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Isotopic finger-printing of active pharmaceutical ingredients by ^{13}C NMR and polarization transfer techniques as a tool to fight against counterfeiting

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ABSTRACT

The robustness of adiabatic polarization transfer methods has been evaluated for determining the carbon isotopic finger-printing of active pharmaceutical ingredients. The short time stabilities of the adiabatic DEPT and INEPT sequences are very close to that observed with the one pulse sequence, but the DEPT long time stability is not sufficient for isotopic measurements at natural abundance or low enrichment. Using the INEPT sequence for ^{13}C isotopic measurements induces a dramatic reduction in the experimental time without deterioration in short time or long time stability. It appears, therefore, to be a method of choice for obtaining the isotopic finger-print of different ibuprofen samples in a minimum time. The results obtained on 13 commercial ibuprofen samples from different origins show that this strategy can be used effectively to determine ^{13}C distribution within a given molecule and to compare accurately differences in the isotopic distribution between different samples of the given molecule. The present methodology is proposed as a suitable tool to fight against counterfeiting.

1. Introduction

Drug counterfeiting is a major problem both for its economic impact and for the safety of the patient population. A variety of types of counterfeiting practice has been defined by the World Health Organization (WHO) and the International Federation of Pharmaceutical Manufacturers Associations (IFPMA) [1]: "A counterfeit drug is one which is deliberately and fraudulently mislabeled with respect to identity and/or source. Counterfeiting can apply to both branded and generic products and counterfeit products may include products with the correct ingredients or with the wrong ingredients, without active ingredients, with insufficient active ingredient". Consequently, the fight against fraudulent medicines uses a wide range of techniques and methodologies. Apart from problems of appearance (packaging, shape and colors of the tablets) which are relatively easy to detect, at least by regulating authorities (much less so by the individual buying via the internet), a large arsenal of analytical tools is available to verify the medicine itself. Thus, techniques such as HPLC, GC, LC-MS, GC-MS, IR and NIR, and NMR (see refs. [2–5] for recent reviews) are able to tackle problems of composition by answering the following questions: (i) is the active molecule the right one? (ii) is the active molecule present in the correct quantity? (iii) is the composition (nature and quantity) of the excipients the expected one? (iv) does the trace analysis correspond to the compounds produced by the side reactions of the manufacturing process of the active pharmaceutical ingredient (API)? For the latter, hyphenated techniques (GC-MS or LC-MS) are the most appropriate [6]. cGMP [7] (current Good Manufacturing Practice) already requires that the impurity profile of a given API should be defined on the basis of an HPLC profile from which any peak higher than 0.1% should be identified. However, it is clear that this impurity profile can be distorted or masked in the final product (tablet, pill, capsule, etc.) by dilution in the other ingredients, making the chromatographic approaches less effective in the fight against counterfeiting. Furthermore, none of the above techniques is able to detect the following counterfeiting practices: (i) the use of synthetic ingredients when a natural origin is labeled, (ii) a deliberately copied process, (iii) patent infringement of current patents for generic medicines, (iv) products that are not made in the specified country of origin (circumvention of anti-dumping taxes or other duties) and (v) diversion (re-importation, for example). To succeed in the detection of such frauds, the molecular probe has to be the active molecule itself, which is not the case for the above-mentioned techniques. The protection of intellectual property rights is of huge interest to pharmaceutical companies in a

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worldwide market [8,9]. In this context, securing the pharmaceutical supply chain [10] is an emerging approach. The goal is to define the pedigree of the drug.

