

Received: 31 October 2019

Revised: 14 December 2019

Accepted: 28 December 2019

DOI: 10.1002/pca.2921

SPECIAL ISSUE REVIEW

WILEY

Low-field benchtop NMR spectroscopy: status and prospects in natural product analysis[†]

Teris André van Beek 

Laboratory of Organic Chemistry, Wageningen University, Stippeneng 4, WE Wageningen, The Netherlands

Correspondence

Teris André van Beek, Laboratory of Organic Chemistry, Wageningen University, Stippeneng 4, 6708 WE Wageningen, The Netherlands.

Email: teris.vanbeek@wur.nl

Abstract

Introduction: Since a couple of years, low-field (LF) nuclear magnetic resonance (NMR) spectrometers (40–100 MHz) have re-entered the market. They are used for various purposes including analyses of natural products. Similar to high-field instruments (300–1200 MHz), modern LF instruments can measure multiple nuclei and record two-dimensional (2D) NMR spectra.

Objective: To review the commercial availability as well as applications, advantages, limitations, and prospects of LF-NMR spectrometers for the purpose of natural products analysis.

Method: Commercial LF instruments were compared. A literature search was performed for articles using and discussing modern LF-NMR. Next, the articles relevant to natural products were read and summarised.

Results: Seventy articles were reviewed. Most appeared in 2018 and 2019. Low costs and ease of operation are most often mentioned as reasons for using LF-NMR.

Conclusion: As the spectral resolution of LF instruments is limited, they are not used for structure elucidation of new natural products but rather applied for quality control (QC), forensics, food and health research, process control and teaching. Chemometric data handling is valuable. LF-NMR is a rapidly developing niche and new instruments keep being introduced.

KEYWORDS

adulteration, benchtop analysis, education, forensics, low-field NMR, quality control

1 | INTRODUCTION

Until 1960, structure elucidation of natural products was tedious and time-consuming requiring skill and persistence. It relied almost exclusively on degradative chemistry and elemental analysis requiring dozens of grams of crystallised material. Often more than 100 years

passed between the first isolation and the final correct structure, examples being morphine, strychnine and patchoulol. With the advent of ultraviolet (UV), infrared (IR), mass spectrometry (MS) and nuclear magnetic resonance (NMR), this all changed and nowadays a new structure is solved within days or weeks instead of a century.

Of those four spectroscopic techniques, NMR is the most powerful one, requiring only sub milligram quantities of amorphous products for a whole range of highly informative two-dimensional (2D) spectra. It provides detailed information on the local chemical environment, connectivity and stereochemistry of individual hydrogen and carbon

[†]Dedicated to Koji Nakanishi, a kind and eminent natural product chemist, an early user of NMR and discoverer of the ginkgolides, who passed away on 28 March 2019 at the age of 93.

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atoms. The basics of NMR were developed in the 1950s and the first commercial instruments, like the Varian A60 (60 MHz) appeared in the early 1960s.¹ Synthetic organic chemists and natural product chemists immediately recognised the usefulness of NMR, even if only for proton (¹H) at 60 MHz in one dimension.

For example, at that time the group of George Büchi was involved in the structure elucidation of terpenes, including patchoulol (Figure 1A). In a 1960 article, NMR was not yet used by them but in 1961 NMR first appeared through courtesy of another laboratory.² In 1964 their own laboratory had already purchased two Varian 60 MHz machines showing the big demand.³ In the case of patchoulol, the application of NMR was mostly limited to methyl groups and olefinic protons of dehydration products but even this was highly useful. Only a few years later, NMR played a bigger and more varied role in the group of Koji Nakanishi. Even with NMR, solving the structures of the ginkgolides (Figure 1B) was a challenging puzzle.⁴ Their spectra were more informative than those of patchoulol, which was also due to the fact that they had access to a – for that time – advanced 100 MHz Varian HR-100. Additionally, coupling constants and nuclear Overhauser effect (NOE) were used to determine stereochemistry and double resonance experiments were carried out to determine proton–proton connectivities.⁵ This illustrates the rapid developments taking place, which were partially catalysed by the desire to solve complex natural products. The rest is history, Fourier-transform (FT)-NMR was introduced, which allowed the recording of carbon-13 (¹³C)-NMR spectra, stronger and stronger superconducting magnets entered the market yielding much more resolution and sensitivity and finally all the 2D NMR techniques were gradually developed leading to the current situation.

However, modern NMR spectrometers are expensive both in terms of initial investment, consumables (liquid helium), maintenance (hardware) and operation (skilled personnel). Thus, they are out of reach for small and medium enterprises (SMEs), governmental quality control (QC) agencies, forensic laboratories and not at all universities students can get hands-on NMR training. This led to the introduction of benchtop NMR spectrometers with permanent magnets (42–100 MHz). They combine a small footprint, a 5–20× lower price, no consumables, almost zero maintenance and easy operation. The

downside is a 5–20× lower resolution and a lower sensitivity. In contrast to low-field (LF)-NMR spectrometers of the 1960s, the new generation is capable of recording 2D spectra.

At first glance modern LF-NMR spectrometers seem to have little merit for natural product or phytochemical analysis as they appear less suitable for structure elucidation and the NMR spectra of even simple natural products exhibit second-order effects. However, natural product analysis is not synonymous with structure elucidation and since 2014 a number of articles have appeared on the application of LF-NMR in natural products analyses, e.g. for QC or forensic purposes. Four reviews on the topic of LF-NMR as a whole have been written by the group of Blümich. Three comprehensively reviewed fundamentals and developments concerning spectroscopy, relaxometry and imaging.^{6–8} A fourth review focussed specifically on NMR spectroscopy.⁹ Rudszuck *et al.* devoted a review to the QC of crude and edible oils by LF-NMR.¹⁰ Finally Grootveld *et al.* summarised applications of LF-NMR in chemical and biochemical analysis.¹¹ The focus of the current review is on spectroscopic LF-NMR applications involving natural products. Additionally, it reviews available instruments. Its appearance is timely as in 2019 many more LF-NMR articles appeared than ever before and in 2019 there were also many exciting hardware introductions: an autosampler (Magritek), first 100 MHz instrument (Nanalysis), first broadband instrument (Oxford Instruments), < 0.2 Hz line width instrument (Magritek) and a new vendor entering the market (Bruker). Combined, this information might act as an eye-opener for the Phytochemical Analysis readership regarding the application niche of LF-NMR.

