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Performance Evaluation of Quantitative Adiabatic ^{13}C NMR Pulse Sequences for Site-Specific Isotopic Measurements

Christophe Thibaudeau, Gérald Remaud, Virginie Silvestre, and Serge Akoka*

University of Nantes, CNRS, Chimie et Interdisciplinarité: Synthèse, Analyse et Modélisation (CEISAM), UMR 6230, 2 rue de la Houssinière, BP 92208, F-44322 Nantes cedex 3, France

$^{2}H/^{1}H$ and $^{13}C/^{12}C$ site-specific isotope ratios determined by NMR spectroscopy may be used to discriminate pharmaceutically active ingredients based on the synthetic process used in production. Extending the Site-specific Natural Isotope Fractionation NMR (SNIF-NMR) method to ^{13}C is highly beneficial for complex organic molecules when measurements of $^{2}H/^{1}H$ ratios lead to poorly defined molecular fingerprints. The current NMR methodology to determine $^{13}C/^{12}C$ site-specific isotope ratios suffers from poor sensitivity and long experimental times. In this work, several NMR pulse sequences based on polarization transfer were evaluated and optimized to measure precise quantitative ^{13}C NMR spectra within a short time. Adiabatic 180° ^{1}H and ^{13}C pulses were incorporated into distortionless enhancement by polarization transfer (DEPT) and refocused insensitive nuclei enhanced by polarization transfer (INEPT) to minimize the influence of 180° pulse imperfections and of off-resonance effects on the precision of the measured ^{13}C peak areas. The adiabatic DEPT sequence was applied to draw up a precise site-specific ^{13}C isotope profile of ibuprofen. A modified heteronuclear cross-polarization (HCP) experiment featuring ^{1}H and ^{13}C spin-locks with adiabatic 180° pulses is also introduced. This sequence enables efficient magnetization transfer across a wide ^{13}C frequency range although not enough for an application in quantitative ^{13}C isotopic analysis.

The detection of counterfeiting is still challenging analytical chemists. Among other issues, there is increasing evidence of counterfeit pharmaceuticals being widely available in both the USA and Europe. Methods based on isotope measurements have been shown to be very efficient for the detection of a number of different types of counterfeiting or patent infringements in the pharmaceutical industry. Following pioneering studies[1] by Martin et al. on $^{2}H/^{1}H$ isotopic ratios that led to the design of the SNIF-NMR-^{2}H method, we have recently shown[2] that $^{13}C/^{12}C$ site-specific isotopic ratios of active pharmaceutical ingredients (API) are correlated with their geographical origin and the synthetic process used in production. Therefore, in conjunction with other data, they may help to identify drug counterfeiting and patent infringements. For structurally complex API, the application of the SNIF-NMR-^{2}H method may not be possible owing to signal overlap in the ^{2}H spectra. It then becomes necessary to resort to other isotopic data, from ^{13}C for instance. At first glance, ^{13}C is a more favorable NMR nucleus than ^{2}H owing to its much wider range of resonance frequencies and a natural abundance 70 times higher. Additionally, it is not subject to chemical exchange. However, quantitative analysis of $^{13}C/^{12}C$ site-specific isotope ratios by NMR represents a serious challenge: (i) The range of values of ^{13}C isotopic deviations ($\delta^{13}C‰$) occurring in natural or synthetic compounds is highly restricted ($\Delta\delta^{13}C‰ \sim 50‰$) compared to ^{2}H ($\Delta\delta^{2}H‰ \sim 500‰$). Thus, an NMR method capable of measuring $\delta^{13}C‰$ values with a very high precision (1‰) is necessary. (ii) To achieve a high degree of precision, ^{13}C NMR spectra must be recorded with a high signal-to-noise ratio (SNR) which is only achieved with extensive signal averaging because of the sensitivity of ^{13}C nuclei. (iii) The NOE (nuclear Overhauser effect) and the partial saturation should be eliminated leading to long experimental times. (iv) Finally, the efficiency of the ^{1}H decoupling sequence used in ^{13}C NMR experiments must be uniform over the whole range of ^{1}H chemical shifts (12 ppm). Studies[3−7] on the optimization of an NMR methodology for accurate and precise measurements of $^{13}C/^{12}C$ isotope ratios have shed light on how the decoupling conditions of ^{1}H in ^{13}C single-pulse NMR experiments strongly affect the precision of measured peak surface areas. A major breakthrough was achieved[6] with the help of an optimized adiabatic ^{1}H decoupling sequence featuring cosine amplitude modulation of the RF field and offset-independent adiabaticity.[8] The single-pulse ^{13}C NMR sequence optimized with adiabatic decoupling was used successfully to measure $\delta^{13}C‰$ values precisely and accurately in a wide

* To whom correspondence should be addressed. E-mail: serge.akoka@univ-nantes.fr. Tel.: +33 2 51 12 57 07. Fax: +33 2 51 12 57 12.

(1) Martin, G. J.; Martin, M. L.; Mabon, F.; Michon, M. *J. Anal. Chem.* **1982**, *54*, 2380–2382.
(2) Silvestre, V.; Maroga Mboula, V.; Jouitteau, C.; Akoka, S.; Robins, R. J.; Remaud, G. S. *J. Pharm. Biomed. Anal.* **2009**, *50*, 336–341.
(3) Caer, V.; Trierweiler, M.; Martin, G. J.; Martin, M. L. *Anal. Chem.* **1991**, *63*, 2306–2313.
(4) Tenailleau, E.; Lancelin, P.; Robins, R. J.; Akoka, S. *Anal. Chem.* **2004**, *76*, 3818–3825.
(5) Tenailleau, E.; Remaud, G.; Akoka, S. *Inst. Sci. Tech.* **2005**, *33*, 391–399.
(6) Tenailleau, E.; Akoka, S. *J. Magn. Reson.* **2007**, *185*, 50–58.
(7) Caytan, E.; Botosoa, E. P.; Silvestre, V.; Robins, R. J.; Akoka, S.; Remaud, G. S. *Anal. Chem.* **2007**, *79*, 8266–8269.
(8) Tannus, A.; Garwood, M. *J. Magn. Res. Ser. A* **1996**, *120*, 133–137.

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variety of samples in a repeatable manner.[9] However, measuring times of several hours associated with the use of this sequence constitute a serious drawback in molecular fingerprinting studies based on $^{13}C/^{12}C$ isotope ratios: the intrinsic low sensitivity of the one-pulse ^{13}C NMR technique is still an issue.

The alternative is to use multi-impulsional NMR sequences based on polarization transfer from highly sensitive ^{1}H to less sensitive ^{13}C nuclei. Three types of polarization transfer sequences mediated by $^{1}J_{CH}$ have been described in the literature, namely DEPT[10] (distortionless enhancement by polarization transfer), INEPT[11] (insensitive nuclei enhanced by polarization transfer), and HCP[12] (heteronuclear cross-polarization). For each scheme, a maximum theoretical signal-to-noise enhancement of about 4 may be achieved [i.e., $\gamma(^{1}H)/\gamma(^{13}C)$]. Moreover, for each of these pulse sequences, the repetition rate between transients is dictated by ^{1}H T_1 relaxation times that are much shorter than ^{13}C T_1 times. DEPT, INEPT, and HCP are thus expected to provide quantitative ^{13}C NMR spectra within a significantly reduced measuring time compared with the one-pulse ^{13}C experiment.

Previous studies[13,14] have shown that parasite signals, resulting from pulse imperfections, modulate the intensity of peaks in standard DEPT and INEPT spectra and prevent their use in quantitative isotopic analysis. These parasite signal modulations could only be partly eliminated[13,14] by replacing 180° hard pulses in DEPT and INEPT by composite pulses. In this work, we use adiabatic pulses[15−25] to modify DEPT, INEPT, and HCP for an application in quantitative ^{13}C isotopic analysis, since they are the best NMR tool for efficient inversion of spins over a wide frequency range.

