Journal of Chromatography, 626 (1992) 25–32
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 407

Review

Hyphenated high-performance liquid chromatography–capillary gas chromatography

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ABSTRACT

Coupled chromatographic techniques involve hyphenation at the front end of the main separation step and will increasingly be required for laboratories to carry out routine analyses of increasing complexity in the future. Thousands of samples have been analysed by automated liquid chromatography–gas chromatography (LC–GC), saving around 15 000 h of sample preparation time and allowed determinations which would otherwise have been out of reach for a small government laboratory to be carried out. The techniques presently applied, however, exploit just a small fraction of the possible LC–GC transfer techniques, of the many LC techniques available, and of auxiliary techniques, such as on-line solvent evaporation and on-line solute derivatization.

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1. SOME CONSIDERATIONS ON HYPHENATION

Hyphenation of techniques may occur at the front or at the rear end of the main chromatographic separation step. At the rear end (outlet of the main chromatograph), it involves sophisticated detectors, whereas the purpose of hyphenation at the front end is usually sample preparation or pre-separation —two totally different methods. Hyphenation just means a close relationship between two devices or techniques, any techniques, and basically it does not

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0021-9673/92/$15.00

even mean on-line coupling. The heterogeneous subject also causes the people involved to be heterogeneous: people involved in sophisticated high technology and "megadollar" equipment have little in common with the "tube artists", directing eluent flows through unfathomable pieces of equipment. So far, the term "hyphenation" does not seem to be a very good choice.

It is difficult to propose a better alternative, however. The term "coupled" is more specific, but also has its limitation: if, for instance, high-performance liquid chromatography (LC) is coupled to capillary gas chromatography (GC), not just two corresponding instruments are merged with tubing connecting the outlet of the first instrument to the inlet of the second. Both techniques are adjusted to each other and the result may strongly differ from their normal way of use. LC, in its normal application, for instance, is mostly used in the reversed-phase mode, with water and salts in the eluent. Many newcomers expect that LC–GC would, therefore, also be reversed phase LC–GC. When hyphenated to GC, however, most applications do not allow reversed-phase LC (*e.g.* because the sample contains too much fat or the derivatives suitable for GC are sensitive to water or alcohols), or there is no advantage in the reversed-phase mode because GC is possible only for relatively non-polar compounds. Adjustment in the hyphenated technique, however, goes further. Smaller LC columns are used with smaller eluent flow-rates, eluents are usually more volatile and requirements on selectivity are often such that no separation is wanted between the members of a class of compounds. This explains why "coupled" is not a particularly suitable choice either: it misses out the fact that the combinations always need adjustment and compromises.

2. ROUTINE ON-LINE LIQUID CHROMATOGRAPHY–GAS CHROMATOGRAPHY

Efforts invested into the development of new techniques must be paid off by time saving during their application and/or by better results. LC–GC has clearly passed this test. It was used in routine analysis from the beginning: Cortes *et al.* [1] brought their first (automated) LC–GC instrument to a production site and let it be operated by untrained users. This allowed a complex method to be performed by non-experts. Gianesello and co-workers [2,3] used several LC–GC instruments for the routine determination of trace amounts of pharmaceuticals in plasma, primarily profiting from a substantially lowered detection limit and a shortened sample preparation procedure.

Working for the government chemist, primarily responsible for the control of foods and drinking water, our laboratory has used LC–GC for many kinds of applications. The largest number of analyses, however, were carried out with four methods: (i) the analysis of mineral oil contamination in foods [4–6]; (ii) determination of sterols in edible oils and fats after cleavage of the esters [7]; (iii) determination of the minor components in oils and fats, leaving the esters intact [8], and (iv) the determination of raffination of oils and fats through the degradation products of sterols and squalene [9]. The first method was used for analysing about 4000 food samples and packaging materials. The latter three methods were used to analyse most of the oils and fats on the Swiss market; summing up the analyses by all three methods, a total of around 2000 is obtained, including 200–300 samples of known origin which served as reference samples.

The previously used (and official) methods for oil analysis involve saponification, a tedious extraction from a soap solution, preparative thin-layer chromatography, derivatization and GC; these widely used methods allow the analysis of about two samples per day per analyst. The proposed method, however, involves making up an oil solution and a derivatization in this solution in two of the three methods; all other steps were replaced by on-line LC. There is no established method for the determination of mineral oil in foods; it is, in fact, difficult to detect about 1–5 ppm of oil by conventional methods. We immersed the sample in pentane and analysed the supernatant by LC–GC.

