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Review

Multiple hyphenation of liquid chromatography with nuclear magnetic resonance spectroscopy, mass spectrometry and beyond

I.D. Wilson*

Drug Metabolism and Pharmacokinetics Department, AstraZeneca Pharmaceuticals, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK

Abstract

The advent of sensitive and reliable HPLC–NMR and HPLC–MS systems has revolutionised the identification of compounds eluting from chromatographic systems. More recently systems have been described wherein both NMR and MS are used together to provide an immensely powerful means of characterising compounds in chromatographic eluents. Here the construction and application of combined HPLC–NMR–MS systems to the analysis of mixtures of pharmaceuticals, drug metabolites in biological fluids and natural products in plant extracts is reviewed. In addition preliminary work with alternative systems such as HPLC–UV–NMR–FTIR–MS is highlighted and the prospects for such complex systems considered. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Multiple hyphenation; Complex mixture analysis; Reviews

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1. Introduction

The rapid and efficient characterisation of analytes present in complex mixtures is an ever present challenge. However, developments in the hyphenation of spectroscopy and chromatography, especially column liquid chromatographic (HPLC) separations and mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy offer the potential

*Tel.: +44-1625-513-424; fax: +44-1625-516-962.
E-mail address: ian.wilson@astrazeneca.com (I.D. Wilson).

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for unknowns to be identified rapidly and without the need for extensive purification. Although more recent, and currently less widely available, than HPLC–MS, HPLC–NMR can now both be considered to be a mature technique with a range of commercially available systems available, of varying degrees of sophistication (HPLC–NMR is reviewed in e.g. Refs. [1–3]). Used individually such HPLC–NMR and HPLC–MS systems are often able to provide practical and efficient solutions to the problems of identifying components in chromatographic eluents. However, there are occasions when both NMR and MS data are needed for structure determination. An oft quoted example of this is the case of positional isomers of substituents on an aromatic ring etc. Thus, although HPLC–MS may provide the molecular mass and even the atomic composition of the unknown it may nevertheless be unable to provide an unequivocal structure in the absence of an authentic standard. In contrast a solution to the sites of substitution might be more easily found using NMR. Conversely NMR can only give part structures for compounds containing NMR ''silent'' substituents. An obvious solution to this type of problem is simply to put both MS and NMR into the same separation system and the first example of this double hyphenation was described in 1995 [4]. This was rapidly followed by further studies describing e.g. HPLC–NMR with ion trap MS for acetaminophen metabolites in urine [5]. Further applications have extended the range of analytes and sample types to cover pharmaceutical mixtures, natural products, drug metabolites, etc. [6–17]. As HPLC–NMR systems become more widespread it would seem inevitable that further examples of HPLC–NMR–MS will be described especially as there are few technical difficulties in assembling these doubly hyphenated installations. Indeed thanks to the introduction of newer HPLC compatible interfaces the ease with which the coupling of many powerful spectroscopic techniques into a single chromatographic system can be achieved has prompted us to question the limit to which HPLC can be combined with spectroscopic detectors. Here the current state of HPLC–NMR–MS is examined and future prospects for even more ambitious combinations such as systems combining UV–diode array and IR spectrometers in addition to NMR and MS are considered.

2. HPLC–NMR–MS: Instrumental layout

Our experiences of the practical aspects of the use of HPLC together with NMR and MS have been described elsewhere, and so will only be briefly discussed here [6]. A schematic showing a ''typical'' HPLC–NMR–MS system is shown in Fig. 1 with, in addition, an FT-IR interface connected in-line with the NMR flow probe to enable off-line FT-IR spectra to be obtained (see later). Such systems are based on conventional HPLC pumps, columns and UV detectors although these can also be combined with a 'sampling unit' where ''interesting'' peaks can be taken off-line into storage loops for NMR at a later stage. The UV detector can be either a variable wavelength instrument or a diode array spectrometer capable of providing UV spectra to complement the NMR and MS spectra. Although not essential the UV detector provides a very convenient means of monitoring the separation and in helping the spectroscopist to accurately determine the delay times between the analyte emerging from the chromatographic system and it reaching the spectrometers. In the set-up shown in Fig. 1 the NMR and MS instruments are connected in parallel via a splitter placed at the outlet of the UV detector. In part this layout results from the deficiencies of early NMR flow probe designs with respect to their ability to tolerate back pressure but there are some advantages to parallel operation of the two spectrometers due to the different sensitivities of NMR and MS. Generally we split the flow to the NMR and MS 95:5 but various ratios have been employed by other workers depending upon the instrumentation. The high stray magnetic field from the NMR magnet (5 or 600 MHz spectrometers are the norm) can have adverse effects on the performance of the mass spectrometer and in practice this means that the mass spectrometer is best sited outside the 5 Gauss line.

One advantage of operating the instruments in parallel is that the delay between the appearance of the peak in the UV detector and its detection by the NMR and MS can be manipulated such that the analyte enters both spectrometers simultaneously or for example is detected in the MS first enabling an informed decision to be taken on obtaining a stopped flow NMR spectrum for a particular peak.