The aim of the work presented here was to develop an NMR method for isotopic fingerprinting of a molecule. As previously explained, this requires being able to distinguish ^{13}C sites with a high degree of precision. In our investigations, we have aimed at correcting for the effects of pulse imperfections and off-resonance on NMR signal intensities in DEPT, INEPT, and HCP. Our goal for each sequence was to achieve maximum precision for the signal intensity, rather than absolute accuracy of the measurements. Indeed, absolute values of $^{13}C/^{12}C$ isotopic ratios only need to be known accurately when the purpose is to elucidate the filiations. The objective of the current study, however, was to explore whether such pulse sequences would enable ^{13}C NMR spectra of organic compounds at natural abundance to be recorded with sufficient precision for an application in quantitative ^{13}C isotopic analysis.

MATERIALS AND METHODS

Chemicals and NMR Samples. A concentrated unlabeled ethanol sample was prepared by adding 0.1 mL DMSO-d_6 (99.8% D, Eurisotop) to 0.6 mL 99.9% commercial ethanol (Dock Des Alcools, France) and filtering the solution. The ibuprofen sample was prepared by initially dissolving 302.6 mg of ibuprofen (*S*-(+)-2-(4-isobutylphenyl)propionic acid, Fluka) into 310 μL DMSO-d_6, subsequently making the ibuprofen salt upon addition of concentrated NaOH (90 μL, 1.03 M), and finally diluting the solution with 190 μL CD_3OH. This preparation allows the full separation of each signal of the ^{13}C spectrum.

NMR Spectroscopy Experiments. NMR experiments were performed on a Bruker DRX 500 spectrometer fitted with a 5 mm i.d. $^{13}C/^{1}H$ dual probe carefully tuned to the recording frequency of 125.76 MHz. The temperature of the probe was set at 308.0 and 303.0 K for experiments performed on ethanol and ibuprofen, respectively. All ^{13}C NMR spectra were recorded with inverse gated adiabatic decoupling[6] of ^{1}H. Typical acquisition parameters for experiments performed to test adiabatic DEPT, INEPT, and HCP pulse sequences (Figure 1) with ethanol were as follows: 90° ^{1}H high power pulse width 11.7 μs; 90° ^{13}C high power pulse width 4.7 μs; repetition delays of 34 s, i.e., 10 times the T_1 value of CH_2 in ethanol; 4, 16, 32, or 64 scans depending on phase cycle. For DEPT pulse sequences, the values of evolution delays τ were set at 3.765 ms, which corresponds to $^{1}/_{2}J_{CHav}$, where J_{CHav} designates the average value of one-bond CH spin−spin coupling constants of CH_2 and CH_3 in ethanol. We found that, upon replacing 180° ^{13}C and ^{1}H hard pulses with adiabatic pulses, the optimal values for τ remained unaffected even for pulse durations of several milliseconds. In the refocused INEPT sequence,[11] τ_1 was adjusted to 1.882 ms and τ_2 to 1.162 ms yielding positive signals for CH_2 and CH_3 of ethanol.

The experimental conditions used to record spectra of ibuprofen with the adiabatic DEPT sequence were as follows: 112 transients, delays τ adjusted to 3.8 ms, and a 15 s delay between the transients, i.e. ~15 T_1 of C7. ^{13}C and ^{1}H offsets were set to 100 and 4 ppm, respectively. The recording time of this experiment was ~30 min. The assignment of ^{1}H and ^{13}C NMR resonances of ibuprofen was performed using Heteronuclear Single-Quantum Coherence Spectroscopy (HSQC) and DEPT experiments and is in agreement with published data.[26]

Adiabatic 180° ^{1}H and ^{13}C Pulses. Adiabatic full passage pulses were generated using Mathcad 8 (MathSoft, Inc.). They were designed with a cosine amplitude modulation of the RF field (ω_2^{max} = 157.1 kHz or 93.89 kHz for ^{13}C or ^{1}H, respectively) and an offset independent adiabaticity (OIA[8]) by optimizing the frequency sweep ΔF (ΔF = 39 kHz or 17 kHz for ^{13}C or ^{1}H, respectively) according to the published procedure.[6] For inversion pulses, adiabatic full passage pulses were used. For refocusing pulses, composite adiabatic pulses were used.[18]

(9) Botosoa, E. P. Protocoles analytiques pour l'étude de la vanilline par RMN ^{13}C isotopique en abondance naturelle: reproductibilté méthodologique, purification, origines du fractionnement isotopique. Ph.D. thesis, University of Nantes, France, 2008.
(10) Doddrell, D. M.; Pegg, D. T.; Bendall, M. R. *J. Magn. Reson.* **1982**, *48*, 323–327.
(11) Morris, G. A.; Freeman, R. *J. Am. Chem. Soc.* **1979**, *101*, 760–762.
(12) Maudsley, A. A.; Müller, L.; Ernst, R. R. *J. Magn. Reson.* **1977**, *28*, 463–469.
(13) Karabulut, N.; Baguet, E.; Trierweiler, M.; Akoka, S. *Anal. Lett.* **2002**, *35*, 2549–2563.
(14) Caytan, E. Determination de la distribution intramoleculaire du ^{13}C en abondance naturelle par RMN quantitative, Ph.D. thesis, University of Nantes, France, 2006.
(15) Bloch, F. *Phys. Rev.* **1946**, *70*, 460–474.
(16) Bloch, F.; Hansen, W. W.; Packard, M. *Phys. Rev.* **1946**, *70*, 474–485.
(17) Garwood, M.; DelaBarre, L. *J. Magn. Reson.* **2001**, *153*, 155–177.
(18) Hwang, T.-L.; van Zijl, P. C. M.; Garwood, M. *J. Magn. Reson.* **1997**, *124*, 250–254.
(19) Kupče, E.; Freeman, R. *J. Magn. Reson.* **2007**, *187*, 258–265.
(20) Freeman, R.; Kupče, E. *NMR Biomed.* **1997**, *10*, 372–380.
(21) Bendall, M. R.; Pegg, D. T. *J. Magn. Reson.* **1986**, *67*, 376–381.
(22) Hardy, C. J.; Edelstein, W. A.; Vatis, D. *J. Magn. Reson.* **1986**, *66*, 470–482.
(23) Silver, M. S.; Joseph, R. I.; Hoult, D. I. *J. Magn. Reson.* **1984**, *59*, 347–351.
(24) Ugurbil, K.; Garwood, M.; Rath, A. R.; Bendall, M. R. *J. Magn. Reson.* **1988**, *78*, 472–497.
(25) Hallenga, K.; Lippens, G. M. *J. Biomol. NMR* **1995**, *5*, 59–66.
(26) Acetti, D.; Brenna, E.; Fronza, G.; Fuganti, C. *Talanta* **2008**, *76*, 651–655.

