers or metal-loaded phases can be used for the selective retention of polar pesticides from environmental samples. Applications with protein-coated phases, immobilized antibodies or different copolymers have also been described for the analysis of different kinds of samples. The combination of two or more pre-columns with complementary separation properties can also been used for handling the clean-up of complex matrices.

Configuration The system design also determines the reliability of the system. In the simplest configuration, only a switching valve is required in addition to a basic chromatograph, as shown in **Figure 4.** It should be noted that, during the sampling and clean-up steps, the eluent in the analytical column is stagnant. Therefore, this configuration may lead to considerable baseline fluctuations depending on the elution conditions, which limits its applicability. However, excellent results are achieved when no buffers are used in the mobile phase. This is illustrated in Figure 4, which shows the chromatogram obtained for a urine sample spiked with the diuretic triamterene processed with the system shown in the figure. More powerful systems can be achieved at a reasonable cost by using an additional pumping system

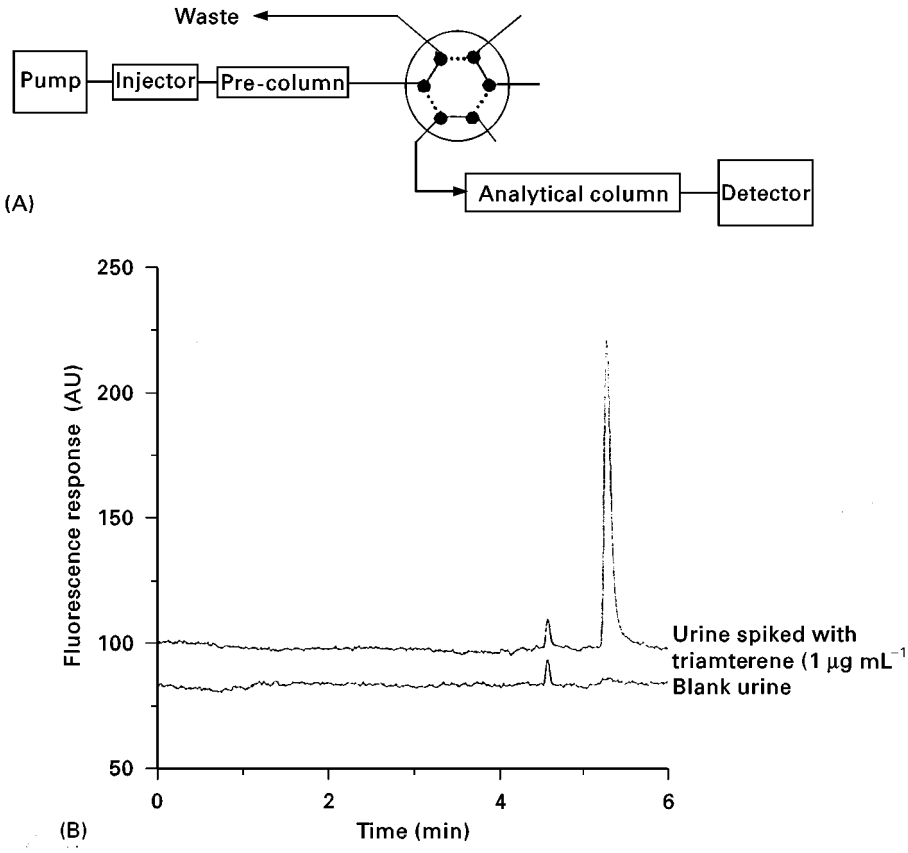

Figure 4 (A) Chromatographic system used for sample clean-up in straight-flush mode and (B) chromatogram obtained for clear and spiked urine sample with triamterene. Conditions: detection, excitation wavelength of 230 nm and emission wavelength of 430 nm; analytical column, LiChrospher 100 RP 18 (5 μm, 125 × 4 mm i.d.); mobile phase, acetonitrile–0.05 mol L^{-1} phosphate buffer (pH 3). (A) Continuous lines, clean-up; dotted lines, transfer and separation.

