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Technical Note

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Internal Referencing for ^{13}C Position-Specific Isotope Analysis Measured by NMR Spectrometry

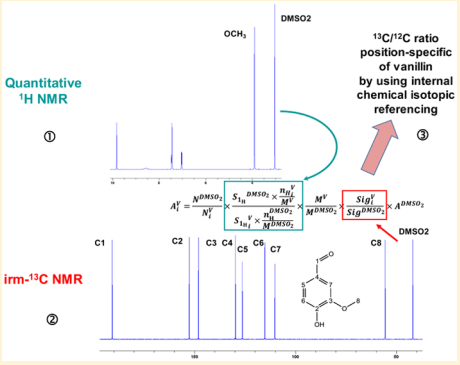
Kevin Bayle,[†] Mathilde Grand,[†] Alain Chaintreau,[‡] Richard J. Robins,[†] Wolfgang Fieber,[‡] Horst Sommer,[‡] Serge Akoka,[†] and Gérald S. Remaud*[,†]

[†]EBSI Team, Interdisciplinary Chemistry: Synthesis, Analysis, Modeling (CEISAM), University of Nantes-CNRS UMR 6230, 2 Rue de la Houssinière, BP 92208, F-44322, Nantes Cedex 3, France

[‡]Firmenich SA, Corporate R&D Division, P.O. Box 239, 1211 Geneva 8, Switzerland

Supporting Information

ABSTRACT: The intramolecular ^{13}C composition of a molecule retains evidence relevant to its (bio)synthetic history and can provide valuable information in numerous fields ranging from biochemistry to environmental sciences. Isotope ratio monitoring by ^{13}C NMR spectrometry (irm-^{13}C NMR) is a generic method that offers the potential to conduct ^{13}C position-specific isotope analysis with a precision better than 1‰. Until now, determining absolute values also required measurement of the global (or bulk) ^{13}C composition ($\delta^{13}C_g$) by mass spectrometry. In a radical new approach, it is shown that an internal isotopic chemical reference for irm-^{13}C NMR can be used instead. The strategy uses ^{1}H NMR to quantify both the number of moles of the reference and of the studied compound present in the NMR tube. Thus, the sample preparation protocol is greatly simplified, bypassing the previous requirement for precise purity and mass determination. The key to accurate results is suppressing the effect of radiation damping in ^{1}H NMR which produces signal distortion and alters quantification. The methodology, applied to vanillin with dimethylsulfone as an internal standard, has an equivalent accuracy (<1‰) to that of the conventional approach. Hence, it was possible to clearly identify vanillin from different origins based on the ^{13}C isotopic profiles.



NMR spectrometry provides access to position-specific isotope analysis (PSIA) because of its double intrinsic property: separation of the signal for each site of the molecule and quantification of the amount of resonating nuclei leading to the peak signal. Originally developed for the measurement of ^{2}H isotope ratios during the 1980s (see refs 1 and 2 for a review), the technique was subsequently developed for the determination of each ^{13}C isotopomer in a given molecule ($\delta^{13}C_i$).[3] The principle of isotope ratio monitoring by ^{2}H NMR (irm-^{2}H NMR) is based on the comparison of the integrals of the areas under the peak for ^{2}H signals for both the molecule of interest and an internal standard present in the sample. However, the transposition of this protocol to irm-^{13}C NMR has proved impractical due to the much lower isotopic variation for ^{13}C in natural compounds: a range of ∼50‰ as against ∼500‰ for ^{2}H on the isotopic composition δ-scale. Thus, the level of precision required for $\delta^{13}C_i$ determination (1‰, per mil or 0.1%) is 10-fold that for $\delta^{2}H_i$ (10‰, per mil or 1%).[4] Consequently, a chemical internal reference for irm-^{13}C NMR would require: (i) that the purity of the studied molecule and of the reference was determined with a precision better than 1‰ and (ii) that the masses used during the preparation of the NMR tube were measured with an accuracy (defined as the contribution of trueness and precision[5]) better than 1‰. While the latter could be achieved by trained operators, the former is a difficult task in the framework of a routine analysis.