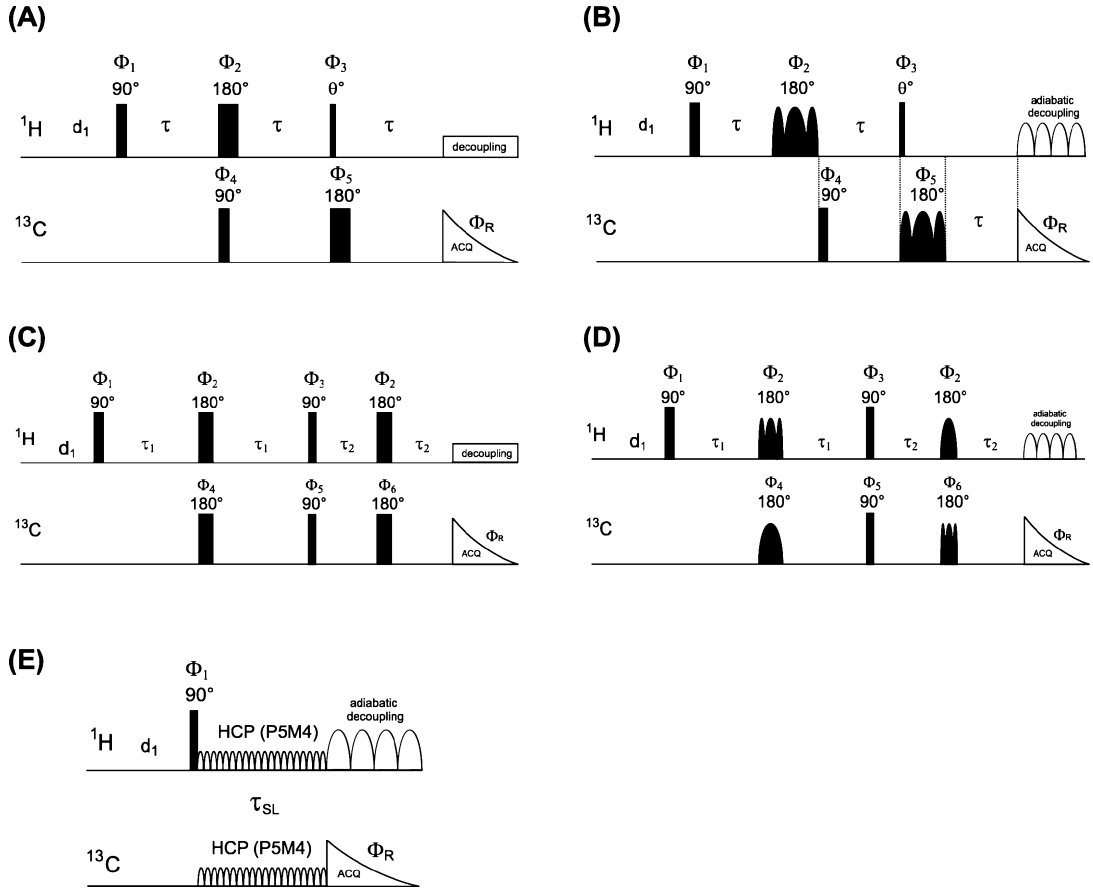

Figure 1. (A) Standard DEPT sequence[10] with hard pulses for 180° ^{1}H and ^{13}C refocusing pulses. (B) Adiabatic DEPT sequence with 180° adiabatic composite refocusing pulses on both ^{1}H and ^{13}C channels. (C) Standard refocused INEPT[11] with hard pulses for 180° ^{1}H and ^{13}C refocusing pulses. (D) Adiabatic refocused INEPT sequence based on ^{1}H and ^{13}C 180° adiabatic composite refocusing pulses and adiabatic full passage inversion pulses with the same shape. (E) Modified HCP[12] sequence with adiabatic spin-locks on both channels. τ_{SL} defines the overall duration of each spin-lock. The same spin-lock supercycles were used for ^{13}C and ^{1}H.

NMR Data Processing and Analysis. For each ^{13}C NMR spectrum, an exponential window function inducing a line broadening of 2 Hz was applied to the free induction decay prior to Fourier transform. An automatic polynomial baseline correction was subsequently applied to the resulting spectra. Surface areas of ^{13}C peaks were determined by the curve-fitting process implemented within Perch (Perch NMR Software, University of Kuopio, Finland).

Five DEPT spectra of ibuprofen were recorded using the optimized adiabatic DEPT pulse sequence. For reference, four ^{13}C NMR spectra were also recorded using a single-pulse NMR experiment. The peak surface areas measured in ^{13}C NMR spectra were corrected to account for the presence of ^{13}C−^{13}C isotopomers in the molecule, which give rise to satellite lines.[27] Reduced ^{13}C isotopic molar fractions were calculated for C4/4′, C5/5′, C6, C7, C8, C9/9′, and C10 in ibuprofen samples according to eq 1:

$$f_{i\mathrm{R}} = S_i/(F_i S_i) \qquad (1)$$

where S_i defines the corrected surface area of the ^{13}C peak of site i in a specific spectrum and $\sum S_i$ represents the sum of the surface areas of C4/4′, C5/5′, C6, C7, C8, C9/9′, and C10 peaks in the same spectrum. F_i corresponds to the statistical molar fraction of site i: $F_i = n/N$, where n corresponds to the number of carbons contributing to the surface area of the ^{13}C peak of site i in the NMR spectrum and N is the total number of CH/CH_2/CH_3 peaks observed in the spectrum (N = 10 for ibuprofen samples).

RESULTS AND DISCUSSION

Description of the Polarization Transfer Mechanism in DEPT, INEPT, and HCP. In the DEPT sequence (Figure 1A), initial ^{1}H longitudinal magnetization is converted by the 90° ^{1}H pulse into in-phase ^{1}H coherence in the transverse plane, which evolves during τ into antiphase ^{1}H coherence. Having $\tau = {}^{1}/_{2}J_{\mathrm{CHav}}$ (where J_{CHav} is the average value of one-bond CH spin−spin coupling constants) ensures efficient coherence transfer. The ^{1}H antiphase coherence is then transformed into zero/double quantum coherence (by the 90° ^{13}C pulse) that evolves during the second delay τ and is converted back into pure antiphase ^{13}C coherence by applying the β pulse. Antiphase ^{13}C coherences are finally refocused into in-phase ^{13}C magnetization during the last delay τ.

In the refocused INEPT sequence (Figure 1C), initial longitudinal ^{1}H magnetization is converted by the 90° ^{1}H pulse into in-phase ^{1}H coherence in the transverse plane. The first spin−echo aims to convert this coherence into antiphase ^{1}H

(27) Zhang, B.-L.; Trierweiler, M.; Jouitteau, C.; Martin, G. *J. Anal. Chem.* **1999**, *71*, 2301–2306.

coherence. Having $\tau_1 = {}^1/_4J_{CHav}$ ensures efficient coherence transfer. Simultaneous ^1H and ^{13}C 90° pulses transform the antiphase ^1H coherence into antiphase ^{13}C coherence. The 180° ^{13}C pulse during this second echo serves to refocus ^{13}C chemical shift evolution during τ_2 delays. Also, having $\tau_2 = {}^1/_6J_{CHav}$ ensures efficient refocusing for CH, CH_2, and CH_3.

In the HCP sequence (Figure 1E), initial in-phase transverse ^1H coherence is created by the 90° ^1H pulse. This coherence is transferred to yield in-phase ^{13}C coherence during the delay τ_{SL} where simultaneous spin-locking fields are applied to both nuclei. The polarization transfer takes place when the Hartmann−Hahn condition is fulfilled ($\omega_{1H} = \omega_{13C}$). The duration of the spin-lock needs to be adjusted to an optimal value that depends on the nature of the resonance observed ($1/J$ for CH, $0.707/J$ for CH_2, and $0.625/J$ for CH_3 spin systems, respectively).

Parameters Used to Evaluate the Performance of the Pulse Sequences. In principle, three parameters may be used to evaluate the performance of an NMR pulse sequence for an application in isotopic analysis, namely, the trueness, the precision, and the robustness. Clear definitions of these terms can be found elsewhere.[28]

Trueness. From the physical description of polarization transfer, it follows that sensitivity gains in DEPT, INEPT, and HCP are only obtained for hydrogen-bearing carbons. In other words, only CH, CH_2, and CH_3 groups will be observed, while quaternary carbons will be missing. Furthermore, with such sequences, ^{13}C intensities strongly depend on the J_{CH} values and on the number of attached protons. As a consequence, the observed relative intensities are no more only governed by molar fractions (as it is shown by mean values given in Table 5). Therefore, the isotopic ^{13}C deviation for each carbon ($\delta_i{}^{13}C‰$) is not accessible by such methods. However, in the case of authentication applications, like the detection of patent infringements in the pharmaceutical industry, the compared samples contain the same molecule with the same J_{CH} coupling constants and the same number of attached protons. The contribution of the pulse sequence to the relative intensities is therefore the same for all the samples. Hence, reduced molar fractions: f_i/F_i (see the Materials and Methods section) can be used to present and discuss the extent of the variability of the NMR response between samples. Therefore, trueness is not an issue for such an application. Isotope fingerprinting from reduced molar fractions, even based on a limited number of carbons, would still give information to characterize unequivocally the industrial process from batch to batch.