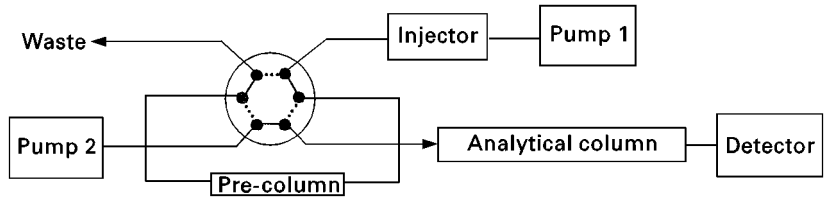

Figure 5 Schematic representation of a conventional chromatographic system with back-flush configuration. Continuous lines, clean-up; dotted lines, transfer and separation.

(**Figure 5**). In such a way, the analytical column is equilibrated during the clean-up stage, and only the fraction of the eluent containing the compounds of interest is transferred to the analytical column.

Different transfer modes can be used. In forward-flush (also called straight-flush) configurations the flow direction in the pre-column is not changed during the transfer stage, which prevents solid particles retained at the head of the pre-column being sent to the second column. However, for samples containing compounds which are strongly retained in the pre-column, backflush configurations (in which the flow direction of the mobile phase through the pre-column is reversed for the transfer on to the second column) are preferable, because the desorption of the most retained components after every injection is easier.

Sample Enrichment

Sample enrichment is based on analyte retention in a pre-column when a large volume of sample is flushed through it. This column is then connected to the main column for separation of the analytes. An essential condition for this set-up is that the sampling solvent is a weak solvent with respect to the retention mechanism of the analytes in the first enrichment column. For example, since water is a noneluting solvent in reversed-phase LC, large volumes of aqueous samples can be passed through columns packed with hydrophobic chemically bonded phases for enrichment of rather apolar analytes. As for the sample clean-up, greater accuracy and precision can be expected in analyte enrichment by multidimensional LC compared with classical enrichment procedures, which are more time-consuming and prone to errors.

According to the basic principles indicated in the above section, the optimization of the chromatographic conditions should include careful selection of the pre-column (packing and dimensions) and system design, in order to achieve maximum analyte enrichment and minimum peak-broadening. The ultimate enrichment factor is determined by the breakthrough volume of the solute in the pre-column. For most applications, rather small columns, similar to those used in sample clean-up, provide successful results. The enrichment of apolar compounds can be performed on C_{18}-bonded silica phases, while carbon-based materials or styrene-divinylbenzene provides sufficient resolution for the enrichment of relatively polar compounds (in environmental analysis, for instance). Maximum analyte detectability is reached by using a backflush configuration, which minimizes band broadening. Under optimized conditions, enrichment factors up to several thousand can be achieved.

Unfortunately, sample enrichment also concentrates components other than the analytes, thus limiting the degree of concentration possible. In this sense, the incorporation of washing stage after sample loading may improve the selectivity of the analysis. In such a case, an additional pump is required to deliver the washing solvent. In fact, many systems are designed to effect analyte enrichment and purification simultaneously.

Apart from the good compatibility of the sample with mobile phases used in reversed-phase LC, multidimensional chromatography is especially successful in the biomedical field, because sufficient sensitivity (high to low ng mL^{-1} level) can usually be reached with the direct injection of 0.1–0.5 mL of sample. However, trace analysis in water samples requires much higher sample volumes (50–100 mL). The employment of an LC pump for sampling allows such volumes to be loaded on to the pre-column. Published results demonstrate that detection limits of a few ng L^{-1} can be reached.

Enrichment via a pre-column can also be used to circumvent analyte dilution introduced by a different purification technique, for example, dialysis. Dilution, which is a serious problem when using online dialysis to remove macroconstituents from the sample matrix, can be overcome by refocusing the analytes in a pre-column before the chromatographic separation.

Improvement of the Separation Process

Multidimensional systems can be used to solve many problems encountered in the analysis of complex samples. Of particular interest are those systems designed to enhance the selectivity and to reduce the time of analysis.