PSIA by NMR has proven valuable in interpreting a number of (bio)chemical[6−11] and physicochemical[12−14] processes, leading to a deeper understanding than previously possible of the underlying phenomena causing isotope fractionation in nature. Prior to the development of imr-^{13}C NMR, PSIA had only been realized by isotope ratio monitoring by mass spectrometry (irm-MS) involving tedious chemical or enzymatic degradations to fragment the molecule, then separation and analysis of the products to give the site specific ^{13}C composition.[15−18] Recently, this has been achieved directly for small molecules such as acetic acid and ethanol[19,20] by the use of a hyphenated technique, irm-Pyrolysis/GC/MS,[21,22] wherein pyrolysis is used to degrade molecules into small volatile fragments, which are separated online by gas chromatography and analyzed separately. Despite the determination of only the global (or bulk) ^{13}C content ($\delta^{13}C_g$) for most molecules, irm-MS has the advantage of a calibration with international reference standards.[23]

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In contrast, irm-^{13}C NMR, while offering much better access to isotopomer composition in a target analyte, has the disadvantage of not being directly linked to referenced international standards. With no internal standard, the current strategy to access the position-specific ^{13}C values ($\delta^{13}C_i$) consists of the determination of the reduced molar fraction f_i/F_i of ^{13}C for each carbon position (for further information on these parameters see ref 24). This is performed by curve fitting based on a total-line shape analysis to obtain the area under each peak. The ratios f_i/F_i are then combined with $\delta^{13}C_g$ obtained by irm-MS to give $\delta^{13}C_i$ (see the Supporting Information for definitions and examples of calculations). This approach has two main drawbacks: (i) the need for both NMR and irm-MS instrumentation and (ii) it fails when all peaks in the NMR spectrum are not well resolved, the integration of each peak is then not possible making the f_i/F_i ratio nonoperating, i.e., not even partial $\delta^{13}C_i$ values can be determined. Thus, the expression of the results on the international isotope composition δ-scale requires an additional measurement, inevitably a source of undesirable error. The aim of the work herein presented was to develop a strategy allowing the determination of $\delta^{13}C_i$ directly on the NMR sample by use of a chemical internal isotopic reference. The two main goals of the present works were (i) to estimate the accuracy of $\delta^{13}C_i$ determination with the aid of the internal reference and (ii) to verify the capability of the protocol to discriminate accurately representative ^{13}C profiles of compounds from different origins. The development of this methodology has been demonstrated and validated using vanillin, a molecule possessing a wide spectral bandwidth, as a molecular probe and dimethylsulfone ($DMSO_2$) as a calibrated internal reference.[25]

EXPERIMENTAL SECTION

Quantitative ^{13}C NMR spectra were recorded at 100.6 MHz using a Bruker 400 NMR spectrometer fitted with a 5 mm i.d. probe: Avance I with a dual ^{1}H/^{13}C dual$^+$ probe. Further details on the origins of chemicals, the NMR pulse sequences, the NMR acquisition conditions, and the isotopic calculations used in this work are given in the Supporting Information.

RESULTS AND DISCUSSION

The calculation of $\delta^{13}C_i$ with an internal reference can be retrieved from eq 1 (for convenience the actual products used are indicated: $DMSO_2$ as reference and vanillin as analyte, but it applies to any other appropriate chemicals), which is the equivalent for ^{13}C NMR to that described in the official method employed for detecting wine chaptalization by ^{2}H NMR.[26] The isotopic abundance A or the isotopic ratio R is easily converted to the isotopic composition δ (see the Supporting Information).

