Journal of Pharmaceutical and Biomedical Analysis 50 (2009) 336–341

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Isotopic ^{13}C NMR spectrometry to assess counterfeiting of active pharmaceutical ingredients: Site-specific ^{13}C content of aspirin and paracetamol

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ARTICLE INFO

Article history:
Received 2 April 2009
Received in revised form 27 April 2009
Accepted 29 April 2009
Available online 7 May 2009

Keywords:
Counterfeiting API
Isotopic ^{13}C NMR spectrometry
Isotopic fingerprint
Aspirin (acetylsalicylic acid)
Paracetamol (acetaminophen)

ABSTRACT

Isotope profiling is a well-established technique to obtain information about the chemical history of a given compound. However, the current methodology using IRMS can only determine the global ^{13}C content, leading to the loss of much valuable data. The development of quantitative isotopic ^{13}C NMR spectrometry at natural abundance enables the measurement of the ^{13}C content of each carbon within a molecule, thus giving simultaneous access to a number of isotopic parameters. When it is applied to active pharmaceutical ingredients, each manufactured batch can be characterized better than by IRMS. Here, quantitative isotopic ^{13}C NMR is shown to be a very promising and effective tool for assessing the counterfeiting of medicines, as exemplified by an analysis of aspirin (acetylsalicylic acid) and paracetamol (acetaminophen) samples collected from pharmacies in different countries. It is proposed as an essential complement to ^{2}H NMR and IRMS.

1. Introduction

It is commonly thought that counterfeit drugs mainly affect developing countries where drug regulatory control and enforcement are weak. Although this is undoubtedly a serious problem in these areas, there is increasing evidence of counterfeit pharmaceuticals being widely available in both the USA and Europe. In early 2005, the US Drug Enforcement Administration concluded a year-long investigation, called Operation Cyber Chase, that dismantled the world's largest online counterfeit prescription drug ring, shutting down 200 illegal e-pharmacies and providing insight into what has become one of the world's most prolific black market industries [1]. The World Health Organization (WHO) and the International Federation of Pharmaceutical Manufacturers Associations (IFPMA) have defined a counterfeited medicine as one which is "deliberately and fraudulently mislabeled, with respect to identity and/or source" [2]. This definition covers a variety of types of counterfeiting practice, among which are: (i) the use of competitor's packaging, (ii) the use of synthetic ingredients or raw materials when a natural origin is labeled, (iii) a deliberately copied process or formulation, (iv) patent infringement of current patents for generic medicines and (v) products that are not made in the specified country of origin (circumvention of anti-dumping taxes or other duties).

In general, most of the published data dealing with the fight against the counterfeiting of pharmaceutical drugs are based on the determination and identification of the organic or inorganic trace impurities that are present, even at the parts per million (ppm) level, in these products. Chromatographic techniques are now the methods of choice for obtaining organic impurity data. A fingerprinting approach using pattern recognition methods is often used to distinguish between authentic and counterfeit drugs from their near-Infrared and/or Raman spectra. Whilst these methods pick up blatant counterfeiting of products, the more sophisticated practices, which are becoming increasing prevalent, add a further level of difficulty and often require a complex combination of chromatographic, spectroscopic and hyphenated techniques [3–6].

Furthermore, all these different methodologies produce only indirect evidence of fraud in the chemical or formulation processes and are not directed at screening the active molecule itself. Recent results obtained using stable isotope analysis, which probes the atomic composition of the molecules themselves, have shown that these methods are extremely valuable for the detection of a number of different types of counterfeiting or patent infringement in the pharmaceutical industry. These analyses include isotope ratio mass spectrometry (IRMS) [7–9] and/or ^{2}H NMR spectrometry (SNIF-NMR) [10–13] techniques. IRMS enables the global value of a given isotope (^{2}H, ^{13}C, ^{18}O, ^{15}N and ^{34}S) to be determined. For this, the sample is "burnt" by oxidation or pyrolysis into a gas prior to its injection into the mass spectrometer source: the isotope content is therefore the average contribution of each atom of a given element,