The improvement in resolution is probably the most important application of multidimensional LC systems in the present context. The coupling of two or more chromatographic modes with complementary retention mechanisms considerably increases the resolution, provided that the separation modes are compatible. The great differences which are attainable due to the wide variety of separation modes available make multidimensional LC a very powerful tool for the resolution of complex mixtures and much more information can be obtained from a single run, especially if a comprehensive approach is used. A typical example is the analysis of proteins for peptide mapping. Reversed-phase LC coupled to mass spectrometry is generally used for peptide mapping, but the analysis of large proteins is often difficult due to the large number of fragments obtained from the enzymatic digestion of the protein. While mass spectrometry detection is useful to identify the presence of overlapping peaks, data interpretation becomes difficult if a large number of peptides co-elute. Difficulties also arise if the masses of the co-eluting peaks are similar and if the ^{13}C, ^{15}N or ^{18}O peaks overlap. A heart-cutting system combining size exclusion chromatography (SEC) and reversed-phase chromatography greatly facilitates the analysis. Two or more peptides co-eluting in the first SEC column could be transferred and separated in the reversed-phase column, because of the unlikely possibility that they would have the same molecular weight as well as the same hydrophobicity. In this approach, only the fractions containing co-eluting peaks are sampled into the reversed-phase column. A comprehensive configuration, however, offers substantially higher resolution, as fractions of the entire effluent from the SEC column are continuously sampled into the reversed-phase column. As a result, the peak capacity of the system is significantly increased, and exhaustive information from each sample can be obtained (three-dimensional data set). The addition of mass spectrometry allows the molecular weight information to identify accurately the chromatographic peaks. This kind of system has been successfully used for the characterization of proteins such as ovoalbumin and serum albumin in a reasonable time. As an example, **Figure 6** shows a typical 2-D chromatogram of a protein sample.

Other interesting applications of two-dimensional systems with part or all of the sample passing into the second dimension deal with the separation of polymers, polymer blends, copolymers or large molecules. The combination of two columns operating under size exclusion and (partition) reversed-phase modes provides complementary information on molecular weight distribution, chemical structure and architecture, thus making possible the complete characterization of complex polymers with a single run. Another example is the separation of groups of compounds in oil samples. The coupling of size exclusion and normal-phase modes enables the separation of oil fractions, and the subsequent resolution of the analytes according to their polarity (if required, the system can be interfaced to a gas chromatograph, for further resolution according to volatility).