$$A_i^{V} = \frac{N^{DMSO_2}}{N_i^{V}} \times \frac{m^{DMSO_2} \times P_m^{DMSO_2\%}}{m^{V} \times P_m^{V\%}} \times \frac{M^{V}}{M^{DMSO_2}} \times \frac{Sig_i^{V}}{Sig^{DMSO_2}} \times A^{DMSO_2} \quad (1)$$

where (same symbols for eqs 2–4): A^{DMSO_2}, ^{13}C isotopic abundance of $DMSO_2$; A_i^V, ^{13}C isotopic abundance of carbon i of vanillin; N^{DMSO_2}, number of carbon associated with $DMSO_2$; N_i^V, number of carbon associated with site i of vanillin; m^{DMSO_2}, mass of $DMSO_2$; m^V, mass of vanillin; $P_m^{DMSO_2\%}$, purity grade of $DMSO_2$; $P_m^{V\%}$, purity grade of vanillin; M^V, molecular mass of vanillin; M^{DMSO_2}, molecular mass of $DMSO_2$; Sig_i^V, ^{13}C signal area of site i of vanillin; Sig^{DMSO_2}, ^{13}C signal area of site i of $DMSO_2$.

To exploit directly eq 1, the purity of vanillin and $DMSO_2$ both need to be known with a precision better than 1‰, so that m^{DMSO_2} and m^V do not introduce significant error. While the masses can be accurately obtained with an adequate balance, a similarly accurate knowledge of the purity could be more challenging. While a certificate of analysis is available for the $DMSO_2$, which has an internal reference status in quantitative NMR,[27] it is extremely difficult to achieve a similar purity assessment for analyte samples extracted from natural sources.

In order to circumvent these constraints, a strategy has been established by which the concentration ratio of both compounds present (analyte and standard) are obtained by ^{1}H NMR. Previous studies performed in relation to irm-^{2}H NMR demonstrated the feasibility of such an *in situ* method,[28] and it is used for current analyses of vinegars.[29] Thus, it has been demonstrated that the mass/purity ratio K' of both standard and compound (eq 2) is equal to ratio K (eq 3), corresponding to the concentration ratio obtained by ^{1}H NMR,

$$K' = \frac{m^{DMSO_2} \times P_m^{DMSO_2\%}}{m^{V} \times P_m^{V\%}} \quad (2)$$

$$K = \frac{S_{^1H}{}^{DMSO_2} \times \frac{n_{H_i}^{V}}{M^{V}}}{S_{^1H_i}^{V} \times \frac{n_H^{DMSO_2}}{M^{DMSO_2}}} \quad (3)$$

providing that appropriate quantitative NMR spectral acquisition conditions are met. It is then possible to incorporate K instead of K' within eq 1 for the determination of A_i^V to give eq 4.

$$A_i^{V} = \frac{N^{DMSO_2}}{N_i^{V}} \times \frac{S_{^1H}{}^{DMSO_2} \times \frac{n_{H_i}{}^{V}}{M^{V}}}{S_{^1H_i}^{V} \times \frac{n_H^{DMSO_2}}{M^{DMSO_2}}} \times \frac{M^{V}}{M^{DMSO_2}} \times \frac{Sig_i^{V}}{Sig^{DMSO_2}} \times A^{DMSO_2} \quad (4)$$

While Faulh and Wittowski[28] achieved the determination of K with the precision required (1%) for irm-^{2}H NMR, the transposition of the methodology to irm-^{13}C NMR is possible only if K can be obtained with a 10-times higher precision.

Some practical considerations have to be taken into account before a potential application can be made for $\delta^{13}C_i$. The strategy requires the determination of both the concentration ratio K by ^{1}H NMR and the $\delta^{13}C_i$ by irm-^{13}C NMR on the same sample. ^{13}C NMR is not very sensitive and so to achieve 1‰ accuracy on the determination of $\delta^{13}C_i$ requires a high signal-to-noise ratio, SNR (typically SNR > 600:1).[4] Therefore, it is required for a realistic analysis time to work with a highly concentrated sample. The much higher sensitivity of ^{1}H NMR relative to ^{13}C NMR is problematic when dealing with concentrated samples, since it is then difficult to avoid signal saturation and, most importantly, the phenomenon of radiation damping.