2 | AVAILABLE BENCHTOP NMR SPECTROMETERS

Currently five brands of benchtop NMR instruments are commercially available. The specifications of available instruments are presented in Table 1 based on information available on the web and no responsibility is taken for deviations from these values. Based on Table 1, not one best instrument emerges, all have pros and cons. Prospective buyers should test the instruments, which best meet their needs with their own samples. For instance, if they would like to be able to measure without deuterated solvents, an external lock should be chosen. Overall there is a trend towards higher field strengths and thus heavier instruments. It is debatable whether instruments over 100 kg should still be considered as “movable”. For non-research uses, e.g. for QC in SMEs, an autosampler is a necessity. The number of times a particular brand and type was used in the discussed research articles was counted and the following percentages were calculated: Magritek 43 MHz – 46%, Magritek 60 MHz – 18%, Oxford Instruments Pulsar 60 MHz – 15%, Nanalysis 60 MHz – 9%, Thermofisher Picospin 45 MHz – 6%, Thermofisher Picospin 82 MHz – 4%, Magritek 80 MHz – 1%, Bruker 20 MHz prototype – 1%, respectively. These percentages should not be confused with actual sales. The majority of uses could well take place within an industrial setting or for academic teaching and such uses will lead to few publications. The Magritek 43 MHz is by far the most popular

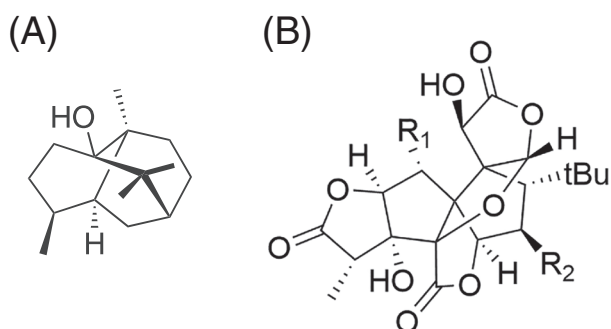


FIGURE 1 Structures of A, patchoulol and B, ginkgolides A–C. They were among the first natural products for which LF-NMR played a key role during structure elucidation in the 1960s. For ginkgolide A: R₁ = R₂ = H; ginkgolide B: R₁ = OH, R₂ = H; ginkgolide C: R₁ = R₂ = OH

TABLE 1 Commercially available LF-NMR spectrometers

Brand	Type	Nuclei	Number of nuclei	MHz (^1H)	Line width 50% (Hz)	Sensitivity ^a	Weight (kg)	Lock	Sample ^b	Dimensions (cm)	Auto-sampler	Reference
Bruker	Fourier	^1H ^{13}C	2	80	< 0.5	> 1500	94	External	Tube	50 × 70 × 60	No	12
Magritek	Spinsolve 43	^1H ^{13}C ^{19}F ^c	2 or 3	43	< 0.5	> 100	55	External	Tube	58 × 43 × 40	Yes	13
Magritek	Spinsolve 60	^1H ^{13}C ^{19}F ^c	2 or 3	60	< 0.5	120	60	External	Tube	58 × 43 × 40	Yes	14
Magritek	Spinsolve 80	^1H ^{13}C ^{19}F ^c	2 or 3	80	0.5	> 200	72	External	Tube	58 × 43 × 40	Yes	15
Magritek	Spinsolve ultra	^1H ^{13}C ^{19}F ^7Li ^{11}B ^{15}N	2 or 3	43 or 60	< 0.2	> 70	—	External	Tube	—	Yes	16
		^{29}Si ^{31}P										
Nanalysis	NMReady-60Pro	^1H ^{13}C ^{19}F ^7Li ^{11}B ^{15}N	2	60	≤ 1.0	100	25	Internal	Tube	30 × 28 × 49	Yes	17
		^{29}Si ^{31}P ^{129}Xe										
Nanalysis	100PRO	^1H ^{13}C ^{19}F ^{31}P ^{29}Si ^{11}B ^7Li ^{15}N ^{129}Xe	2	100	< 1.0	220	97	Internal	Tube	37 × 41 × 65	Yes	18
Oxford Instruments	X-pulse ^d	^1H ^{13}C ^{19}F ^{31}P ^{29}Si ^{23}Na ^{11}B ^7Li	2, 3 or 8	60	< 0.35	> 120	172	Internal	Tube	38 × 54 × 42	No	19
ThermoFisher	picoSpin 45 II	^1H (^{19}F)	1	45	< 1.8	> 1000	5	Internal	Cap	18 × 15 × 29	No	20
ThermoFisher	picoSpin 82 II	^1H (^{19}F)	1	82	< 1.6	> 4000	19	Internal	Cap	43 × 36 × 25	No	20

^aSensitivity: signal/noise ratios given are dependent on test conditions; Magritek, Nanalysis and Oxford use 1% ethylbenzene, Bruker 10% ethylbenzene and ThermoFisher 100% water.

^bAll spectrometers use standard 5 mm tubes (tube) with the exception of the picoSpins, which use a 0.4 mm capillary (cap) with 40 μL sample volume.

^cOther nuclei on request.

^dSample temperature 20–70°C.

research LF-NMR. This is partially caused by the fact that the key players in the LF-NMR field, Bernhard Blümich and Patrick Giraudeau, have been using this instrument.

Two more manufacturers of low-field or cryogen-free instruments exist: Anasazi and HTS-110. However, as their spectrometers cannot be considered as benchtop they are not included in Table 1.

3 | LF-NMR APPLICATIONS

This review is restricted to articles making use of NMR spectroscopy and commercially available LF-NMR spectrometers. In the following paragraph, all LF-NMR applications involving natural products or plant matrixes have been subdivided in five categories: (1) quality control and adulteration detection, (2) forensic applications, (3) food and health applications, (4) process control, and (5) teaching. In Table S1 there is a listing of relevant papers making use of non-spectroscopic NMR approaches such as relaxation, diffusion and imaging, or home-built equipment.

3.1 | LF-NMR in quality control and adulteration detection

This is an important field and the application of NMR in general to detect food fraud was recently separately reviewed. Some attention to LF-NMR was given and according to the authors benchtop NMR is potentially a breakthrough technology in food authentication.²¹

Parker *et al.* in 2014 were the first to apply LF-NMR in phytochemical analysis.²² They wished to detect adulteration of olive oil with hazelnut oil. This was highly challenging as these two vegetable oils possess an almost identical fatty acid (FA) composition, the only difference being the ~6% higher content of double unsaturated fatty acids (UFAs) of hazelnut oil. Thus, the peak area ratio of olefinic peaks (~5.3 ppm, also including H2 of glycerol) versus H1 and H3 of glycerol (~4.2 ppm) was determined for both oils. This ratio was 1.70 and 1.52 for hazelnut and olive oil, respectively, but due to natural variation the most extreme values were almost identical. The final result was that 13% (w/w) hazelnut adulteration could be detected with 95% confidence. Additionally, they used a chemometric approach using the shape of the entire spectrum. This allowed for the detection of 11% adulteration with 95% confidence. Thus, the method is not fool-proof, false negatives and especially false positives will occur occasionally. Compared to an FT-IR method, the 60 MHz NMR method performed better.²²

The earlier-mentioned study was summarised in a subsequent article by this group together with new results.²³ Vegetable oil samples were diluted 1:1 with chloroform (CHCl₃) and measured at 60 MHz. Integration of various triacylglycerol (TAG) peaks (0.9 ppm = ω -3 CH₃; 2.7 ppm = bis-allylic CH₂; 5.2 ppm = olefinic peak; remaining FAs are saturated) allowed to calculate the percentage of ω -3 FAs, polyunsaturated fatty acids (PUFAs), monounsaturated fatty acids (MUFAs) and saturated fatty acids (SFAs), respectively, and an excellent correspondence was found with gas chromatography flame ionisation detector (GC-FID) data. Even for a whole range of complex foods, such as rolls,