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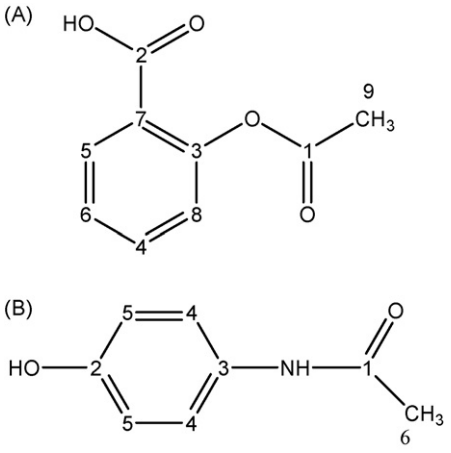
doi:10.1016/j.jpba.2009.04.030



Scheme 1. Molecular structures of (A) acetylsalicylic acid and (B) acetaminophen with carbon positions numbered in order of decreasing ^{13}C chemical shift.

hiding the internal isotopic distribution. NMR has a double intrinsic property: separation of the signal of each site of the molecule and quantification of the resonating nuclei under the peak. Thus, ^{2}H NMR has been used for the last 25 years for the measurement of ($^{2}H/^{1}H$) ratios in several molecules and matrices for such areas as authentication [14], metabolism, and counterfeiting [15]. On the one hand, IRMS requires little compound, is relatively cheap and rapid and gives access to isotope ratios for several nuclei, but only gives one parameter for each element. On the other hand, NMR requires large amounts of product, a long analysis time, has only been applicable to ^{2}H so far, is restricted to molecules of relatively low molecular weight (<300), but produces multiple parameters by measuring different sites within the molecule simultaneously.

Very recently, we have shown the feasibility of using quantitative ^{13}C NMR for measuring the absolute site-specific ^{13}C content at natural abundance. Methodological conditions have been established for sufficiently high trueness and precision to make routine measurements possible at natural abundance [16]. Thus, the ^{13}C content of each carbon can be determined with a long-term repeatability of the order of 1‰ on the absolute isotopic deviation scale δ (‰) [17]. This is accurate enough to observe natural variation from a statistical distribution in the internal $^{13}C/^{12}C$ ratio, i.e. quantification of each ^{13}C isotopomer of a given molecule. Consequently, isotopic ^{13}C NMR is becoming a tool of interest for discriminating the origin of natural molecules [18] and for studying isotope effects [19]. The isotopic composition of multistep-synthesized products, such as active pharmaceutical ingredients (API), depends on: (i) the isotopic composition of the raw materials, (ii) the types of chemical reaction involved (for example, esterification induces no significant isotope fractionation, while oxidation does [20,21]), (iii) the yield of each reaction (yields significantly below 100% are susceptible to creating an isotope effect during the transformation of a substrate to a product), (iv) the purification step (distillation and chromatographic separation generate normal or inverse isotope effects when not complete [19] while recrystalization is neutral [22]). Therefore, it can be expected that each API batch has a highly specific isotopic composition leading to a characteristic isotopic fingerprint. It can also be predicted that when more isotopic parameters are available from the API, then a more reliable characterization will be achieved. The goal of the present work is to illustrate this on two "common" over-the-counter medicines: aspirin and paracetamol, the APIs of which are acetylsalicylic acid and acetaminophen, respectively (Scheme 1). ^{13}C NMR spectrometry gives access in the former to nine independent parameters, which are the $\delta^{13}C$ values of the nine carbon atoms of the molecule. In the latter, although there are eight carbons, only six $\delta_i{}^{13}C$ values are obtained due to the symmetry in the acetaminophen structure. This resolution compares very favorably to only one global $\delta^{13}C$ value as measured by IRMS ($\delta_g{}^{13}C$).

Table 1
Identification of the aspirin samples according to their origin.

