LC-NMR for Natural Products Analysis: A Journey from an Academic Curiosity to a Robust Analytical Tool

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Abstract: LC–NMR combines the advantage of the outstanding separation power of liquid chromatography (LC) and the superior structural elucidating capability of nuclear magnetic resonance (NMR). NMR has proved that it is a standout detector for LC by providing maximum structural information about plant originated extracts particularly in its isolating ability of isomeric (same molecular formula) and/or isobaric (same molecular weight) compounds as compared to other detectors. The present review provides an overview of the LC–NMR developmental trends and its application in natural products analysis. The different LC–NMR operational modes are described, as well as how technical improvements assist in establishing this powerful technique as an important analytical tool in the analysis of complex plant-derived compounds. On-flow, stop-flow and loop-storage modes, as well as the new offline mode LC–SPE–NMR and capLC-NMR configurations that avoid the ingestion of expensive deuterated solvents throughout the experiment are mentioned. Utilization of cryogenic probe and microprobe technologies which are the other important promising approaches for guaranteeing the sensitivity issues are also described. Concluding remarks and future outlooks are also discussed.

Keywords: separation technique; spectroscopic technique; hyphenated techniques; LC-NMR; natural products

1. Introduction

The extraordinary technological advancement and technical improvements done so far in innovative analytical hyphenated techniques helps to enhance the full chemical characterization of natural products. The study of complex biological matrices such as crude plant extracts need efficient detection and rapid characterization route. At the early stage of separation, the initial identification of target constituents is essential for further selective identification procedures [1].

Although, liquid chromatography (LC) was conceived by the Russian Botanist, Mikhail S. Tswett in 1903, high-performance liquid chromatography (HPLC), comprising variety of packed columns integrated with high-pressure pumps, was achieved in the late 1960s. An extensive advancement of HPLC, involving automated computer controlled system with highly sensitive detectors has been extended the limits of detection to femtogram level. These advances directed HPLC to became as an
indispensable analytical instrument with a countless of applications in both industry and academia [2]. However, the search for a universal detector for HPLC in separating a complex mixtures, such as analysis of natural products, can still be challenging, requiring an extensive work.

Initially, nuclear magnetic resonance (NMR) spectroscopic technique was discovered in 1945 by physicists Edward Purcell at Harvard [3] and Felix Bloch at Stanford University [4] to determine the magnetic moments of all elements, however, the ability of NMR to provide structural information through chemical shift at the molecular level was demonstrated in 1951 by an organic chemist, Dharmatti and coworkers [5] in structure elucidation using the organic molecule ethanol. Later on, this discovery was rapidly involved by organic chemists in elucidating chemical structure of natural products and their metabolites as well as other complex chemical matrices.

Chemists have long searched for alternative detectors that can replace the traditional LC UV-detector in natural product analysis that leads to the renaissance of hyphenated techniques. While UV-detectors are very sensitive, they only provide limited information about the structure of the eluting compound. Hence, an extended development of verities of hyphenated techniques have been rapidly emerged and integrated as analytical tools for complicated mixtures like crude plant extracts [6,7].

In chemical analysis, detectors such as Infrared-spectroscopy (IR) and Mass Spectroscopy (MS) that provide better sensitivity and selectivity would be preferred [8]. However, these spectroscopic detectors do not permit a full identification, except for some well-known natural products. Hence, a harmonized on-line detection technique is needed to analyze complex mixtures like crude plant extracts. In this regard, coupling NMR as a detector with HPLC where generally abbreviated as LC-NMR would be a sounding hyphenated technique for getting detailed on-line structural information [6,9,10]. Indeed, the increasingly developmental progress in NMR technology has given a new motivation to LC-NMR [11] as shown in Scheme 1 presenting the time line of major events associated with the development of LC-NMR. Therefore it can be expected that the developmental progress of LC-NMR is steadily increasing (Scheme 2). Hence in this review, the developmental trend and application of different modes (continuous flow mode alternatively called measurement under dynamic conditions, stop-flow as well as loop/cartridge storage modes collectively and alternatively called measurement under static conditions) of operation of LC-NMR will be discussed with illustrative examples and applications in natural product analysis available for this technique.