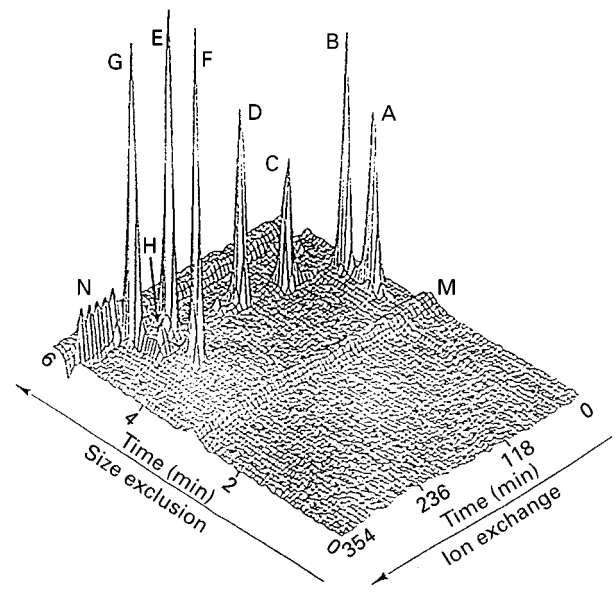

Figure 6 2-D chromatogram of protein sample. Peak A, glucose oxidase; B, ovalbumin; C, β-lactoglobulin A; D, trypsinogen; E, α-chymotrypsinogen A; F, conalbumin; G, ribonuclease A; H, haemoglobin; M, exclusion volume pressure ridge; N, inclusion volume salt ridge. Ovalbumin and α-chymotrypsinogen A at 2%, other proteins at 0.3% (w/v). C1 conditions: 5 μL min^{-1}, 0–100% buffer B from 20 to 260 min; buffer A, 0.2 mol L^{-1} NaH_2PO_4, pH 5; buffer B, 0.2 mol L^{-1} NaH_2PO_4/0.25 mol L^{-1} Na_2SO_4, pH 5. Valve activated 4 every 6 min; detection at 215 nm, data collection rate 0.5 points per s; plot shows every other point collected for injections 1–60. Each line perpendicular to the ion exchange chromatography time axis represents one injection on the SEC column. (Reproduced with permission from Bushey MM and Jorgenson JW (1990). Automated instrumentation for comprehensive two-dimensional high-performance liquid chromatography of proteins. *Analytical Chemistry* 62: 161–167. Copyright 1990 American Chemical Society.)

An area of special interest is the resolution of enantiomers. Although different combination have been described, in the most commonly used configuration the first column (achiral) effects a separation of the compounds of interest, whereas only the fraction of the eluent containing the optical isomers is transferred to the second chiral column for enantioresolution. This simplifies analysis, particularly in the biomedical field, because several matrix compounds can lead to pairs of peaks, thus making the resolution of the compounds of interest difficult. In addition, the

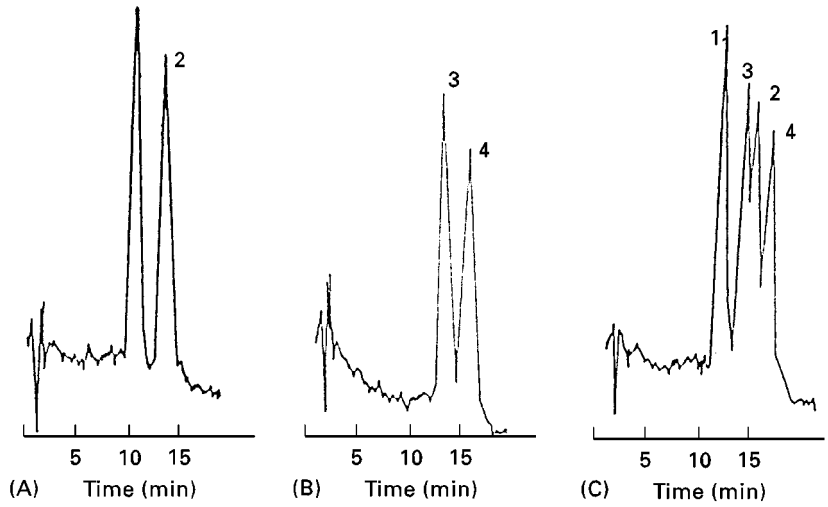

Figure 7 Representative chromatograms for the separation of separation of racemic verapamil (*R,S*-VER) and racemic norverapamil (*R,S*-NOR) on the AGP-CSP. (A) *R,S*-VER; (B) *R,S*-NOR; (C) *R,S*-VER and *R,S*-NOR. 1, *R*-VER; 2, *S*-VER; 3, *R*-NOR; 4, *S*-NOR. (Reprinted from Fried F and Wainer IW (1997) Column-switching techniques on the biomedical analysis of stereoisomeric drugs: why, how and when. *Journal of Chromatography B* 689: 91–104. Copyright 1997, with permission from Elsevier Science.)

chiral column is better protected. An example of the kind of problems encountered in chiral analysis is the inability of many chiral stationary phases to separate parent drugs from metabolites. This is illustrated in **Figure 7**, which shows the chromatograms obtained for the calcium channel-blocking agent verapamil and its major metabolite norverapamil on an AGP chiral column. This column allows the enantioresolution of both compounds but overlap between *S*-verapamil and *R*-norverapamil is observed. The problem can be overcome by using an achiral–chiral system, where verapamil and norverapamil are initially separated from each other on the achiral (reversed-phase) column. The eluent fractions containing verapamil and norverapamil are then selectively transferred to the chiral AGP column for further separation of the enantiomers. A multidimensional approach can also be used to enhance the selectivity by effecting peak compression on an achiral column, after enantioresolution in a primary chiral column. This approach appears as an elegant alternative in chiral analysis of drugs in biofluids or in multiresidue pesticide analysis.