Precision. To obtain relative isotope profiles, the analyses should be performed in a repeatable manner. As a result, the predominant issue of the present work is to assess the precision in the f_i/F_i for each optimized pulse sequence. In fact, the repeatability of isotopic measurements by NMR may also be studied on a simple model compound where the area of only two peaks is compared. We chose ethanol for this purpose. The advantages of this molecule as a model are due to (i) the fact that it contains two proton-bearing carbons, (ii) its low molecular weight, which allows a large number of moles to be introduced into the NMR tube, leading to a high sensitivity, and (iii) the fact that it has been extensively studied by the classical one-pulse sequence. The modifications, optimizations, and comparison of the pulse sequences presented herein were achieved using the same sample of pure ethanol.

^{13}C peak areas measured with DEPT[10] and INEPT[11] are intrinsically less precise than those measured using single-pulse experiments owing to the presence of 180° hard RF pulses. Imperfect 180° pulses lead to incomplete refocusing of chemical shifts and modulate NMR peak intensities by resonance frequencies.[10,11] A simple calibration procedure cannot correct for this modulation: a small change in the temperature, for instance, may result in changes in resonance frequencies of some of the NMR peaks. Peak intensities may, in turn, be modulated to a different extent. Such a nonsystematic contribution of 180° pulse imperfections to signal intensity in DEPT and INEPT must be eliminated before applying these techniques to quantitative ^{13}C isotopic analysis. In this work, the influence of ^{13}C and ^1H 180° RF pulse imperfections on the precision of the measured ^{13}C peak areas and, therefore, on the repeatability of the isotopic analysis, was studied for INEPT and DEPT by performing a series of experiments where either ^{13}C or ^1H offset was varied throughout a narrow range of values (for ^{13}C, the range of offset values covered about 18 ppm or 2250 Hz; for ^1H, it covered 1.4 ppm or 700 Hz). Because of the value of the frequency range covered, these series of measurements do not take into account the off resonance effect and only caracterised the impact of pulse imperfections coming from RF inhomogeneity or maladjusted pulse length.

Robustness. Before applying an NMR pulse sequence in routine quantitative ^{13}C isotopic analysis, it is also necessary to ensure that the technique is sufficiently robust. For instance, it should be easily transferable between several NMR spectrometers operating at different resonance frequencies and/or based on older or state-of-the-art hardware components (e.g., amplifiers with different powers, resulting in different lengths for the 90°/180° hard pulses). Such differences may lead to a different contribution of off-resonance effects to the precision of the measured peak areas. In this work, we estimated the influence of ^{13}C and ^1H off-resonance on the precision of ^{13}C peak surface areas measured with DEPT and INEPT by recording a series of spectra in which the ^{13}C or ^1H offset was varied to cover a much wider range of values and therefore to simulate the situation of resonances spread across an entire NMR spectrum.

Optimization of the Polarization Transfer Pulse Sequences. *DEPT.* In the present work, a two-step procedure was applied to modify the standard DEPT sequence (Figure 1A) using adiabatic pulses. In step I, only the 180° ^{13}C refocusing pulse was replaced by a 180° ^{13}C adiabatic pulse. Two profiles of adiabatic pulse shapes were implemented in the ^{13}C adiabatic DEPT sequence to perform experiments: either a cosinus (COS)/OIA shape optimized according to the procedure previously proposed by our laboratory[6] or a smoothed frequency-modulated pulse (CHIRP) shape.[29]

In step II, a ^1H 180° adiabatic composite pulse was also incorporated into the DEPT sequence to give the adiabatic

(28) Ermakov, V. L.; Bohlen, J.-M.; Bodenhausen, G. *J. Magn. Reson.* **1993**, *A103*, 226–229.

(29) Menditto, A.; Patriarca, M.; Magnusson, B. *Accred. Qual. Assur.* **2007**, *12*, 45–47.

DEPT sequence shown in Figure 1B. In this sequence, a COS/OIA 180° composite adiabatic ^{1}H pulse was used as it was the most efficient to eliminate the influence of RF field inhomogeneity and off-resonance effects on the ^{13}C peak intensities (see below). The influence of the duration and power of the ^{1}H 180° composite adiabatic pulse on the ^{13}C peak surface areas measured with the adiabatic DEPT sequence was thoroughly investigated.

It is noteworthy that, in the adiabatic DEPT pulse sequence shown in Figure 1B, neither the 90° nor the 45° (β angle) pulses are adiabatic. In DEPT (or INEPT), refocusing of ^{13}C and ^{1}H chemical shifts is only achieved using 180° pulses, which must be adiabatic for the reasons explained above. If either the 90° or β pulse is not fully accurate, this will only induce a slight sensitivity loss in the experiment, not an improper refocusing of chemical shift. Thus, incorporating adiabatic 90° and/or 45° pulses into DEPT (or INEPT) provides no direct improvement in precision. The incorporation, in a further step, of calibration-free broadband excitations pulses[30,31] could improve the robustness of the method.

INEPT. The adiabatic INEPT pulse sequence (Figure 1D) was designed by incorporating 180° adiabatic composite refocusing pulses for ^{1}H and ^{13}C following the strategy applied to the DEPT sequence. We also introduced single adiabatic 180° inversion pulses for ^{1}H and ^{13}C into the sequence to replace 180° high power inversion pulses. COS/OIA adiabatic pulse shapes were systematically incorporated into adiabatic INEPT pulse sequences. The same methodology as used for DEPT was applied to evaluate the effect of 180° pulse imperfections and of off-resonance on the precision of ^{13}C peak surface areas.

HCP. As an alternative to DEPT and INEPT, heteronuclear cross-polarization[12] (HCP) may be used to enhance the sensitivity of ^{13}C NMR experiments. Although HCP has been widely employed in solid-state NMR, it has only recently gained popularity in liquid-state applications.[32,33]

A comparison[34] of INEPT and HCP has shown that the sensitivity to RF field inhomogeneity is reduced overall in HCP compared to INEPT. Initial tests confirmed that the intensity of the NMR peaks in ^{13}C spectra of ethanol recorded with the standard HCP sequence (MLEV-16 spin-locks) is extremely sensitive to Hartmann−Hahn mismatch and to ^{13}C offset. To circumvent these shortcomings, a modified HCP pulse sequence (Figure 1E) was created, by implementing adiabatic 180° pulses and supercycles within the ^{1}H and ^{13}C spin-locks. To the best of our knowledge, in liquid-state NMR experiments, no spin-lock by adiabatic pulse trains has yet been used to transfer magnetization from ^{1}H to a heteronucleus through HCP. However, adiabatic spin-locks have already been successfully applied in the case of ^{1}H,^{1}H or ^{13}C,^{13}C homonuclear spin−spin transfer such as Total Correlation Spectroscopy (TOCSY).[35,36]

A series of spectra of ethanol were recorded using the adiabatic HCP sequence and varying the ^{13}C spin-lock power to cover a wide range of values. For each experiment, the P5M4 or M4P5 supercycle was implemented in the ^{1}H and ^{13}C spin-locks together with a specific adiabatic pulse shape (COS/OIA, Tanh/Tan or WURST-100/OIA), and a selected duration for the adiabatic 180° pulses (50, 125, and 250 μs), yielding an overall spin-lock duration $\tau_{SL} = 5$ or 7.5 ms. We found that the sensitivity of spectra recorded using Tanh/Tan adiabatic 180° pulses in ^{1}H and ^{13}C spin-locks was strongly degraded in comparison with the same spectra obtained with COS/OIA and WURST-100 pulses. For COS/OIA and WURST-100/OIA, as the duration of the adiabatic pulse increased, the intensity of CH_2 and CH_3 peaks in NMR spectra decreased over the whole range of ^{13}C spin-lock power values explored. This is in agreement with the results previously published on adiabatic TOCSY experiments.[35] Among all the combinations of pulse durations/pulse shape/supercycle tested, experiments performed using a 7.5 ms mixing time, the P5M4 supercycle and COS/OIA pulses provided the NMR response most insensitive to the change in ^{13}C spin-lock power (see Figure 4A).

