

Carbon Isotope Site-Specific Measurement by Nuclear Magnetic Resonance in Vanillin CRM Production

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ABSTRACT

- The National Research Council of Canada is engaged in producing Certified Reference Materials (CRMs) of two vanillin materials to provide analysis tools for quality control and promote conformity of measurement.
- In this study, a combined carbon isotope monitoring by nuclear magnetic resonance and mass spectrometry (irm-NMR and irm-MS) approach was applied and successfully measured the carbon isotope position-specific content of all carbons with high accuracy in the two commercial vanillin materials.
- Detailed irm-NMR measurements, homogeneity, stability, and uncertainty were assessed in this poster.

INTRODUCTION

- Vanillin is one of the most important flavors used in the food and pharmaceutical industries.

- There are two main sources of vanillin:

(i) **Natural vanillin**: scarce product and very expensive
- obtained from **vanilla bean** and **natural precursors** [1]

(ii) **Synthetic vanillin**: large quantities and relatively cheap
- obtained from **chemical synthesis** [1]

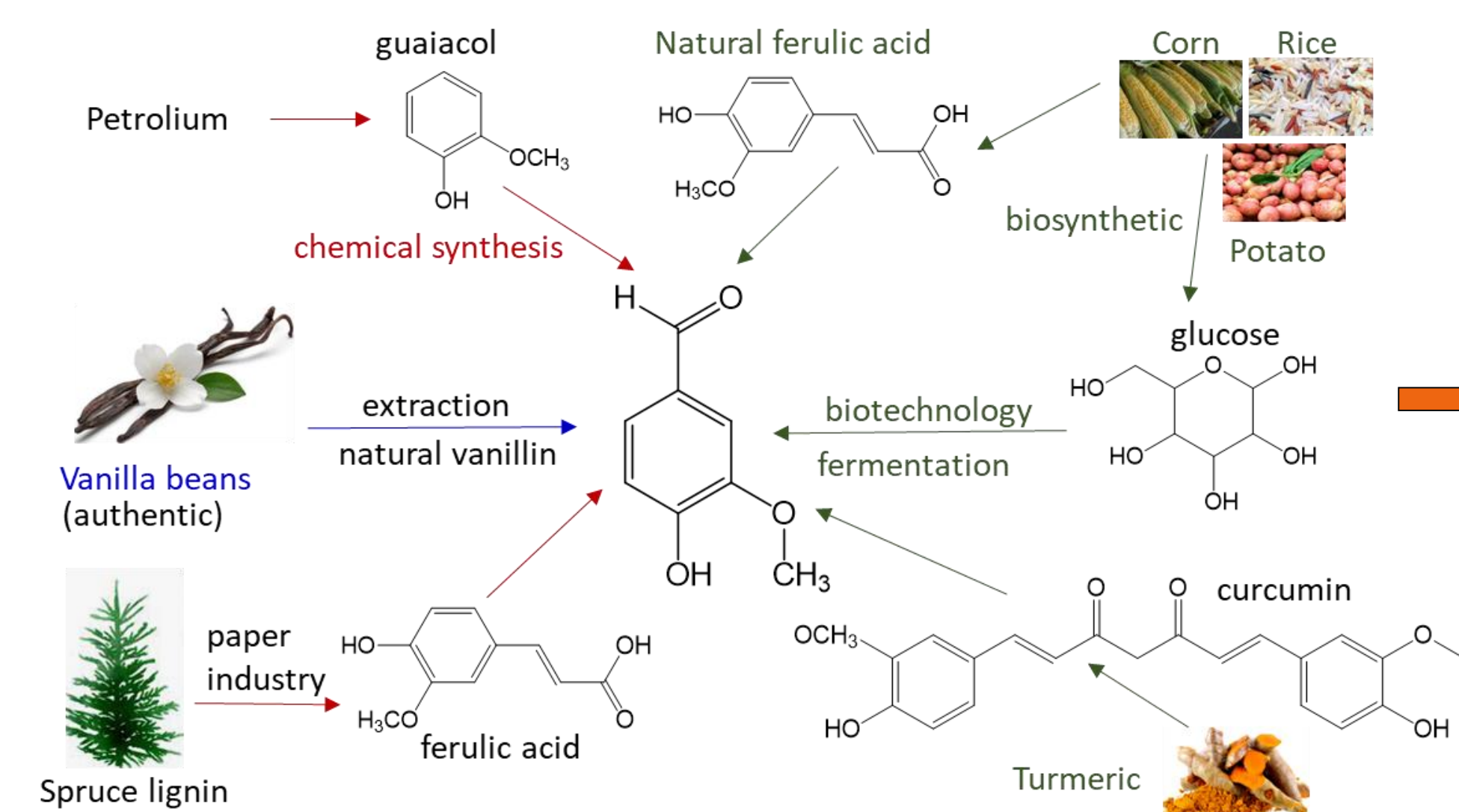