Another area of application is the analysis of derivatized analytes. In this case, pre-separation or trapping in the first column allows a large amount of unreacted reagent or secondary products to be excluded for the second (analytical) column (**Figure 8**). Solid-phase reagents prepared by immobilizing detection-sensitive reagents on solid supports such as organic polymers can be used as column packings and integrated into the liquid chromatography system in order to form derivatives online. This methodology has been successfully applied to the derivatization of a variety of drugs. During the last 5 years, our research group has been studying the possibility of carrying out derivatizations with conventional octadecylsilica stationary phases. A possible online set-up for this purpose is shown in Figure 4 or 5. The system uses a 20×4.6 mm i.d. pre-column packed with an unmodified octadecylsilica stationary phase. This column is used to purify the sample and

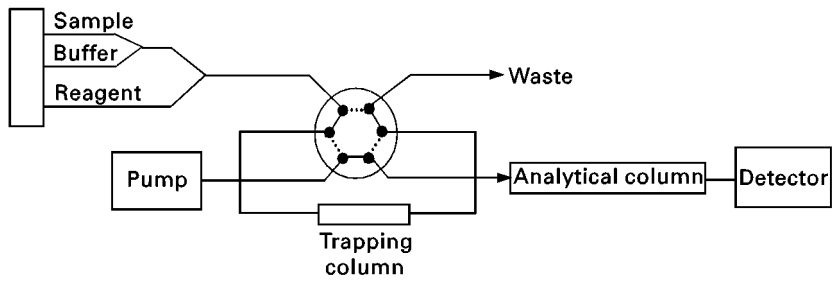

Figure 8 Configuration used for online sample clean-up and derivatization. Continuous lines, derivatization; dotted lines, transfer and separation.