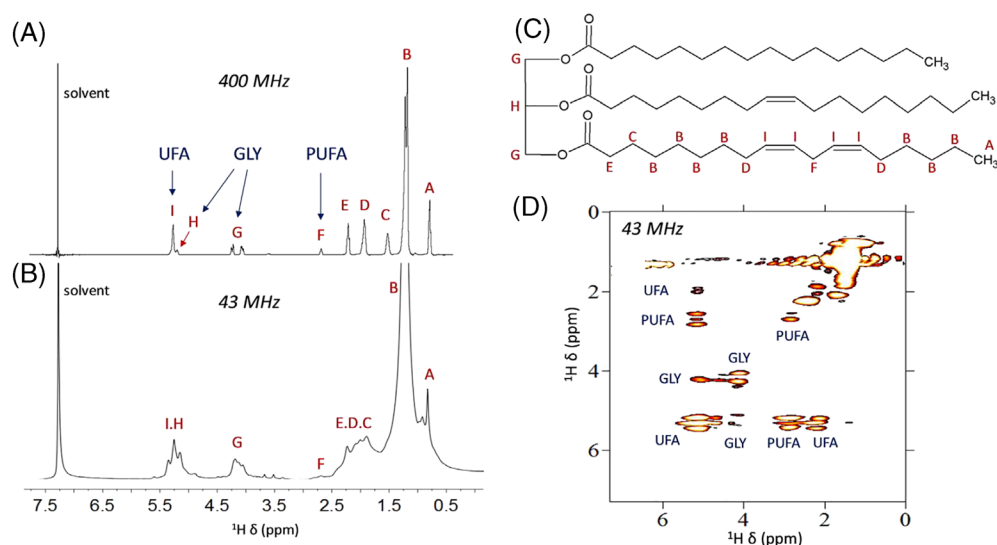
pies and crisps, a fair correspondence with the labelled SFAs was found. Through chemometric treatment [principal component analysis (PCA)] of the LF-NMR data, 10 different vegetable oils could be clearly distinguished. Not surprisingly, as the analytes (TAGs) are the same, the methodology could also distinguish between different types of meats and detect adulteration of sunflower oil with lard.²³ The meat application was also separately published.²⁴

Riegel investigated adulteration of olive oil with soybean oil.²⁵ To this purpose, solutions in CDCl₃ of olive oils spiked with 5–60% of soybean oil were measured at 60 MHz. As soybean oil has a much higher content of PUFAs, plotting the percentage of bis-allylic protons against the soybean oil percentage gave a straight line. By means of this curve the extent of adulteration could be determined. Possible pitfalls are that beforehand it is not always known which vegetable oil is used for adulteration and the natural variation in FA composition of both olive and soybean oil will influence the calibration curve.

The adulteration of edible oils was further investigated by the group of Giraudeau.^{26,27} They convincingly showed that 2D NMR at 43 MHz, can provide more reliable information regarding vegetable oils than one-dimensional (1D) NMR at 43 or 60 MHz (Figure 2). To allow a faster recording of correlated spectroscopy (COSY) spectra, they added a gradient coil along the B₀-axis. In combination with a homemade pulse sequence, high quality COSY spectra could be obtained with only 72 scans in 2.4 min. However, to reduce integration errors, each sample was measured five times. Sample preparation was simple: mixing roughly equal volumes of non-deuterated chloroform and oil. Due to the two dimensions, signals specific for UFAs and PUFAs as well as H1/H3 and H2 of glycerol (GLY) could be separately integrated (Figure 2D). By looking at both the ratios PUFA/UFA and GLY/UFA, a fair separation of five different vegetable oils was possible although the number of samples was relatively small.²⁷ The distinction between olive and hazelnut oils remained difficult. In a follow-up article, they applied different chemometric methods on the 1D and ultrafast 2D spectra. The PCA using 2D data was able to separate the six investigated oils. The adulteration of olive oil with hazelnut oil could be detected better than by 1D NMR although below 20% adulteration users might still encounter false negatives and positives.²⁶ This article by Gouilleux and others by him cited in this review were also published as part of his PhD thesis.²⁸

A LF-NMR method for adulteration detection of *Perilla frutescens* edible oil with 30 times cheaper soybean oil was developed.²⁹ Samples (100 μ L, in triplicate) were dissolved in 500 μ L of CDCl₃ containing 0.03% TMS (tetramethylsilane), measured at 43 MHz and the ranges of 0.5279–1.0454, 1.0643–1.664, 1.6641–2.5532, 2.5720–3.1742, 4.0046–4.5009 and 4.8631–5.8652 ppm were integrated (ranges 1–6). Ranges 2, 6, 4 and 3 were most significant for detecting adulteration of the highly unsaturated *Perilla* vegetable oil with the much more saturated soybean oil. Due to the presence of fewer methylene units (-CH₂-) in PUFAs, the range 2 peak became larger upon adulteration. In contrast, peaks corresponding with ranges 6 and 4 became smaller upon adulteration as these ranges are correlated with double bonds and PUFAs, respectively. Adulteration with \geq 6% (v/v) soybean oil was detectable.²⁹

FIGURE 2 One-dimensional ^1H -NMR spectra of a vegetable oil sample in chloroform recorded at 400 MHz A, and 43 MHz B, together with the structure and nomenclature used for the glycerol and fatty acid protons C, D, Ultrafast two-dimensional NMR spectrum recorded in 2.4 min on the same oil sample. Reprinted from Gouilleux et al.,²⁶ copyright (2016), with permission from Elsevier [Colour figure can be viewed at wileyonlinelibrary.com]



Patchouli essential oil (PEO) is an important and expensive fragrance commodity and therefore frequently adulterated with either synthetic chemicals or natural products like cheaper essential or vegetable oils.³¹ NMR spectra (60 MHz) were recorded of 75 genuine PEOs as well as 17 adulterants, 10 commercial PEOs and 10 other essential oils.³⁰ All samples (600 μL) were measured neat after the addition of 25 μL TMS (32 scans, 2 min). The NMR fingerprint of

genuine PEO was remarkably constant. This made it easy to detect most adulterants when PEO was spiked at 20%. These included non-volatile ones (ricinus oil, paraffin), which could not be observed by qualitative GC-MS (Figure 3A). Chemometric data treatment (Mahalanobis distance) based on integration from 0.1 to 8.1 ppm in 0.01 ppm increments identified 15 out of 17 adulterants including gurjun balsam, which was difficult to detect by the human eye (Figure 3B). The model showed a linear correlation between the Mahalanobis distance and 10, 20 or 30% of adulteration with gurjun balsam. All adulterated commercial PEOs as well as all other essential oils were recognised by the software. The authors concluded that LF-NMR is complementary to GC-MS and refractive index measurements for the QC of essential oils.³⁰

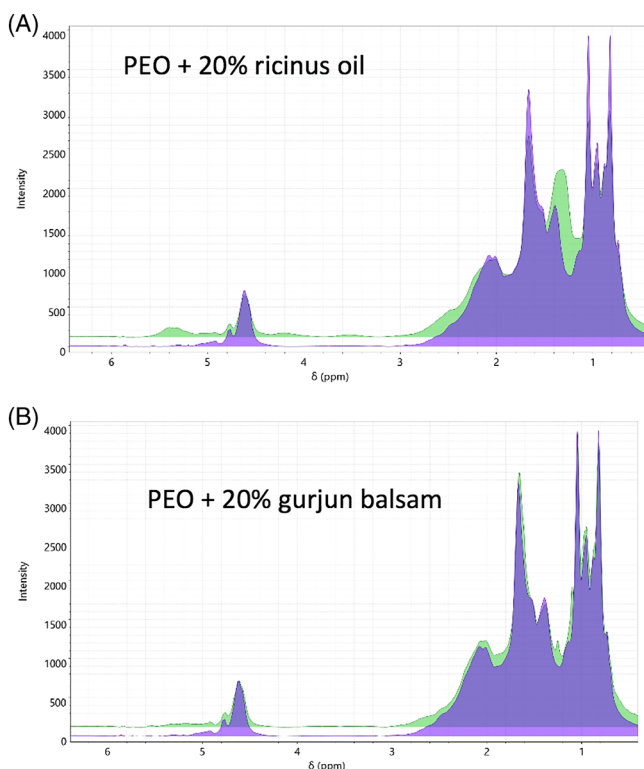


FIGURE 3 Overlaid 60 MHz ^1H -NMR spectra of patchouli essential oil (PEO) pure (in purple) and PEO spiked at 20% with ricinus oil (in green) (A) and gurjun balsam (in green) (B). All samples were measured neat. Reprinted from Krause et al.,³⁰ with permission from Thieme [Colour figure can be viewed at wileyonlinelibrary.com]