Sample number	Manufacturer	Supplier	Country where purchased
1	Bayer Germany	Bayer	France
2		3M Santé France	France
3		Sanofi- Synthelabo	Spain
4		Bayer	Greece
5		Pharmachem	Great Britain
6	Bayer USA	Bayer	USA
7		Albertsons Inc.	USA
8	Bayer Turkey	Bayer	Turkey
9[a]		Polpharma	Poland
10		Bayer	Italy
11	Perrigo USA		Poland
12	Boots Company PLC GB	Boots	Great Britain
13	Boots Company PLC GB	Boots	Great Britain
14			USA
15		Laboratory synthesis	Germany
16	Bayer Germany	Bayer	Poland
17	Bayer Mexico	Bayer	Mexico
18[b]		Woolworths	Australia
19[b]		Woolworths	Australia
20[a]		Polpharma	Poland

[a] Same supplier, but batch N° is different.
[b] Same batch, but from different packaging.

2. Experimental

2.1. Sample collection

Twenty samples of aspirin and 16 samples of paracetamol were bought from pharmacies in several countries. The supplier, manufacturing pharmaceutical company indicated on the packaging (when known) and country of purchase are given in Tables 1 and 2 for aspirin and paracetamol samples, respectively. The numbering of the samples has been randomized to emphasize the individual aspect of the isotope fingerprint.

2.2. Chemicals

Hexadeuterated dimethylsulfoxide (DMSO-d_6) was purchased from Eurisotop (http://www.eurisotop.fr). Chloroform was purchased from VWR (http://fr.vwr.com). Ethanol (99.9%) was supplied by Docks Des Alcools (France).

Table 2
Identification of the paracetamol samples according their origin.

Sample number	Manufacturer	Supplier	Country where purchased
1	Bristol Myers Squibb	Bristol Myers Squibb	France
2		Herbapol	Poland
3	M&A Pharmachem	Christy Products	Great Britain
4		Kern Pharma	Spain
5	GlaxoSmithKline Ireland	GlaxoSmithKline	Mexico
6	GlaxoSmithKline Ireland	GlaxoSmithKline	Greece
7		Polfa-Lodz SA	Poland
8		Aflofarm Fabryka Lekow	Poland
9		GlaxoSmithKline	Poland
10	US Pharmacia	US Pharmacia	Poland
11		Biofarm	Poland
12	Aventis	Théraplix	France
13	Opodex-Industrie	Biogaran	France
14	SMB Technology Belgium	Biogaran	France
15	Boots Company PLC	Boots	Great Britain
16		Atabay	Turkey

2.3. Extraction—purification protocol

The same method was applied to aspirin and to paracetamol. A number of tablets or capsules sufficient to contain 1 g of API were powdered and/or blended. Boiling ethanol (20 mL) was added to the solid and the suspension was filtered through a fritted glass funnel to remove the major excipients. The resulting filtrate was evaporated to dryness using a rotary evaporator. The residue was taken into a minimum volume of ethanol and recrystalized: the crystals were collected by filtration through a fritted glass funnel, dried overnight at 70 °C and further dried under vacuum over P_2O_5. The extraction yield could be as low as 40%, but since there is no isotopic fractionation using solvent extraction [22 and references therein], the purity of the final product was considered as the primary criterion.

2.4. IRMS measurements

The global value of $\delta_g{}^{13}C$ (‰) for compounds of interest was determined by encapsulation and analysis using AE-IRMS with an AE Flash HT coupled with a spectrometer Delta-V Advantage (ThermoFinnigan, http://www.thermo.com). Between 1 and 2.5 mg of compound was sealed in a tin capsule and the $\delta^{13}C$ determined by reference to a working standard of glutamic acid standardized against calibrated international reference material (IAEA-N1 or IAEA-N2; IAEA, Vienna) [23]. The results are expressed relative to the international reference PeeDee Belemnite (PDB), now designated V-PDB.

2.5. NMR spectroscopy experiments

Samples were prepared as follows: for acetylsalicylic acid, 300 mg dissolved in 400 μL DMSO-d_6 + 200 μL $CHCl_3$; for acetaminophen, 250 mg dissolved in 600 μL DMSO-d_6. The solution was then carefully filtered into a 5 mm o.d. tube.

