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Review

Solid-phase extraction or liquid chromatography coupled on-line with gas chromatography in the analysis of biological samples

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Abstract

This review provides an overview of the on-line coupling of solid-phase extraction or liquid chromatography with gas chromatography for the analysis of biological samples. Principles relevant to techniques are briefly presented and selected applications are described. Benefits of the coupled systems are discussed.

Keywords: Reviews; Biological samples; On-line coupling

Contents

1. Introduction

More sensitive methods to analyse trace amounts of compounds in biological matrices are continually required by industry and the research community. Because the number of samples to be analysed tends to be large, the methods are also expected to be automated and as rapid as possible. At present, trace-level analysis relies heavily on chromatographic techniques such as liquid and gas chromatography (LC and GC). GC is often the method of choice for the analysis of complex samples with volatile target analytes because of the speed of analysis, separation efficiency and the wide range of selective and sensitive detectors available. Mass spectrometry (MS) is a particularly important detection method: it is easy to couple with gas chromatography and allows reliable and selective identification and detection. Because the ionisation of compounds in GC–MS is highly reproducible, spectral libraries for electron impact ionization have been compiled for the identification of unknown compounds. The same is not true in LC–MS systems, in which the ionisation is greatly affected by the choice of eluent. A major drawback of the GC is that it is not suitable for thermolabile or insufficiently volatile analytes without a derivatization step. A particular problem with chromatographic methods in general is the tedious sample preparation that most samples require, especially biological samples where the analytes of interest are present in highly complex matrices. Sample pretreatment is aimed at improving the

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selectivity, detectability, reliability, accuracy and repeatability of the analysis, which is done by selectively removing matrix compounds that might disturb the analysis. The most widely applied sample preparation techniques for biological liquids are liquid–liquid extraction (LLE) and solid-phase extraction (SPE).

The inadequacy of the classical instrumental methods used in the analysis of biological, and other complex samples, usually involving lack of sensitivity, has spurred the development of multidimensional techniques. Several studies have already been appeared, describing the on-line combination of sample pretreatment with GC for the analysis of complex samples, including biological ones. The idea of on-line coupling is to perform sample clean-up, concentration and fractionation as an integral part of the analysis in a closed system. The whole sample then become available for the analysis. On-line techniques do not require handling of the samples between the trace-enrichment and the separation steps and are highly suitable, therefore for full automation. Manual sample preparation is minimised or even totally avoided. Another benefit is that the sample clean-up usually is much more effective. In on-line methods, none of the sample material is wasted and sensitivity is accordingly improved. Yet, further advantages are the shortened time of analysis and better reliability and repeatability. For biological and pharmaceutical samples, both SPE and LC have been coupled on-line with GC [1–19]. Also other on-line techniques, such as membrane extraction and on-line LLE, have been directly coupled with GC, but these methods are not covered in this review.

Selection of a suitable on-line SPE–GC or LC–GC method for the analysis of biological samples must take into account the type of sample matrix and the purpose of the analysis. Liquid samples, such as urine and serum, are typically analysed by SPE–GC or reversed phase-LC–GC, although a few normal phase LC–GC applications have been applied as well. Tissue samples, however, typically require extraction with organic solvent before analysis, and NPLC–GC is then the more straightforward technique. The main difference between SPE–GC and LC–GC is that in SPE–GC the whole extract is transferred to the GC, while in LC–GC only part of the sample is transferred. SPE–GC methods are thus better suited for the screening of unknowns whereas LC–GC methods are suitable for target analysis and the analysis of complex samples, which require highly efficient and selective clean-up procedures.

2. Instrumentation

The instrumentation required for SPE–GC and LC–GC is fairly similar. Both systems require special methods, because the volumes of extracts transferred to GC are typically several hundred microlitres, or even millilitres. Aqueous samples, too, call for special procedures in the on-line coupling because water is not a suitable solvent for GC as it hydrolyses the siloxane bonds in GC columns causing re-activation of silylated surfaces and deterioration of the stationary phase.

Figs. 1 and 2 present typical instrumentations for SPE–GC and LC–GC. Both instruments consist of a solvent delivery system, two or more multiport valves, and a GC system usually equipped with a solvent vapour exit (SVE). A drying gas is often required in SPE–GC. In LC the separation is frequently monitored with a UV detector.