Radiation damping is a dynamic process that results from the nonlinear coupling between the transverse magnetization and the radio-frequency (rf) coil. The precession of the transverse magnetization after an rf pulse induces an oscillating current in

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the rf coil which in turn generates a low but significant magnetic field acting upon the nuclear spins. The action of this field is to bring the system back to equilibrium more quickly than by mere relaxation phenomena (for a more detailed description of radiation damping see ref 30). The amplitude and the phase of rf pulses are thus altered and, accordingly, result in deformation/widening of the shape of peaks as well as in a shift of the relative phase of multiplets. Consequently, the accuracy of peak area measurements is greatly affected. This effect is in most cases very small and can be neglected; however, it appears significant when concentrated samples are analyzed in an intense magnetic field. Although several methods have been proposed to control radiation damping,[30] none were found appropriate to both control the effect and achieve highly precise quantification in concentrated samples. A novel ^{1}H NMR sequence named DWET (for double WET) inspired by the WET NMR sequence[31] has therefore been designed to acquire ratio concentration with a precision higher than 1‰. The DWET sequence consists of two trains of WET pulse saturating two different regions in the frequency range (see the Supporting Information). It manages to control radiation damping by reducing the spatial area and therefore the number of spins contributing to the signal detected by the receiver. Quantitative tests in which K was determined using solutions with well-defined concentrations were conclusive, with accuracy better than 1‰. This work will be described in a specialist paper.

In order to answer the question: "is it possible to implement an internal isotopic reference in irm-^{13}C NMR?" and to validate this proof of concept, a model compound was selected as well as a suitable chemical reference. Vanillin has previously been identified as a good candidate for developing irm-^{13}C NMR techniques,[32] as its properties cover the main characteristics which have to be controlled in quantitative NMR: (i) a solid with a finite solubility in organic solvents, (ii) a broad ^{1}H frequency (from 3 to 11 ppm) requiring the most efficient decoupling, (iii) a broad ^{13}C frequency range, and (iv) different and characteristic profiles can be observed as a function of its origin. Because of its exigent properties, positive results obtained for this compound would indicate that the protocol could be applied to a wide range of chemical structures of suitable molecular mass. The commercial origins used in the present work are labeled below: (i) ex-lignin originating from a semisynthetic transformation of lignin from wood and (ii) ex-bean directly extracted from vanilla pod. These two origins are the most difficult to discriminate by irm-^{13}C NMR because both have an essentially natural origin. Thus, they give a similar intramolecular ^{13}C relative profile, while the absolute $\delta^{13}C_i$ values are distinct.[32]

The criteria for both ^{1}H and ^{13}C NMR for a compound to be fit to serve as an internal standard can be divided in two categories: (a) chemical properties and (b) NMR properties. For its chemical properties (a), the suitable candidate should be (i) soluble in different NMR solvents, (ii) nonreactive, (iii) nonvolatile, and (iv) nonhygroscopic. For its NMR properties (b) it should have (i) a large number of equivalent protons and carbons, (ii) a relaxation time close to that of the analyte, and (iii) ^{1}H and ^{13}C chemical shifts in opaque zones of the analyte spectrum. Furthermore, while not a strict requirement of the strategy, an added advantage for the internal standard is that it should be easily weighable as well as having a certificate of analysis attesting to its purity. On the basis of these criteria, dimethylsulfone ($DMSO_2$), a molecule previously proposed as a universal standard for ^{1}H NMR,[25] was selected. $DMSO_2$ does not contain adjacent carbon atoms, thus does not display satellite peaks due to C−C coupling on its ^{13}C NMR spectrum. This represents an added advantage as it avoids the use of a correction factor (see the Supporting Information).

With these tools in hand, we have assessed (i) the precision and robustness of the DWET ^{1}H pulse sequence, (ii) the operator effect, (iii) the consistency of the $\delta^{13}C_i$ results obtained with an internal referencing versus the classical approach using f_i/F_i combined with the $\delta^{13}C_g$ from irm-MS, and (iv) the ability to discriminate the origin of vanillin.

The precision of the NMR contribution (^{1}H and ^{13}C) was assessed by recording five independent spectra (within-sample variability) and by repeating four times the same experiment: same compound, different preparations, and two operators. The key parameter for the performance assessment of the whole protocol lies in the comparison between the $\delta^{13}C_i$ obtained from the f_i/F_i calculation, and the $\delta^{13}C_i$ calculated from the internal reference (from eq 4). For an application in routine, this difference, named "Δ", should be lower than the standard deviation of the accuracy required for irm-^{13}C NMR: 1‰. The variability within-experiments over the five ^{1}H spectra is lower than that observed for the five ^{13}C spectra from the same tube. This is expected because SNR for the ^{13}C spectra and ^{1}H spectra is higher than 600:1 and 2000:1, respectively. Furthermore, integration of the signal in the ^{1}H spectrum was performed on well-resolved singlet peaks: the methoxyl group of vanillin and the internal reference $DMSO_2$.