One problem with the use of deuterated solvents such as D_2O with MS is that the deuterium can

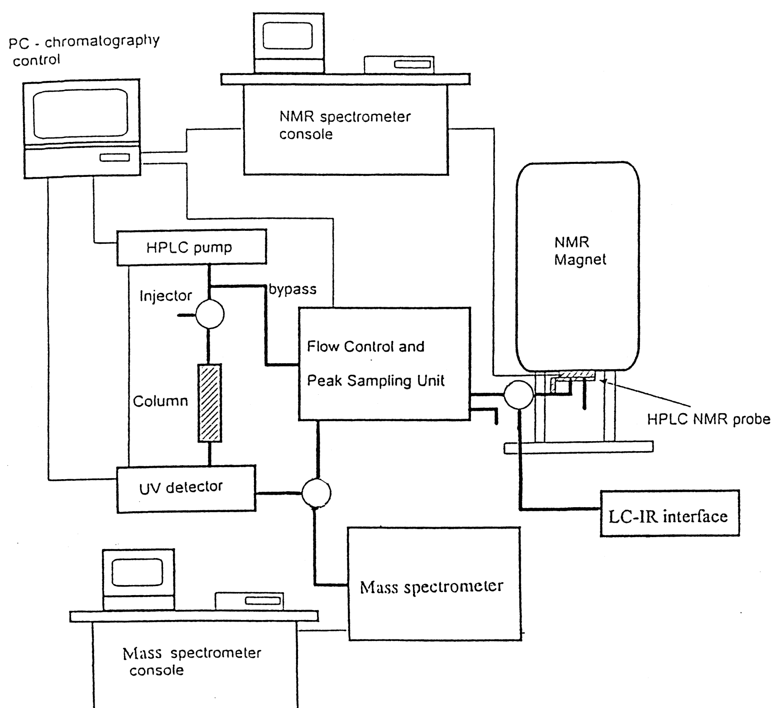

Fig. 1. A schematic of an HPLC–NMR–MS–IR system.

exchange with protons on ionisable groups. The practical consequence of this is that the wrong molecular mass will be determined unless the mass spectrometerist takes exchange into account. Of course this can be turned to advantage in that if the run is repeated using protonated solvents (i.e. H_2O) it is a very simple matter to determine the number of exchangeable protons in an unknown, which can provide valuable structural information. Another way of achieving this result is to mix that portion of the effluent from the column that is directed towards the mass spectrometer with a suitable solvent for back exchange. This type of system has been described for the study of drug metabolites by several groups [8,9].

3. Applications of HPLC–NMR–MS

The published applications of HPLC–NMR–MS currently cover a variety of sample types including mixtures of pharmaceuticals and related substances, endogenous and drug metabolites in samples such as urine, herbicide metabolites in plant extracts and natural products.

3.1. Pharmaceuticals

As noted in the introduction, the first published HPLC–NMR–MS system was described in 1995 [4] and detailed the on-flow MS and NMR of a simple mixture of fluconazole and two related triazole structures. A Varian Unity*plus* 500 MHz NMR instrument and Fisons Trio 1000 mass spectrometer, equipped with a particle beam interface in chemical ionisation mode, were used. The isocratic separation used a mobile phase of acetonitrile and D_2O (25:75) at 1 ml min^{-1} and was performed on a Xorbax RX-C_{18} column (150×4.6 mm I.D.). In addition to NMR and MS the separation was monitored by UV at 254 nm. The effluent from the HPLC column was split 6:4 enabling on-flow ^1H NMR and mass spectra to be obtained for all three components.

A second, rather more detailed, example from this group also used a Varian Unity*plus* 500 MHz NMR spectrometer in combination with a Trio 1000 mass spectrometer (originally manufactured by Fisons Instruments), fitted this time with an electrospray interface [7]. This system was used to obtain MS and ^1H NMR data on a mixture of ten model peptides in

a simulated "library" of the type produced by combinatorial chemistry. The spectrometers were operated in parallel, with the flow from the column split immediately after the UV detector (220 nm) in the ratio 20:1 in favour of the NMR. The separation was performed on an Inertsil C_{18} column (ODS 2, 25×4.6 mm I.D.) using reversed-phase gradient chromatography from 5 to 50% acetonitrile against D_2O containing 0.1% TFA over 50 min (0.5 ml min^{-1}). Approximately 100 μg of each component was injected on-column enabling on-flow NMR spectra to be obtained, and the combination of NMR and MS data enabled the identification of components even when they co-eluted, as in the case of Trp-Gly and p-Glu-Gly-Arg-Phe-amide.

A rather more unusual example of the use of HPLC–NMR–MS for pharmaceuticals has recently been described as part of an investigation on the use of superheated water (or in this case superheated D_2O) for a number of analgesics and caffeine [10]. Chromatography was performed at 180°C on a PS-DVB column (150×4 mm I.D.) at a flow-rate of 1 ml min^{-1} and HPLC–NMR–MS was performed on the model compound salicylamide. The use of superheated water has a number of advantages for HPLC–NMR with D_2O compared to mobile phases containing an organic modifier. Thus the absence of a large signal, for example, acetonitrile means that the region of the spectrum normally obscured by its resonance is clear. In addition even the best available HPLC grades of acetonitrile seem to contain small amounts of impurities such as propionitrile which has the potential to interfere with the NMR of analytes present in the eluents in low concentration and the use of D_2O alone eliminates this problem.