Quantitative ^{13}C NMR spectra were recorded using 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 303 K. The experimental parameters for ^{13}C NMR spectral acquisition were the following: pulse width 4.3 μs (90°), sampling period 0.7 s. The offsets for both ^{13}C and ^{1}H were set at the middle of the frequency range for each molecule. For acetylsalicylic acid: 152 scans using a repetition delay of 64 s leading to a signal-to-noise ratio (SNR) ≈500. For acetaminophen: 232 scans using a repetition delay of 30 s leading to an SNR ≈750. Inverse-gated decoupling techniques were applied in order to avoid NOE. The decoupling sequence employed an adiabatic pulse with appropriate phase cycles, as described in [24]. Each measurement consisted of the average of five independently recorded NMR spectra.

Free induction decay was submitted to an exponential multiplication inducing a line broadening of 1.9 and 1.6 Hz for acetylsalicylic acid and acetaminophen, respectively. The curve fitting was carried out with a Lorentzian mathematical model using Perch Software (Perch NMR Software, University of Kuopio, Finland).

2.6. Isotopic data processing

The isotopic distribution in a molecule is characterized by the actual ^{13}C molar fraction f of a specific site i: $f_i = S_i/S_T$ where S_i is the area of the ^{13}C NMR signal of i and S_T is the sum of the areas of all the signals for the molecule. Each S_i is corrected in order to compensate for intensity losses due to satellite signals caused by ^{13}C–^{13}C coupling by multiplying by $(1 + n0.011)$ where n is the number of carbons directly connected to carbon site i, and 1.1% is the mean natural abundance of ^{13}C (see [25] for a detailed explanation). Thus, the corrected ^{13}C molar fraction $f_{i(c)}$ for a specific site i is expressed as: $f_{i(c)} = (1 + n0.011)S_i/S_T$. The statistical molar fraction for the distribution of ^{13}C in the molecule is designated as F_i (i.e. the molar fraction if no site-specific variation has been introduced). Hence, the deviation in the site-specific isotopic distribution from the statistical distribution (Δf_i), is the reduced molar fraction defined as: $\Delta f_i = f_{i(c)}/F_i$. From ($\Delta f_i$) using the global value for $\delta_g{}^{13}C$ (‰) obtained by IRMS, the $\delta_i{}^{13}C$ (‰) for each peak can be calculated as follows: (i) the isotopic abundance ($^{13}C/(^{13}C + {}^{12}C)$) of each NMR peak (carbon) A_i is defined from the global abundance A_g: $A_i = \Delta f_i \times A_g$; (ii) A_g is determined from δ_g obtained by IRMS through: $R_g = ((\delta_g/1000) + 1) \times R_{PDB}$, where R_g is the global isotope ratio $^{13}C/^{12}C$ and R_{PDB} is the isotope ratio $^{13}C/^{12}C$ of the international reference PeeDee Belemnite ($R_{PDB} = 0.0112372$) [23]; (iii) $R_{PDB} = (A_{PDB})/(1 - A_{PDB})$ and $A_{PDB} = 0.01111233$; (iv) $A_g = R_g/(1 + R_g)$ and $R_i = A_i/(1 - A_i)$; then (v) the site-specific carbon content for each carbon i, $\delta_i = ((R_i/R_{PDB}) - 1) \times 1000$ (‰).