Recent results obtained using stable isotope analysis, which probes the molecules themselves, have shown that these methods are extremely valuable for the detection of a number of different types of counterfeiting: the isotopic profile of a given molecule appears as a label of origin (see refs. [11–16] and refs. therein). The stable-isotopic composition observed is the result of three variables: (i) the isotopic composition of the raw materials used in the synthesis, (ii) the isotopic fractionation which may occur during the synthesis and (iii) the purification process of the API. There are two main techniques for measuring isotope ratios for the elements constituting organic matter. The oldest one is the “isotope ratio measured by mass spectrometry”, known as IRMS or irm-MS [17]. With IRMS, $^{2}H/^{1}H$ (D/H), $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$ and $^{34}S/^{32}S$ ratios can be routinely determined in pure molecules or in a mixture when coupling with either GC or HPLC is used (GC-IRMS and LC-IRMS). However, this technique is based on the complete combustion of the sample molecules, thus providing only average values of the content of the heavy isotope. Valuable information is inevitably lost. Ideally, the quantification of each isotopomer of a given molecule should provide more data for a greater discrimination. Nuclear magnetic resonance spectrometry offers this advantage. Indeed, the direct access to site-specific natural isotope fractionation opened up by the SNIF-NMR method [18,19] is recognized as a powerful technique for verifying the origin of commercial products [20]. A previous study [21] demonstrated the potential of using ^{2}H-SNIF-NMR to identify the synthetic process used in the production of the compounds and to provide market intelligence about products sold commercially. However, certain limitations of the method were highlighted: (i) a long measurement time; even for the relatively simple molecular structure, obtaining a sufficiently resolved ^{2}H NMR spectrum required several hours of spectrometer time and (ii) large molecules could not be studied by ^{2}H NMR because of the complexity of the NMR spectrum. The extension of the SNIF-NMR method to nuclei other than deuterium thus provides major advantages such as (i) an increase in the number of parameters allowing the determination of the molecular fingerprint and (ii) the possibility of analyzing molecules for which ^{2}H NMR is particularly difficult or even impossible.

In the case of carbon-13, a precision of about 1‰ is necessary for isotopic analysis because of the narrow range of specific isotopic deviations (less than 50‰ instead of 500‰ for deuterium). Therefore, although carbon-13 is 70 times more abundant than deuterium, the determination of the isotopic relationship with a high degree of accuracy (sum of the trueness and precision, a clear definition of these terms is given in ref. [22]) is difficult for several reasons: significant values of the relaxation times, the nuclear Overhauser effect, homogeneity of decoupling [23,24].

From a statistical point of view, the maximum error σ on the signal area S is inversely proportional to the signal-to-noise ratio (SNR) [25]. High precision requires a large number of acquisition scans inducing a long experimental time in ^{13}C NMR. This difficulty can be circumvented by the application of multi-pulse sequences which enhances spectral sensitivity by the transfer of polarization from a more sensitive nucleus (*i.e.* ^{1}H) to a scalar coupled less sensitive nucleus (*i.e.* ^{13}C) [26,27]. Furthermore, the pulse repetition rate now depends on the longitudinal relaxation times T_1 of the proton nuclei, which are considerably shorter than those of the carbon nuclei. As a result, partial signal saturation can be avoided with a significantly shorter relaxation delay.

However, these techniques can generate a bias in the isotope ratio measurement since the signal intensity now also depends on several experimental parameters such as pulse flip angles, coupling constants, relaxation times and evolution periods [28]. Nevertheless, these distortions are reproducible and can be calculated. Another source of error disturbing the polarization transfer sequences is the loss of signal intensity resulting from relaxation during the evolution periods. According to the usual values of ^{1}H and ^{13}C T_2, this kind of distortion is around a few thousands. However, in the case of authentication applications, like the detection of patent infringements in the pharmaceutical industry, the compared samples contain the same molecule at the same concentration and temperature and in the same solvent, so with the same relaxation times. The contribution of the NMR pulse sequence to the relative intensities is therefore the same for all the samples.

The major problem that influences the accuracy of quantitative analysis is the deviation of pulse flip angles from their ideal values due to inhomogeneity in the RF field. In a previous work [29], we optimized the DEPT and INEPT sequences in order to eliminate the dependence of the ^{13}C signal intensity on RF pulse flip angles, frequency offsets and evolution periods, thus improving the accuracy of quantitative determinations. The elimination of the observed distortions was achieved by replacing the proton and carbon π pulses of the sequence by adiabatic pulses. A significant increase in the precision was obtained which is now sufficient to satisfy the criteria of carbon isotopic NMR.

The aim of the work presented here was to evaluate the robustness of these adiabatic sequences and their ability to provide an isotopic profile of ibuprofen samples from different sources in a short time, thus creating a specific origin label which can be used by the manufacturer to protect their product.