3.2. Drug metabolites

Not long after the first publication on HPLC–NMR–MS a second application, based around a Bruker DMX-500 NMR spectrometer and a Finnegan MAT LCQ ion trap mass spectrometer with positive ion electrospray was described. Acetaminophen (paracetamol) metabolites in an extract of human urine were characterised [5] using gradient reversed-phase chromatography to separate the metabolites from endogenous materials with the separation monitored by UV at 210 nm. A flow-rate of 1 ml min^{-1} was employed with chromatography carried out on a Waters Symmetry C_{18} bonded column (150×3.9 mm I.D.) using an initial mobile phase composition of 100% D_2O containing 0.1% TFA (pH 2) rising to 50% deuteroacetonitrile over 30 min. Acetaminophen provides a useful example of the value of the combination with regard to the sulphate metabolite. The NMR spectrum of this metabolite quite clearly shows the presence of a para-disubstituted, drug-related, substance but unequivocal identification is not possible based simply on the basis of the NMR spectrum it since the sulphate moiety provides no diagnostic NMR resonances. Mass spectrometry however, easily resolves this difficulty on the basis that the mass has increased by 80 Da, consistent with sulphation, confirmed by the fragmentation of the metabolite to give diagnostic fragments. In addition the combined use of HPLC–NMR–MS also enabled the rapid identification of an "unknown" endogenous substance (*N*-acetylphenylglycine) that is not normally present in high concentration in urine to any great extent. More recently similar studies on the identification of acetaminophen metabolites in human urine employing a Finnigan Navigator single quadrupole instrument to obtain the mass spectral data have been published [8]. In this work the metabolites were separated using gradient reversed-phase HPLC with acetonitrile and D_2O containing 0.1% TFA on a C_{18} bonded phase (5 μm ODS Sperisorb-II, 4.6×250 mm). The initial mobile phase composition was 1% acetonitrile rising linearly to 35% over 35 min, with the separation also monitored by UV detection at 210 nm. In this experimental set-up the flow was split 50:1 in favour of the NMR, with the timing so arranged that peaks reached the mass spectrometer 30 s before arriving in the NMR flow probe. These authors also employed the addition of a protonated solvent (methanol plus 1% acetic acid) in order to obtain back exchange prior to MS in order to determine the number of exchangeable protons.

A further application of HPLC–NMR–MS, again with a 500 MHz NMR spectrometer but this time employing a single quadrupole instrument was for ibuprofen metabolites [11]. Over the course of a 45 min reversed-phase HPLC gradient (5 μm C_{18} Hypersil BDS column 250×4.6 mm I.D., 20 to 60% acetonitrile, 0.2% formic acid in D_2O at 1 ml

min^{-1}), run a total of nine drug related compounds were detected in a solid-phase extract of human urine. This study was particularly useful in highlighting the complementary information provided by NMR and MS. In general both instruments readily detected the abundant phase II glucuronide metabolites of ibuprofen and its phase I oxidation products but in the case of the aglycones this was not the case. Thus, some of these phase I metabolites, that were readily observed by NMR, and that were present in high concentration, remained obstinately refractory to MS detection. In contrast one glucuronide (probably an artefact resulting from the dehydration of another metabolite during the isolation procedure) was present only as a very minor component and would not have been detected without MS. Having detected this metabolite by MS the sample was re-run and an NMR spectrum obtained. It should also be remembered that ibuprofen is a chiral compound, dosed as a racemate. Although both NMR and MS were unable to distinguish between enantiomers NMR was able to detect the presence of diasterioisomeric metabolites in the peaks of drug-related material eluting from the column, which MS was not. This example also provides a good illustration of the ability of even a modest single quadrupole instrument in combination with NMR to provide extensive metabolic data.

More challenging than the identification of known metabolites were studies on the metabolic fate of 2-trifluoromethyl-4-bromoaniline [12,13] and the related 4-chloro [14] substituted analogue in the rat. HPLC with ^{1}H NMR and MS rapidly facilitated the identification of the major metabolites of the two halogenated anilines as sulphate conjugates of a ring-hydroxylated metabolite with a host of minor metabolites (sulphates and glucuronides). An example of the metabolic pathway for the bromoaniline is shown in Fig. 2 as an illustration of the type of information that can be obtained, rapidly and efficiently by HPLC–NMR–MS. It is also noteworthy that the presence of fluorine in these compounds allowed the use of ^{19}F NMR for the detection of metabolites. The use of ^{19}F NMR spectroscopy can therefore be used to provide yet another means for determining the presence of compound related material and direct the spectroscopist to compound related peaks. An example of the wealth of information that can be provided is shown in Fig. 3. As in the case of the ibuprofen studies the bulk of this work [13,14] was undertaken using a combination of reversed-phase gradient HPLC (5 μm BDS Hypersil C_{18}, 5 to 65% acetonitrile over 45 min, pH 7 with 0.01 *M* ammonium acetate) with a Bruker 500 MHz NMR spectrometer and a Micromass Platform single quadrupole mass spectrometer (although the preliminary studies used on the bromoaniline used a slightly different setup, [12]).

The use of HPLC–NMR–MS in the study of drug metabolism has not been restricted to our group. As well as the acetaminophen study mentioned above studies using HPLC–NMR, HPLC–MS and HPLC–NMR–MS on the novel non-nucleoside reverse transcriptase inhibitor GW 420867, where a number of hydroxylated and glucuronidated metabolites were identified, have recently been described [9]. This group used the in-line configuration (with MS subsequent to NMR) and employed a Bruker DRX- 600 MHz NMR spectrometer and a Bruker Esquire ion-trap mass spectrometer. Reversed-phase chromatography was performed on a Phenomenex Magellan C_{18} column (250×3.2 mm I.D.) at 0.6 m min^{-1} using a linear gradient starting with 100% D_2O (containing 0.1% formic acid) to 80 acetonitrile over 35 min. The separation was monitored at 254 nm and peaks of interest eluting from the column were collected into the peak sampling unit and stored there until taken for NMR. When a suitable NMR spectrum had been obtained the peak was then transferred to the mass spectrometer. Mass spectrometry was performed in two ways. Thus, in one method the flow to the MS was split 1:12, with the minor portion directed into the interface of the mass spectrometer to give $[M+D]^{+}$ ions. Alternatively a make up flow (1 ml min^{-1}) of 0.1% aqueous formic acid was added to promote back exchange in order to obtain the $[M+H]^{+}$. In the latter experiment the flow was split 1:30.