The interface between SPE or LC and GC allows for large volume sample introduction to GC. On-column, loop-type and vaporiser interfaces (programmable temperature

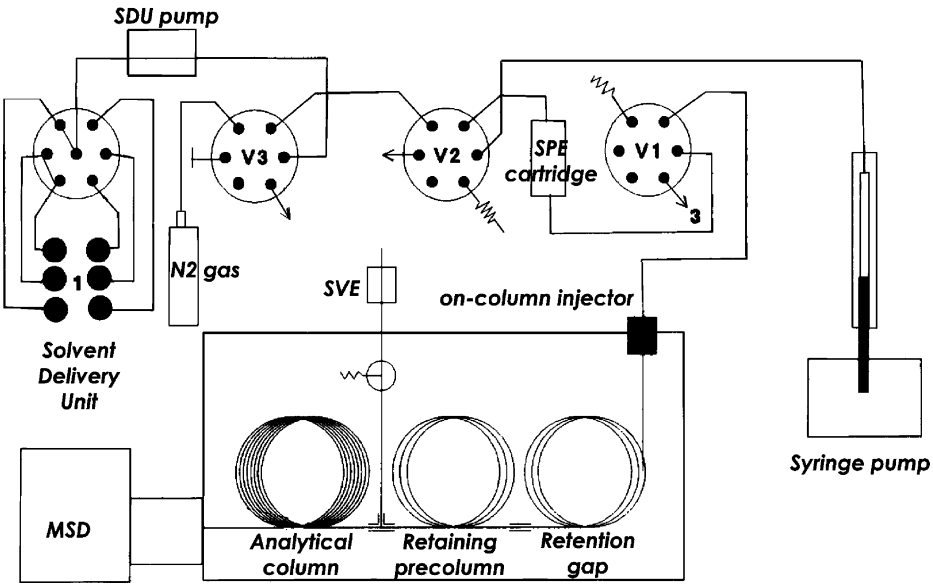

Fig. 1. Construction of an on-line SPE–GC system consisting of three switching valves (V1–V3), two pumps (SDU pump and syringe pump) and a GC system equipped with an SVE, an MS detector, a retention gap, a retaining precolumn and an analytical GC column (from [40]).

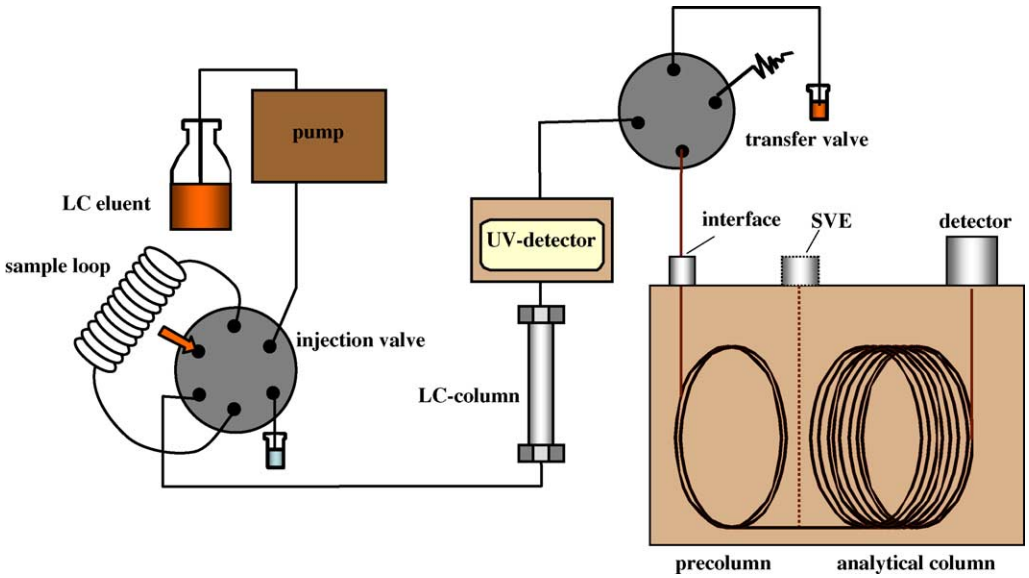

Fig. 2. A schematic drawing of an on-line coupled LC–GC system.

vaporiser, PTV) have been employed with both SPE–GC and LC–GC, as have been reviewed earlier [19–26]. In large-volume transfer the solvent evaporation technique is critical for obtaining sharp peaks. On-column and loop-type interfaces are usually employed with retention gap techniques and fully concurrent solvent evaporation (FCSE) techniques, respectively. Generally, a solvent vapour exit (SVE) is used to accelerate the solvent evaporation. With a PTV as the interface, the SVE is not always necessary because the solvent can be removed via a split exit valve. The interfaces each have their own advantages and disadvantages as has been described in [1–5]. Briefly, the loop-type interface with FCSE is the simplest to optimise and use, but it is not suitable for highly volatile analytes. The on-column interface with the retention gap technique requires more optimisation, but it also allows the analysis of volatiles as well as non-volatiles. The main drawback of this interface is that it does not tolerate dirty samples or LC fractions. The vaporiser interface is suitable for dirty samples but the transfer of volatiles requires careful optimisation.