The between-sample variability is dependent on the performance of the spectrometer. The instrument used for the present work has been qualified according a recent protocol:[33] thus the standard deviation for the repeatability is less than 1‰. The parameter Δ is an evaluation of the trueness of the internal referencing approach. Table 1 shows that Δ remains between the interval [−1,+1]‰: this is very satisfactory. As expected there is no position-effect on the values of Δ: they are very similar, whichever carbon of vanillin is considered (see Figure 1 for the numbering of the carbons in vanillin).

Table 1. Difference Δ (‰) Observed between $\delta^{13}C_i$ Calculated from the Classical Approach and Using the Internal Isotopic Reference for Each Carbon of Vanillin

	Δ (‰)				
	Ex-lignin				Ex-bean
carbon no.[a]	1	2	3	4	5
C-1	−0.35	−0.34	−0.92	0.92	−0.17
C-2	−0.34	−0.33	−0.91	0.91	−0.17
C-3	−0.34	−0.33	−0.91	0.90	−0.17
C-4	−0.35	−0.34	−0.92	0.91	−0.17
C-5	−0.35	−0.34	−0.92	0.91	−0.17
C-6	−0.34	−0.34	−0.92	0.91	−0.17
C-7	−0.35	−0.34	−0.92	0.92	−0.17
C-8	−0.34	−0.33	−0.90	0.89	−0.17

[a]See Figure 1 for the numbering of the carbons in vanillin.

It can therefore be deduced that for the present protocol, circumventing the need for a precise determination of the purity of both the analyte and the reference makes possible the use of an internal chemical standard for ^{13}C PSIA by irm-^{13}C NMR. Moreover, the sample preparation is simple since there is

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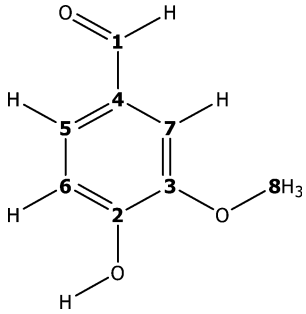

Figure 1. Molecular structure of vanillin with carbon atoms numbered on the basis of their decreasing ^{13}C chemical shift in the NMR spectrum.

no need to weigh very precisely the analyte or the reference. Application of the protocol to vanillin using $DMSO_2$ as isotopic internal standard has given results at least as accurate as those obtained by the classical method for distinguishing origins of vanillin,[6] while avoiding a number of sources of error in the classical approach.

CONCLUSION

The approach described here offers new possibilities that significantly broaden the scope of potential application of irm-^{13}C NMR in several research domains, such as plant physiology, enzyme reaction mechanisms, forensic, and environmental investigations. Critically, it makes possible the analysis of impure compounds or mixtures because accurate determination of the $\delta^{13}C_g$ on a pure analyte is no longer required. In these circumstances it is also possible to analyze congested spectra in which only resolved peaks could be measured bearing still some level of information and thus making possible the study of molecules of greater mass than can be tackled at present. This has great potential, as mixtures of compounds are often difficult or impossible to separate without causing isotopic fractionation. A calibrated internal standard could be useful as a tool to attest accuracy of both techniques (irm-MS and irm-NMR) and spectrometers employed, allowing the intercalibration of machines. Finally, as for irm-^2H NMR, the electronic referencing method[34] ERETIC is theoretically applicable to the measurement of ^{13}C isotope ratios thanks to the previously developed method.[35] The main constraint for its implementation is similar to that encountered with the internal reference: ensuring that the quality of the electronic signal is sufficiently precise and reproducible to enter into the calculation of K, eq 4. Furthermore, ongoing developments should also make it possible to apply the strategy developed for the determination of $\delta^{13}C_i$ by exploiting multipulse NMR methods such as 2D NMR. This is not possible with the currently applied strategy, as this needs the measurement of f_i/F_i of all the carbon atoms of the molecule and multipulse NMR acquisitions only allow the observation of carbon positions bearing at least one proton.[36] Similarly, emerging approaches on the monitoring of pollutants in the environment consisting of enantioselective stable isotope analysis (ESIA)[37] could be greatly refined by the proposed concept being able to determine the enantiomeric fraction and then the PSIA on each enantiomer by irm-^{13}C NMR. It should also be beneficial to other domains than isotopes, such as metabolomics or fluxomics for which ^{13}C NMR shows a steady increase in applications.[38]