A second series of tests was conducted on the adiabatic HCP sequence to explore the effect of ^{13}C offset changes on the intensity of ^{13}C peaks in the NMR spectra of ethanol. These experiments were conducted using the P5M4 supercycle and several COS/OIA (or WURST-20/100) adiabatic pulse durations ranging from 50 to 350 μs, with either a 5 ms or a 7.5 ms mixing time. Representative peak surface areas, as measured in these experiments, are displayed in Figure 4B as a function of ^{13}C offset. For all the experiments in Figure 4B, the ^{1}H offset was set close to the chemical shift of CH_3 protons. The following conclusions can be drawn: (i) maximum signal intensity is always reached when the ^{13}C offset is near the CH_3 chemical shift. As the adiabatic pulse duration changes in the order 250, 125, 83, and 50 μs, the maximum intensity of the NMR signal gradually increases, despite the fact that the adiabaticity of the pulse simultaneously decreases: (ii) When short adiabatic pulses are used ($\leq$125 μs), the intensity of the CH_3 peak remains reasonably stable (within ~10%) within a 35 ppm range (~4400 Hz) of ^{13}C offset values. Outside this range, the intensity of the NMR signal decreases almost linearly as a function of offset. (iii) For longer adiabatic pulses ($\geq$250 μs), the dependence of the peak surface areas on offset is even more accentuated and the graphs show several minima and maxima throughout the range of the offset values explored. The adiabatic HCP sequence provides a rather precise (within several percent) measurement of ^{13}C peak surface areas only within a rather limited range of offset values and may therefore not be applied in quantitative ^{13}C NMR isotopic analysis but might be useful for other NMR applications where other spin-lock patterns are inefficient. Further work would be needed to improve the offset dependence properties of the sequence.

Additional Comments. A single 180° adiabatic pulse cannot be used as a chemical shift refocusing pulse in DEPT or INEPT as it would induce a phase roll across the spectrum.[24,25] The current study has clearly demonstrated that both adiabatic DEPT and INEPT sequences featuring composite adiabatic 180° pulses are much less sensitive to ^{1}H and ^{13}C pulse imperfections and off-resonance effects than the parent sequences based on hard 180° pulses. As an alternative to composite adiabatic 180°

(30) Skinner, T. E.; Kobzar, K.; Luy, B.; Bendall, M. R.; Bermel, W.; Khaneja, N.; Glaser, S. J. *J. Magn. Reson.* **2006**, *179*, 241–249.
(31) Kobzar, K.; Skinner, T. E.; Khaneja, N.; Glaser, S. J.; Luy, B. *J. Magn. Reson.* **2008**, *194*, 58–66.
(32) Nolis, P.; Parella, T. *Curr. Anal. Chem.* **2007**, *3*, 47–68.
(33) Sklenar, V.; Dieckmann, T.; Butcher, S. E.; Feigon, J. *J. Biomol. NMR.* **1996**, *7*, 83–87.
(34) Majumdar, A.; Zuiderweg, E. R. P. *J. Magn. Res. Ser. A.* **1995**, *113*, 19–31.
(35) Kupče, E.; Schmidt, P.; Rance, M.; Wagner, G. *J. Magn. Reson.* **1998**, *135*, 361–367.
(36) Peti, W.; Griesinger, C.; Bermel, W. *J. Biomol. NMR* **2000**, *18*, 199–205.

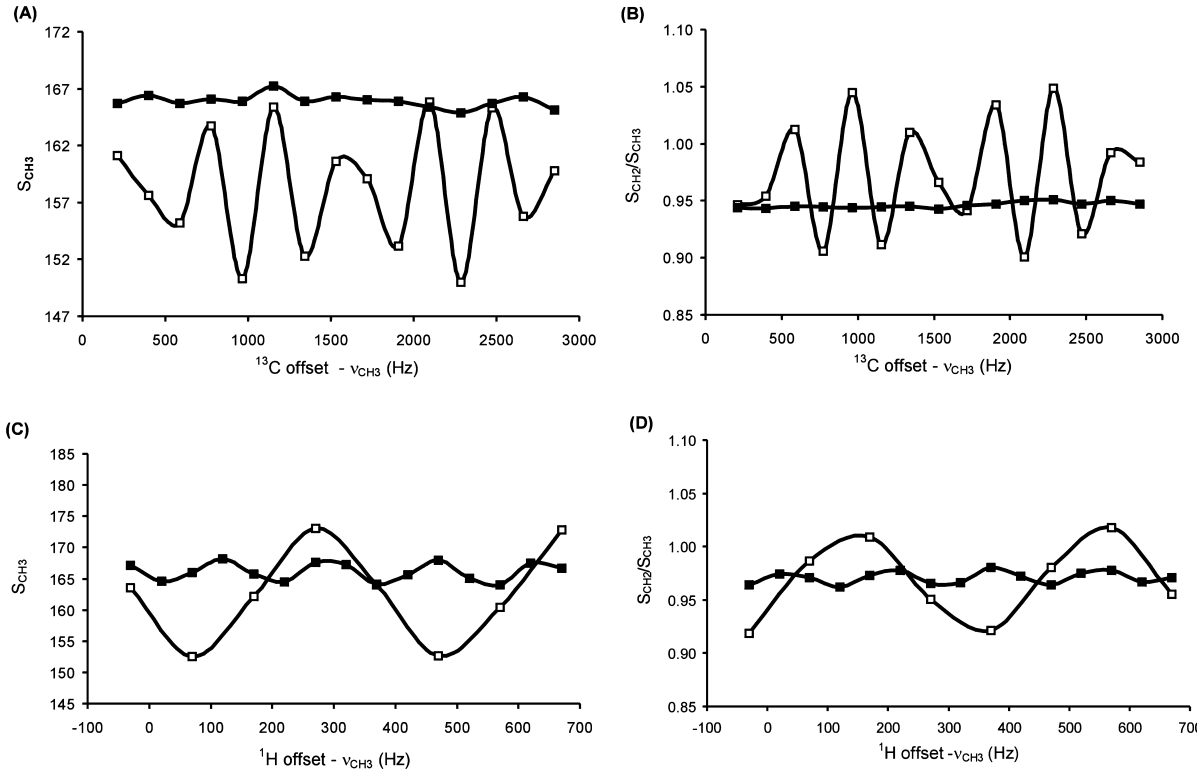

Figure 2. Influence of ^{13}C and ^{1}H 180° RF pulse imperfections on the precision of peak surface areas measured with DEPT sequences on ethanol. The offset was varied over 2625 Hz, in steps of 187.5 Hz (1.5 ppm) for ^{13}C and over 700 Hz in steps of 50 or 100 Hz (0.1 or 0.2 ppm) for ^{1}H. Standard DEPT sequence □; adiabatic DEPT sequence (COS/OIA) ■. (A) Surface area of CH_3 peak (S_{CH3}) as a function of difference between ^{13}C carrier frequency and chemical shift of CH_3. (B) Ratio S_{CH2}/S_{CH3} of peak surface areas as a function of difference between ^{13}C carrier frequency and chemical shift of CH_3. (C) CH_3 (S_{CH3}) peak surface areas in DEPT spectra as a function of the difference between ^{1}H offset and chemical shift of CH_3. (D) Ratio of peak surface areas S_{CH2}/S_{CH3} as a function of difference between ^{1}H offset and chemical shift of CH_3.

Table 1. Effect[a–c] of ^{13}C 180° RF Pulse Imperfections and Off-resonance Effect on the Precision and the Robustness of Peak Surface Areas Measured with DEPT Sequences for Two Kinds of 180° RF Pulses: Hard Pulses and Adiabatic Pulses

	pulse imperfections			off-resonance		
^{13}C RF pulse	$(S_{CH2}/S_{CH3})_{av}$	$\sigma\ S_{CH2}/S_{CH3}$	RSD (%) S_{CH2}/S_{CH3}	S_{CH3av}	$\sigma\ S_{CH3}$	RSD (%) S_{CH3}
hard pulse	0.972	0.051	5.2	158.60	9.3	5.9
adiabatic pulse	0.946	0.003	0.3	163.52	1.0	0.6

[a] S_{CH3av} and $(S_{CH2}/S_{CH3})_{av}$ are average CH_3 peak surface areas and average ratios of CH_2 and CH_3 peak surface areas. [b] $\sigma\ S_{CH3}$ and $\sigma\ S_{CH2}/S_{CH3}$ designate their respective standard deviations. [c] RSD represents the precision (in percent) calculated according to RSD $S_{CH2}/S_{CH3} = 100\sigma\ S_{CH2}/S_{CH3}/(S_{CH2}/S_{CH3})_{av}$ and RSD $S_{CH3} = 100\sigma\ S_{CH3}/S_{CH3av}$. For the pulse imperfections part, 15 spectra were measured by varying the ^{13}C offset over 2625 Hz. For the off-resonance part, spectra were recorded with the ^{13}C offset arrayed over 25 kHz (for hard pulses; 5 spectra) and 12.5 kHz (for adiabatic pulses; 11 spectra).

pulses, we also generated a second series of DEPT and INEPT adiabatic sequences with double-echoes of ^{13}C 180° pulses instead of hard 180° pulses (sequences not shown), following the strategy recently discussed by Kupče and Freeman.[19] The precision and robustness of ^{13}C peak surface areas measured using double-echo DEPT or INEPT sequences were either almost equal to or lower than those provided by counterparts featuring adiabatic composite 180° pulses.