2. Materials and methods

2.1. Chemicals and samples

Acetone–d_6 was purchased from Eurisotop (Paris, France). Methanol, pentane, diethyl ether, P_2O_5 and silica gel were purchased from Sigma–Aldrich (Saint Quentin-Fallavier, France). 13 samples of ibuprofen (commercial packaged form) were purchased from 5 countries.

2.2. Extraction–purification protocol

The tablets (equivalent to 1 g of API) were first powdered, then dissolved in 20 mL of methanol and filtered under vacuum through filter paper to remove the major excipients. The solvent was reduced to a maximum of 5 mL using a rotary evaporator. The sample was loaded onto the head of a silica gel (35–70 μm) column (3 cm o.d.) and eluted by a 75/25 (v/v) mixture of pentane/ether. 50 mL fractions were collected and checked by TLC. All the fractions containing the product of interest were pooled in a round-bottomed flask and the solvent was removed using a rotary evaporator. Then, ibuprofen ((4-isobutylphenyl) propionic acid) was dried overnight at 40 °C and stored under vacuum over P_2O_5 in a desiccator. The purity of the ibuprofen samples (including the commercial origin) was checked by ^{1}H NMR.

2.3. NMR spectrometry experiments

For quantitative ^{13}C NMR, samples were prepared by dissolving 300 mg of ibuprofen in 600 μL acetone–d_6. The solution was then carefully filtered into a 5 mm o.d. tube. NMR experiments were performed on a Bruker Avance 400 spectrometer fitted with a 5 mm i.d. $^{13}C/^{1}H$ dual^{+} probe was carefully tuned to the recording frequency of 100.61 MHz. The temperature of the probe was set at 303 K. All ^{13}C NMR spectra were recorded with inverse gated adiabatic proton decoupling [30]. Typical acquisition parameters were as follows: ^{13}C and ^{1}H offsets were set at 100 ppm and 4 ppm, respectively, 90° ^{1}H and ^{13}C high power pulse width 10 μs. The

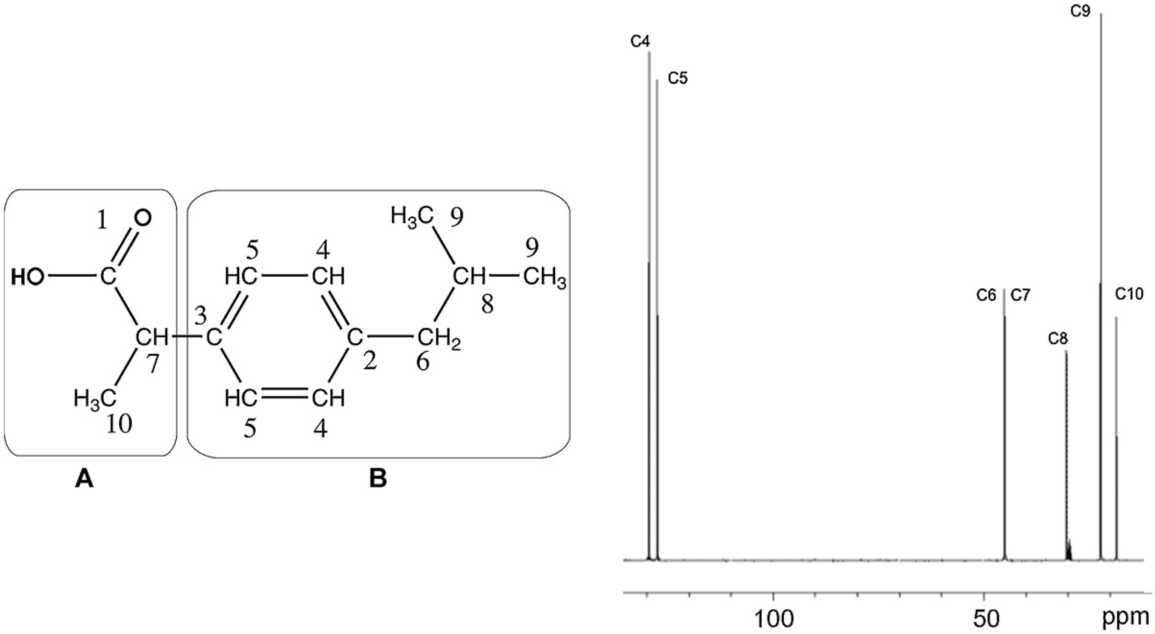

Fig. 1. Ibuprofen molecule with carbon sites numbered in decreasing chemical shift (left) and ^{13}C NMR {^{1}H} spectrum of ibuprofen in acetone–d$_6$ recorded at 100.61 MHz at 303 K with an INEPT sequence. Part B is the common raw material and part A is the side chain which is added according to the main industrial synthetic pathways [15].

pulse lengths were calibrated at the beginning of each "measurement session" and the probe tuning and matching were adjusted for each sample.