2.1. On-line coupling of solid-phase extraction with gas chromatography

On-line coupled solid-phase extraction-gas chromatography (SPE–GC) is basically simple. The coupled SPE procedures are essentially the same as off-line ones, in both cases involving conditioning of the SP material before loading of the sample. Typically, after trapping, and before elution of the analytes, the SPE column is dried [25,27–30]. The extract is then directly transferred to the GC interface where the final separation takes place. The solid-phase extraction takes place in a short column (10–20 mm × 1–4.6 mm i.d.) packed with suitable stationary phase, with particle sizes ranging from 10 to 30 μm [5–7,24,25]. Normally, large sample volumes (over 10 ml) can be handled with this type of SPE column.

Alkyl bonded silicas (C8, C18) or styrene-divinylbenzyl copolymers with a particle size of 10–40 μm are the main solid-phase materials used for trapping in SPE–GC [5–7,24,25]. Also immunosorbents have been utilised [31,32]. Most applications make use of cartridges or small precolumns, but also membrane discs, known as Empore discs, can be used as well [33,34]. The advantages of discs over SPE columns are higher sample throughput, shorter and more efficient removal of water and smaller volumes of the solvents needed for desorption. A major drawback is the small sample capacity of the discs.

Three parameters should be taken into consideration in choosing the sorbent material for on-line techniques. First, the sorbent should have satisfactory breakthrough volume. Second, drying of the sorbent should be easy, because GC cannot tolerate large amounts of water. Third, it should be possible to elute the analytes easily and in small volume with a solvent suitable for GC analysis.

Breakthrough is seldom a problem in SPE–GC applications because the target analytes tend to be nonpolar or only relatively polar compounds with have reasonably large breakthrough volumes. It should be noted that copolymers of PLRP-S type typically offer 20–30-fold more retention than C18-bonded silicas and breakthrough volumes are in the range 10–100 ml [24,31].

Even though drying of the sorbent is easily achieved with a gas flow, effective elimination of water will always be time-consuming at ambient temperature. The SPE column can be heated during the drying process, but this increases the risk of losing volatile analytes. Another approach is to remove water after the SPE column using a special drying column packed with copper sulphate or silica [28,32].

Copolymer sorbents are more easily dried, again making them advantageous in on-line coupling.

Elution is done with a solvent suitable for GC analysis, often ethyl acetate or *n*-propanol. The benefit of ethyl acetate is that it is slightly soluble in water, and forms an azeotropic mixture with water (8:92, v/v), which minimises problems if small volumes of water happen to be transferred together with the organic solvent into the GC column.

It is also possible to perform SPE–GC making use of thermal desorption in SPE (SPETD) instead eluting with solvent [35,36]. In SPETD–GC the analytes are thermally desorbed from the stationary phase by heating the trap. Often the trap is located directly at the GC injector. The carrier gas supply can be adjusted so that there is a counterflow from the GC column to the injector, preventing water from entering the GC column during sampling and drying. As many common solid-phase materials cannot withstand elevated temperatures, the packing materials used in SPETD and in SPE are often different. Tenax is a common packing material for SPETD since it has sufficient retention power for analytes, good thermal stability and poor enough interaction with water to allow optimal drying. One drawback of SPETD is that some analytes are too efficiently trapped on the stationary phase and do not desorb upon heating.

2.2. On-line coupling of liquid chromatography with gas chromatography

On-line coupled liquid chromatography–gas chromatography is used in heartcut analyses, when more selective cleanup is required than is possible by techniques such as SPE–GC. LC provides excellent separation efficiency and selectivity because high efficiency columns can be used. Moreover, the facility to monitor the separation with the LC detector allows conditions to be optimised quickly and accurately. In addition, fraction(s) containing the analytes of interest can be accurately cut and transferred to the GC.

Both NPLC–GC and RPLC–GC methods have been developed for the analysis of biological samples. The coupling systems for NPLC–GC are mature, robust and suitable for routine analyses. Relative to the coupling of NPLC to GC, the coupling RPLC to GC is a demanding task [24–26]. The problems arise from the aqueous eluents required in RPLC. A particular disadvantage of aqueous eluents is that salts and non-volatile matrix constituents will be introduced to the GC, where they will interfere with the performance of the system.

The problems of RPLC–GC coupling have been tackled in two ways: direct solutions to the problem of aqueous eluents rely on special techniques, whereas indirect solutions avoid them by phase switching, i.e. replacing the water with suitable organic solvent before the GC analysis. Direct approaches are generally more tempting, especially for routine applications, as they are simple and instrumentally less complicated than indirect solutions. Of the many solutions devised to overcome the problems of direct injection, only the vaporiser-type systems can be considered robust enough for routine applications. The indirect phase switching approaches, in turn, utilise continuous liquid–liquid extraction, solid-phase extraction or open tubular trapping. Among these techniques, the SPE-type interface is the most promising. The techniques are well described in [24–26].