![Scheme 1. Timeline of major events connected with the development of LC-NMR.](image-url)
Scheme 2. Number of articles published on LC-NMR in 1996–2017 in all application areas (available online on Sciencedirect website: https://www.sciencedirect.com/ (accessed on 20 December 2018, 6:24 P.M.)).

2. LC–NMR Instrumentation and Principal Modes of Operations

2.1. LC–NMR Instrumentation

Analytical methods that integrate or connect chromatographic with spectrometers online are called hyphenated techniques [12], and they have attracted attention in recent years as high-throughput analytical methods that provide separation of mixtures as well as the spectra of the structural or compositional of various components at the same time. Besides that the term hyphenation or coupling also refers to the researches that have been done to combine these techniques resulting improved standards that are unreachable with the individual techniques. Consequently, LC–NMR is a hyphenated technique that merges LC (separation techniques) with NMR (structurally informative spectroscopic detection method). The general instrumentation of an LC-NMR system is given in Figure 1. The main component of an LC-NMR is the isolation zone (column), interface zone and the detection zone i.e., probe for recording NMR spectra. The HPLC is directly connected to the NMR under computer controlled data acquisition system with automated harmonization of the different operations. A sensitive detector, such as UV and/or MS, is usually coupled in parallel with proper splitting ratio in order to monitor the main detector (NMR) measurements [13].
2.2. LC–NMR Principal Modes of Operation

Several operational modes of LC-NMR have been employed in order to deal with the major sensitivity difficulties of NMR. Generally, LC-NMR experiments can be performed mainly in three modes namely, continuous flow (measurement under dynamic conditions), direct stop-flow and loop/cartridge storage (measurement under static conditions) (Figure 2) [14]. All these modes of LC-NMR experiments have been employed in several studies depending on the interest of the analyst and type of analysis where they have their own advantages and disadvantages as described below.
2.2.1. Online-Flow Mode

In online-flow mode, the outlet of the LC detector is connected directly to the NMR probe (Figure 2a) and while the compound peaks are eluting, the spectra are continuously acquired. The chromatographic system is used to separate and move the components through the NMR probe for detection. Continuous flow is the easiest setup since it does not involve with any synchronization between the separation and the detection system, and thereby maintains a good separation resolution. However, it has a poor sensitivity due to the fact that, the eluted peaks get a short duration of exposure time in the detection cell i.e., NMR flow cell [15]. Additionally, the chemical shifts of the sample as well as the solvent depend on the solvent properties which may cause shifting of the position of NMR peaks, while the solvent composition is changing during elution [16].

2.2.2. Stop-flow Mode

Similarly, in the stopped-flow mode, the NMR probe is directly connected to the outlet of the LC-detector at the interface (Figure 2b). It has a better sensitivity as compared to the online-flow mode with better signal-to-noise ratio as it is clearly indicated in the illustrative example given in Figure 3 [1]. It also permits the detection of only selected peaks of our interest with the help of the valve found in the interface. After the eluted peaks detected by LC-detector and reached the NMR detection cell, the chromatographic working conditions (like: pump and gradient) should be stopped until the NMR signals are acquired, then the chromatographic working conditions continue until the next peak reaches the NMR detection cell again [14]. The time-slice mode is a modified extension of the stopped-flow mode, where the flow is stopped at regular and programed intervals. An LC-NMR experiments involving this type stopped-flow have been also employed in several studies [17,18]. The major advantage of time-slice mode is that, it can be used when the separation is poor, for example, in identifying two analytes having closer retention times. Generally, the major disadvantage of stop-flow mode may be it is dependent for separations resolved >2 min retention time [19].