The study of metabolism using this technology has not been restricted only to animal systems and recently the fate of a model herbicide, 5-trifluoromethyl pyridone (2-hydroxy-5-fluoromethylpyridine) in hydroponically grown maize has been studied by HPLC–NMR–MS [15]. Aqueous extracts of plant material were subjected to gradient reversed-phase HPLC (5 μm Hypersil BDS C_{18} 250×4.6 mm I.D.

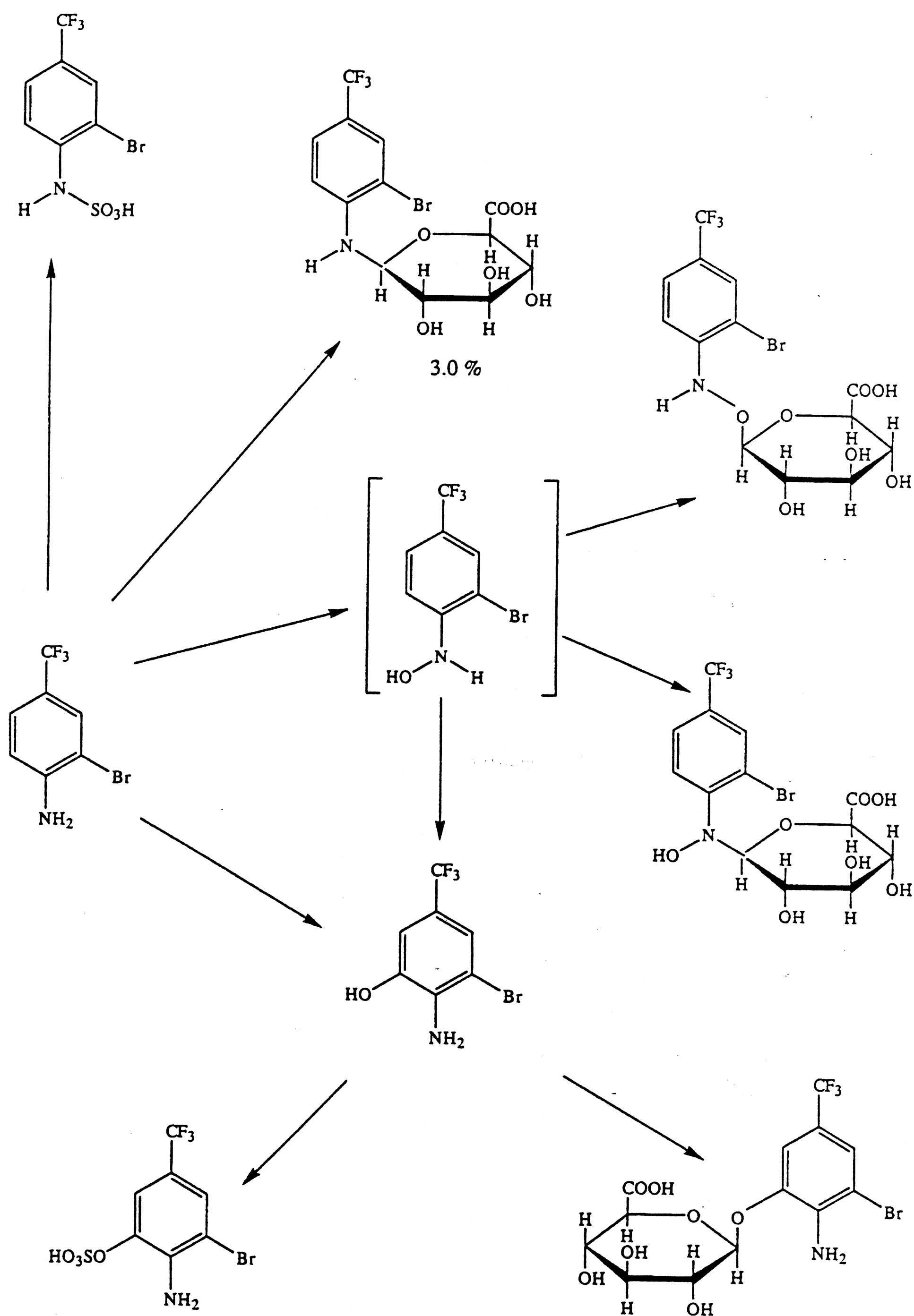

Fig. 2. The metabolic pathway if 2-bromo-4-trifluoroaniline as determined by HPLC–NMR–MS (for further details see Refs. [12,13]).