Cold pressed rapeseed oil (CPRO) is an expensive new culinary oil. Adulteration with cheaper industrially produced refined rapeseed oil (RRO) or refined sunflower oil (RSO) is a possibility. McDowell et al. investigated whether NMR (60 and 400 MHz) is able to detect such fraud.³² However, this proved difficult as – especially in the case of RRO – the TAG composition is highly similar leading to LF-NMR spectra, which are difficult to distinguish by a human observer. Chemometric data treatment gave better results for RSO. The LODs (limits of detection) were 24% and 8% admixture of RSO at 60 and 400 MHz, respectively. NMR was less suitable for detecting adulteration with RRO.³²

A remarkable case of an inadvertent process error (or willful adulteration) in the production of two synthetic peptides was reported by Choules et al.³³ Based on their QC measurements by means of LC-UV, the manufacturer claimed the peptides to be 98.44% and 98.34% pure. However, several large, unexplainable peaks were discovered by the customer in both NMR spectra. 800 MHz qualitative and quantitative NMR showed the additional compound to be mannitol and the true peptide purities to be 80.5% and 56.9%. Also 60 MHz ^1H -NMR was used and mannitol could be easily observed. This impurity was missed by routine reversed-phase high-performance liquid chromatography (RP-HPLC)-UV as it elutes at the dead time (t_m) and is UV transparent. It might have been missed by LC-MS too as there

are often unexplained ions observed around t_m and ionisation can be poor. Using an only 57% pure biochemical without being aware of this, will produce unreliable results in expensive tests. The authors urged manufacturers of fine chemicals to include also a LF-NMR spectrum as part of their certificates of analysis. Combining orthogonal analytical methods like LC-UV, IR, infusion electrospray ionisation (ESI)-MS and LF-NMR, provides a better guarantee that the bought chemical is indeed the declared chemical with the declared purity.³³

3.2 | LF-NMR in forensic applications

Pàges *et al.* qualitatively (22.5 min measuring time) and quantitatively (3 × 45 min) analysed “100% natural” dietary supplements (DSs) for sexual enhancement and weight loss for QC purposes by 60 MHz NMR.³⁴ Sample preparation was simple: vortexing one powdered tablet or capsule during 15 s in 1 mL of deuterated methanol (CD_3OD) containing 0.03% TMS and, after sedimentation, transfer to an NMR tube. The quantitative extraction took ~30 min and TSP (trimethylsilylpropanoic acid) was used as internal standard. Comparative 500 MHz analyses were carried out. Ten out of the 11 sexual enhancement DSs contained one or two synthetic adulterants such as sildenafil. By comparison with reference spectra, six different adulterants could be detected. Three DSs contained two adulterants (Figure 4) besides caffeine, FAs, citrate and unidentified compounds. Four out of five weight loss DSs were adulterated with sibutramine or/and phenolphthaleine. Results from quantitative analyses of sildenafil were within 10% on LF and HF (high-field) instruments. The LOQ (limit of quantitation) was 2.0 mM. The authors concluded that LF-NMR is an excellent technique to uncover DS adulteration. It is relatively cheap, requires little sample preparation and provides structural and quantitative information. It is proposed as a routine screening tool.

The structure of strychnine could be confirmed by interpreting several 1D (1H and ^{13}C , incl. 3 × DEPT) and 2D (COSY, HETCOR, HSQC, HMBC, *J*-resolved) NMR spectra at 43 MHz (total recording time 90 h).³⁵ For comparison, 400 MHz spectra were recorded. In

spite of the significant signal overlap and second-order effects, it proved possible to assign all protons and carbons as well as many coupling constants. Still it is a misconception to think that the full structure of strychnine including stereochemistry at its six chiral centres could have been elucidated without prior knowledge of the structure with only 43 MHz spectra. Apart from the free base, also two salts of strychnine (hemisulphate and hydrochloride) were measured in three solvents. Several protons and carbons gave different chemical shifts depending on the counter ion. This could prove useful for forensic purposes.³⁵

Smokable herbal mixtures sold as “incense” may contain one or more synthetic cannabinoids. LF-NMR has been applied as a qualitative pre-screening method to rapidly identify nine cannabinoids.³⁶ Reference spectra were recorded. Thus, 40 mg of a herbal mixture was extracted during 2 × 5 min with 1 mL of $CDCl_3$ containing 0.5 mM dimethyl maleate and 600 μL of supernatant was then measured in 8 min. Each cannabinoid gave specific signals and if one of them was present in a herbal mixture it could be identified. However, if more compounds are present, the identification becomes cumbersome due to many matrix compounds, spectral overlap and lower concentrations. Confirmation and quantitation after initial screening by LF-NMR is possible by HF-NMR and MS.³⁶

Pseudoephedrine and ephedrine can be used as starting materials for methamphetamine (“meth”) synthesis.³⁷ LF-NMR spectra (43 MHz) of both ephedrines and methamphetamine were recorded. A distinction could be made between the free base and HCl salt of the ephedrines and this proved useful for identifying reaction mixtures and extracts thereof. Phosphorus-31 (^{31}P) LF-NMR at 17.5 MHz was applied for the first time. During the methamphetamine synthesis from ephedrine, phosphoric acid, phosphorous acid and hypophosphorous acid can occur in various ratios. These acids could be distinguished both in their neutral form and as anions by ^{31}P -NMR. The combination of 1H and ^{31}P LF-NMR made it possible to reliably and reproducibly analyse liquids found in clandestine laboratories, which is forensically valuable.

Zhong *et al.* created a 1H -NMR spectral database of 12 illegal drug substances including morphine and codeine at 600 MHz (in D_2O)

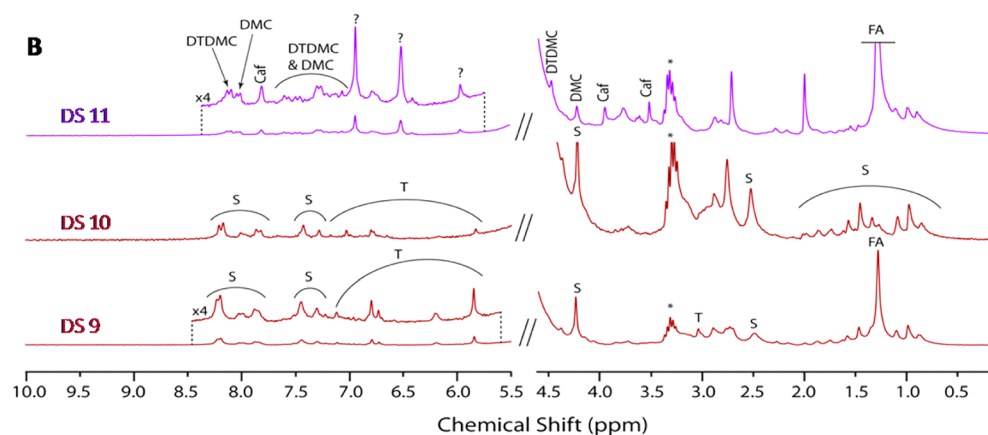


FIGURE 4 60 MHz 1H -NMR spectra in deuterated methanol (CD_3OD) of three sexual enhancement dietary supplements containing two adulterants. DMC, desmethylocarbodenafil; DTDMC, dithiodesmethylocarbodenafil; S, sildenafil; T, tadalafil; Caf, caffeine; FAs, fatty acids; * = CD_2HOD ; ? = unknown. Reprinted from Pàges *et al.*,³⁴ (<https://doi.org/10.1021/ac503699u>) with permission from the ACS [Colour figure can be viewed at wileyonlinelibrary.com]