![Figure 3](image-url)  
Figure 3. The comparison of stop-flow (A) and online-flow (B) modes of LC-1H-NMR spectra of sweroside in a dichloromethane extract of *Swertia calycina* depicting S/N ratio enhancement in stop flow mode (Reproduced from Ref. [1] with permission. Copyright © 1997, John Wiley & Sons, Ltd.).
2.2.3. Loop/Cartridge Storage Mode

In this type of mode, the outlet of the LC detector must be connected directly to sample storage loops or cartridges, i.e., loop storage or cartridge (solid phase extraction-SPE) storage (Figure 4). Usually, an LC detector (commonly UV and/or MS) is employed to monitor eluted peaks from the column in parallel. Then, the detected peak is directed and collected in either the sample loops or SPE cartridges with the help of the chromatographic working conditions. When the separation is completed, the LC pump again can be used to push the pre-collected peaks into the NMR flow cell by using a valve as shown in the general instrumentation block diagram in Figure 2c. In the new offline mode (SPE/cartridge storage mode) also called LC-SPE-NMR demonstrated by Exarchou and co-workers in 2003 [20], non-deuterated solvents are used in the HPLC system, and the separated peaks will be collected in SPE cartridges. After the SPE cartridges are dried using nitrogen, the deuterated solvent is used to push it to the NMR flow cell. Loop storage gives better peak resolution as compared to direct stop-flow mode (Figure 5) [16]. Several research groups have been used LC-SPE-NMR mode in natural products identification for instance; Lambert and his coworkers reported ten new isoflavonoids in addition to the seven previously reported constituents from the roots of Smirnowia iranica (S. iranica) and two compounds are described in Figure 6 as an illustrative example to show the practicability of this mode [21]. Even though, the loop/cartridge storage mode avoids the consumption of expensive deuterated solvents throughout the experiment, there might be sample decomposition/alteration during storage and also it requires additional special pumping equipment to elute samples from the temporary storage [19]. Based on the current and future needs of the end-user, literature survey as well as in our opinion; LC-SPE-NMR mode will still have put strong emphasis due to its cost effectiveness by avoiding consumption of expensive deuterated solvents throughout the experiment.

Figure 4. (a) Removable loop cassette for 36 sample loops, (the position of eluted peaks in each loop is monitored by memory board sits in the center), (b) Two units of Spark Holland SPE-Unit with 96 SPE cartridges (robot gripper is used to monitor the eluted peaks position in SPE cartridges).
Figure 5. (a) Comparison of peak resolution of the direct stop-flow and (b) loop-storage/loop transfer procedures (Reproduced from Ref. [16] with permission. Copyright © 2002, John Wiley & Sons, Ltd.).

Figure 6. (I) Structure of extracted compounds of 3 and 4. (II) Instrumentation diagram of the HPLC-SPE-NMR used. (III) HPLC-SPE-NMR experiments heteronuclear correlations spectra of compounds 3 and 4 fragments obtained from peak 2 where (A) HSQC spectrum (total acquisition time 9 h 40 min) and (B) One-bond correlations in HMBC spectrum (total acquisition time 15 h 7 min). (IV) HPLC chromatogram of compounds 1–10 from the ethanolic extract of *S. iranica* roots on a C18 column; acetonitrile gradient profile in water with average absorbance at 254 and 300 nm (Reproduced from Ref. [21] with permission. Copyright © 2005, American Chemical Society).
3. Technological Progresses and Limitations of LC-NMR

The complex nature of natural products and their metabolites isolation and quantification have led to the development of LC-NMR. After demonstrating NMR that can be used as a detector for HPLC by the end of the 1970s [22], it attained continuous technological improvements. Recently LC-NMR becomes, among the most powerful analytical methods for the separation and structural elucidation of unknown compounds in mixtures. The major technological advancements of LC-NMR, like use of super conducting magnets [23], solvent suppression [24], strong field superconducting magnets [25], microprobes [26] and cryoprobes [27] technologies, which help to improving the sensitivity and resolution [26–28]. Undeniably, the current advances and improvements of NMR play an important role in making LC-NMR, practically a useful method.