For one pulse acquisitions [26], 232 scans were accumulated with a repetition time of 152 s. For DEPT and INEPT acquisitions [25], 64 scans were accumulated with a repetition time of 17.8 s. For the DEPT pulse sequence, the values of evolution delays τ were set at 3.23 ms and the variable ^{1}H flip angle was fixed at 60°. For the refocused INEPT sequence [29], τ_1 was adjusted to 2.3 ms and τ_2 to 1.3 ms. The assignment of ^{1}H and ^{13}C NMR resonances of ibuprofen was performed using HSQC and DEPT experiments.

Adiabatic full passage pulses were generated using Mathcad 8 (MathSoft, Inc.). They were designed with a cosine amplitude modulation of the RF field ($\omega_2{}^{\max}$ = 157.1 kHz or 93.89 kHz for ^{13}C or ^{1}H, respectively) and an offset independent adiabaticity (OIA [31]) by optimizing the frequency sweep ΔF (ΔF = 39 kHz or 17 kHz for ^{13}C or ^{1}H, respectively) according to the published procedure [30]. For inversion pulses, adiabatic full passage pulses were used. For refocusing pulses, composite adiabatic pulses were used [32].

For each measurement, five spectra of ibuprofen were recorded. Five measurements were performed over three months with the DEPT and INEPT protocols.

The T$_1$ values were determined by using an inversion recovery sequence, with 35 inversion-time values ranging from 50 ms to 150 s for ^{13}C and with 40 inversion-time values ranging from 50 ms to 30 s for ^{1}H, and the T$_1$ processing software of the spectrometer.

2.4. 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. Peak areas of ^{13}C peaks were determined by the curve-fitting process implemented within Perch (Perch NMR Software, University of Kuopio, Finland).

The peak 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 [33]. Partial reduced molar fractions were calculated for each visible site according to Eq. (1) [23]:

$$f_{i\mathrm{R}} = \frac{S_i}{F_i S_\mathrm{T}} \qquad (1)$$

where S_i defines the corrected peak area of the ^{13}C peak of site i in a specific spectrum and S_T represents the sum of the peak areas of sites 1–10 for one pulse acquisitions and 4–10 for DEPT or INEPT acquisitions (see Fig. 1 for atom numbering). 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 peak area of the ^{13}C peak of site i in the NMR spectrum, and N is the total number of carbons observed in the spectrum (N = 13 for one pulse acquisitions and N = 10 for DEPT or INEPT acquisitions).

Deviation (in ‰) from the average value of the partial reduced molar fractions was calculated according to $\Delta_i = 1000 \times (f_{i\mathrm{R}} - (f_{i\mathrm{R}})_\mathrm{av})$ where $f_{i\mathrm{R}}$ is the partial reduced molar fraction of site i and $(f_{i\mathrm{R}})_\mathrm{av}$ is its average value calculated over the 13 samples.

3. Results and discussion

3.1. Experimental conditions and analysis duration

In a previous study [29], we chose to dissolve ibuprofen in a mixture of DMSO–d$_6$, NaOH 1 M solution and CD$_3$OH. This preparation allows a full separation of each signal of the ^{13}C spectrum. However, the stability-over-time study revealed that chemical degradation of ibuprofen can affect the isotopic results. In the present study, acetone–d$_6$ was chosen as solvent because it provides a high solubility without chemical degradation and the samples can be easily recovered. The only drawback is a partial overlapping of the bases of the C6 and C7 peaks. ^{1}H and ^{13}C T$_1$ values for ibuprofen in acetone–d$_6$ at 303 K are listed in Table 1. In the DEPT and INEPT sequence, the proton magnetization is transferred to the bounded carbons and the signal coming from the direct excitation of carbons is removed by the phase cycling [25–27]. The intensity of the detected signal is therefore only dependent on the ^{1}H longitudinal magnetization before the first 90° pulse and no longer depends on

Table 1
Chemical shifts (δ_{13C} and δ_{1H}) and longitudinal relaxation times ($T1_{13C}$ and $T1_{1H}$) for a solution of ibuprofen in acetone–d$_6$ (500 mg mL^{-1}) at 303 K.