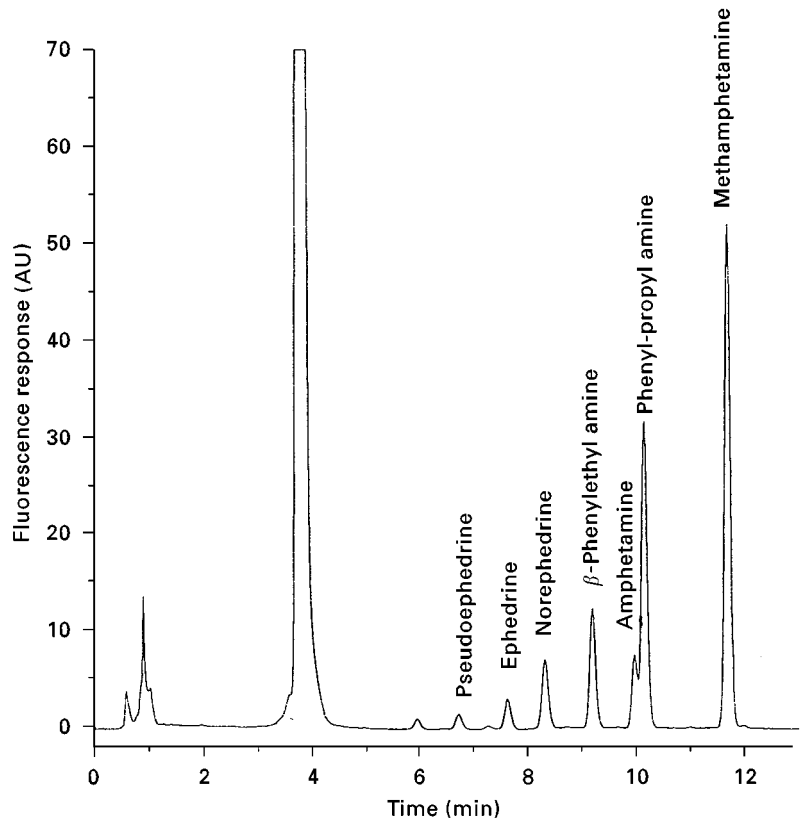

Figure 9 Chromatogram of an aqueous mixture of the amphetamines (5 μg mL^{-1}) derivatized online by using the configuration shown in Figure 5. Conditions: derivatization reagent, 9-fluorenylmethyl chloroformate; detection, excitation wavelength 264 nm and emission wavelength 313 nm; analytical column, LiChrospher 100 RP 18 (5 μm, 125 × 4 mm i.d.); mobile phase, acetonitrile–water.

concentrate the analytes; the trapped analytes are derivatized by injection of the derivatization reagent into the pre-column. Finally, the derivatives are transferred to the analytical column by means of a switching arrangement. Some applications based on this methodology have been described for the determination of amphetamine and related compounds in urine, by using reagents such as 1,2-naphthoquinone 4-sulfonate, 9-fluorenylmethyl chloroformate and *o*-phthaldialdehyde. **Figure 9** is an example of the chromatograms obtained by this methodology.

Multidimensional systems can also be used to minimize the separation time of mixtures containing compounds of considerably different retention factors. In a column of high selectivity and under optimized conditions, compounds eluting at the start of the chromatogram can be generally well resolved, but later eluting compounds with long retention times may be difficult to detect. Conversely, if the column has low selectivity, the separation time will be shorter, but early eluting components will be poorly resolved. The coupling of two columns is well suited for such kinds of problems. The first column (short or low selectivity) is used to resolve the late eluting components in a short time. These compounds are sent directly to the detector, whereas the poorly retained components are transferred to the second column (longer or more selective) for complete resolution. The overall effect is a considerable reduction of the time of analysis. This approach is a good alternative to gradient elution in those cases where a large number of plates is required, where a large number of samples will be analysed (as full automation is possible), or where detectors not compatible with gradient elution will be used.

An interesting form of this alternative is the so-called box car chromatography. The box car configuration involves partial separation of the compounds of interest in the first column, and subsequent transfer to a second column, where they are injected with the maximum frequency permitted by the resolution of the column. In this way, the separation power of the second column is fully utilized, and this considerably increases sample throughput. An example of the box car technique is the analysis of primidone and phenobarbitone in plasma. When utilizing a box car configuration, up to 40 samples per hour can be analysed, and this illustrates the potential of this technique.

Future Developments

Looking to the future, evolutionary development can be expected because the separating power of multidimension liquid chromatography is greatly increased over single-dimension liquid chromatography. The simultaneous improvements on both the software and the chromatographic apparatus will lead to a system capable of automatically developing analytical methods for a wide range of analytes in many different matrices. Other desirable aspects are a reasonably short analysis time and flexible operating conditions.

The thrust of multidimensional research will most probably be to improve the combination of separation methods, including coupling to alternative separation techniques.

See also: **II/Chromatography: Thin-layer (Planar):** Modes of Development: Conventional. **Electrophoresis:** Two-Dimensional Electrophoresis. **III/Chiral Separations:** Liquid Chromatography. **Pharmaceuticals:** Chiral Separations: Liquid Chromatography.

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Normal Phase Chromatography: Mechanisms

See **II / CHROMATOGRAPHY: LIQUID / Mechanisms: Normal Phase**

Nuclear Magnetic Resonance Detectors

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Introduction

In many fields of chemistry, biology, pharmacy and medicine, progress is often limited by the ability to resolve complex analytical problems. To this end analytical techniques have been developed in recent decades dealing with an integrated approach to the separation of mixtures together with structural elucidation of unknown compounds. High performance liquid chromatography (HPLC), gel permeation chromatography (GPC) and supercritical fluid chromatography (SFC), as well as the capillary separation techniques capillary HPLC (CHPLC), capillary electrophoresis (CE) and capillary electrochromatography (CEC) are the most powerful techniques