ASSOCIATED CONTENT

Supporting Information

Further details on the experimental methods. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.5b02094.

AUTHOR INFORMATION

Corresponding Author

*Fax: 33 2 51 12 57 12. E-mail: gerald.remaud@univ-nantes.fr.

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Martin, G. J.; Akoka, S.; Martin, M. L. In *Modern Magnetic Resonance*; Webb, G. A., Ed.; Springer: Berlin, Germany, 2006; pp 1651−1658.
(2) Martin, G. J.; Martin, M.; Remaud, G. In *Modern Magnetic Resonance*, Webb, G. A., Ed.; Springer: Berlin, Germany, 2006; pp 1647−1658.
(3) Caytan, E.; Botosoa, E. P.; Silvestre, V.; Robins, R. J.; Akoka, S.; Remaud, G. *Anal. Chem.* **2007**, *79*, 8266−8269.
(4) Caytan, E.; Remaud, G. S.; Tenailleau, E.; Akoka, S. *Talanta* **2007**, *71*, 1016−1021.
(5) Menditto, A.; Patriarca, M.; Magnusson, B. *Accredit. Qual. Assur.* **2007**, *12*, 45−47.
(6) Botosoa, E. P.; Blumenstein, C.; MacKenzie, D. A.; Silvestre, V.; Remaud, G. S.; Kwiecień, R. A.; Robins, R. J. *Anal. Biochem.* **2009**, *393*, 182−188.
(7) Gilbert, A.; Robins, R. J.; Remaud, G. S.; Tcherkez, G. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 18204−18209.
(8) Gilbert, A.; Silvestre, V.; Robins, R. J.; Tcherkez, G.; Remaud, G. S. *New Phytol.* **2011**, *191*, 579−588.
(9) Gilbert, A.; Silvestre, V.; Segebarth, N.; Tcherkez, G.; Guillou, C.; Robins, R. J.; Akoka, S.; Remaud, G. *Plant, Cell Environ.* **2011**, *34*, 1104−1112.
(10) Thomas, F.; Randet, C.; Gilbert, A.; Silvestre, V.; Jamin, E.; Akoka, S.; Remaud, G.; Segebarth, N.; Guillou, C. *J. Agric. Food Chem.* **2010**, *58*, 11580−11585.
(11) Bayle, K.; Akoka, S.; Remaud, G. S.; Robins, R. J. *J. Biol. Chem.* **2015**, *290*, 4118−4128.
(12) Botosoa, E. P.; Caytan, E.; Silvestre, V.; Robins, R. J.; Akoka, S.; Remaud, G. S. *J. Am. Chem. Soc.* **2008**, *130*, 414−415.
(13) Botosoa, E. P.; Silvestre, V.; Robins, R. J.; Rojas, J. M. M.; Guillou, C.; Remaud, G. S. *J. Chromatogr. A* **2009**, *1216*, 7043−7048.
(14) Höhener, P.; Silvestre, V.; Lefrançois, A.; Loquet, D.; Botosoa, E. P.; Robins, R. J.; Remaud, G. S. *Chemosphere* **2012**, *87*, 445−452.
(15) Monson, K.; Hayes, J. *Geochim. Cosmochim. Acta* **1982**, *46*, 139−149.
(16) Rossmann, A.; Butzenlechner, M.; Schmidt, H.-L. *Plant Physiol.* **1991**, *96*, 609−614.
(17) Weber, D.; Kexel, H.; Schmidt, H. L. *J. Agric. Food Chem.* **1997**, *45*, 2042−2046.
(18) Weilacher, T.; Gleixner, G.; Schmidt, H.-L. *Phytochemistry* **1996**, *41*, 1073−1077.
(19) Yamada, K.; Tanaka, M.; Nakagawa, F.; Yoshida, N. *Rapid Commun. Mass Spectrom.* **2002**, *16*, 1059−1064.
(20) Hattori, R.; Yamada, K.; Kikuchi, M.; Hirano, S.; Hirano, S.; Yoshida, N. *J. Agric. Food Chem.* **2011**, *59*, 9049−9053.
(21) Corso, T.; Brenna, J. *Proc. Natl. Acad. Sci. U. S. A.* **1997**, *94*, 1049−1053.