2.3. On-line derivatisation

Many analytes need to be derivatised before GC separation, either to increase their volatility and thermal stability or to decrease their adsorptivity. In particular, many drugs and pharmaceuticals require derivatisation. Typically, derivatisation is performed before the LC–GC analysis but it is also possible to include on-line derivatisation in the analysis, a procedure that often offers advantages over off-line methods. One notable advantage of on-line derivatisation is that the enrichment or preseparation can then be based on underivatised functional groups of the analytes or of the matrix compounds. If the analytes are present in water, a matrix that in many cases does not allow derivatisation, off-line derivatisation is not even possible before the RPLC–GC analysis. The on-line derivatisation needed in LC–GC can be carried out in the LC system, between LC and GC, or in the inlet of the GC column.

A post-column reactor between the LC and GC columns has been applied to triglycerides, but the reaction kinetics proved to be too slow for viable assay [37]. Derivatisation also has been combined with the on-line extraction step, although not in actual LC–GC analysis. The approach for derivatisation of organic acids and anilines has included either simultaneous derivatisation and extraction [38] or derivatisation in a segmented stream followed by extraction [39].

Derivatisation in the inlet of the GC precolumn not only requires fast reactions but also volatility and high purity of the reagent. Any side products formed in the reaction must also be volatile. Furthermore, neither the reagent itself nor the side products should damage the surface of the precolumn or the analytical column. On-line derivatisation in the GC precolumn has been accomplished with both the loop-type interface and the on-column interface. In the latter case, silylation, acylation and methylation reactions have been demonstrated for NPLC–GC [16]. The procedure was either to premix the reagent and the analytes in the HPLC effluent before the GC precolumn, or to deliver the reagent independently after the transfer and the evaporation process. No negative effects were reported on the surfaces of the columns, although the derivatisation reagent was used in large excess. The loop-type interface, combined with on-line LLE to extract the analytes into organic solvent, has been used for on-line silylation, and it is well suited for on-line derivatisation in RPLC–GC systems [9–14].

3. Comparison of coupling techniques

In on-line systems, the parameters than need to be optimised are generally the same than those in off-line methods. These include the selection of conditions in preseparation in SPE or HPLC: type and amount of adsorbent, sample amount, elution solvent and flow rate during sample introduction and elution. In addition, in SPE–GC, drying of the trap must be considered. The advantage over off-line methods is that in the optimisation the facility to monitor the separation with the LC detector allows the conditions to be optimised quickly and precisely. In the interface system, the optimal conditions are largely dependent on the type of the interface and the volatility of the analytes. The loop-type interface is the simplest to optimise, as only temperature must be considered. However, it is not suited for the analysis of very volatile analytes. In on-column interface and PTV, more parameters must be taken into account, namely, transfer flow rate, temperature, carrier gas pressure and flow rate and closure of SVE or PTV split valve. Choosing the parameters for the transfer is relatively straightforward in most cases, however, as there is a lot of data available in literature. Fine-tuning of the conditions may be then required for very volatile analytes.

For aqueous samples, two on-line approaches are available, namely SPE–GC and RPLC–GC. Of these, the on-line RPLC–GC has not yet become a routine technique. Direct systems in RPLC–GC coupling are at least yet not of practical use, mainly because of many of the systems are still not rugged enough for large scale use. In addition, the optimisation in direct coupling is demanding and requires special knowledge. Among the indirect solutions, the phase switching with SPE technique would seem to be most promising. The main benefit compared with phase switching with on-line LLE is the simplicity. No phase separator is needed, and also the pre-concentration with SPE is more efficient than that with LLE. SPE–GC system is a simplified version of the RPLC–SPE–GC, and it is applicable also for routine use. Automated systems have been developed and the applicability of SPE–GC has been demonstrated in several studies. The use of polymeric materials for SPE allow introduction of large sample volumes, and therefore, an efficient enrichment of even relatively polar analytes. Optimisation of SPE conditions (sample amount, flow rates, drying and elution) is simple, because UV detection can be used in the optimisation. Also the conditions for transferring the sample fraction from SPE to GC (i.e. elution solvent, elution volume, interface type and GC conditions) are rather well characterised in literature. The main drawback of the system is the rather time-consuming drying of the SP trap. The drying is, however, essential, because even small amounts of water can decrease the lifetime of the GC column system substantially. Typically, elution is performed with ethyl acetate, which has relatively high boiling point. Therefore, even with on-column interface utilising retention gap techniques, the analysis of very volatile analytes is not possible. Thus, the system is best suited for the analysis of medium volatile to (relatively) nonvolatile analytes (>C12). Both on-column interface and loop-type interface work well as interfaces for SPE and GC. SPE with thermal desorption has not been utilised widely, probably because efficient desorption of high boiling compounds from the adsorbent is difficult, and the analysis of high-boiling compounds is then restricted.