DEPT sequences make use of a 32-step phase cycle in which the 180° ^{1}H pulse phase Φ_2 is increased by 90° with a simultaneous change in the receiver phase Φ_R by 180°. This step partially compensates for 180° ^{1}H pulse imperfections, as described in our previous study.[13] In the adiabatic DEPT sequence (Figure 1B), this step was removed to yield a 16-step phase cycle and our results clearly show that 180° adiabatic composite pulses themselves are sufficient to ensure insensitivity of the pulse sequence to RF pulse imperfections and off-resonance effects. As a result, if the ^{13}C NMR spectrum of a sample can be recorded with a sufficient signal-to-noise ratio within 16 transients (for instance on a modern spectrometer equipped with a cryoprobe), the adiabatic DEPT pulse sequence will provide quantitative ^{13}C NMR data without any need to perform a full 32-step phase cycle as in standard DEPT. This provides a significant advantage in terms of overall measuring time for series of ^{13}C spectra, as required in fingerprinting studies of pharmaceuticals.

As we have previously mentioned, our strategy is only useful for authentication applications because of the pulse sequence contribution to relative intensities. The polarization transfer efficiency in DEPT and INEPT sequences is indeed highly dependent on the J_{CH} value and on the number of attached protons. Several strategies have been proposed[37,38] in order to overcome this problem and increase the quantitative application field of the polarization transfer techniques. However, because the sensitivity gain is only obtained for hydrogen-bearing carbons,

(37) Jiang, B.; Xiao, N.; Liu, H.; Zhou, Z.; Mao, X.; Liu, M. *Anal. Chem.* **2008**, *80*, 8293–8298.
(38) Mäkelä, A. V.; Kilpeläinen, I.; Heikkinen, S. *J. Magn. Reson.* **2010**, *204*, 124–130.

Table 2. Effect[a–c] of ^{1}H 180° RF Pulse Imperfections and Off-resonance Effect on the Precision and the Robustness of Peak Surface Areas Measured with DEPT Sequences for Two Kinds of 180° RF Pulses: Hard Pulses and Adiabatic Pulses

	pulse imperfections			off-resonance		
^{1}H RF pulse	$(S_{CH2}/S_{CH3})_{av}$	σ S_{CH2}/S_{CH3}	RSD (%) S_{CH2}/S_{CH3}	S_{CH3av}	σ S_{CH3}	RSD (%) S_{CH3}
hard pulse	0.9675	0.0374	3.9	160.83	10.2	6.4
adiabatic pulse	0.9707	0.0057	0.6	165.78	0.4	0.3

[a] S_{CH3av} and $(S_{CH2}/S_{CH3})_a$ are average CH_3 peak surface areas and average ratios of CH_2 and CH_3 peak surface areas. [b] σ S_{CH3} and σ S_{CH2}/S_{CH3} designate their respective standard deviations. [c] RSD represents the precision (in percent) calculated according to RSD $S_{CH2}/S_{CH3} = 100\sigma\ S_{CH2}/S_{CH3}/(S_{CH2}/S_{CH3})_{av}$ and RSD $S_{CH3} = 100\sigma\ S_{CH3}/S_{CH3av}$. For the imperfection pulses part, 8 and 15 spectra were performed with the ^{1}H offset varied over 700 Hz for adiabatic and hard pulses, respectively. For the off-resonance part, six spectra were performed with the ^{1}H offset varied over 5 kHz for both types of pulses.

it is always impossible to obtain isotopic deviations from such methods and they do not present any additional advantage (for isotopic analysis) in comparison to the adiabatic sequences presented here. Furthermore, the precision and repeatability of these pulse sequences has not yet been thoroughly evaluated.

In this study, we have used cosine adiabatic pulses with offset-independent adiabaticity because we have shown in a previous work[6] that these pulses give very uniform inversion over the target frequency range. Other strategies have been proposed to perform broadband inversions[30,31,39] and shorter pulse durations could be achieved by using these approaches.

Precision of the Pulse Sequences for ^{13}C Isotopic Fingerprinting. *DEPT.* In the case of a perfectly accurate 180° ^{13}C pulse in DEPT, the measured peak surface areas should be completely insensitive to ^{13}C offset. Figure 2A provides experimental evidence showing that this is not the case for the standard DEPT sequence. As the ^{13}C offset is gradually increased, the surface area of the CH_3 peak of ethanol oscillates rapidly around an average value. The signal modulation is over 5% of its average intensity as shown by the relative standard deviation (RSD) determined for the ratio of CH_2 and CH_3 peak surface areas (S_{CH2}/S_{CH3}) (Figure 2B, Table 1). The observed modulation may be attributed to ^{13}C 180° RF pulse imperfections that induce incomplete refocusing of ^{13}C chemical shift evolution. Incorporation of a 180° ^{13}C adiabatic composite pulse in DEPT dramatically improves the situation: the RSD value for S_{CH2}/S_{CH3} is reduced to 0.4% (Table 1), and the signal intensity is nearly independent of ^{13}C offset. It should also be pointed out that 180° ^{13}C adiabatic composite pulses with cosine amplitude modulation and OIA were more efficient than CHIRP pulses (results not shown).

Similar conclusions could be drawn from our investigations of the influence of ^{13}C off-resonance on the precision of NMR signal surface areas measured with DEPT upon varying the offset over a broad chemical shift range (Table 1). In spectra recorded using the standard DEPT sequence, the robustness of the peak surface areas was rather poor (RSD ~ 6%) whereas a minimum contribution of ^{13}C off-resonance was observed for the adiabatic DEPT sequence and a COS/OIA ^{13}C 180° adiabatic composite pulse