3.1. NMR Flow-Probe Design

The most significant technical amendment done was by fabricating a new probe called flow cell having a different RF coils and probe structural geometry from the conventional probe. In conventional experiment, the sample in the NMR probe rotates inside a Helmholtz coil to avoid magnetic field inhomogeneity (Figure 7A). A new and improved design having an optimum geometry with continuous flow-cell, where both ends are open (Figure 7B) was fabricated in cooperation with Bruker Company, Karlsruhe, Germany [8,23]. The flow cell was designed with a U-type glass tube shape elongated by polytetrafluoroethylene tubing called a “saddle”-shaped geometry to compromise the hyphenation between LC and NMR. The practical demonstration of this type of improved design using superconducting magnets were published during 1980s [25,29].

![Figure 7. Schematics of NMR cryoprobes (A) conventional (B) saddle/U-shaped continuous-flow NMR probe and (C) solenoidal design called Microprobe with microcoil.](image)

Another modification of NMR-flow probe, a novel type of flow cells called cryoprobes has been introduced by nitrogen/helium cooling system to reduce the resistance of RF coil and preamplifiers by lowering the temperature cryogenically. Cryogenic cooling also helps to increase the sensitivity in detection of trace quantities of components [26,27]. Hence, this new cryoprobe (e.g., Cryoprobe Prodigy, Bruker Biospin) is commonly used in many laboratories and several reports in the analysis of phytochemical profile of natural products have been appeared in the past decades [30,31].

Additionally, the reduction of the NMR probes volume is another appropriate milestone in flow probe design modification to increase mass sensitivity. This improvement was achieved by introducing flow cells called microprobes, where their actual volume is typically between 30 and
120 µL. Further modification was also achieved by employing of microprobes having capacity of several microliters (<5 µm) [15,26,32,33]. These types of probes are also structurally different from the saddle-shaped or standard Helmholtz probes because of their probe and RF coil geometry. Their design is called solenoidal-shaped capillary probes where the coil is wrapped directly on the capillary probe (Figure 7C) and this geometrical and size improvement makes them more sensitive than the saddle-shaped flow cells [34]. This kind of approach is called capillary LC-NMR (capLC-NMR), where a number of reports have proven that it is becoming a promising approach for high throughput natural products and their metabolites analysis having a better spectral quality when compared to the other LC-NMR modes [35,36]. Nowadays commercial microprobes are available at a reasonable cost which is comparable with the other probes. However, the application of microprobes is also limited for small volume and fully soluble compounds and in such occasions, utilization of cryoprobes could be a better solution.

3.2. Solvent Suppression

In LC–NMR experiment, sometimes the solvent signal is much larger than those of the sample and this signal must be suppressed. For example, in the case of solvents such as, acetonitrile (CH₃CN or CD₃CN), the solvent peaks are higher than the sample signals and that’s why solvent suppression technique is required. After the optimized solvent suppression technique called Watergate Excitation Technique (WET) that has been developed by Smallcombe and co-workers [24], the quality of spectra of LC–NMR was greatly improved. Practically, the WET method is a standard technique for LC–NMR due to its capability in suppressing solvent peaks efficiently, as compared to other solvent suppression techniques like presaturation and excitation sculpting [37]. Their merits and demerits are summarized in Table 1. The general drawback of all solvent suppression methods are, when the analyte signals are overlapping with the solvent signal, they are also suppressed together. The possible remedy for this type of problem is to execute the isolation process by using fully deuterated solvents in microcolumns to avoid consumption of excess deuterated solvent [38].