Site	δ_{13C} (ppm)	$T1_{13C}$ (s)	δ_{1H} (ppm)	$T1_{1H}$ (s)
1	175.76	15.1		
2	139.85	9.4		
3	138.12	10.4		
4	128.87	3.0	7.16	2.3
5	126.95	2.9	7.30	2.4
6	44.48	1.9	3.78	2.4
7	44.36	2.5	2.50	1.2
8	29.76	3.8	1.90	2.2
9	21.61	2.2	0.93	1.2
10	17.94	1.3	1.49	0.9

the carbon longitudinal relaxation. This avoids any influence of the nOe on the detected signal. The recovery delay is therefore only governed by the ^{1}H proton relaxation and must be greater than 7 times the longest ^{1}H T_1. Here, this condition induces TR = 17.8 s (*i.e.* ~AQ + 7 T_1 of H5 or H6). The occurrence of the polarization transfer increases the sensitivity, thus only 64 scans were required to obtain a signal-to-noise ratio higher than 700, which is the minimum value for a precision of the order of 1‰. The measurement time was therefore 1 h 35 min for five spectra.

As a comparison, the recovery delay in the one pulse sequence must be greater than 10 times the longest ^{13}C T_1 [34]; this condition is required to avoid the influence of the nuclear Overhauser effect (nOe). Here, it induces TR = 152 s (*i.e.* AQ + 10 T_1 of C1). Furthermore, 232 scans are needed with this sequence to obtain a signal-to-noise higher than 700. The measurement time was therefore 49 h for five spectra. However, it must be noted that the polarization transfer methods allow only the measurement of signal from hydrogen-bearing carbons. If the repetition time is calculated to obtain quantitative measurements for only hydrogen-bearing carbons (using the one pulse experiment), the optimum TR value becomes 39 s (*i.e.* AQ + 10 T_1 of C8) and the experimental time is reduced to 12 h 34 min for five spectra, which is still much longer than that observed with the DEPT or INEPT sequence.

Clearly, using the DEPT or INEPT sequence for ^{13}C isotopic measurements induces a dramatic reduction in the experimental time. In the case of ibuprofen, it is divided by a factor of 7.9 even if only hydrogen-bearing carbons are quantified by the one pulse sequence.

However, only hydrogen-bearing carbons are observed and the relative intensities are no longer governed by only molar fractions [29]. As a result, specific isotopic deviations that are calculated from overall mean ^{13}C abundance – measured by IRMS – and from total molar fractions [23] cannot now be obtained.

In the case of isotope-profile 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, partial reduced molar fractions can be used to obtain an isotopic finger-print [29].

3.2. Short time stability

Table 2 shows the typical intra-sample variations in the partial molar fractions measured with the three NMR experiments for one sample of ibuprofen. The short time stability which, in turn, corresponds to the intra-assay evaluation, was assessed by the relative standard deviation (RSD) calculated over five successive spectra. Values obtained on other samples were not significantly different (data not shown).

Table 2
Short time stability of partial reduced molar fractions for the different ^{13}C NMR acquisition protocols tested in this work.

Site	Short time stability (‰)[a]		
	One pulse	DEPT	INEPT
10	0.4	2.0	1.4
9	1.0	1.8	0.8
8	4.2	1.9	3.1
7	2.3	1.7	1.8
6	3.0	1.5	1.2
5	0.5	1.4	0.9
4	1.2	1.4	0.8
3	1.1		
2	0.5		
1	2.0		
Mean[b]	1.6	1.7	1.4

[a] Short time stability was calculated according to the formula: $1000 \times \sigma_i/(f_{iR})_{av}$, where $(f_{iR})_{av}$ and σ_i are the average value and the standard deviation of the partial reduced molar fraction of the site *i* calculated over five consecutive experiments.