NPLC–GC is the obvious choice for fat-containing biological samples such as tissue samples, since such samples usually require extraction with organic solvent. NPLC–GC is, however, not usually suitable for direct injection of aqueous samples. The advantage of NPLC–GC is that several methods have already been developed and they are fairly simple to use, and automation is easy. The on-column interface has been widely used, as it also allows the analysis of relatively volatile analytes. Because LC enables very efficient clean up of the sample, the transferred fraction is generally clean and contamination of the column inlet is not a problem. The on-column interface can also be used with concurrent solvent evaporation. If a loop-type interface is used, the oven temperature must be higher, restricting analysis of the volatiles.

4. Applications

Several interesting applications involving SPE–GC and LC–GC systems have been developed for the analysis of biological samples (Table 1). The on-line systems are feasible for the biological samples because the sensitivity of such systems is high, and the amount of sample required is much less than in conventional analysis. Compared with the applications in environmental analysis, however, the use in the biomedical area is much less widespread. One reasons for this is that many of the analytes of interest in biological matrices require derivatisation before GC analysis. Often, derivatisation cannot be done before the analysis because the aqueous matrix would interfere with the reaction. Although an on-line derivatisation step can be added to the procedure, this makes the procedure more complex and less attractive.

4.1. Liquid samples

At-line SPE in combination with on-line SPE–GC has been developed for the determination of trazodone and benzodiazepines in plasma [4–7]. The procedure utilised automated sample preparation with extraction unit (ASPEC) for at-line SPE of the plasma sample, which was eluted into the vial of the ASPEC [6]. The methanolic extract was on-line diluted with water before injection to the on-line SPE–GC system, consisting of a PLRP-S column and an on-column interface. The limit of detection was 3 ng/l for trazodone and 30 ng/l for the benzodiazepines using a FID as detector.

The system was subsequently modified to obtain better sensitivity for the benzodiazepines [7]. The trapping column

Table 1
Applications of SPE–GC and LC–GC systems in the analysis of biological samples (ACN, acetonitrile; DEA, ditehylamine; DEE, diethyl ether; MeOH, methanol)

Sample	Method	LC/SPE column; eluent, flow rate	Interface, evaporation technique	Analysis time (min)	LOD	Ref.
Stilbene hormones in meat	NPLC–GC with on-line derivatisation	250 mm × 2.1 mm i.d. Lichrosorb Diol; methanol/pentane (15/85), 0.14 ml/min	On-column with FCSE at 100 °C	25	0.17–0.47 g/kg	[16]
Diazepam in urine	μ-RPLC–GC	150 mm × 0.32 mm i.d. RoSil-C18 column; MeOH/H_2O (80/20), 4 μl/min	On-column with PCSE at 65 °C	20	–	[1]
DDE and PCBs in adipose tissue	NPLC–GC–ECD	50 mm × 1 mm Hypersil; *n*-hexane, 0.15 ml/min	On-column with PCSE at 77 °C	75	0.1 ng/ml	[19]
β-Blockers in serum and urine	RPLC–LLE–GC–FID with on-line derivatisation	20 × 2.1 CapcellPak C18 SG-120 column; 0.05 M boric acid/ACN (88/22) at pH 10.2, 0.8 ml/min	Loop-type, FCSE, at 92 °C	45	18–44 ng/ml	[12,14]
Opiates in serum and urine	RPLC–LLE–GC–FID with on-line derivatisation	20 mm × 2.1 mm i.d. C18 column; boric acid/ACN (80/20)	Loop-type, FCSE, at 90 °C	60	61–62 ng/ml	[13]
Polychlorinated biphenyls in plasma	NPLC–GC-ECD	100 mm × 3 mm i.d. dinitroanilinepropylsilica; pentane, 0.5 ml/min	Loop-type, FCSE at 57 °C	70	<1 pg/g	[17]
Heroin metabolites in urine	NPLC–GC–FID	100 mm × 2 mm i.d. silica column, DEE/MeOH/DEA (91.5/8/0.5), 0.4 ml/min	Loop, FCSE at 100 °C	ca. 50	<800 ng/ml	[18]
PCBs in fish tissue	NPLC–GC-ECD	100 mm × 2.1 mm i.d., cyano column; hexane, 0.25 ml/min	Loop, FCSE at 105 °C	ca. 50	1 pg/ml	[2]
Fungicides in pepper, strawberry, citrus, soybean	NPLC–GC–ECD	Nucleosil CN5 column; hexane/ethanol (8/2)	Loop type, FCSE	–	<0.01 mg/kg	[3]
Levoprolol in plasma	NPLC–GC–ECD	250 mm × 4 mm i.d. Hibar Lichrosorb CN, pentane/DEE (55/45), 1 ml/min	Loop-type, FCSE at 79 °C	18	0.2 ng/ml	[15]
Trazodone in plasma	SPE–GC–FID	10 mm × 2 mm i.d. PLRP-S; ethylacetate, 0.08 ml/min	On-column, FCSE at 80 °C	45	3 ng/ml	[6]
Benzodiazepines in plasma	ASPEC–GC–NPD	10 mm × 2 mm i.d. LC-18; ethylacetate	Loop-type, FCSE at 110 °C	20	0.5–2 ng/ml	[7]
Benzodiazepines in plasma	Dialysis–SPE–GC–NPD	10 mm × 2 mm i.d. PLRP-S; ethylacetate, 0.5 ml/min	Loop-type, FCSE at 110 °C	40	5–25 ng/ml	[5]