3. Results and discussion

3.1. Isotopic ^{13}C NMR spectrometry

Since isotopic ^{13}C NMR is an emerging methodology, a summary of the approach is considered pertinent. The ^{13}C equivalent to the ^{2}H-SNIF-NMR method has been established relatively recently because of difficulties associated with the high analytical performance required for site-specific ^{13}C content ($\delta_i{}^{13}C$). The variation in the ^{13}C content of natural products is within a range of about 5%, compared with 50% for ^{2}H. Therefore, small changes of the order of 0.1% have to be detected, i.e. the method must be able to detect a difference of 1‰ in the peak areas, a real challenge for the spectroscopist. To reach such a performance in terms of precision and trueness, a detailed study of the sources of error in the methodology and instrumentation has been required. Proton broad-band decoupling, found to be the most significant source of error [26], was minimized by setting an adiabatic decoupling procedure [24]. The need for an internal reference for the calculations of the site-specific ^{13}C deviations was overcome by using the global ^{13}C content determined by IRMS, and the molar fractions of the ^{13}C NMR peak areas. A correction for the ^{13}C–^{13}C satellite signals also has to be taken into account [25] (see Section 2). The whole methodology shows a good internal reproducibility on compounds at natural abundance ^{13}C, such as vanillin and ethanol [17]. Once instrumental stability is achieved through appropriate decoupling, the improvement in precision is then solely based on the SNR and therefore on the analysis time. A large reduction in NMR time is obtained by the addition of relaxation agents, which decrease the T1 relaxation times of carbons in the molecule without affecting the accuracy [16].

This methodology was applied to aspirin and paracetamol samples extracted from various over-the-counter sources (Tables 1 and 2). The attribution of the ^{13}C chemical shifts in each spectrum of the purified compounds, acetylsalicylic acid and acetaminophen, was made using data from the literature and from two-dimensional NMR spectra. The numbering of the carbon atoms (decreasing ^{13}C chemical shift) is shown in Scheme 1. To ensure that the internal reproducibility previously established [17] is maintained with these analytes, a control was performed on the purified samples. Thus, the repeatability was assessed in both the preparation part, by analyzing three replicate purifications from the same batch for acetylsalicylic acid, and the instrumental part by performing three NMR measurements on the same acetaminophen sample (Table 3). It is shown that the standard deviation (SD) from the mean value is similar to that found for vanillin from the internal reproducibility [17]: <1.5 ‰, for an SNR ≈500. Furthermore, a careful inspection of the ^{13}C NMR spectra confirmed the absence of impu-

Table 3
Mean isotopic value ($\delta^{13}C$) for each carbon (see Scheme 1 for carbon numbering) over three measurements by ^{13}C NMR spectrometry from three independent purifications from the same batch for acetylsalicylic acid and from three NMR replicates from the same sample for acetaminophen. The standard deviation (SD) from the 3 determinations is expressed on the δ-scale (‰).

	$\delta^{13}C$ (‰)								
	C1	C2	C3	C4	C5	C6	C7	C8	C9
Acetylsalicylic acid	−50.1	−35.6	−34.2	−27.3	−29.1	−30.8	−25.3	−25.1	−38.0
SD (‰)	1.0	0.8	0.7	1.4	0.3	0.5	0.5	1.2	0.9
Acetaminophen	−23.4	−41.0	−24.2	−24.1	−21.8	−28.6			
SD (‰)	0.9	1.0	0.4	0.2	0.2	0.6			

rities, which would falsify the results if their signals were to overlap with those of the analyte molecule.

3.2. Site-specific ^{13}C content in aspirin and paracetamol samples

The site-specific ^{13}C content ($\delta_i{}^{13}C$) measured by ^{13}C NMR and the global ^{13}C content ($\delta_g{}^{13}C$) measured by IRMS are presented for acetylsalicylic acid and acetaminophen samples in Tables 4 and 5, respectively. As can be seen, the $\delta_g{}^{13}C$ values are comprised within a SD of about 3‰ for both compounds. In contrast, when $\delta_i{}^{13}C$ values are examined, a much larger variability is seen for both acetylsalicylic acid and acetaminophen, both for inter-carbon-position comparison and for inter-sample comparison. For the latter, sites C1, C2 and C9 for acetylsalicylic acid and C1, C3 and C6 for acetaminophen show particularly wide ranges. Even if these values show significance only for the pool of samples studied, it is clear that individual $\delta_i{}^{13}C$ contents show a much greater variation than $\delta_g{}^{13}C$ values. The isotopic characterization of each compound, and therefore of each manufactured batch of API, is emphasized by the availability of these very variable parameters.