[b] The mean values were calculated over sites 1–10 for the one pulse acquisitions and over sites 4–10 for the DEPT and INEPT acquisitions.

One pulse sequence. The mean value for RSD is 1.7‰. The highest values are obtained for carbons 6, 7 and 8. This can be explained by the partial overlapping of the C6 and C7 peaks and the proximity of the C8 peak to the acetone residual signal.

DEPT and INEPT sequences. The mean short time stability observed is of the same order with these two sequences and close to that observed with the one pulse sequence. However, it should be noted that RSD values are lower for carbons 6, 7 and 8 with the DEPT or INEPT sequences. The short time stability of the three methods is therefore sufficient for isotopic finger-printing and this parameter does not show performance differences between these methods.

3.3. Long time stability

The NMR measurements for all the ibuprofen samples discussed were acquired over a period of 3 months. In order to monitor any drift with time, a control measurement was performed with the DEPT and the INEPT sequences on the same sample before each series of measurements. The data obtained for this sample were therefore analyzed to evaluate measurement stability with time over a 3-month period; this corresponded to an inter-assay comparison. Site-specific relative standard deviations were calculated for the partial molar fractions obtained from the five sets (each of 5 repetitions) of ^{13}C NMR spectra. The results are presented in Table 3. It should be noted that the long time stability using the one pulse sequence has already been assessed for other molecules: it is of the order of the short time stability [35].

Those obtained with the INEPT sequence show that the repetition of measurements over a 3-month period produces a scatter within the sample set of results which is no larger than that obtained with five successive experiments. The highest RSD values are always lower than 1.4‰. On the other hand, the RSD values obtained with the DEPT sequence are significantly higher and the maximum value reaches 8.9‰ (C10). Statistically, this is usually the case when comparing intra-assay and inter-assay results, because a larger number of data is available for the latter. For practical considerations, long time stability is the main goal when studying the robustness of an analytical method.

The overall stability of the NMR measurement is strongly governed by spectrometer instabilities, a component of which is the stability of the RF pulse amplitude. The 180° pulses implemented in the sequences evaluated in this paper are adiabatic pulses. They are therefore partially immune to transmitter variations. For other

Table 3
Long time stability of partial reduced molar fractions for DEPT and INEPT acquisitions.

Site	Long time stability (‰)[a]	
	DEPT	INEPT
10	8.9	0.8
9	8.1	0.5
8	8.0	1.2
7	7.5	1.3
6	4.3	0.3
5	3.4	0.2
4	3.1	0.5
Mean[b]	6.2	0.7

[a] Long time stability was calculated according to the formula: $1000 \times \sigma_i/(f_{iR})_{av}$, where $(f_{iR})_{av}$ and σ_i are the average value and the standard deviation of the partial reduced molar fraction of the site *i* calculated over five measurements performed over three months.

[b] The mean values were calculated over sites 4–10.

pulses, such as 90° pulses or the θ pulse of the DEPT sequence (last ^{1}H pulse), the measured signal intensity depends on $\cos(\theta)$ so a variation in θ induces a signal variation proportional to $\sin(\theta)$. This means that 90° pulses are naturally immune to transmitter variations. However, for any pulses different from 90°, small variations in the transmitter power can induce significant signal intensity variation owing to the above $\sin(\theta)$ dependency. While the INEPT sequence contains only 90° and 180° pulses, the DEPT sequence contains a θ pulse with $\theta = 60°$. This could explain the poor results observed for the long time stability with the DEPT sequence.

In conclusion, the DEPT long time stability is not sufficient for isotopic measurements. The isotopic finger-printing of different ibuprofen samples was therefore obtained from the INEPT sequence.

3.4. Site-specific ^{13}C content in ibuprofen samples

^{13}C-INEPT measurements (mean of five successive spectra) were performed on 13 commercial samples of ibuprofen purchased from 5 countries (see legend of Fig. 2 for details). In order to obtain a clear display, the influence of the sequence on the partial reduced molar fractions was eliminated by calculating the deviation from the average value (Δ_i) as described in Section 2. The results are plotted in Fig. 2. It must be noted that inter-sample differences are the same for a given Δ_i as for the corresponding partial reduced molar fraction f_{iR}.