was eliminated from the system, while the on-column interface was changed to a loop-type interface and the FID to NPD. The modified system was not strictly on-line, therefore, but rather at-line SPE–GC. The SPE cartridge was eluted with ethyl acetate and, since no drying step could be incorporated in this set-up, a small amount of water was transferred to the GC together with the ethyl acetate, requiring very careful optimisation of the transfer conditions. The LODs improved with this system to 0.5 ng/l. However, the clean-up procedure was not fully satisfactory and as a next step it was developed further to comprise on-line dialysis coupled to on-line SPE–GC [5]. Dialysis is the most widely used membrane-based sample pretreatment technique and it is typically performed in continuous mode in trace analysis. A pre-concentration column is then almost always used. Automated systems for on-line dialysis –SPE are available and it was relatively easy, therefore, to extend the system by adding on-line dialysis. The sample clean-up by combined dialysis-SPE was very efficient and provided highly sensitive determination (ng/l) of the benzodiapines (Fig. 3).

An RPLC–LLE–GC system has been developed for the analysis of beta-blockers in human serum and urine [12,14]. The system enables direct injection of the biological fluids to the RPLC–GC. An indirect technique relying on continuous liquid–liquid extraction was used in the LC–GC coupling, because on-line derivatisation of the analytes was required under non-aqueous conditions. Derivatisation of the polar analytes was also accomplished on-line, by co-injection of silylation reagent during transfer of the analyte fraction via a loop-type interface. Analysis of human urine and serum is shown in Fig. 4. Fully concurrent eluent evaporation was used during the transfer because the analytes were not particularly volatile. The total analysis time (less than 45 min) was considerably less than with traditional methods (2–3 h). A similar method has been utilised for the determination of opiates in urine samples [13] and for structure elucidation of degradation products of drug substances [9–11].

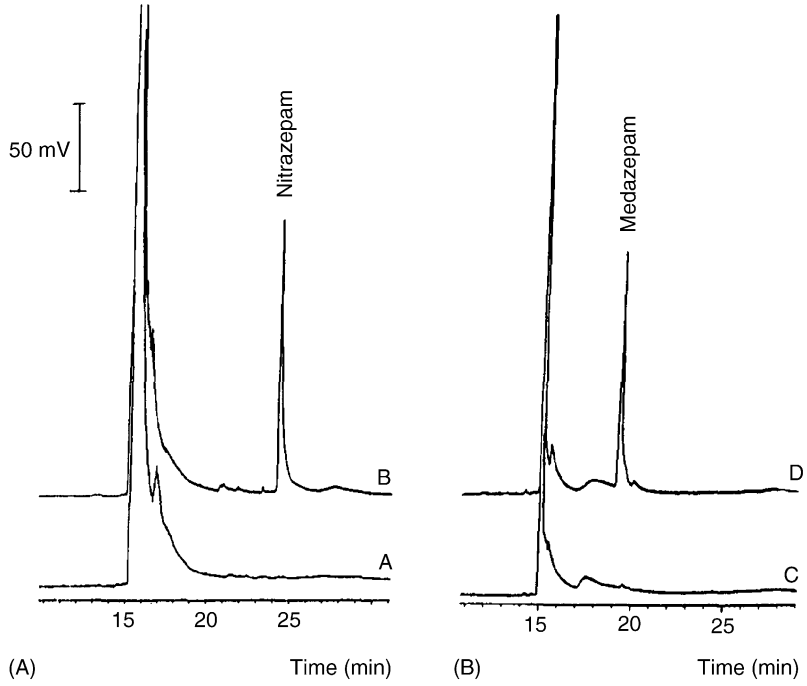

Fig. 3. On-line dialysis-SPE–GC–NPD analysis of (A) untreated blank plasma and (B) untreated blank plasma spiked with 1 μg/ml of nitrazepam (C) acidified blank plasma and (D) acidified blank plasma spiked with 1 μg/ml of nitrazepam. Analytical conditions: 10 mm × 2 mm i.d. PLRP-S precolumn; eluent ethylacetate with a flow rate of 0.5 ml/min. Interface: loop-type, FCSE at 110 °C. GC columns: 3 m × 0.32 mm i.d. DPTDMS deactivated retention gap +3 m × 0.32 mm i.d. retaining precolumn (SE-54, 0.25 μm) +15 m × 0.32 mm i.d. analytical column (SE-54, 0.25 μm). Temperature program 110 °C (8 min), 10 °C/min to 300 °C (10 min). Carrier gas helium (from [5]).