and partially at 82 MHz (in H₂O).³⁸ Two case samples were analysed by GC-MS as well as HF and LF-NMR. One sample was shown to contain heroin and acetylcodeine while the other consisted of methamphetamine and 3,4-methylenedioxymethamphetamine (MDMA), ketamine and caffeine. Although it was concluded that LF-NMR is an effective qualitative method for the analysis of drugs, the study appears somewhat superficial and more evidence should have been produced to substantiate this claim.

Assemat *et al.* checked six artesunate containing tablets (antimalarial) as well as other medicines both qualitatively and quantitatively.³⁹ None contained any artesunate according to LF- and HF-NMR although one tablet did contain some artemisinin. Qualitative analysis involved vortexing 100 mg of powdered tablet with 1 mL of CD₃OD and some TSP. After centrifugation, the solution was measured at 60 MHz in 5.5 min. For the quantitative analysis in triplicate both extraction and NMR recording took longer, 35 min and 45 min respectively for a single extraction. The authors concluded that LF-NMR has potential for uncovering drug falsification. It quickly provides information on whether or not the active ingredient is present at all. If it is, subsequent quantitation is straightforward.³⁹

The degradation of compounds present in fingerprints (squalene, oleic acid, nonanoic acid) as a function of light exposure was studied to determine the age of human fingerprints.⁴⁰ Irradiated pure compounds were dissolved in CDCl₃ and investigated by 60 and 400 MHz NMR. No details on LF-NMR spectra were given.

To deal with more and more seized samples containing psychoactive substances, LF-NMR (60 MHz) was employed to screen samples.⁴¹ A maximum of 10 mg of sample was dissolved in 1 mL of deuterated dimethyl sulfoxide (DMSO-d₆). Subsequent NMR measurement and chemometric analysis took 5 min. The spectra were subdivided in two regions: one identifying the class (0.46–1.54 ppm) and one identifying the substance ("fingerprint-region", 3.90–12.50 ppm). DMSO was used as internal standard. The spectra were compared with a library of 302 spectra. The substances were of natural (cocaine, caffeine, cannabis, sugars) and synthetic origin. A Pearson's correlation was calculated for the match between a sample and library spectrum (1.000 = perfect match). Validation took place and resulted in class being correct > 99% and compound > 95% correct. For mixtures, these percentages dropped but still for 65% of the samples both compounds were correctly identified. Subsequently 432 seized samples were analysed by both LF-NMR and GC-MS. In 93% of the cases the results matched and in another 6% there was a partial match with one component of a binary mixture identified. Only for 1% of the samples contradictory results were obtained. Interestingly, the correct class identification of an unknown drug, eventually led to its identification.⁴¹

Keizers *et al.* reported on qualitative and quantitative investigations of falsified medicines and illegal drugs by LF-NMR.⁴² The emphasis was on synthetic medicines like aspirin and diclofenac and the only natural products investigated were cocaine, caffeine and 4-hydroxybutyric acid (GHB). Qualitative identification occurred through chemometrics in combination with a spectral database. For quantitation of illegal medicines, internal standardisation was used

and validation was by LC-MS. GHB and cocaine in street drugs could be identified by LF-NMR.

3.3 | LF-NMR in food and health related applications

LF-NMR was used to distinguish more expensive arabica from cheaper robusta coffees.⁴³ Only robusta coffee contains 16-O-methyl-cafestol (16-OMC). This compound exhibits a 3H singlet at 3.16 ppm due to its methyl ether moiety and no other coffee constituents show resonances in this region. Thus, it could serve as an indicator for the inadvertent or deliberate occurrence of robusta in arabica coffees. The procedure was simple: extraction of 1 g of ground coffee with 3.0 mL of CDCl₃ in 5 min with agitation followed by direct filtration through cotton into an NMR tube. The 16-OMC content was given relative to the glyceride region (3.9–4.6 ppm) to correct for a variable extraction efficiency. As the 16-OMC content of robusta varies, a precise detection limit could not be given but the adulteration limit was estimated to lie between 10 and 20%. A survey of 27 "100% arabica" coffees in the UK revealed no fraud.⁴³

In a follow-up article, the sensitivity of the method mentioned earlier was increased by extracting 10 g of coffee with 30 mL of CHCl₃, filtration, evaporation, re-dissolving in 800 μ L of CDCl₃ and filtration directly into an NMR tube.⁴⁴ This gave a much better signal/noise (S/N) ratio for the 16-OMC peak. Due to the higher sensitivity, it was discovered that arabica beans also contain small amounts of 16-OMC, on average 1.5% of the content of robusta coffee. However, arabica coffee from some marginal coffee-growing areas contained significantly higher concentrations of 16-OMC, potentially increasing the risk of false positives. The authors claim that with the adapted methodology the occurrence of 2% robusta in arabica will be detected in 90% of the cases, so the risk of false negatives is low. Sixty arabica coffees were screened and eight samples were marked as suspicious with the most adulterated sample containing ~33% of robusta.⁴⁴

Hop (*Humulus lupulus*) is an important beer ingredient. Its bitter acids impart the specific beer flavour. Killeen *et al.* used various techniques, including 400 MHz NMR, to quantify bitter acids in hops.⁴⁵ Lupulin glands (~20 mg) were sonicated with 800 μ L of CDCl₃ containing 0.05 M dimethylformamide (DMF) (internal standard) during 15 min, filtered, diluted and measured. They compared the region from 18.0 to 19.4 ppm (enolic protons of bitter acids) with a 43 MHz NMR spectrum (Figure 5). At 43 MHz α -acids (18.8–19.1 ppm) were separated from β -acids (19.1–19.4 ppm) so α/β acid ratios could be calculated. Cohumulone was not resolved from adhumulone but chemometrics might still provide useful information. The authors concluded that LF-NMR might be used by hops breeders at field stations for screening hops bitter acid profiles.⁴⁵

Gouilleux *et al.* used 2D ¹H/³¹P NMR to probe phospholipids at the mM level at 43.5 and 80.5 MHz (for ¹H).⁴⁶ By means of a total correlated spectroscopy (TOCSY) experiment (60 mg α -lecithin, 12 h), they were able to separate phosphadityl-ethanolamine,