A general trend is that the site-specific ^{13}C content exhibits a large variability both for inter-carbon-site comparison and inter-sample comparison. Furthermore, the overall ^{13}C content was measured by IRMS (Isotope Ratio Mass Spectrometry) for all these samples. Values ranged from −25.8‰ to −29.9‰ with an average of −28.4‰. It is therefore clear that site-specific ^{13}C content in ibuprofen samples shows a much larger variation than the overall content, which is consistent with previous observations on other compounds [14,23,36,37].Samples Tesco and Wallis from the United Kingdom have similar profiles, except on carbons 6 and 7, whose partial reduced molar fraction values are very scattered. It is interesting to note that samples from the same distributor do not have exactly the same profile. Samples purchased in the U.S. also have very similar profiles, with the exception of carbons 6 and 7, which again have partial reduced molar fractions dispersed from one sample to another. Furthermore, the McNeil sample, whose partial reduced molar fractions 6 and 7 are radically opposed, is very clearly different from the other American samples. In the case of European samples, a slight trend emerges. Once again, carbons 6 and 7 show the greatest dispersion of partial reduced molar fractions. In addition, Abbott samples do not have the same profile.The goal of the present work is to propose a new analytical tool which can be used by the manufacturer and/or the trader to set up an isotopic profile as a fingerprint to characterize the process and the raw material used for the API. In fact, each batch could lead to a characteristic isotope profile which could ensure a production label to counteract counterfeiting. The large dispersion of the intramolecular ^{13}C distribution in ibuprofen samples, as observed in Fig. 2, reflects that there may be an almost infinite number of combinations of different raw materials, from different geographical origins and different synthetic pathways.At the present stage of the study, it is clear that the proposed methodology fulfills the above aim. A