Another type of RPLC–GC procedure has been utilised in the determination of diazepam in urine [1]. Direct transfer of water/methanol mixture to the GC was possible because the volume of the fraction containing the target analyte was only a few microlitres. A major drawback of this system was the rather low sample capacity of the micro LC column allowing injection of maximum 1 μl of the sample. Moreover, the retention gap had to be changed after just 35 injections.

A few NPLC–GC methods have been developed for the determination of drugs in plasma. An NPLC–GC –ECD method has been applied for the determination of levomoprol [15] and an NPLC–GC–MS method for polychlorinated biphenyls [17]. The latter method enabled a selective determination of toxic non-*ortho*-chlorobiphenyls in the presence of less toxic PCBs at a pg/g level. This was possible through use of a selective NPLC column (dinitroanilino propyl silica) for selective clean-up of the plasma samples.

4.2. Tissue samples

Tissue samples typically require extraction to a suitable organic solvent before clean-up and analysis. As a rule, organic solvents are utilised in the extraction, and NPLC–GC is thus obvious choice when the analysis is carried out by multidimensional techniques.

LC–GC is particularly useful for bioanalysis of tissue of living patients where the amount of sample is limited and the sensitivity of the method needs to be extremely high. In addition, the number of samples may be very high in bioanalysis, and automated methods are desirable. In a multi-centre control study of breast cancer the concentrations of DDE and PCBs were determined in subcutaneous fat aspirated from the buttocks of breast cancer patients and of age-matched controls [19]. The number of samples was >600 and the sample amount was limited to 200–800 μl of extract of the fat. Because conventional methods would have been too tedious and of too low sensitivity, an on-line coupled NPLC–GC method was developed for the analysis. Clean-up of the organic extract was performed by NPLC on a silica column with hexane as the eluent, and with a use of an on-column interface in the transfer. A chromatogram of the tissue extract is shown in Fig. 5. The total analysis time was 80 min and the recoveries of target compounds exceeded 97%. Conventional methods for this kind of determination involve several steps, including collection and extraction of xenobials, removal of coextractives by appropriate clean-up methods using alumina, florisil, silica or GPC, and finally the analysis.

NPLC–GC has also been used in the analysis of stilbene hormones in meat extracts [16]. The NPLC–GC method that was developed involved an on-line derivatisation step. Two approaches were studied in the derivatisation, namely derivatisation in-between LC and GC using acylation with triethylamine in acetic anhydride, methylation with methanolic trimethylanilinium hydroxide or dimethylformamide dimethyl acetal, and derivatisation in the GC precolumn by silylation with *N*-methyl-*N*-(*tert*-