The remaining carbons have SDs similar to or lower than the global value: C3–C8 for acetylsalicylic acid, C2, C4 and C5 for acetaminophen. It is notable that these are the carbons that constitute the aromatic ring, the wider ranges being found in the carbon positions in the substituent groups (Scheme 1). Hence, while the latter could be used for discriminating between batches, the former indicate constancy in the ^{13}C composition of the raw material, consistent with the probable involvement of phenol as the ini-

Table 5
Isotopic value ($\delta^{13}C$ in ‰) for each carbon (C1–C6 Scheme 1) measured by ^{13}C NMR spectrometry and the global value determined by IRMS for acetaminophen samples from sources as described in Table 2. The mean and the standard deviation (SD) values were calculated from the 16 samples.

Sample number	$\delta^{13}C$ (‰)						
	C1	C2	C3	C4	C5	C6	Global
1	−23.4	−41.0	−24.2	−24.1	−21.8	−28.6	−26.1
2	−43.8	−34.8	−28.3	−23.4	−22.3	−35.4	−29.2
3	−27.2	−36.2	−27.7	−23.9	−22.0	−32.9	−27.0
4	−26.1	−31.9	−37.0	−25.5	−25.6	−33.3	−28.8
5	−41.5	−33.2	−29.1	−22.6	−21.5	−48.7	−30.1
6	−25.5	−38.2	−26.9	−23.9	−22.4	−32.0	−26.9
7	−68.8	−34.0	−28.0	−24.4	−23.0	−48.5	−34.3
8	−27.1	−31.5	−37.2	−23.5	−24.4	−35.0	−28.3
9	−70.3	−35.9	−30.2	−22.6	−22.5	−49.0	−34.4
10	−25.5	−30.9	−36.9	−24.7	−25.3	−34.0	−28.4
11	−28.4	−29.4	−36.2	−24.3	−24.8	−31.8	−28.0
12	−26.0	−41.7	−24.9	−22.2	−19.8	−30.9	−25.9
13	−70.1	−32.1	−29.5	−26.2	−24.7	−48.4	−35.2
14	−26.3	−30.9	−37.0	−24.7	−24.5	−33.3	−28.2
15	−28.9	−38.7	−28.8	−23.4	−22.4	−15.2	−25.4
16	−27.5	−33.5	−31.7	−24.0	−23.9	−34.5	−27.9
Mean	−36.7	−34.7	−30.8	−24.0	−23.2	−35.7	−29.0
SD	17.3	3.8	4.6	1.0	1.6	9.0	3.1

Table 4
Isotopic value ($\delta^{13}C$ in‰) for each carbon (C1–C9, Scheme 1) measured by ^{13}C NMR spectrometry and the global value determined by IRMS for acetylsalicylic acid samples from sources as described in Table 1. The mean and the standard deviation (SD) values were calculated from the 20 samples.