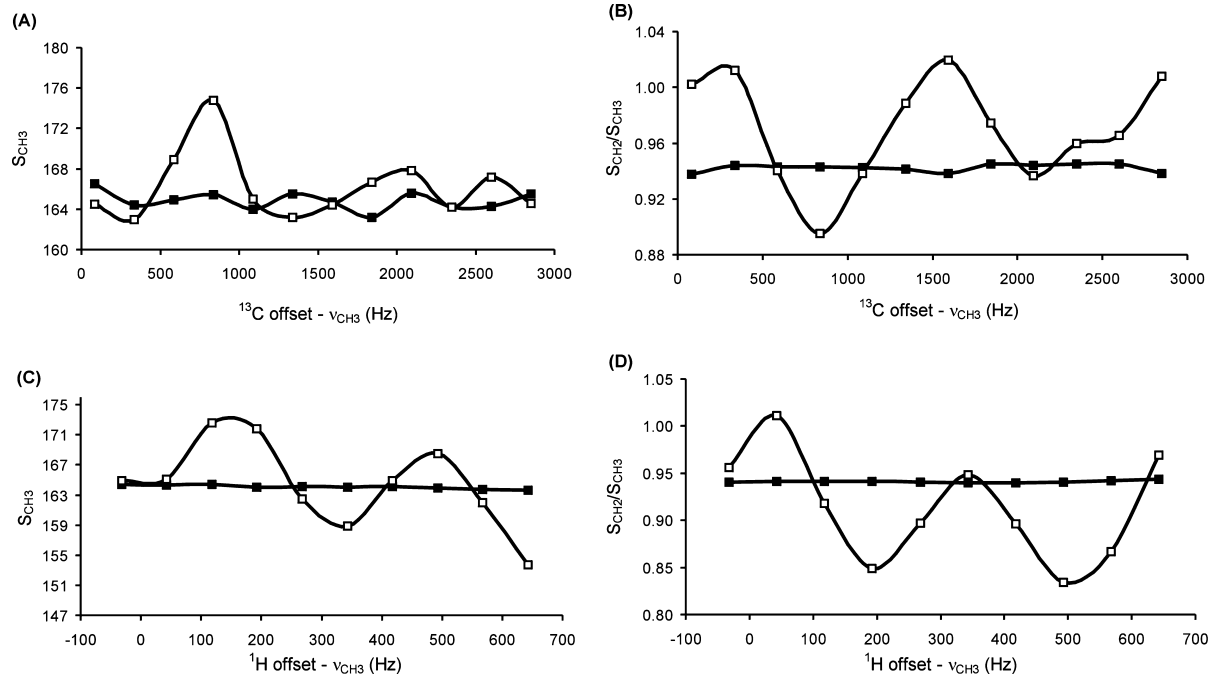

Figure 3. Influence of ^{13}C and ^{1}H 180° RF pulse imperfections on the precision of peak surface areas measured with INEPT sequences. For each pulse sequence, the offset was varied systematically over 2750 Hz in steps of 250 Hz (2 ppm) for ^{13}C and over 675 Hz in steps of 75 Hz (0.15 ppm) for ^{1}H. Standard INEPT sequence □; adiabatic INEPT sequence with composite refocusing pulses (COS/OIA) ■. (A) Dependence of CH_3 (S_{CH3}) ^{13}C peak surface areas in INEPT spectra on the difference between ^{13}C offset and chemical shifts of CH_3. (B) Ratio S_{CH2}/S_{CH3} of peak surface areas as a function of difference between the ^{13}C carrier offset and the chemical shift of the CH_3 group. (C) Dependence of CH_3 (S_{CH3}) peak surface areas in INEPT spectra on the difference between ^{1}H offset and CH_3 chemical shift. (D) Ratio S_{CH2}/S_{CH3} of peak surface areas as a function of difference between the carrier ^{1}H offset and the chemical shift of the CH_2 group.

Table 3. Effect[a–c] of ^{13}C 180° RF Pulse Imperfections and Off-resonance Effect on the Precision and the Robustness of Peak Surface Areas Measured with INEPT Sequences for Two Kinds of 180° RF Pulses: Hard Pulses and Adiabatic Pulses

	pulse imperfections			off-resonance		
^{13}C RF pulse	$(S_{CH2}/S_{CH3})_{av}$	$\sigma\ S_{CH2}/S_{CH3}$	RSD (%) S_{CH2}/S_{CH3}	S_{CH3av}	$\sigma\ S_{CH3}$	RSD (%) S_{CH3}
hard pulses	0.9700	0.0378	3.9	160.88	8.1	5.1
adiabatic pulses (composite)	0.9423	0.0028	0.3	165.43	0.40	0.2

[a] S_{CH3av} and $(S_{CH2}/S_{CH3})_{av}$ are average CH_3 peak surface areas and average ratios of CH_2 and CH_3 peak surface areas. [b] $\sigma\ S_{CH3}$ and $\sigma\ S_{CH2}/S_{CH3}$ designate their respective standard deviations. [c] RSD represents the precision (in percent) calculated according to RSD $S_{CH2}/S_{CH3} = 100\sigma\ S_{CH2}/S_{CH3}/(S_{CH2}/S_{CH3})_{av}$ and RSD $S_{CH3} = 100\sigma\ S_{CH3}/S_{CH3av}$. For the pulse imperfections part, 12 spectra were measured by varying the ^{13}C offset over 2750 Hz. For the off-resonance part, six spectra were recorded with the ^{13}C offset varied over 12.5 kHz.

Table 4. Effect[a–c] of ^{1}H 180° RF Pulse Imperfections and Off-resonance Effect on the Precision and the Robustness of Peak Surface Areas Measured with INEPT Sequences for Two Kinds of 180° RF Pulses: Hard Pulses and Adiabatic Pulses

	pulse imperfections			off-resonance		
^{1}H RF pulses	$(S_{CH2}/S_{CH3})_{av}$	$\sigma\ S_{CH2}/S_{CH3}$	RSD (%) S_{CH2}/S_{CH3}	S_{CH3av}	$\sigma\ S_{CH3}$	RSD (%) S_{CH3}
hard pulses	0.9145	0.0567	6.2	158.46	7.4	4.7
adiabatic pulses	0.9412	0.0012	0.1	163.38	1.7	1.1

[a] S_{CH3av} and $(S_{CH2}/S_{CH3})_{a}$ are average CH_3 peak surface areas and average ratios of CH_2 and CH_3 peak surface areas. [b] $\sigma\ S_{CH3}$ and $\sigma\ S_{CH2}/S_{CH3}$ designate their respective standard deviations. [c] RSD represents the precision (in percent) calculated according to RSD $S_{CH2}/S_{CH3} = 100\sigma\ S_{CH2}/S_{CH3}/(S_{CH2}/S_{CH3})_{av}$ and RSD $S_{CH3} = 100\sigma\ S_{CH3}/S_{CH3av}$. For the imperfection pulses part, 10 spectra were performed with the ^{1}H offset varied over 675 Hz. For the off-resonance part, 11 spectra were recorded by varying the ^{1}H offset over 2.5 kHz.

(RSD ~ 0.6%). The stability of the NMR response was again better for COS/OIA than for CHIRP pulse shapes.

In the above investigations, the DEPT sequence making use of a ^{13}C 180° composite adiabatic pulse with a COS/OIA profile provided the most precisely determined peak surface areas. A ^{1}H 180° adiabatic composite refocusing pulse with a COS/OIA shape was therefore also implemented in the DEPT sequence to give the adiabatic DEPT sequence shown in Figure 1A.

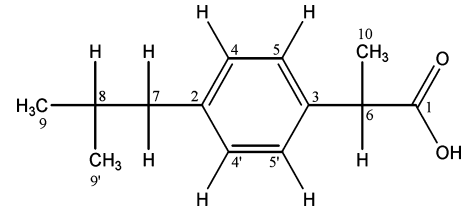

Figure 5. Structure of ibuprofen with the atom numbering indicated according to decreasing ^{13}C chemical shift.

Figure 2C and D shows the influence of varying the ^{1}H offset within a small range of values on the precision of CH_2 and CH_3 peak surface areas measured using DEPT sequences. ^{1}H RF field inhomogeneity results in a periodic modulation of S_{CH3} and of the S_{CH2}/S_{CH3} ratio as observed for ^{13}C (RSD ~ 4%, Table 2), and this oscillation is almost completely eliminated in the fully adiabatic DEPT sequence (RSD ~ 0.6%, Table 2). Similarly, an examination of the effect of ^{1}H off-resonance revealed a dramatic improvement in the robustness of ^{13}C peak surface areas measured with the fully adiabatic DEPT sequence compared with a DEPT sequence featuring a 180° ^{1}H hard pulse (RSD ~ 0.3% and 6.4%, respectively, Table 2).