A conservative estimate shows that about 15 000 working hours were saved by using the LC–GC methods, more than was invested into the development of the technique. In addition, sensitivities were far higher and for sterol analysis the relative standard deviations of the quantitative results were approximately ten times lower, lower, in fact, than those obtained by GC analysis alone when using usual injection [7,10].

3. THE NEED FOR AUTOMATED METHODS

If all three people in our group had carried out the above analyses using conventional, manual methods, the work would have taken about four years—which would, of course, have been considered unacceptable. There was, in fact, not a choice of performing the analyses by one or another method, but whether such control analyses were feasible at all.

At this point it is necessary to explain briefly the situation in a government chemist's laboratory, such as that of the Kanton of Zürich. About 35 people work in the laboratory, which is supposed to be capable of controlling all foods, the water of swimming pools, etc. Olive oils, for instance (about 80 products are available on the local market) are just a very small segment. Owing to their high price, adulteration of olive oils has a long tradition. Easily detectable admixtures, *e.g.* of rape seed oil, however, are now rarely used. The people carrying out the adulteration are well equipped with analytical methods. Harmless components are removed from certain olive oils (by unsavoury chemistry) because control laboratories, such as the government chemists, use them as markers, *e.g.* for the detection of cheaper solvent-extracted oils in pressed oils. Control, therefore, requires methods of increasing complexity, which rapidly overtax the government chemists' laboratories. Together with the necessary blanks, recoveries and confirmations, checking of the 80 olive oils by the conventional sterol method would require about 150 analyses, or 75 days of work for a single person; using LC–GC, the work was carried out in less than 10 days (about 30 analyses during a successful day).

The situation is similar in other fields: the analysis of pesticide residues keeps an unreasonably large proportion of our staff busy. It is boring work, and the results would be better if most of this work was carried out by a hyphenated technique, for example by a kind of a pesticide analyser, the development of which is overdue. Chemistry misused for rearing animals with less feed or providing meat with less fat is another problem: in the Kanton of Zürich, about 1.5 million pigs and cattle are slaughtered every year and each animal is a sample. If 0.1% of these samples were analysed, this would involve 1500 samples a year. With present methods, a few tens of samples are analysed for a small number of chemicals, and this is carried out at the limit of our capabilities. Only hyphenated analysers will be capable of handling the number of samples sufficient for a serious control programme, as long as chromatography is the analytical method.

4. TECHNIQUES FOR TRANSFER FROM LIQUID CHROMATOGRAPHY TO GAS CHROMATOGRAPHY

Routine analysis of large numbers of samples has confirmed usefulness of LC–GC in practice. Only a very small section of the potential of such a technique is, however, used at present; numerous ideas are available, suggesting a broad field into which it could expand. Some of the ideas are discussed here.

4.1. On-column transfer techniques

At present, nearly all LC–GC transfers are carried out by a kind of on-column technique. The eluent is introduced into the oven-thermostated inlet of the GC column, using retention gap techniques, the technique applied by Cortes *et al.* [1], or concurrent eluent evaporation [11,12]. On-column techniques allow extremely precise and accurate results to be produced, but also have two limitations: first, involatile material introduced into the oven-thermostated column rapidly builds up enough retention power to cause peak broadening. As up to several tens of milligrams of sample are injected into the LC part of the system, a very small fraction of the material injected is sufficient to ruin the GC system (one part out of ten thousand probably destroys it at once). Second, water attacks the pre-column if it enters in the liquid phase [13]; some humidity in the eluent (*e.g.* ethyl acetate containing up to 4% water) is sufficient to destroy the deactivation on the uncoated pre-column. Despite some disagreement about whether or not there are GC pre-columns resisting water [14–16], there seems to be little hope for the reliable LC–GC transfer of water-containing eluents by on-column techniques.

4.2. Transfer via programmed temperature vaporizing injector

The use of a programmed temperature vaporizing (PTV) injector for LC–GC transfer has been discussed, but, surprisingly, has not yet been put into practice. A solvent split technique was intended, as used for the syringe injection of large volumes [17].