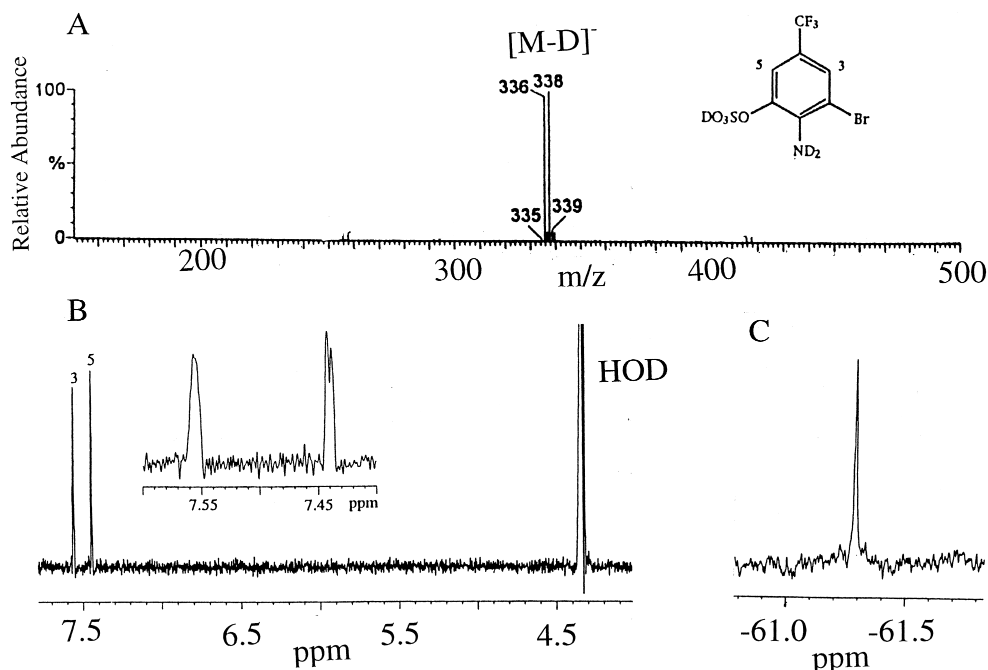

Fig. 3. Spectroscopic data for the major metabolite of 2-bromo-4-trifluoromethylaniline obtained by HPLC–NMR–MS (see Refs. [12,13]). (A) mass spectrum, (B) ^{1}H-NMR spectrum, (C) ^{19}F-NMR spectrum. HOD=residual water.

column, 0 to 2% ACN over 20 min then to 20% ACN in 40 min, 1 ml min^{-1}) with NMR performed with a Bruker DRX-500 NMR spectrometer and mass spectrometry via a Micromass Quattro LC (using positive ion electrospray). In this study the unchanged parent compound and two metabolites were identified. One metabolite was identified as the O-malonylglucoside, despite co-eluting with unchanged parent compound, whilst the other was shown to be an *N*-glucoside. The property of D_2O as an aid to structure determination was nicely illustrated in this example as, in addition to exchange with the hydroxyl groups on the conjugating sugar moieties there was also replacement of the exchangeable proton of the NH in metabolite 2 with deuterium to give ND. This resulted in an m/z of 166 instead of the 164 that would have resulted had conjugation been via the nitrogen and thus enabled the site of conjugation with glucose to be unequivocally assigned as being through the oxygen. Conversely the absence of a similar exchangeable proton in metabolite 1 helped to show that conjugation must be with the N.

3.3. Natural products

Natural products are an important source of new drugs, but many active extracts contain compounds which have been described before. Thus techniques to rapidly screen extracts in order to eliminate known compounds (“dereplication”) and characterise previously unknown substance are potentially of immense value. HPLC–NMR–MS could well be such a tool and has recently been applied to two studies of plant extracts. In the first of these it was used for determining the identity of the phytoecdysteroids an extract of the plant *Silene otites* [16]. Reversed-phase HPLC on a C_{18} bonded HPLC column (Waters Symmetry 150×3.9 mm I.D.) was performed with a gradient of D_2O and deuteroacetonitrile. The separation, which was also monitored at 254 nm, was achieved with a shallow gradient from 20% acetoni-

trile to 25% over 20 min at a flow-rate of 0.7 ml min^{-1}. The extract was sufficiently concentrated to enable on-flow ^{1}H NMR and MS spectroscopy, and stopped flow 2-dimensional NMR spectroscopy to be performed. This confirmed the presence 20-hydroxyecdysone, 2-deoxy-20-hydroxyecdysone and 2-deoxyecdysone in the extract but further examination of the MS data however, indicated that another ecdysteroid might be present as a minor component. The mass spectral data itself was insufficient to identify the new compound, although it did significantly reduce the number of possibilities. A second analysis, with stopped-flow NMR of the MS detected peak, enabled the ‘‘unknown’’ to be identified as integristerone A. The pseudo 2-dimensional HPLC–NMR chromatogram for this extract is shown in Fig. 4, with representative NMR and mass spectra for 20-hydroxyecdysone illustrated in Fig. 5.

A more recent example of the use of HPLC–NMR–MS for natural product work is an application to an extract of *Hypericum preforatum* L. [17]. Here the separation and identification of a range of natural products was performed, including the galacturonide (tentative identification), rutinoside, glucoside, arabinoside, rhamnoside and galactosides of quercetin, and the aglycone itself together with hypericin, protohypericin, pseudohypericin, protopseudohypericin amongst others. The arabinoside and galacturonide have not previously been described as constituents of extracts of this plant. The system used to generate these data comprised a 500 MHz Bruker DRX-500 NMR spectrometer and a Micromas Quattro LCZ triple quadrupole mass spectrometer. The separation was performed on a conventional 120×4.6 mm I.D. column with gradient HPLC (acetonitrile-D_2O ammonium acetate, 1 ml min^{-1}) with a 95:5 split in favour of the NMR flow probe. NMR spectra were obtained in stopped-flow mode on the major components but not all of the components were present in sufficient quantity to enable NMR spectra to be obtained in a reasonable time. The chromatogram obtained from this extract is