<table>
<thead>
<tr>
<th>Solvent Suppression Method</th>
<th>Operating Set Up</th>
<th>Benefits</th>
<th>Drawbacks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presaturation</td>
<td>Irradiation of solvent signal for a period of time with a continuous wave RF field</td>
<td>Easy set up and effective for eliminating single solvent signal</td>
<td>Not easy to suppress multiple solvent peaks simultaneously</td>
</tr>
<tr>
<td>Excitation sculpting</td>
<td>Extraction of the solvent signals with selective pulses</td>
<td>Could be automated and multiple solvent signals can be suppressed</td>
<td>The pulse sequences need to be optimized and it could suppress analyte signals that overlap with the solvent</td>
</tr>
<tr>
<td>Watergate excitation technique (WET)</td>
<td>The solvent signals are suppressed at the beginning of the experiment.</td>
<td>Could be automated and multiple solvent peaks can be suppressed</td>
<td>Suppresses analyte signals that overlap with the solvent.</td>
</tr>
</tbody>
</table>

4. Application of LC–NMR in Natural Product Identification

NMR is a technique of choice for rapid determination of the complete structure of natural compounds. The coupling of this technique with the HPLC having high separating abilities allows an online and complete identification of compounds in a plant extract [15,39–41]. LC–NMR provides that information without needing particular handling to treat the eluted peak for an NMR analysis, which could be used for identification of labile compounds. This is why LC–NMR is a technique under dynamic development since its first application demonstrated at the end of the 1970s [22]. Several publications demonstrated the suitability of LC–NMR to examine different complex mixtures of natural products however, the first application of LC–NMR to natural products extracts chemotaxonomic investigation was presented by Spring and co-workers in 1995 with the characterization of sesquiterpene
lactones from the Mexican plant *Zaluzania grayana* and then a new lactone was already identified in on-flow mode [41].

The first LC–NMR practical application for natural products analysis was reported in mid of 1990s. Since then, several applications to characterise natural product extracts have been presented. Different researchers have been investigated various plant originated natural product compounds such as, isoflavonoids from roots of *Smirnowia iranica* H., [21] prenylated flavonones from whole plants of *Munotes engleri* GILG, [9] secoiridoids from whole *S. calycina* and *G. ottonis* plants, [1] pyrroloidizine alkaloids from *Senecio vulgaris* L., *Senecio maritettia* M. and *Senecio venosus* H. extracts [42] and naphthoquinones from roots of *Cordia linnaei* Stearn [43]. Other groups have been also reported different natural products like; antibacterial sesquiterpene lactones, [10,41,44] naphthylisoquinoline alkaloids, [7,45,46] lignanes, [47] triterpene saponins, [48] fasciculol triterpenes, [49] taxanes, [50] tocopherols and tocotrienols, [51] phenylphenalenones, [52] polyhydroxy steroids, [53] carotenoid isomers, [54] flavonoids [55,56] as well as hop bitter acids [52,57]. Mostly, applications of LC-NMR published so far were the characterization of plant-derived natural products and their metabolites, while applications to micro-organisms or marine based natural products are still rare [49,58–60].

Heteronuclear (HSQC and HMBC) on-flow mode of LC–NMR experiment has been also reported by Garo and his coworkers [9] however, this was found to be practical only for a highly enriched fraction of a natural product showing the limitation of this approach. In natural products analysis, the stop-flow mode is more popular and employed one to acquire 1 H spectra, or if further structural information is required to perform two-dimensional 1 H NMR spectra, such as COSY, TOCSY, NOESY or ROESY. In many cases, an on-flow NMR chromatogram (usually at flow rates 0.3–1 mL min$^{-1}$) is recorded earlier for screening of particular groups of compounds or to gain a general overview on the sample composition. Moreover, time-sliced stop-flow [7,45,46] and a modified on-flow approaches at low flow rates [59,60] have been also applied to natural product extracts in order to combine the advantages of both on-flow (dynamic condition) and stop-flow (static condition which allows the samples to get sufficient acquisition time for trace compounds) modes. According to our literature review LC-SPE-NMR which is the latest hyphenated one and seems to be the promising technique as compared to the other modes considering the cost issues, sensitivity as well as compatibility with 2D NMR techniques. Table 2 describes the early starting breakthrough real practical applications of LC-NMR which can be considered as an illustrative example in natural product analysis field.