Vaporization in a PTV injector chamber instead of in an oven-thermostated capillary pre-column allows the introduction of far larger amounts of involatile by-products [18], as the retention power of this material can be overcome by a high temperature. It is expected, furthermore, that a packing, *e.g.* Tenax, would not be attacked by water, enabling the introduction of water-containing eluents. PTV solvent split injection will not, however, be a simple technique for the adjustment of conditions, it will not allow the analysis of volatile solutes, and it will hardly produce results of a precision and reliability comparable with the on-column techniques.

Some of the drawbacks of transfer via the PTV solvent split technique could be overcome by using PTV vapour overflow [19]: instead of driving the eluent vapours out of the vaporizing chamber by a carrier gas flow, the vapours leave it on their own, as a result of their expansion during evaporation. To create the necessary vapour pressure, the vaporizer temperature must exceed the solvent boiling point at the current pressure. PTV vapour overflow largely regulates itself: the vapours escape at a rate corresponding to the evaporation rate and at the end of the evaporation process, the escape automatically stops. This reduces the loss of volatiles, as no carrier gas drives the solutes through the packed bed. Method development should be facilitated as the input flow-rate, the carrier gas flow-rate, and the vaporizer temperature do not need to be adjusted to each other. As an additional advantage, evaporation may occur under reduced pressure, as the carrier gas supply may be cut off during eluent evaporation. Reduced pressures allow using lower vaporizing temperatures, which increases the retention of the volatile components in the injector and reduces the aggressivity of water. The technique has, however, not yet been put into practice.

4.3. Splitless injection of large volumes

Another technique, which has again not been tried for LC–GC transfer, could involve splitless injection with a conventional vaporizing injector and a packed insert [20]. Cooling by the evaporating solvent is exploited to create an island in the normally heated vaporizer, which remains at the solvent boiling point until all the solvent is evaporated. All but the most volatile components remain in this cooled zone until the latter resumes the injector temperature. The solutes are then transferred to the column in the splitless mode. Injection by syringe allows the introduction of at least 500-μl volumes, and a 200-μl injection of water is not the upper limit. The method is simple to handle and resists involatile and aggressive dirt or water.

4.4. Extraction into packed bed: water-containing eluents

Evaporation of large volumes of solvent is always accompanied by a loss of volatile solutes because the solvent vapours act as a carrier gas and advance the solute material, *e.g.* through a retaining packed bed. Losses are, of course, particularly high if the solvent has a high boiling point. If the solute material could first be extracted from the eluent into a solid phase, at least a large proportion of the solvent could be removed without evaporation, avoiding the corresponding losses (although possibly in exchange with losses by poor extraction). This approach is particularly promising for the transfer of water-containing eluents, because it could provide a method for coupling reversed-phase LC to GC without introducing water into the GC system.

Vreuls *et al.* [21] extracted aqueous samples into various packed beds, evaporated the residual water from the packing, and thermally transferred the solute material into the GC system. Tenax was the most thermostable packing material, but alkylated silica gels extracted the aqueous phase better.

Extraction into a solid phase is not necessarily followed by thermal transfer to GC: transfer with a small volume of a convenient solvent may be an interesting alternative because an adsorbent with good extraction properties often has insufficient thermostability. Vreuls *et al.* [22] described the trapping of solutes in a small packed bed followed by transfer to GC with ethyl acetate, using partially concurrent eluent evaporation. This approach seemed to work, but has two problems: as most components of interest will be eluted near the interface between the organic and the aqueous phase, accurate cutting of the transferred fraction is crucial. If the cut is slightly early, the GC system is flooded by water; if it is slightly late, substantial amounts of solute material are lost. The transfer of some water cannot be completely avoided; at best it only involves the water dissolved in the ethyl acetate (which, however, is sufficient to damage the de-

activation of the precolumn when run routinely).

It is the feeling of the author that the basic possibilities for transferring water-containing eluents to the GC system have been determined and that the breakthrough depends only on the ingenious combination of these means. However, the breakthrough has not yet been made.

5. LIQUID CHROMATOGRAPHY FOR COUPLING TO GAS CHROMATOGRAPHY

LC offers an enormous wealth of possibilities to pre-separate or enrich samples. The ingenious hyphenated methods will probably be those with an inventive LC part. LC for LC–GC has been reviewed [23], emphasizing the special requirements of LC if it has to serve sample preparation for GC. Discussion was centred on the use of raw and derivatized silica gel, for which considerable experience is available. Work with size-exclusion chromatography (SEC) is continuing. SEC removes the high-molecular-mass material which disturbs GC and should, therefore, facilitate on-column transfer. An attempt to perform on-line SEC–GC for the determination of chlorinated pesticides in fat-containing foods has been described [24].