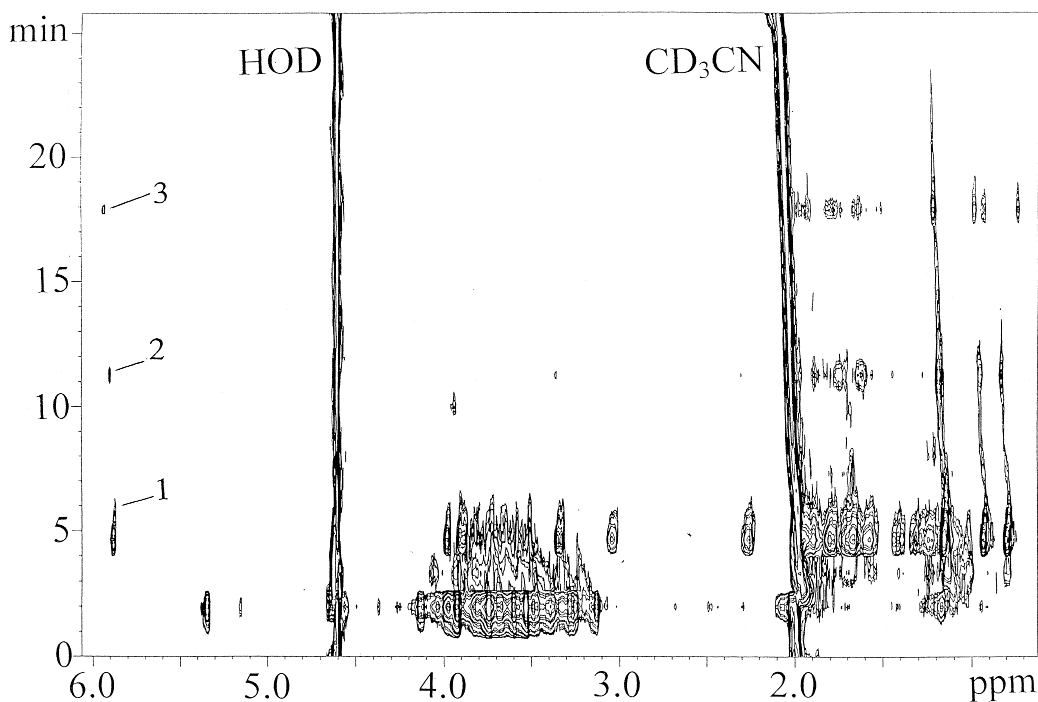

Fig. 4. A pseudo 2-D HPLC–NMR plot showing the separation of ecdysteroids in an extract of the plant *Silene otites*. Key to peaks, 1, 20-hydroxyecdysone, 2, 2-deoxy-20-hydroxyecdysone, 3, 2-deoxyecdysone. The resonance identified here is for the C7 proton of the analytes (see Fig. 5). Reproduced from Ref. [16] with permission.

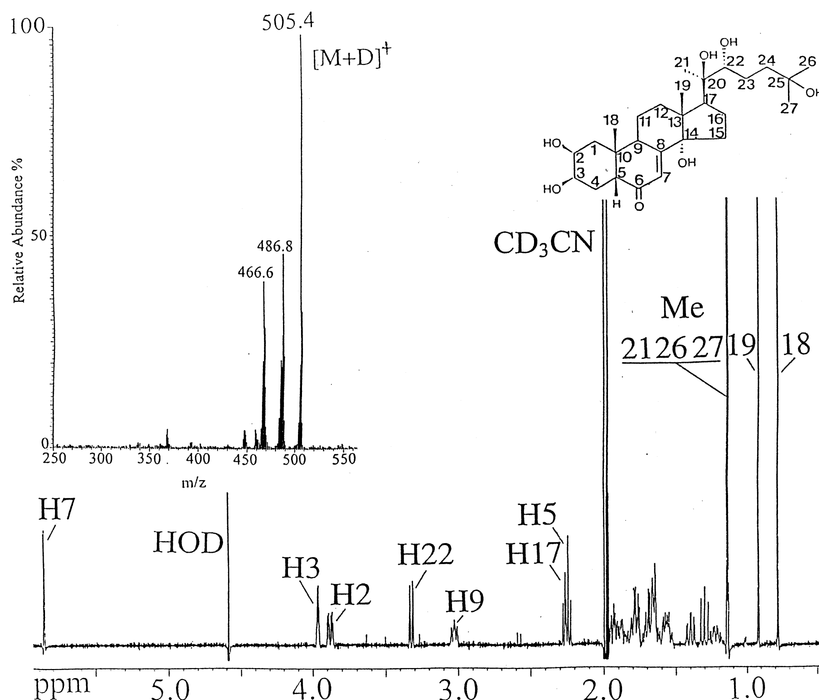

Fig. 5. ^{1}H-NMR and mass spectral data obtained from peak 1 of Fig. 4 for 20-hydroxyecdysone. CD_3CN=deuteroacetonitrile, HOD= residual water.

shown if Fig. 6, whilst the NMR and MS data for 13′-II8-biapigenin are shown in Fig. 7.

4. Multiple hyphenation of HPLC–NMR and HPLC–NMR–MS with other spectroscopic techniques

4.1. SEC–NMR–IR

A further double hyphenation that we have recently examined is the linking of size exclusion chromatography with on-line NMR and on-line collection onto a special interface for off-line FT-IR [18]. The application was to the characterisation of a number of model polymer additives (2,6-di-*tert*.-butyl-4-methylphenol (BHT), octadecyl-3-(3,5-di-*tert*.-4-hydroxypheny)propionate (Irganox 1076) and di-iso-octylphthalate (DIOP)) following separation on a size exclusion column. The separation was accomplished using deuterochloroform, at 1 ml min^{-1}, as the mobile phase and two "Mixed E" SEC columns (3 μm, 300×7.5 mm I.D., Polymer Laboratories) columns connected in series. Following on-flow ^{1}H NMR the effluent from the NMR flow probe was directed towards a dedicated HPLC–FT-IR interface (the Viscotec LC-Transform Model 300). Here the solvent was evaporated and the compounds deposited on a slowly rotating germanium composite disc. After all of the compounds of interest had been collected they were then taken for subsequent FT-IR. This procedure enabled good quality NMR and IR spectra to be obtained for all three model compounds, the former in "real time", whilst the latter, being off-line were obviously subject to a delay.