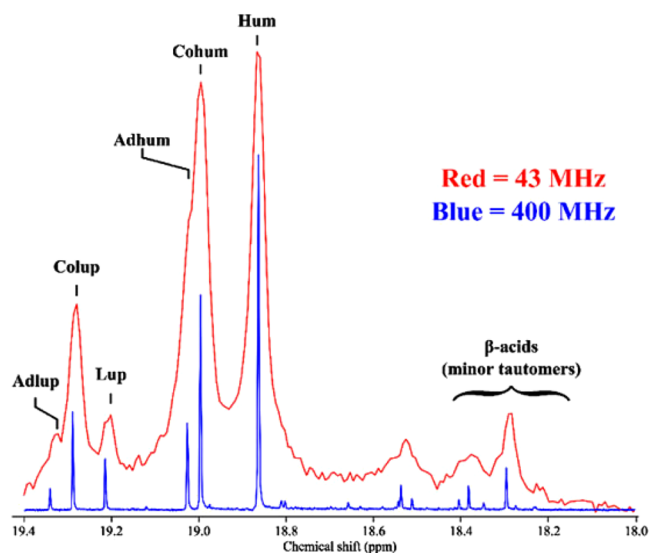


FIGURE 5 The 18.0–19.4 ppm range of 400 MHz and 43 MHz ^1H NMR spectra of an extract of lupulin glands from hops in CDCl_3 . Adlup = adlupulone; Colup = colupulone; Lup = lupulone; Adhum = adhumulone; Cohum = cohumulone; hum = humulone. Reprinted from Killeen *et al.*,⁴⁵ with permission from Wiley [Colour figure can be viewed at wileyonlinelibrary.com]

phosphatidylinositol and phosphatidylcholine and determine their relative concentrations. Absolute quantitation proved possible by two different methods (relaxation reagent or ^1H excitation and magnetisation transfer to ^{31}P) both overcoming long ^{31}P relaxation times. Both methods had their pros and cons but both yielded fair accuracy (maximum 0.5–9% deviation) and relative standard deviations (RSDs) (2–5%) making LF-NMR a promising tool for lipidomics.⁴⁶

Matviychuk *et al.* developed a quantitative method for determining relative concentrations of known components, e.g. the main

sugars in fruit juices.⁴⁷ They did not make use of internal standards or integration values. First, they quantum mechanically calculated spectra of the individual components at 60 MHz based on all known chemical shifts and coupling constants. Then, using the simulated spectra of all components, software calculated the mole fractions for which the combined spectrum gave the best fit with the real-life spectrum of the food under investigation. The advantage of this approach is that independent of the field strength of the used instrument, the combined spectrum can be predicted. A disadvantage is that the NMR data of any component to be measured must first be completely unravelled at high field. The same approach was followed by Duffy *et al.* in their differentiation of fentanyl analogues by LF-NMR.⁴⁸ and by Choules *et al.* for QC of fine chemicals.³³ An example is given in Figure 6 for kiwi juice at 43 MHz. For sugars in juices, the methodology was insensitive to pH changes but this may not be the case when analytes contain acidic and basic groups. Due to its lower resolution LF-NMR was actually less sensitive to small chemical shift deviations than HF-NMR. The methodology compared well with HF-NMR analyses and actual values.⁴⁷

Percival *et al.* applied 60 MHz NMR to detect α - and β -glucose in human urine as markers of diabetic control.⁴⁹ Briefly, 450 μL of centrifuged urine was mixed with 50 μL phosphate buffer pH 7 containing sodium azide (NaN_3) and 50 μL 0.05% TSP (internal standard) in D_2O . Employing presaturation at 4.95 ppm to suppress the water signal, a spectrum was collected (Figure 7). Total glucose was calculated based on H1 of α -glucose (5.25 ppm), which was resolved from the water signal taking into account the α/β ratio. Calibration curves were linear and the LOQ was established at 8 mM. The identity of glucose was confirmed through 2D NMR. There was a good correspondence of the glucose level (92.9 mM) determined by LF-NMR with the values obtained via 400 MHz NMR and a spectrophotometric assay. According to the authors LF-NMR has potential to be used as a “point-of-care” diagnostic and prognostic screening facility.⁴⁹

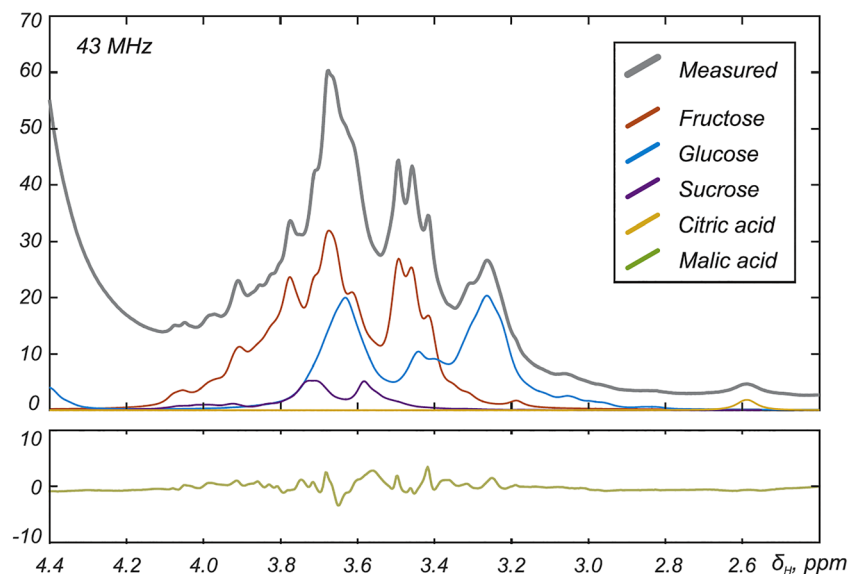
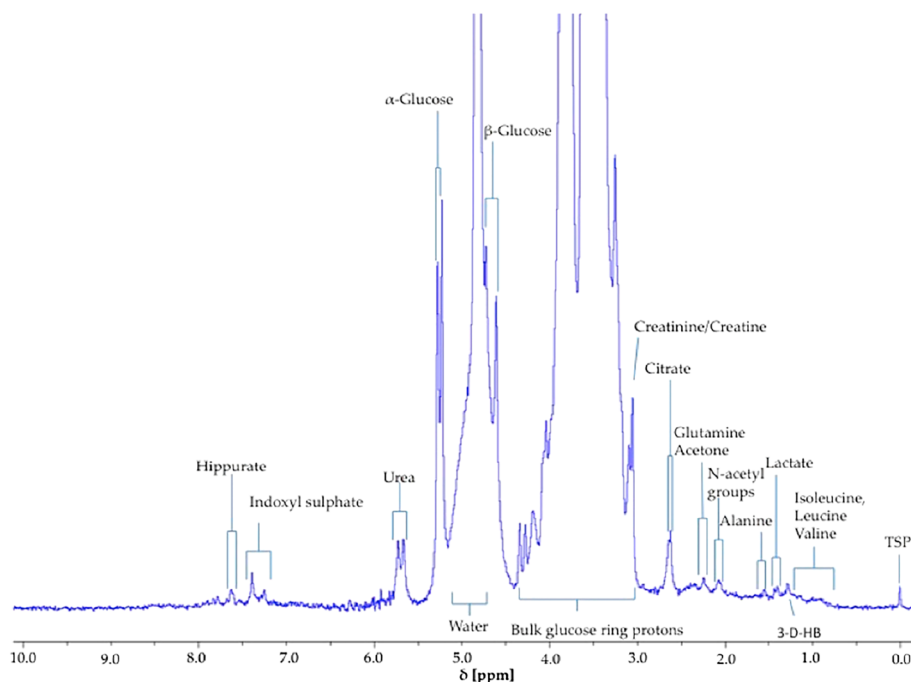