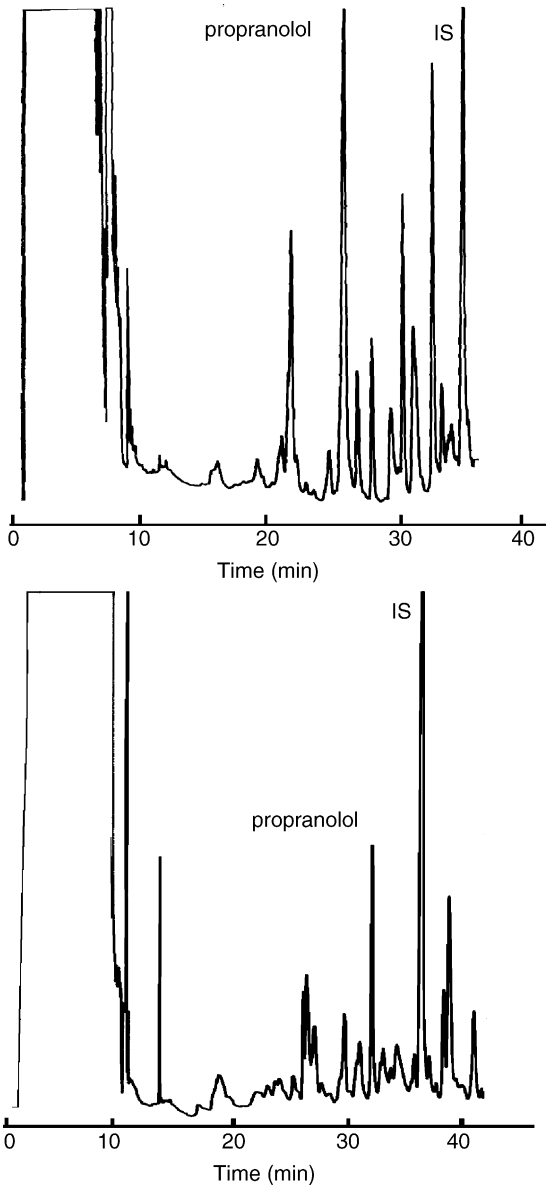

Fig. 4. Analysis of beta-blockers in human urine (A) and serum (B) by RPLC–LLE–GC–FID with on-line derivatisation. Samples were collected 4 h after a 10 mg dose was administered. Amounts of propranolol found were 1.46 μg/ml in urine and 0.50 μg/ml in serum. Internal standard was codeine Analytical conditions: LC column 20 × 2.1 CapcellPak C18 SG-120 column; eluent 0.05 M boric acid/ACN (88/22) at pH 10.2, with a flow rate of 0.8 ml/min, on-line LLE to dichloromethane (0.8 ml) min, loop-type interface with FCSE at 110 °C, GC columns: 3 m × 0.32 mm i.d. DPTDMS deactivated retention gap +3 m × 0.32 mm i.d. retaining precolumn (BGP-5, 0.25 μm) + 12 m × 0.32 mm i.d. analytical column (BGP-5, 0.1 μm). Temperature program 92 °C (12 min), 15 °C/min to 120 °C, 3.5 °C/min to 220 °C, 15 °C/min to 280 °C. Carrier gas helium (from [12]).

butyldimethylsilyl)trifluoroacetamide. The advantage of the on-line derivatisation was that formation of potentially interfering derivatives was avoided since only the fraction containing the target analytes was derivatised. With on-line silylation in the GC precolumn the LODs were below 4 μg/g.

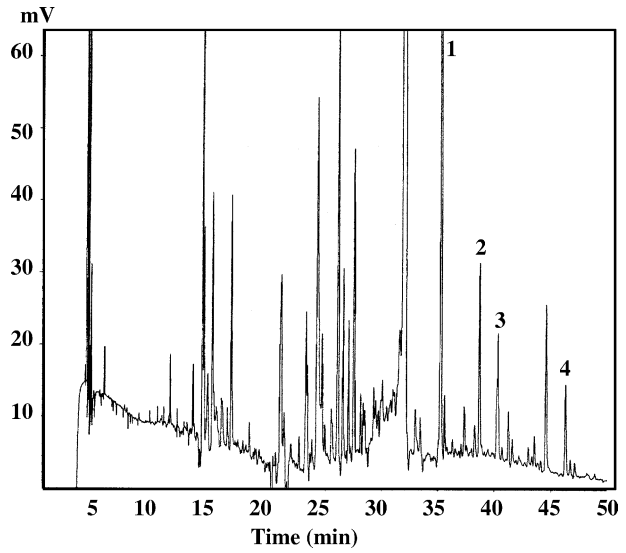

Fig. 5. Analysis of adipose tissue extract by NPLC–GC–ECD. Peaks: (1) *p,p′*-DDE; (2) PCB 153; (3) PCB 138; and (4) PCB 180. Analytical conditions: LC column 50 mm × 1 mm Hypersil; *n*-hexane, 0.15 ml/min, interface: on-column with PCSE at 77 °C. GC columns: 10 m × 0.53 mm i.d. DPTDMS deactivated retention gap +3 m × 0.32 mm i.d. retaining precolumn (DB-5MS) + 27 m × 0.32 mm i.d. analytical column (DB-5MS, 0.5 μm). Temperature program 77 °C (5 min), 15 °C/min to 160 °C, 3 °C/min to 290 °C (10 min). Carrier gas helium (from [19]).

5. Conclusions

SPE–GC and LC–GC techniques are a powerful combination in terms of sensitivity and reliability and they have been applied to a number of highly demanding biological applications. The NPLC–GC and SPE–GC systems already available are fairly simple to use, they can be totally automated, and their performance is usually superior to traditional off-line methods. These coupled methods still are not widely used in routine applications, however, probably because they are considered to be too difficult. It is true that optimisation is more demanding than for conventional methods and requires considerable knowledge of chromatography. Better training is needed, and the analyst needs to be encouraged to adopt new techniques and innovations in their method development. The benefits of on-line coupling techniques are nevertheless clear and the time invested in optimisation of the conditions is quickly repaid in shorter analysis time, better repeatability, improved detection limits and lower consumption of harmful organic solvents.

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