Following the success of HPLC–NMR with an on-line interface (the LC-Transform) for collecting the eluent for subsequent FT-IR we further expanded the system to give the triple hyphenation of HPLC–NMR–IR–MS [19]. The instrumental layout is shown in Fig. 1. In this system a slightly different interface (the Viscotek LC Transform Series 400) was used with the interface in line with the NMR flow probe a 1:1 split to the NMR and LC-Transform

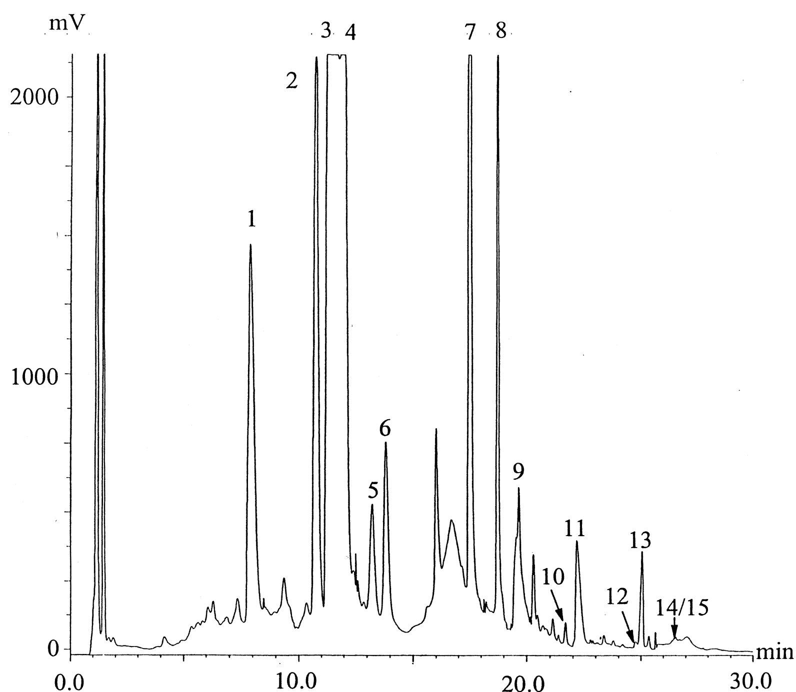

Fig. 6. A reversed-phase gradient separation of the constituents in an extract of *Hypericum perforatum* L. Key 1, Quercetin-galacturonide, 2, quercetin-rutinoside, 3, quercetin-galactoside, 4, quercetin-glucoside, 5, quercetin-arabinoside, 6, quercetin-rhamnoside, 7, quercetin, 8, 13′-II8-biapigenin, 9, 13-II8-biapigenin, 10, protopseudohypericin, 11, pseudohypericin, 12, protohypericin, 13, hypericin, 14, hyperforin, 15, adhyperforin (further details in Ref. [17]).

was employed. This triple hyphenation proved to be somewhat more problematic than the ‘‘simple’’ HPLC–NMR–FT-IR set-up described above as the dry $CDCl_3$ used did not allow sufficient ionisation in the electrospray interface to provide mass spectra. This difficulty was circumvented by including 1% ammonium acetate and 5% deuteromethanol as additives to the $CDCl_3$. This expedient did however, result in an increase in the background interferences in the NMR spectra and there is no doubt that it would have been preferable to add these ionisation promoters post chromatography directly into the stream of effluent to the MS (this was tried but resulted in other difficulties). However, whatever the deficiencies of the system it was still possible to obtain on-flow NMR and MS spectra and off-line IR spectra (see Fig. 8) and whilst it would be wrong to claim that the operation of such a system was trivial this example nevertheless illustrates that such multiple hyphenations are technically feasible. We have also used this type of interface with reversed-phase type aqueous based eluents (Ludlow et al., unpublished observations).

Whilst the on-line collection of the effluent as described above enables very sensitive FT-IR to be performed there is no doubt that the on-line collection of IR spectra would have some benefits. In addition, it should always be remembered that such a multiply hyphenated system is, in practice only as sensitive as its least sensitive detector. Currently this will most likely be the NMR spectrometer. It is therefore possible to trade off sensitivity in the case of the IR detector to gain the advantages of on line detection. Recently therefore we have investigated the possibilities of HPLC–NMR combined with fully on-line MS (a Micromass LC–TOF time-of-flight mass spectrometer) FT-IR and Diode Array UV (DAD-UV) spectroscopy with some success (Louden et al., in preparation). The FT-IR detector was equipped with a TATR flow cell and the overall