FIGURE 6 43 MHz ^1H -NMR spectrum of yellow kiwi juice along with fitted models of sugars and one acid (malic acid concentration is too low). The residual signals between the combined model spectra and the true spectrum are shown in the lower box. Reprinted from Matviychuk *et al.*,⁴⁷ copyright (2019), with permission from Elsevier [Colour figure can be viewed at wileyonlinelibrary.com]

FIGURE 7 60 MHz ^1H NMR spectrum (64 scans, repetition time 10 s) of a type 2 diabetic urine sample. Reprinted from Percival et al.,⁴⁹ (open access) [Colour figure can be viewed at wileyonlinelibrary.com]



3.4 | LF-NMR in process control

Three articles discussed the use of 42 MHz NMR to monitor the transesterification reaction for producing biodiesel from vegetable oil.^{50–52} Biodiesel consists of fatty acid methyl esters (FAMES). The percentage of unsaturated FAMES in biodiesel could be determined by LF-NMR. Values were ~5% lower than the HF-NMR values.⁵⁰ The reaction mixture could also be pumped through the NMR with the recording of spectra either every 10–15 s or in stopped-flow mode every 3 min without pretreatment.^{51,52} For comparison, also 400 MHz NMR was used. Early on, the spectrum of the reaction mixture is dominated by TAGs [1.30 (B), 2.00 (F), 4.24 (G) and 5.32 ppm (H + I)] and methanol [3.30 (CH₃) and 3.40 ppm (OH); capital letters refer to Figure 2c]. During the reaction methanol is consumed and new peaks at 3.60 ppm (glycerol and methyl esters) and between 5.3 and 5.6 ppm (OH of methanol and glycerol) emerge. Eventually two phases form: upper FAME phase and lower glycerol phase. For data analysis, three different methods were applied: (1) deconvolution of 42 MHz spectra to determine peak areas of methyl esters and olefinic protons; (2) multivariate calibration (partial least squares regression) using both LF and HF data to build a calibration model; (3) a model based on the shift of the OH peak in upper and lower phases during the conversion. The two latter methods were more accurate but required a secondary method and are possibly sensitive to process changes.⁵¹ The authors concluded that LF-NMR can be used for on-line monitoring of chemical processes.⁵¹ In their third article, they further investigated the catalyst, mechanism and kinetics.⁵²

In a biotechnological application, the growth of a yeast and a fungus was followed via on-line LF-NMR (42.5 MHz) measurements of consumed and produced chemicals.⁵³ The culture broth was pumped

through the magnet [polyurethane tube, 3 mm inner diameter (i.d.), 5 mm outer diameter] and 12 or 32 scans (repetition time of 15 s for quantitative results) were combined leading to a temporal resolution of 3 or 8 min, respectively. In case of *Hansenula polymorpha*, the consumption of glycerol (3.58 ppm, broad singlet) could be monitored by NMR and there was an excellent correspondence with HPLC data. The conversion of glucose (3.4–3.9 ppm, multiplet) to itaconic acid 3.25 ppm (singlet) by *Ustilago maydis* could also be followed by LF-NMR during 60 h of culturing. Due to interference of a peak at 3.8 ppm, the glucose signal was integrated from 3.32 to 3.71 ppm. Once again there was excellent correspondence with HPLC data, including lag phase and total consumption of glucose. The increasing concentration of intracellular glycolipids (taken as the alkyl signal from 0.6 to 1.75 ppm) showed a correlation with the dry cell weight. Thus LF-NMR appears capable of following microbial growth with high temporal resolution, fair chemical resolution and without needing sample preparation such as removal of cells or extraction.

Gomes *et al.* electrochemically converted biomass-derived valeric acid into the more valuable octane inside a 5 mm NMR tube residing in a 43 MHz NMR.⁵⁴ The reaction was conducted in methanol with potassium hydroxide (KOH) as supporting electrolyte. The strong magnetic field increased the reaction rate through a better mass transfer and reduced the required energy. The reaction could be followed by monitoring the regions from 0.6 to 0.9 ppm (n-octane) and 1.6 to 2.0 ppm (valeric acid). Calibration curves were linear. The real-time NMR analysis contributed significantly to understanding and optimizing the reactions.⁵⁴

Soyler *et al.* showed that the enzymatic conversion of sucrose into fructose and glucose can be monitored on-line by 43 MHz NMR. Several parameters were optimised such as selection of appropriate signals (H1 of sucrose and α -glucose), water suppression, repetition

time (6 s), temperature (29°C), flow rate (0.5 mL/min, i.d. flow cell 4 mm) and integration procedure. The authors concluded that quantitative LF-NMR is an easy and rapid method for the on-line monitoring of enzymatic reactions.⁵⁵

3.5 | LF-NMR in teaching

Although NMR can be learned solely by theory, spectra and movies, gaining hands-on experience with preparing an NMR sample, introducing it into a spectrometer, recording your own spectrum in real-time and interpreting it afterwards definitely has added value for students. The field strength of the spectrometer is of lesser importance. Thus, when its 400 MHz research NMR was logistically no longer available for BSc students, my university acquired a 60 MHz instrument exclusively for teaching. The author of this review was taught NMR with a book published in 1965⁵⁶ containing only 60 MHz spectra and recorded his first spectrum in 1974 on a 60 MHz machine and is none the worse for it. With the right choice of compounds, 60 MHz is as instructive as 400 MHz. The websites of LF-NMR manufacturers give dozens of examples of applications in education and it can be concluded that many LF instruments are used for hands-on teaching of NMR.

Riegel and Leskowitz have nicely summarised the important application of LF-NMR in academic teaching.⁵⁷ There were no phytochemically oriented experiments. Several experiments for teaching NMR to undergraduates have been published.⁵⁸⁻⁶⁶ One of them deals with common OTC (over-the-counter) formulations including the natural products ascorbic acid and caffeine.⁶¹ Another experiment is centred around caffeine.⁶⁴ At the university of the author, natural products play a significant role for introducing NMR to students. In the basic course, all students quantitatively analyse the alcohol content of different liquors according to a quantitative protocol published earlier by our group.⁶⁷ Further students check the identity and purity of natural products such as piperine, anethol and xanthorrhizol isolated by themselves through interpretation of the spectra. In another course, also 2D spectra are recorded.

4 | CONCLUSIONS

4.1 | Application area

From the earlier cited articles, it is obvious that there are many opportunities for modern LF-NMR for the purpose of natural products analysis. QC uses have until now focussed primarily on TAG profiles of vegetable oils. TAGs are surely an important group but an additional reason for their high occurrence may have been that vegetable oils have functioned as an easily available test substrate to evaluate the potential of LF-NMR. There only two non-TAG applications, namely adulteration detection of patchouli essential oil with other oils or synthetic aromas,³⁰ and detection of an undeclared sugar in fine chemicals.³³ The forensic use is more varied. The non-research real-

life application of LF-NMR as a first screening tool of seized drugs with a large library and automated data treatment is the way to go.⁴¹ Food and health applications are varied too, ranging from fraud detection, metabolite screening of food ingredients,⁴⁵ lipidomics by means of ¹H- and ³¹P-NMR,⁴⁶ and point-of-care metabolomics.⁴⁹ Potentially healthcare applications are a huge market for LF-NMR. However, the demands of high-quality metabolomics (80 MHz, narrow line width, high sensitivity, autosampler, i.e. heavy and > \$100000) and point-of-care diagnostic use by a general practitioner (hand-held, fast and automated read-out, i.e. light and < \$5000) are unfortunately diametrically opposed. To bridge this gap is challenging^{68,69} and in the near future a shared LF-NMR instrument in a hospital or local healthcare centre is a more realistic outcome. Even then, to quantify minor metabolites enrichment and a deuterated solvent may be needed. LF-NMR can be used to optimise chemical or biotechnological processes. It allows continuous real-time measurements of reaction kinetics and products and several examples were given. Finally teaching is an obvious and frequent application area and several articles in the *Journal of Chemical Education* describe NMR experiments for undergraduate students.