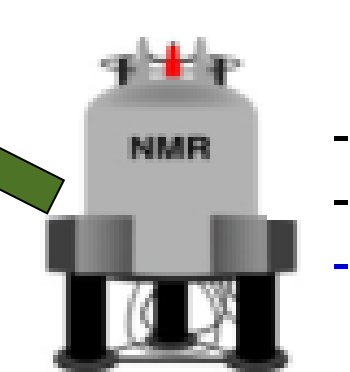
Fig1: Principal sources for the production of vanillin

- Global $\delta^{13}C_9$ of a compound
- high sensitivity
- no intramolecular information



irm-MS

Vanillin authentication



irm-NMR

- site-specific $^{13}C_i$ molar fractionation
- less sensitivity
- intramolecular information

- Can authenticate natural, biosynthetic and chemically synthetic vanillin

- No authentication of bio- or chemical- synthetic vanillin obtained from the same precursor
- Limitation for detection of adulterated natural vanillin

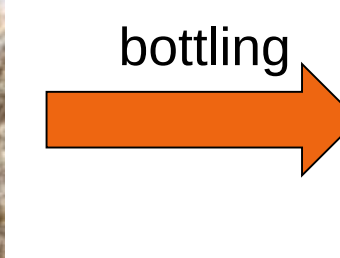
- Absolute $\delta^{13}C_i$ values established and traced to the $\delta^{13}C_9$ by irm-MS
- Authentication of vanillin from different origins
- Can detect vanillin adulteration via addition of synthetic vanillin to natural vanillin.

➡ We are interested in using a combined irm-MS & irm-NMR method for production of vanillin isotopic reference materials

MATERIAL



2 commercial vanillin materials



bottling



VANA-1 $\xrightarrow{\text{irm-MS}}$ $\delta^{13}C_9$: - 31.31 $\pm$ 0.08 ‰
10 vials, n=3

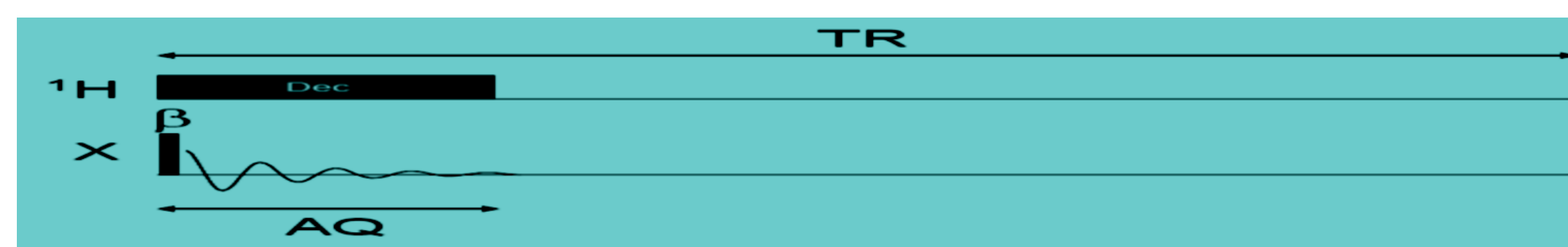
VANB-1 $\xrightarrow{\text{irm-MS}}$ $\delta^{13}C_9$: - 25.87 $\pm$ 0.08 ‰
10 vials, n=3