DOI: 10.1021/acs.analchem.5b02094
Anal. Chem. 2015, 87, 7550−7554

(22) Brenna, J. T. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 1252−1262.
(23) Muccio, Z.; Jackson, G. P. *Analyst* **2009**, *134*, 213−222.
(24) Martin, G. J.; Martin, M. L. *Annu. Rep. NMR Spectrosc.* **1995**, *31*, 81−104.
(25) Wells, R.; Cheung, J.; Hook, J. *Accredit. Qual. Assur.* **2004**, *9*, 450−456.
(26) EEC. *Council Regulation No. 2676/90*, Official Journal of the European Communities 1990; *L272*, pp 64−73.
(27) Weber, M.; Hellriegel, C.; Rueck, A.; Wuethrich, J.; Jenks, P. *J. Pharm. Biomed. Anal.* **2014**, *93*, 102−110.
(28) Fauhl, C.; Wittkowski, R. *Z. Lebensm.-Unters. -Forsch. A* **1996**, *203*, 541−545.
(29) Perini, M.; Paolini, M.; Simoni, M.; Bontempo, L.; Vrhovsek, U.; Sacco, M.; Thomas, F.; Jamin, E.; Hermann, A.; Camin, F. *J. Agric. Food Chem.* **2014**, *62*, 8197−8203.
(30) Krishnan, V. V.; Murali, N. *Prog. Nucl. Magn. Reson. Spectrosc.* **2013**, *68*, 41−57.
(31) Ogg, R. J.; Kingsley, R. B.; Taylor, J. S. *J. Magn. Reson., Ser. B* **1994**, *104*, 1−10.
(32) Chaintreau, A.; Fieber, W.; Sommer, H.; Gilbert, A.; Yamada, K.; Yoshida, N.; Pagelot, A.; Moskau, D.; Moreno, A.; Schleucher, J.; Reniero, F.; Holland, M.; Guillou, C.; Silvestre, V.; Akoka, S.; Remaud, G. S. *Anal. Chim. Acta* **2013**, *788*, 108−113.
(33) Bayle, K.; Gilbert, A.; Julien, M.; Yamada, K.; Silvestre, V.; Robins, R. J.; Akoka, S.; Yoshida, N.; Remaud, G. S. *Anal. Chim. Acta* **2014**, *846*, 1−7.
(34) Remaud, G. S.; Silvestre, V.; Akoka, S. *Accredit. Qual. Assur.* **2005**, *10*, 415−420.
(35) Ziarelli, F.; Viel, S.; Caldarelli, S.; Sobieski, D. N.; Augustine, M. P. *J. Magn. Reson.* **2008**, *194*, 307−312.
(36) Martineau, E.; Akoka, S.; Boisseau, R.; Delanoue, B.; Giraudeau, P. *Anal. Chem.* **2013**, *85*, 4777−4783.
(37) Badea, S.-L.; Danet, A.-F. *Sci. Total Environ.* **2015**, *514*, 459−466.
(38) Clendinen, C. S.; Lee-McMullen, B.; Williams, C. M.; Stupp, G. S.; Vandenborne, K.; Hahn, D. A.; Walter, G. A.; Edison, A. S. *Anal. Chem.* **2014**, *86*, 9242−9250.

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Anal. Chem. 2015, 87, 7550−7554