Sample number	$\delta^{13}C$ (‰)									
	C1	C2	C3	C4	C5	C6	C7	C8	C9	Global
1	−50.1	−35.6	−34.2	−27.3	−29.1	−30.8	−25.3	−25.1	−38.0	−32.8
2	−54.8	−16.3	−33.4	−26.9	−29.3	−30.1	−25.9	−26.1	−49.1	−32.4
3	−52.5	−13.1	−33.6	−25.1	−28.1	−30.1	−27.8	−24.3	−45.4	−31.1
4	−51.9	−35.1	−34.7	−25.2	−26.7	−30.7	−25.3	−24.7	−40.7	−32.8
5	−55.0	−23.0	−35.7	−23.8	−24.9	−27.0	−24.9	−21.2	−44.8	−31.1
6	−40.3	−27.2	−32.8	−26.1	−26.8	−29.4	−27.9	−25.8	−37.2	−30.4
7	−51.4	−38.0	−32.7	−26.4	−28.6	−27.2	−24.2	−23.4	−42.8	−32.8
8	−38.8	−14.2	−31.2	−29.0	−29.4	−29.6	−26.2	−26.9	−39.0	−29.4
9[a]	−56.3	−31.5	−35.9	−28.0	−27.2	−29.4	−26.6	−23.6	−45.0	−33.7
10	−53.7	−37.7	−34.6	−23.6	−25.9	−27.8	−25.8	−22.1	−48.0	−33.2
11	−37.0	−9.3	−35.2	−25.8	−26.7	−28.3	−27.1	−22.9	−44.5	−28.5
12	−60.1	−22.8	−34.6	−24.2	−27.9	−26.1	−26.7	−24.0	−48.4	−32.8
13	−59.4	−16.1	−36.0	−25.1	−26.9	−28.8	−26.2	−23.6	−42.3	−31.6
14	−59.2	−31.2	−35.7	−21.6	−24.5	−25.9	−24.3	−19.0	−49.5	−32.3
15	−37.5	−27.6	−22.6	−27.3	−27.6	−27.8	−30.2	−18.3	−18.6	−26.4
16	−54.1	−35.2	−35.2	−27.9	−29.3	−29.7	−25.8	−24.5	−43.1	−33.9
17	−46.2	−29.3	−34.5	−26.0	−27.6	−29.6	−23.9	−24.8	−38.6	−31.1
18[b]	−17.3	−29.9	−35.2	−22.8	−24.2	−25.3	−22.4	−19.3	−32.0	−25.4
19[b]	−15.8	−28.6	−35.7	−23.6	−25.3	−26.3	−22.3	−20.3	−30.4	−25.3
20[a]	−38.1	−34.4	−34.2	−26.7	−30.1	−29.4	−27.8	−23.0	−34.0	−38.1
Mean	−46.5	−26.8	−33.9	−25.5	−27.3	−28.5	−25.9	−23.2	−40.5	−30.9
SD	12.8	8.8	2.9	1.9	1.7	1.7	1.9	2.5	7.6	2.6

[a] Same supplier "Polpharma", but batch N° different.
[b] Same batch, but from different packaging.

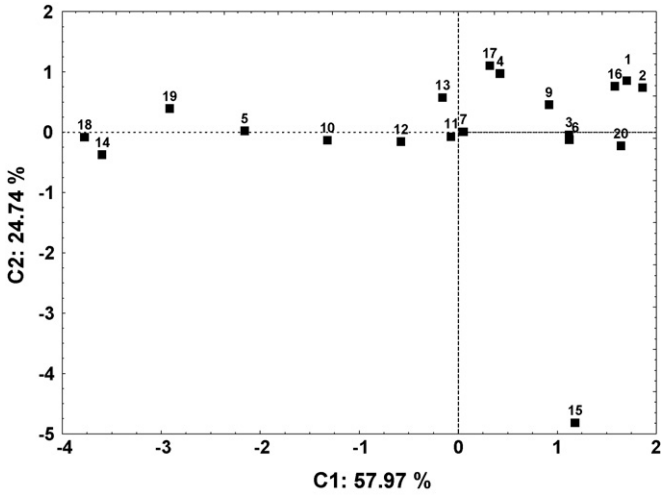


Fig. 1. Principal component analysis (PCA) on the acetylsalicylic acid data set using the δ^{13}C of the aromatic carbons C3–C8 as variables: component 1 (C1) vs. component 2 (C2). Note that sample N° 15 is very atypical.

tial starting material in the synthetic process for both molecules. Even so, some variability exists that could help distinguish different batches of phenol used in the synthesis. This is illustrated by Figs. 1 and 2, where PCA were performed on the aromatic $\delta_i{}^{13}$C as variables (there are 6 and 4 for acetylsalicylic acid and acetaminophen, respectively). It is clear that, even using only the ^{13}C content of each aromatic carbon, each sample is individually characterized.