WORK FLOW OF irm- ^{13}C -qNMR APPLIED ON VANILLIN

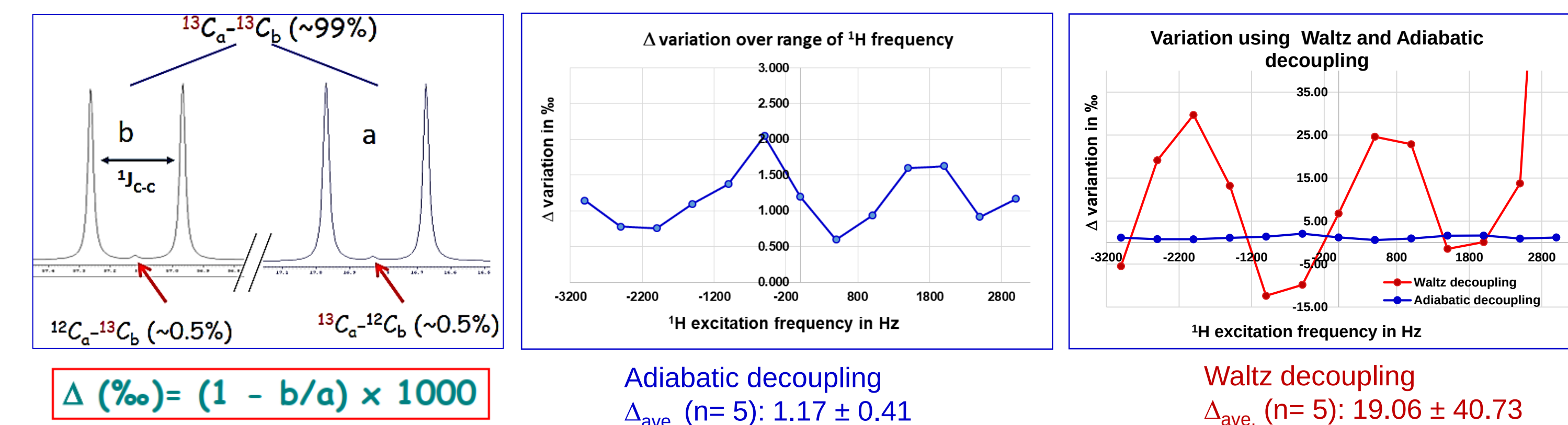
A very high precision of 1-2 (‰) is required to analyse the ^{13}C profile of a given compound at natural abundance

Important keys for the success of the high precision of irm- ^{13}C NMR:

- The uniformity of ^1H decoupling over the whole ^1H chemical range $\rightarrow$ use of ^{13}C -NMR adiabatic pulse [4]

Fig. 2: Scheme of single-pulse ^{13}C NMR with adiabatic ^1H decoupling

- Spectrometer adjustment: determination of ^1H decoupling power using 10% $CHCl_3$ in Acetone- d_6
- Spectrometer evaluation using bi-labelled ethanol with 99% of ^{13}C as a molecule probe for sensitivity and accuracy test