<table>
<thead>
<tr>
<th>Mode of Operation</th>
<th>Plant Species</th>
<th>Class of Compounds Analyzed</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>On-flow</td>
<td><em>Orophea enneandra</em></td>
<td>Lignans, Tocopherol, Polyacetylene</td>
<td>[61]</td>
</tr>
<tr>
<td></td>
<td><em>Iris domestica</em></td>
<td>Isoflavonoids</td>
<td>[62]</td>
</tr>
<tr>
<td></td>
<td><em>Ancistrocladus guineensis</em></td>
<td></td>
<td>[46]</td>
</tr>
<tr>
<td></td>
<td><em>E. vacciniifolium</em></td>
<td>Crude alkaloid</td>
<td>[31]</td>
</tr>
<tr>
<td></td>
<td><em>Urtica dioica</em></td>
<td>Phytosterols</td>
<td>[63]</td>
</tr>
<tr>
<td></td>
<td><em>Vitis species</em></td>
<td>Anthocyanin composition</td>
<td>[64]</td>
</tr>
<tr>
<td></td>
<td><em>Bobgunnia madagascariensis</em></td>
<td></td>
<td>[65]</td>
</tr>
<tr>
<td>Stop-flow (including loop storage)</td>
<td><em>Monotes engleri</em></td>
<td>Prenylated flavanones</td>
<td>[9]</td>
</tr>
<tr>
<td></td>
<td><em>Schizanthus graharnii</em></td>
<td>Tropane alkaloids</td>
<td>[66]</td>
</tr>
<tr>
<td></td>
<td><em>Swietenia macrophylla</em></td>
<td>Limonoids</td>
<td>[67]</td>
</tr>
<tr>
<td></td>
<td><em>E. vacciniifolium</em></td>
<td>Crude alkaloid</td>
<td>[31]</td>
</tr>
<tr>
<td></td>
<td><em>Isoplexis species</em></td>
<td>glycosides</td>
<td>[68]</td>
</tr>
<tr>
<td></td>
<td><em>Ravensara crassifolia</em></td>
<td>6-alkylated a-pyrones</td>
<td>[69]</td>
</tr>
<tr>
<td></td>
<td><em>Heliotropiu ovalifolium</em></td>
<td>tetrahydrophenanthrene</td>
<td>[70]</td>
</tr>
<tr>
<td></td>
<td><em>Schizanthus graharnii</em></td>
<td>Isomeric tropane alkaloids</td>
<td>[66]</td>
</tr>
<tr>
<td></td>
<td><em>Nandina domestic</em></td>
<td>Alkaloids, crude extracts and cultured cells</td>
<td>[71]</td>
</tr>
<tr>
<td></td>
<td><em>Vitis species</em></td>
<td>Anthocyanin composition</td>
<td>[64]</td>
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Table 2. Cont.