INEPT. The implementation of 180° adiabatic composite refocusing pulses in INEPT results in dramatically improved ^{13}C chemical shift refocusing throughout the pulse sequence: the surface areas of CH_3 (and CH_2) peaks in INEPT spectra were much less sensitive to ^{13}C offset changes (over a narrow range of values) when the measurements were performed using adiabatic sequences compared with the standard sequence (Figure 3A–B; compare also the respective RSD values for the S_{CH2}/S_{CH3} ratio in Table 3). Once the ^{13}C offset was varied over the entire range of values as in a whole ^{13}C spectrum, ^{13}C peak surface areas measured with the standard INEPT sequence were also very much dependent on the offset (RSD ~ 5.9%) (Table 3). The ^{13}C off-resonance effect was almost completely eliminated with only the adiabatic refocused INEPT (Figure

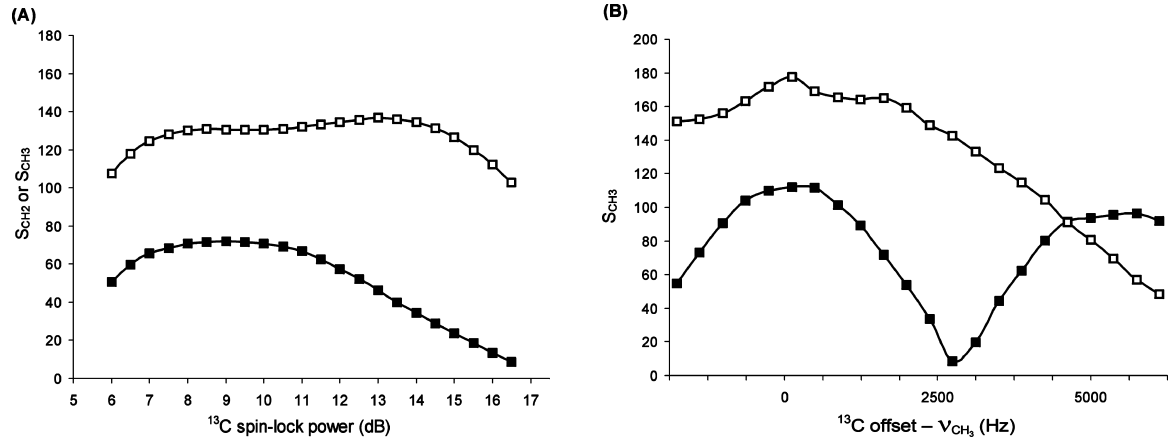

Figure 4. Influence of ^{13}C spin-lock power and ^{13}C offset on CH_2 or CH_3 peak surface areas in NMR spectra of ethanol recorded with the adiabatic HCP pulse sequence. (A) Effect of ^{13}C spin-lock power. Experiments were performed using the P5M4 supercycle and 7.5 ms ^{1}H and ^{13}C spin-locks with 180° COS/OIA adiabatic pulses (pulse length: 125 μs). CH_2 ■ and CH_3 □. (B) Effect of ^{13}C offset. CH_3 peak surface areas are shown as a function of the difference between ^{13}C offset and ^{13}C chemical shift of CH_3. 5 ms spin-lock with 180° adiabatic pulse lengths of 250 μs (■) and 50 μs (□).

Table 5. Calculation of ^{13}C Isotopic Molar Fractions[a] for Ibuprofen from Peak Surface Areas Measured in DEPT NMR Spectra

	f_R C10	f_R C6	f_R C5,C5′	f_R C4,C4′	f_R C7	f_R C8	f_R C9,C9′
				DEPT spectra			
f_{iR} av	1.2231	0.9090	0.8416	0.8471	1.2210	0.7709	1.2492
σ	0.0015	0.0011	0.0009	0.0008	0.0012	0.0010	0.0010
RSD (%)	0.12	0.12	0.11	0.09	0.10	0.13	0.08
				one-pulse spectra			
f_{iR} av	0.9959	1.0030	0.9984	0.9954	1.0017	0.9938	1.0089
σ	0.0006	0.0013	0.0007	0.0007	0.0013	0.0013	0.0006
RSD (%)	0.06	0.13	0.07	0.07	0.13	0.13	0.06

[a] f_R designates the reduced isotopic molar fractions of ^{13}C sites in ibuprofen and is calculated using eq 1 for each recorded spectrum. f_{iR} av, σ, and RSD (%) designate average values of reduced isotopic molar fractions of each site, and their respective standard deviations and variation coefficients (RSD = $100\sigma/f_{iR}$ average), calculated for each type of NMR experiment (with the adiabatic DEPT pulse sequence or the reference one-pulse ^{13}C experiment).

1D) (RSD ~ 0.4%). The study of the influence of ^1H RF pulse imperfections and off-resonance on the precision of ^{13}C peaks in spectra recorded with INEPT sequences was carried out using the same methodology as used for DEPT sequences. The standard INEPT sequence is highly sensitive to ^1H RF pulse imperfections and off-resonance effects, as shown in Figure 3C and D and in Table 4. The effects of ^1H 180° RF pulse imperfections were almost completely eliminated with the help of the ^1H 180° adiabatic composite refocusing pulse and the ^1H 180° adiabatic inversion pulse in the adiabatic INEPT sequence (Figure 1D) which from this viewpoint performs even better than the adiabatic DEPT sequence (Figure 1B). However, the robustness of ^{13}C peak surface areas, measured using the adiabatic INEPT sequence by increasing the ^1H offset throughout the whole range of experimentally observed values, is worse than for the adiabatic DEPT sequence.

Application of the Adiabatic DEPT Sequence to the ^{13}C Fingerprinting in an API: Ibuprofen. In this last part of our study, we selected a very common drug, ibuprofen (Figure 5), to verify whether the adiabatic DEPT sequence (Figure 1B) would enable a precise map of the distribution of ^{13}C within this molecule to be drawn. Ibuprofen sample conditions were initially optimized for ^{13}C NMR measurements (See the Materials and Methods section). Series of adiabatic DEPT spectra were subsequently recorded to evaluate the precision and robustness of the sequence. Each DEPT spectrum was used to build a map of site-specific ^{13}C isotopic molar fractions in ibuprofen. This map was finally compared to an analogous map made from spectra recorded with conventional one-pulse experiments.

The minimum SNR value of 824 was determined for C8 in the spectra of ibuprofen recorded using the adiabatic DEPT experiment. For comparison, the minimum SNR value was 890 for the corresponding peaks in the ^{13}C spectrum of ibuprofen recorded using 560 transients and a delay of 20 s between the transients for a total experimental time greater than 3 h.

For all spectra of ibuprofen measured using the adiabatic DEPT sequence, reduced ^{13}C isotopic molar fractions f_{iR} of each protonated carbon are compiled in Table 5. For each series, average ^{13}C isotopic molar fractions were calculated as well as the associated standard deviations and coefficients of variation (see f_{iR} av, σ, and RSD (%) values in Table 5). From the detailed analysis of the data in Table 5, the following conclusions can be drawn: (i) f_{iR} values of each carbon site within ibuprofen can be determined with a high degree of precision from DEPT spectra. Small coefficients of variation were consistently calculated (RSD < 0.14%) for all sets of spectra. C9/9′, C10, and C7 have the highest f_{iR} av values among all carbons, and they appear as enriched, whereas for all other carbons within the molecule, ^{13}C is less or much less abundant, with the smallest f_{iR} av value observed for C8. (ii) Values determined from NMR spectra recorded using the reference one-pulse ^{13}C experiment are also very precise (RSD < 0.14%). From the viewpoint of ^{13}C isotopic distribution within ibuprofen, the data derived from spectra recorded with the one-pulse experiment and the adiabatic DEPT sequence agree qualitatively, except for C10 and C6. However, the primary goal of this study was not to determine absolute $\delta^{13}C$‰ values within the sample. Thus, in these initial tests, the adiabatic DEPT sequence appears able to provide precise NMR spectra that may be used in quantitative ^{13}C isotopic analysis.

CONCLUSIONS

Our approach consisted in modifying original DEPT, INEPT, and HCP pulse sequences with the help of adiabatic pulses in order to obtain an isotopic fingerprint with a high repeatability.

We have clearly shown that adiabatic ^1H and ^{13}C 180° pulses significantly improve the repeatability of the measurements with DEPT, INEPT, and HCP by minimizing the influence of 180° pulse imperfections and therefore of RF field inhomogeneity as well as the contribution of off-resonance effects to the detected NMR signals. Among all the sequences tested, both the adiabatic DEPT and INEPT sequences have the potential to be used in quantitative ^{13}C site-specific isotopic measurements by NMR, as confirmed by our preliminary results on ibuprofen by DEPT. Further work needs to be done, however, to test systematically whether either or both these sequences will provide reproducible data for measurements performed over a longer period of time on a wide range of experimental and environmental setups.

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(39) Smith, M. A.; Hu, H.; Shaka, A. J. *J. Magn. Reson.* **2001**, *151*, 269–283.