$$\Delta (\text{‰}) = (1 - b/a) \times 1000$$

Adiabatic decoupling
 $\Delta_{\text{ave.}} (n=5): 1.17 \pm 0.41$

Waltz decoupling
 $\Delta_{\text{ave.}} (n=5): 19.06 \pm 40.73$

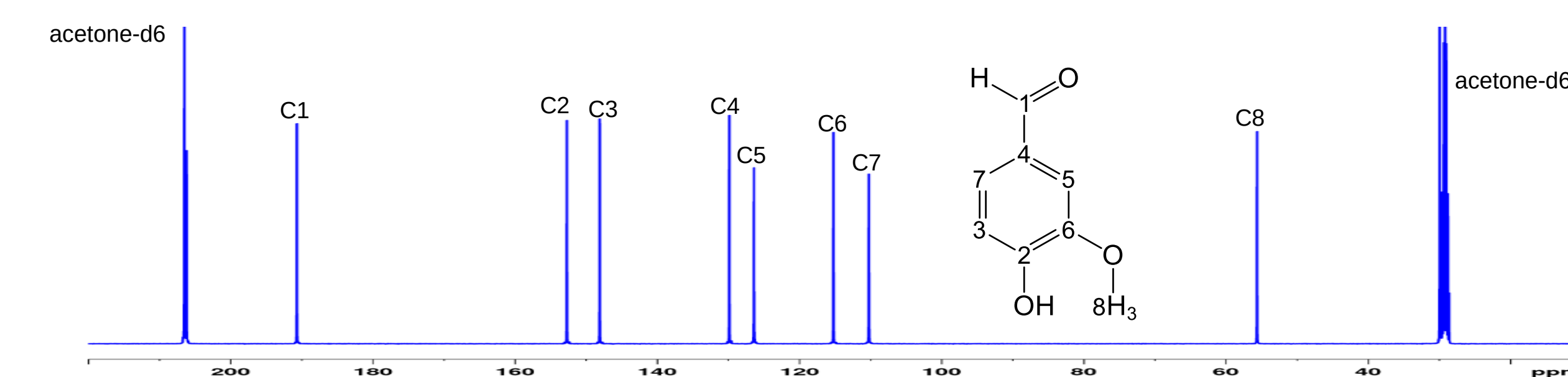
Fig. 3: Spectrometer qualification using adiabatic and waltz decoupling pulses on bi-labelled ethanol

- To achieve a high degree of ^{13}C -qNMR precision: NMR parameters should be meticulously optimized [5]

- Signal to noise ratio (SNR) > 700
- Elimination of nOe and the partial saturation
- Reasonable acquisition time

solution $\rightarrow$

- Use ^{13}C -zgig pulse with 90° flip angle, increase NS
- A large inter-pulse delay with respect of $10 \times T_1^{\text{max}}$
- Relaxation reagent solution $\rightarrow T_1^{\text{max}} < 1.6$ s

Fig. 4: ^{13}C -NMR spectra of Vanillin in acetone- d_6 on Bruker Avance III-400 MHz, BBFO probe

- Data processing
- 1-2 ‰ of precision and/or trueness cannot be obtained by a simple integration or by basic deconvolution protocols
- Lineshape, linewidth, Gaussian-to-Lorentzian ratio, phase and baseline influence on the peak areas detected.
- $\rightarrow$ use of specific program: R-nmrfit or Perch [6]

References

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RESULTS

- ^{13}C -NMR sequence-adiabatic decoupling were applied to analyse the ^{13}C molar fraction profile of vanillin.
- Carbon site specific content $\delta^{13}C_i$ for each vanillin was established.



Fig. 5: Carbon isotope site-specific of VANA-1 (left) and VANB-1 (right) in ‰

- VANA-1 shows the consistency of $\delta^{13}C_i$ values between five measurements (STDEV of 0.4 - 2.0 ‰) for the same sample and from sample to sample (STDEV of 0.8 - 1.2 ‰; n= 8).
- VANB-1 shows some discrepancy between samples on values of $\delta^{13}C_5$ and $\delta^{13}C_7$.
- Inter-laboratory study shows the similar carbon isotope profile of VANA-1 and VANB-1 obtained between two labs, combined results