<table>
<thead>
<tr>
<th>Mode of Operation</th>
<th>Plant Species</th>
<th>Class of Compounds Analyzed</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC-SPE-NMR</td>
<td><em>Taraxacum officinale</em></td>
<td>4-hydroxyphenylacetic acid derivatives of inositol</td>
<td>[72]</td>
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<tr>
<td></td>
<td><em>Hypericum perforatum</em></td>
<td>Phenolic acids, Phloroglucinols, Flavonoids</td>
<td>[73]</td>
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<tr>
<td></td>
<td><em>Warburgia salutaris</em></td>
<td>Sequisterpenes</td>
<td>[74]</td>
</tr>
<tr>
<td></td>
<td><em>Salvia grahamii</em></td>
<td>Isomeric tropane alkaloids</td>
<td>[66]</td>
</tr>
<tr>
<td></td>
<td><em>Smirnowia iranica</em></td>
<td>Isoflavonoids</td>
<td>[21]</td>
</tr>
<tr>
<td></td>
<td><em>Neolitsea sericea</em></td>
<td>Flavonoid glycosides</td>
<td>[75]</td>
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<tr>
<td></td>
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<td>[76]</td>
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<tr>
<td></td>
<td><em>Strychnos usambarensis G.</em></td>
<td>Akagerine, Palicoside</td>
<td>[77]</td>
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<tr>
<td></td>
<td><em>Steganotaenia aralia</em></td>
<td>Cytotoxic stem bark extract</td>
<td>[78]</td>
</tr>
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<td></td>
<td><em>Ormocarpum kirki</em></td>
<td>Minor natural products</td>
<td>[79]</td>
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</table>

5. Conclusions and Future Outlooks

The technological improvements in NMR achieved during the past three decades have made it to become a practically versatile and reliable LC detector in different application areas like natural products analysis. NMR offers advantages over other detectors as it enables to get better structural information. Major overall performance improvements have been achieved through technological advances such as (a) selective solvent signal suppression, (b) cryogenic probes, (c) miniaturization of probes (microprobes) and (d) automation of the different working modes. In addition, the ability to accumulate multiple analytes using storage loops (peak parking) and SPE cartridges (peak trapping) has improved the sensitivity to the nanogram level. The combination of these technical developments has taken LC-NMR from an academic curiosity to a practically useful analytical tool. Based on the current and future needs of the end-user, literature survey as well as in our opinion; LC-SPE-NMR and capLC-NMR modes will still have put strong emphasis due to their cost effectiveness by avoiding consumption of excess amount of expensive deuterated solvents throughout the experiment. Since the plant kingdom is endless in producing potential natural products, further developments in (a) finding compatible solvents for sensitivity, (b) simplicity and cost issues as well as (c) overall miniaturization are still required to become indispensable technology throughout analytical laboratories in both industry and academia. Additionally, technological advancements in both hardware and software aspects of the isolation (LC) and detection (NMR) parts are also needed.

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Conflicts of Interest: The authors herein declare that there is no conflict of interest.

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>1D</td>
<td>one dimension</td>
</tr>
<tr>
<td>2D</td>
<td>two dimension</td>
</tr>
<tr>
<td>COSY</td>
<td>correlated spectroscopy</td>
</tr>
<tr>
<td>HMBC</td>
<td>heteronuclear multiple bond correlation</td>
</tr>
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<td>HMQC</td>
<td>heteronuclear multiple quantum correlation</td>
</tr>
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<td>HPLC</td>
<td>high-performance liquid chromatography</td>
</tr>
<tr>
<td>HSQC</td>
<td>heteronuclear single quantum correlation</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared-spectroscopy</td>
</tr>
<tr>
<td>LC</td>
<td>liquid chromatography</td>
</tr>
<tr>
<td>LC–NMR</td>
<td>liquid chromatography-nuclear resonance spectroscopy</td>
</tr>
<tr>
<td>LC–SPE–NMR</td>
<td>liquid chromatography-solid phase extraction-nuclear magnetic resonance</td>
</tr>
<tr>
<td>MS</td>
<td>Mass Spectroscopy</td>
</tr>
</tbody>
</table>
NMR  nuclear magnetic resonance
NOE  nuclear Overhauser experiment
NOESY  nuclear Overhauser experiment spectroscopy
RF/rf  radio frequency
ROESY  rotating frame nuclear Overhauser experiment spectroscopy
SPE  solid phase extraction
TOCSY  total correlated spectroscopy
UV  Ultra-Violet
WET  Watergate excitation technique

References


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