The combination of SEC and LC provides preseparation by two totally different selectivities: SEC removes the molecules larger and smaller than that of interest, whereas LC separates the isolated fraction according to polarity. Owing to the efficient removal of by-products, an on-line combination of the two should enable a trace analyser with a large field of application to be built. Some results in this direction were reported by De Paoli *et al.* [25] for the determination of organophosphorus pesticides in fruits: extracts were pre-separated by a 25 cm × 3 mm I.D. SEC column and filtered through a silica gel column before being transferred to the GC system. As the mobile phase in SEC on a polystyrene-type column could not be weaker than dichloromethane and the fraction from SEC was directly transferred to the silica gel column, the function of the silica gel column was restricted. The first components of interest broke through during introduction of the SEC fraction and the initial bands were broad; the removal of by-products less polar than the insecticides was therefore impossible. The components disturbing the GC were, however, more polar than the pesticides and could be eliminated completely. Detection limits by this SEC–LC–GC method with flame photometric detection were around 1 μg/kg, and there were no peaks other than the insecticides of interest in the gas chromatogram. An on-line eluent evaporator, positioned between the SEC and the LC column, would have allowed heart cutting (see below).

No work has been published on coupling ion-exchange sample enrichment or pre-separation to GC. Ion exchangers strongly and selectively bind some components and are therefore of interest for the determination of acidic and basic components. Such techniques often presuppose, however, on-line derivatization.

5.1. Example: determination of degradation of edible oils

One of the highly successful routine applications of LC–GC is the determination of degradation products from sterols and squalene in edible oils for the determination of whether oils or fats have been refined or subjected to other thermal stress (Fig. 1) [9]. Sterols are dehydroxylated, forming a hydrocarbon with at least two double bonds (3,5-stigmastadiene is the degradation product of sitosterol); squalene isomerization products are formed. Previous methods involved lengthy saponification and

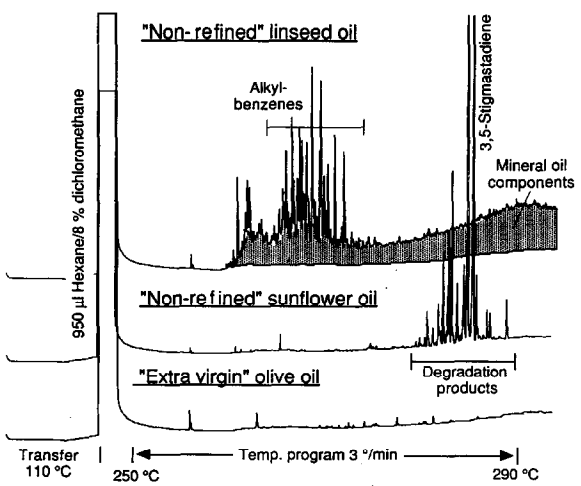

Fig. 1. Example of a routine application of LC–GC determination of degradation products of sterols and squalene in edible oils. For detailed explanation, see text.

clean-up. The LC–GC method reduces sample preparation to making up a 20% solution of the oil in hexane and gives an increased sensitivity by one to two orders of magnitude. LC removes the large amounts of triglycerides and separates the alkenes from alkanes and squalene (which presupposes a high separation efficiency).

For an extra virgin olive oil (bottom chromatogram), only pression and filtration is allowed. In this instance, no degradation products are visible (a small peak corresponds to about 50 μg/kg), confirming the quality of the oil; for most of the olive oils sold in Switzerland, the declaration corresponded to the oil. The sunflower oil sold as non-refined, however, contained a substantial amount (about 30 mg/kg) of degradation products (centre chromatogram) and obviously did not correspond to the declaration. Disregarding the olive oils, hardly more than half of the oils declared as non-refined were what they stated. The cold pressed and non-refined linseed oil (top chromatogram) contained no degradation products, *i.e.* the declaration is not violated. However, the chromatogram shows numerous other peaks, which have nothing to do with the oil: they were identified as alkylbenzenes, which are used, for example, in paints or (after sulphonation) as detergents (about 15 mg/kg referring to the oil). The increase in the baseline is not due to column bleed (dotted area; the real baseline, taken from a blank run, is shown), but to contamination with mineral oil. The *n*-alkanes, removed by LC, ranged from C_{17} to higher than C_{35}. The residues in the fraction shown consist of alkenes with more than one double bond and (primarily) alkyl aromatics with more than one ring. Owing to the enormous number of isomers, no peaks are distinguishable (which is typical for aromatics from mineral oils [26]). The concentration of mineral oil in this linseed oil approached 0.1%; its origin is still unknown.