4.2 | Structure elucidation

The applications confirm that LF-NMR is not that useful for structure elucidation of unknown compounds other than the simplest ones. At best one can get an idea about the identity of a major adulterant or product, which needs to be further proven by HF-NMR, GC-MS or LC-MS.^{30,41} LF-NMR can be used to detect known products if one has previously recorded LF spectra,⁴¹ or if one can simulate the LF spectrum on the basis of chemical shifts and coupling constants obtained through detailed HF-NMR studies.^{33,47}

4.3 | Sensitivity

In most studies, simply 1D ¹H-NMR spectra are recorded and then sensitivity is not a big issue unless one is dealing with low concentrations. For example, it is less likely that LF-NMR will be applied in the near future for detecting drugs or minor metabolites in biofluids without enrichment or signal enhancement. The recording of ¹³C-NMR or 2D spectra is possible on modern LF instruments but can take 24 h or more. However, COSY spectra can be recorded in less than an hour for concentrated samples or even in a few minutes on a modified LF instrument.²⁷ Research is on-going to increase the sensitivity 10000-fold or more through hyperpolarisation. This would accomplish a ¹³C spectrum in one scan,⁷⁰⁻⁷⁴ or enable metabolite analysis in sec.⁷⁵ SABRE (signal amplification by reversible exchange) is such a promising technique but it is not universal for all analytes, requires additional chemicals and logistically it is rather complicated.⁷⁰⁻⁷² This means LF-NMR would lose one of its strongest points: simplicity. Interestingly, at the time of submission of this review a PhD project was being advertised on sensitivity-enhanced benchtop NMR spectroscopy.⁷⁶

4.4 | Resolution

A way to diminish signal overlap is to use 2D diffusion-ordered spectroscopy (DOSY) experiments.^{77,78} Compounds are “separated” in the second dimension based on their difference in diffusion coefficients. Although added resolution can be obtained for some mixtures, for now the approach suffers from several drawbacks such as considerably longer measurement times, non-standard hardware (gradient coil), higher complexity, incorrect spectra in case of overlapping spectral regions of different compounds, and not being applicable when compounds have near similar diffusion coefficients. Thus, it does not appear to be the holy grail for increasing LF-NMR resolution. However, at higher field strengths (80 MHz) and after further improvements, the methodology is likely to find application for selected samples. An alternative approach to increase resolution would be to remove all couplings, like in a projection of a 2D *J*-resolved spectrum. Various pulse sequences for this purpose were compared but the results are disappointing in terms of sensitivity (1% of 1D sensitivity) and resolution due to the appearance of artefacts.⁷⁹ Also, apart from 2D *J*-resolved spectra, all other pulse sequences require hardware modifications and simplicity is lost, which again is one of advantages of LF-NMR. Thus, also this approach appears short term doubtful. A simpler approach is the addition of Lanthanide shift reagents to disperse overlapping signals.⁸⁰ Two convincing examples after adding Eu(FOD)₃ were shown. Disadvantages are the additional signals of the shift reagent and the fact that some polar group must be present to interact with the shift reagent. Febrian *et al.* obtained increased resolution in 60 MHz spectra of oligopeptides by adding various salts.⁸¹

4.5 | As on-line detector

If benchtop NMR could be used as an on-line detector in preparative chromatography, this would constitute a highly relevant application for natural product chemists. It has now been shown many times that LF-NMR is effective in following at-line or – more frequently – on-line the output of synthetic chemistry (flow reactors) and only few, mostly recent, articles are cited here.^{82–89} See the reviews by Giraudeau and Felpin, and Grootveld *et al.* for more references.^{11,90} In some cases, even the on-line recording of 2D NMR and ¹³C-NMR spectra proved possible. However, in synthetic applications concentrations are usually 100× higher than in chromatography. Four articles describe LF-NMR as a detector after size exclusion chromatography (SEC)-NMR.^{91–94} Höpfner *et al.* used CHCl₃ as solvent in combination with 20 mm i.d. columns. With solvent suppression, analytes could be observed after data treatment.⁹² In a follow-up article, they optimised sensitivity and selectivity.⁹⁴ Sabatino *et al.* experienced considerable band broadening in the NMR detector cell with their 7.5 mm i.d. SEC columns. They concluded that 20 mm i.d. columns are preferred.⁹³ If LF-NMR is to be used after preparative column chromatography on silica, solvent problems will worsen as often a gradient is used of proton-rich solvents like petroleum ether or hexane and ethyl acetate. A gradient from CHCl₃ to acetone or acetonitrile would work better

but CHCl₃ is less desirable from an environmental and toxicological point of view. Preparative RP-HPLC, e.g. with 21 mm i.d. columns and a gradient from water to acetonitrile constitutes probably a more workable approach. This would be similar to LC-NMR in the late 1990s but without D₂O in the eluent. Solvent suppression in LF-NMR applications has been investigated by Gouilleux *et al.*⁹⁵

4.6 | Prospects

Finally, what will natural products chemists and phytochemists be using LF-NMR for in the near future? Due to limited sample preparation requirements, absence of chromatography, universal detection and the innate quantitative abilities of NMR, there is a bright future for screening, quality control and adulteration detection of herbal drugs, plant extracts and pure compounds. Currently, in the field of plant-based drugs there is only a preliminary communication on using LF-NMR for QC of Liu Wei Dihuang Wan pills, a TCM (traditional Chinese medicine).⁹⁶ More applications are bound to follow.

Future publications will mostly originate from academic users but LF-NMR is of particular interest for SMEs and governmental control laboratories, which until now could not afford NMR. Potentially this is a big market. Forensics is another area due to the many natural products (cocaine, morphine, ephedrine, cannabis) or derivatives thereof (heroin, synthetic cannabinoids) used as illegal drugs. A further use is in industrial organic synthesis through flow chemistry where the synthesis can be adapted by means of feedback from the NMR detector. An example is the synthesis of the natural product carpanone.⁸⁵ In all such applications chemometric processing of acquired data will prove essential. It may reveal in an objective manner spectral differences, which are otherwise difficult to discern. In terms of hardware, 80–100 MHz will remain the maximum but through the development of more homogeneous magnetic fields, narrower line widths can be expected, also leading to higher *S/N* ratios. Finally, it is prudent to realise the main limitation of LF-NMR, i.e. its limited resolution. For instance, in the very first LF-NMR article it was tried to detect the adulteration of olive oil with near-identical hazelnut oil based on TAG profiles.²² This is almost impossible as the two oils are highly similar. However, if NMR had not been used as the single analytical technique but rather in combination with refractive index or GC-FID measurements, the combined analytical information might have been convincing. In other words, LF-NMR should in some instances not be used as a stand-alone technique but rather as a *complementary* technique.

ORCID

Teris André van Beek  <https://orcid.org/0000-0002-9843-7096>

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: van Beek TA. Low-field benchtop NMR spectroscopy: status and prospects in natural product analysis. *Phytochemical Analysis.* 2020;1-14. <https://doi.org/10.1002/pca.2921>