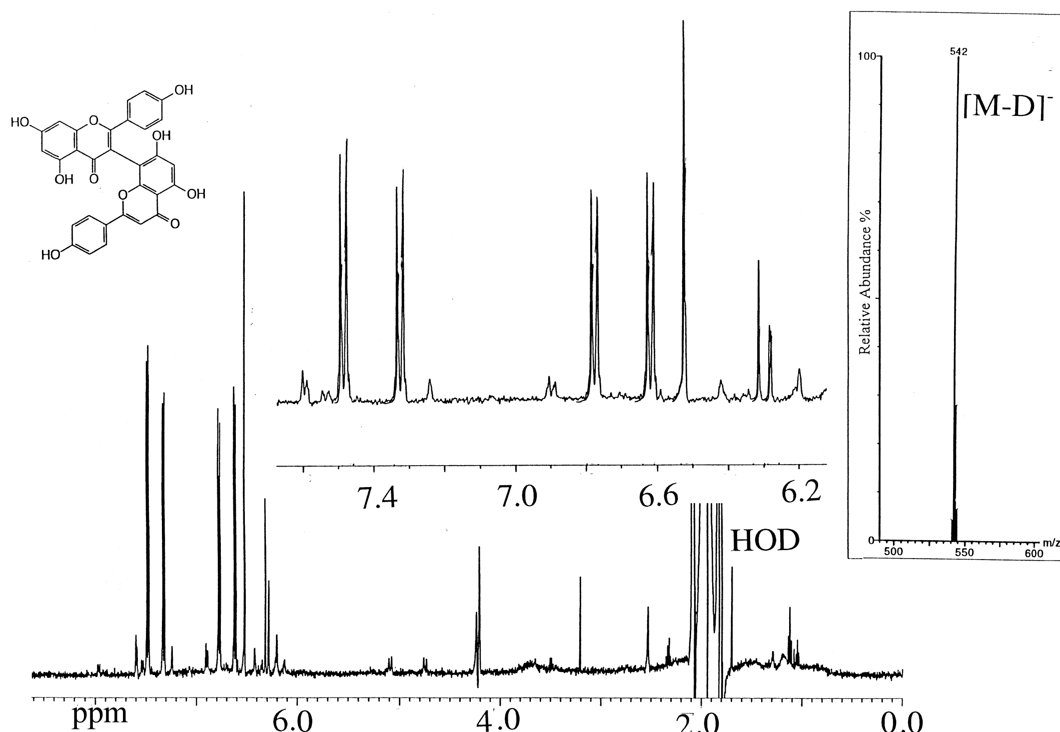

Fig. 7. MS and ^{1}H-NMR spectra of 13′II8-biapigenin, peak number 8 in Fig. 6 (further details in Ref. [17]. The inset shows an expansion of the aromatic portion of the spectrum. HOD=residual water.

sensitivity of the system for on-flow detection was ca. 50 μg/compound, with this limit due to the requirements of the NMR and IR spectrometers. However, we do not believe that we have yet reached the practical limit of for the current technology.

5. Conclusions

Clearly the multiple hyphenations described above by no means exhausts all of the possible combinations and other spectroscopic detectors are available for HPLC (for example spectrofluorimetry or circular dichroism measurements). These instruments could also be used in combination with NMR and MS detectors to provide the basis for even more complex systems to be built for complex mixture analysis. For such complex systems to become truly viable problem solving tools however, significant effort will have to be devoted to providing suitable methods for their control, and for data storage and manipulation.

It should also be recognised that most problems do not require LC–NMR–MS making the assembly of dedicated instrumentation an inefficient use of resources. However, provided that the spectrometers are carefully sited it should be possible to rapidly configure the required multi-hyphenated system when needed with the minimum of delay.

The concept of multiple hyphenation follows naturally from the use of HPLC–MS or HPLC–NMR systems singly and several groups have now demonstrated the combination of HPLC with both NMR and MS. This leads to the possibility at least of a ‘‘total organic analysis device’’ for the separation and structural identification of the components of complex mixtures of e.g. natural products or drug metabolites etc. Given the potential for such instrumentation to rapidly and efficiently provide com-

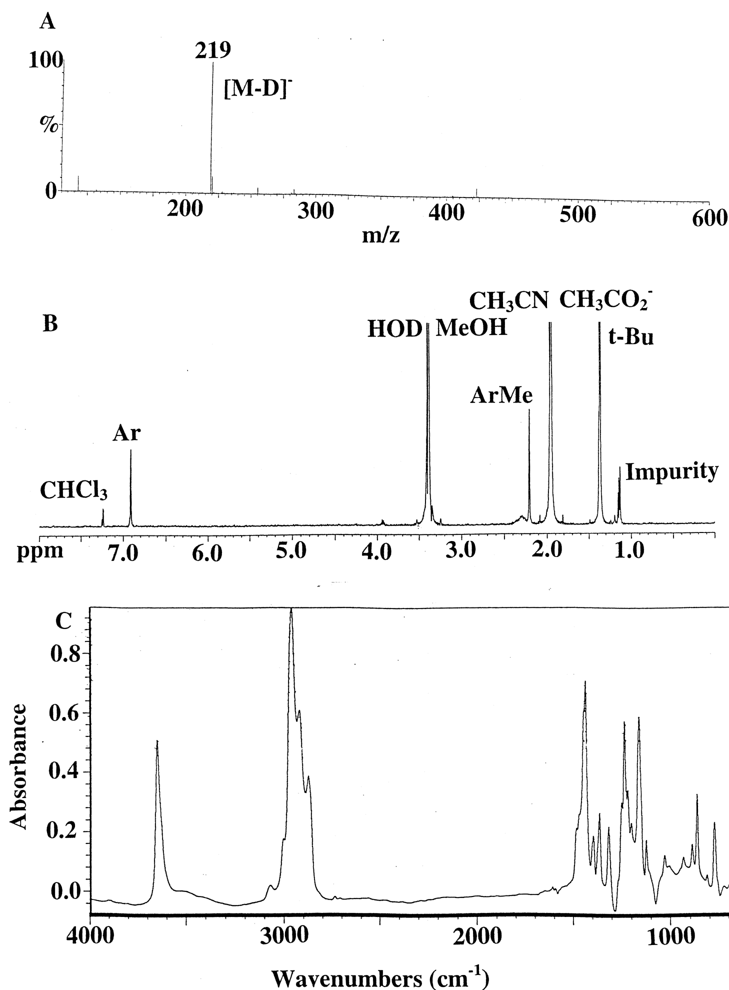

Fig. 8. Spectra obtained for 2,6-di-*tert*.butyl-4-methoxyphenol (BHT) following SEC, (A) MS, (B) NMR and (C) FT-IR [19].

prehensive spectroscopic information on samples the future of multiple hyphenation should be exciting.

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