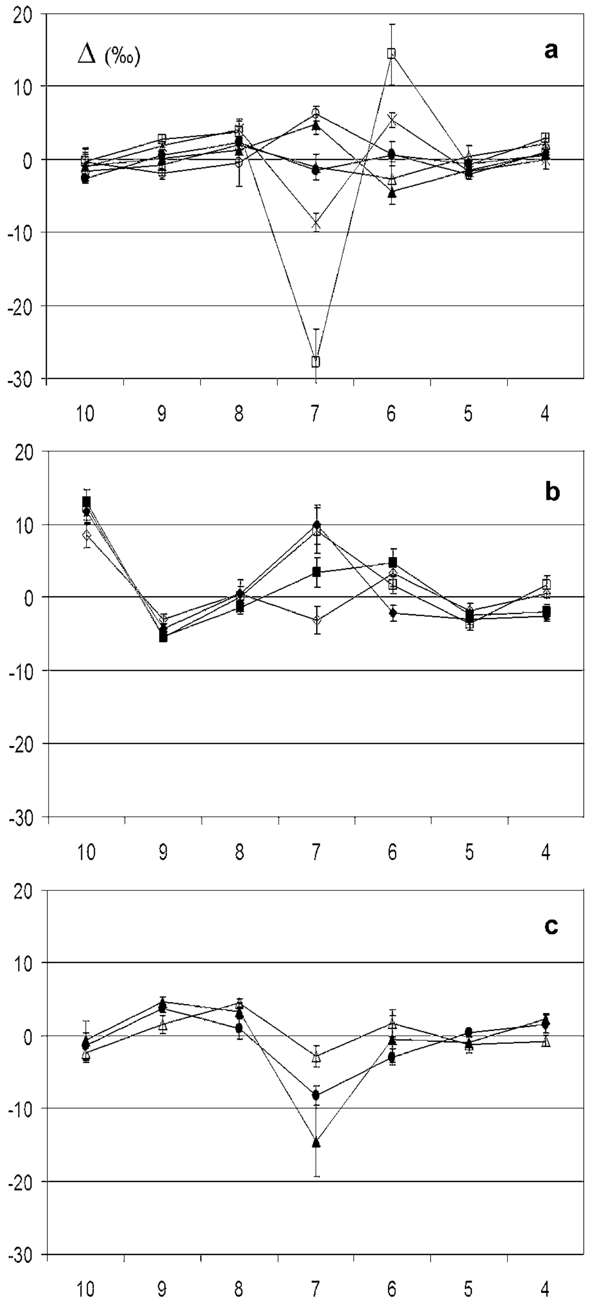

Fig. 2. Deviation (in ‰) from the average value (Δ_i) of the partial reduced molar fractions obtained with the INEPT sequence for sites 4 to 10 of ibuprofen samples from different origins: (a) United States (▲ and △ Wal-Mart-Stores; ● and ○: Walgreens; ✕ : Wyeth; □: McNeils), (b) United Kingdom (♦ and ◊: Tesco; □ and ■: Wallis), (c) continental Europe (▲: Belgium Abbott; △: Portugal Abbott; ●: France). Δ_i is calculated according to $\Delta_i = 1000 \times (f_{iR} - (f_{iR})_{av})$, where f_{iR} is the partial reduced molar fraction of site *i* and $(f_{iR})_{av}$ is its average value calculated over the 13 samples.

detailed explanation of the sources responsible for the isotope fractionations cannot be established because the required information about the raw materials used and the processes is not available. The name on the packaging is more often the distributor and not the manufacturer. It is not within the scope of this work to predict the final isotope profile for a given molecule according to its manufacturing process. Nevertheless, a preliminary discussion is possible based on the major industrial synthetic pathways which have already been described in ref. [15]. Accordingly, part B of the molecule of ibuprofen in Fig. 1 is the common synthon for these pathways. The variations in the ^{13}C content of this part should be associated with the origin of this starting material. Among the carbon atoms, it is expected that C-4 and C-5 should show the lowest variability due to their origin from crude oil. The partial reduced molar fractions of carbons 9 seem to depend strongly on the geographical origin. This could be explained by the difference in raw materials used in each geographic region. Indeed, the various treatments carried out on crude oil to obtain chemical compounds induce isotopic fractionations and thus specific profiles. The hypothesis of greater sensitivity of CH_3 treatments used for the production of raw materials has therefore to be considered. A large range is observed for C-6 which could be rationalized by the fact that it is the carbon where the addition on the aromatic ring occurs. Therefore, several types of reaction and reagent are possible to create the bond, leading to a large set of isotope profiles.The processes used for making ibuprofen, as described in [15], differ from one another by the building of the side chain A (Fig. 1). Nevertheless, one part remains constant: the “acetaldehyde” or “acetate” synthon, constituted by carbons 7 and 10. Previous works have shown that the ^{13}C content of this “ethane fragment” could display a very large range of isotope values, especially carbon 7, including fossil origins (ethylene, acetylene, methanol, and ethanol) but also biotechnological sources [38,39]. This carbon potentially contains a significant part of the information for the isotopic discrimination of samples according to their synthetic pathways.

Thus, the analysis of Fig. 2 indicates that, while the partial reduced molar fractions of carbons 6, 8 and 9 can be used to discriminate the raw materials, those of carbon 7 and 10 can discriminate the synthetic pathways.

4. Conclusions

The short time stabilities of the DEPT and INEPT sequences used in this work are very close, but the DEPT long time stability is not sufficient for isotopic measurements at natural abundance or low enrichment. Using the INEPT sequence for ^{13}C isotopic measurements induces a dramatic reduction in the experimental time without a deterioration in short time or long time stability. It is therefore a method of choice for obtaining the isotopic finger-print of different ibuprofen samples in a minimum time.

The results obtained on ibuprofen show that this strategy can be used effectively to determine ^{13}C distribution within a given molecule and to compare accurately differences in the isotopic distribution between different samples of the given molecule, in a very short analytical time, even if absolute values of specific isotopic deviations cannot be provided. A test of the identity (a part of the pedigree) of the active ingredient, defined as its isotope profile, may be used as a tag which could characterize (i) a batch to batch production, (ii) a specific manufacturing process and (iii) the origin of the raw materials used in the process. Further improvements can now be considered: (i) using ^{2}H and ^{13}C NMR together in order to increase the number of parameters constituting the molecule-isotope finger-print and (ii) analyzing larger molecules for which ^{2}H NMR is particularly difficult or even impossible. Work is currently in progress to ascertain the influence on the site-specific ^{13}C content of the raw materials *versus* the manufacturing process.

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