Nevertheless, the main difference between samples for both acetylsalicylic acid and acetaminophen lies in the side chains. In particular, the acetyl group, which is added during synthesis, shows the widest variation. This isotopic discrimination could be due to a number of parameters, including the origin of the acetate/acetic acid used (biotechnological or fossil source) and fractionation introduced during the chemical reaction via kinetic and/or thermodynamic isotope effects [27]. Three features of particular interest can be highlighted. First, in both compounds, the mean values of the ^{13}C content $\delta_{acet}{}^{13}$C of the acetyl groups are −43.5‰ (SD = 9.5) and −36.2‰ (SD = 12.5) for aspirin and paracetamol, respectively. These values fall within the range usually observed for acetic acid synthesized from natural gas (about −45‰) and petrochemical sources (about −26‰). In contrast, for aspirin samples 18 and 19 (Table 3), a natural origin may be suggested as the values lie within the range of biotechnologically produced acetic acid [28]. Secondly, some carboxyl carbons are very impoverished, having values in the −50 to −70‰ range. These are likely to reflect a fossil source. Thirdly, the insertion of the C2 of acetylsalicylic acid by the carboxylation of phenol is a potentially important source of variability, which will depend on the origin of CO_2 and the reaction yield.

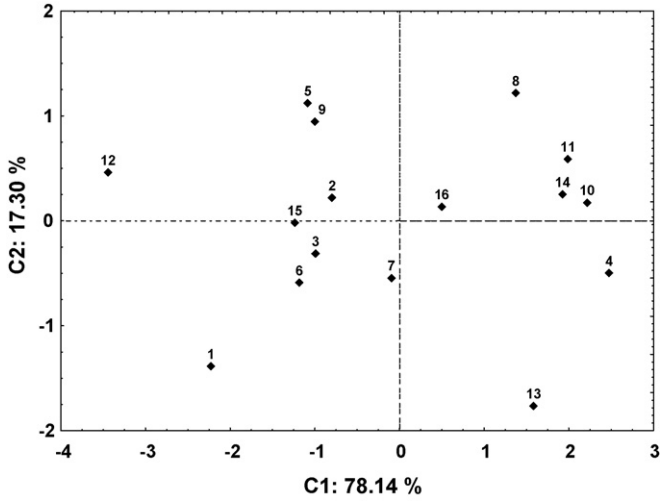


Fig. 2. Principal component analysis (PCA) on the acetaminophen data set using the δ^{13}C of the aromatic carbons C2–C5 as variables: component 1 (C1) vs. component 2 (C2).

4. Conclusions

The present work shows the power of quantitative isotopic ^{13}C NMR to define the internal site-specific isotopic profile of a pharmaceutically active substance. As the measurement is made on the core structure of the molecule, there is an intrinsic difficulty in manipulating the values measured fraudulently (see [29] for a development of this problem). Indeed, the isotopic fingerprint can be seen to be individual to each batch, as illustrated by comparing aspirin samples 9 and 20, which are from the same supplier but from different batches. Conversely, the same batch from different packaging shows the same isotope profile, as seen for aspirin samples 18 and 19 (Tables 1 and 4). Hence, this method has the potential to make a major contribution to the arsenal of isotopic techniques available for detecting counterfeiting and patent infringement in the pharmaceutical industry.

The present work effectively demonstrates the feasibility of the approach. Nevertheless, further improvements may be envisaged in three areas: (i) reduction of the NMR analysis time by using relaxation agents or by establishing new pulse sequences (DEPT, INEPT or HCP) that enhance sensitivity [30–32]; (ii) differentiation of batches produced by different synthetic pathways (measurements of the isotopic profiles of the starting materials and of the fractionation associated with differing synthetic routes); (iii) application of a variety of chemometric tools with which to link the isotope data to the processes.

Acknowledgements

The following are thanked for providing samples of aspirin and/or paracetamol: John Hamill and Jane King (Australia), Piotr Paneth, Wieslaw Oleszek and Renata Kwiecień (Poland), Fatima Tosun (Turkey), Maike Petersen (USA and Germany), Vassilios Roussis (Greece), Cristina García-Viguera (Spain). We thank Carol Wrigglesworth (Scientific English, Nantes, France) for linguistic correction.

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