6. AUXILIARY TECHNIQUES

Standard LC for LC–GC consists of one LC pump, one LC column, two switching valves and the accessory for backflushing the LC column. To this system, however, many steps could be added, which would render the system more versatile. LC–LC–GC has been described for the determination of food irradiation products [27].

6.1. On-line extraction

On-line extraction, originating in flow injection analysis, was introduced in GC as a method for analysing organic components in water [28,29]. It allows fully automated on-line water analysis, but could also be used as a first step for analysing other samples.

As there is still no method for the direct transfer of water-containing eluents to a GC system, a number of workers have experimented with on-line extraction aiming at exchanging the solvent. The water-containing eluent was mixed with an organic solvent of low polarity, passed through extraction coils, the organic phase was separated, and finally transferred to the GC system through a loop-type interface [30–33]. Such methods produced interesting results, but might become obsolete as soon as better alternatives become available. Extraction yields are often far below 100% and there usually remains enough water in the organic extract to cause problems in GC.

6.2. On-line derivatization

Complex sample preparation procedures can hardly be used without on-line derivatization. There are two important reasons for this: first, enrichment or pre-separation may need to be based on underivatized functional groups of the components of interest or of those to be removed. If the components need to be derivatized before they are amenable to GC, *e.g.* those containing amino or carboxyl groups, derivatization must occur between the LC sample preparation step and GC. Second, samples may be in a matrix not allowing derivatization, *e.g.* in water, which must be removed before derivatization.

There are two options to achieve on-line derivatization: reactions within the LC system, *i.e.* on the LC column or within an interface, or reactions in the inlet of the GC column. Raglione and Hartwick [34] isolated triglycerides from biological samples by LC, methylated them on-line on a cation exchanger (acid catalysis) and analysed the esters by GC. Derivatization in an uncoated GC pre-column was achieved for pentachlorophenol, though not in an LC–GC system. The methyl ester was formed with diazomethane and the acetate with acetanhydride–pyridine [35].

6.3. On-line evaporator

Multiple step LC often creates the problem that the volume of eluent from the first column is excessively large for a direct transfer to the second column. This is particularly true in LC–GC because the first column typically needs to be large to offer the capacity required for removing large amounts of by-products, and the second column should be smaller to produce fractions small enough for easy transfer to GC. In other instances the mobile phase from the first column does not suit the second column, *e.g.* because it is excessively strong (see on-line SEC–LC discussed earlier). To overcome such incompatibilities, an on-line eluent evaporator was constructed [36], evaporating the mobile phase, *e.g.* from a first LC column, but retaining the solutes. When evaporation is completed, the solute material is carried into, *e.g.* a second LC column by a mobile phase suiting the second LC step.

7. LC–GC INSTRUMENTATION

Instrumentation allowing complete automation of LC–GC is needed. Automation is required for the analysis of large numbers of samples, but also because LC often presupposes reproducible chromatographic cycles: retention times may not be sufficiently reproducible otherwise.

Scientists often tend to underestimate the work carried out by instrument manufacturers, perhaps because they have to pay for the instrument. The work carried out by F. Munari at Carlo Erba/Fisons deserves special recognition. Over a number of years a totally new type of instrument has been developed. The first two instruments of this type in this laboratory analysed the many thousands of samples mentioned earlier, often running over weekends. They did their job without major problems, and thus fulfilled the expectations.

This instrument offers a broad range of capabilities, including multitransfer, *i.e.* the transfer of several fractions from a liquid chromatogram to GC, "GC scanning", *i.e.* GC analysis of a larger section of a liquid chromatogram, segment by segment, and LC–GC transfer by peak recognition, adjusting the transfer to the signals observed by the LC detector. It allows automated backflush of the LC column, steps in the LC part (*e.g.* on-line eluent evaporation) to be performed at increased temperature, and a variety of LC–LC techniques. With respect to the more sophisticated techniques discussed here, it is obvious, however, that the development of the "Dualchrom" instrument will not end in the near future: more valves will be needed, additional LC–GC interfaces may need to be integrated, and the software will have to follow these developments.

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