in ‰	$\delta^{13}C_9$	C1	C2	C3	C4	C5	C6	C7	C8
VANA-1	-31.31 $\pm$ 0.08	-21.68 $\pm$ 1.04	-31.96 $\pm$ 0.88	-32.29 $\pm$ 0.82	-28.24 $\pm$ 1.09	-29.81 $\pm$ 1.20	-29.15 $\pm$ 1.00	-23.72 $\pm$ 1.18	-53.63 $\pm$ 0.85
VANB-1	-25.87 $\pm$ 0.08	-20.48 $\pm$ 0.86	-29.93 $\pm$ 0.97	-32.87 $\pm$ 0.95	-24.30 $\pm$ 0.85	-23.58 $\pm$ 1.33	-26.22 $\pm$ 0.82	-18.74 $\pm$ 1.18	-30.84 $\pm$ 1.13

- The carbon isotope site-specific profile of VANA-1 and VANB-1 shows the main difference between two vanillin materials on the C8 position with $\delta^{13}C_8$ of -53.63 $\pm$ 0.85 ‰ and -30.84 $\pm$ 1.13 ‰, respectively.

- Carbon isotope site-specific of the mixture of VANA-1 and VANB-1 were analysed by irm-NMR and irm-MS.

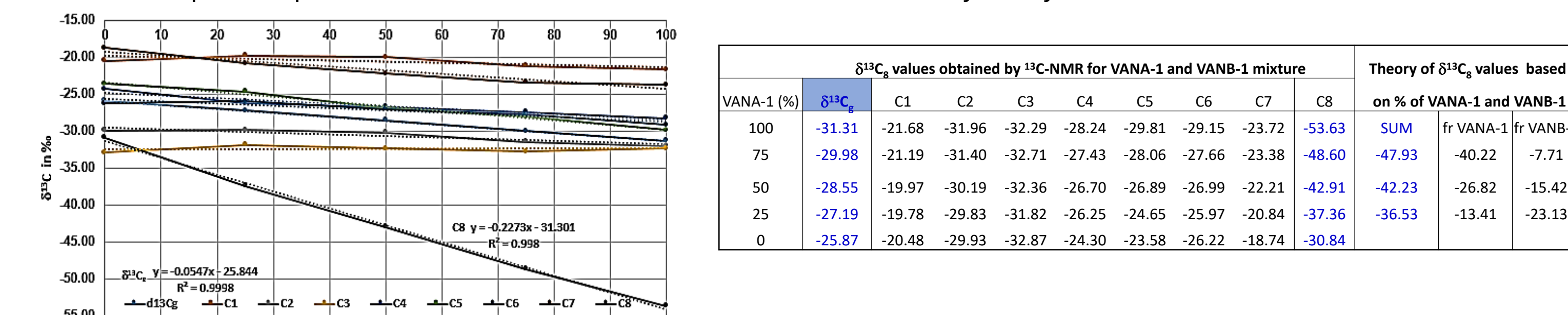


Fig. 6: Carbon isotope site-specific of vanillin mixture

- $\delta^{13}C_9$ and $\delta^{13}C_8$ have a good correlation with the weight ratio of VANA-1 and VANB-1 in the mixture with the R^2 factor of 0.9998 and 0.9989, respectively.
- No significant correlation observed between $\delta^{13}C_i$ and the weight ratio of VANA-1 and VANB-1 for other carbon position.

- The homogeneity of VANA-1 and VANB-1 materials was evaluated using the $\delta^{13}C_i$ values of analysed samples by the DerSimonian-Laird random effects model. U_{hom} of VANA-1 and VANB-1 are 0.94 and 1.10 ‰, respectively.

- The stabilities of VANA-1 and VANB-1 material were observed based on $\delta^{13}C_9$ and $\delta^{13}C_i$ of several samples over 6 months.

CONCLUSION

- The combined irm- ^{13}C qNMR and irm-MS was successfully applied and allowed measurement of the carbon isotope site-specific content of all carbon from the two vanillin CRMs with high accuracy from 0.3 to 2 ‰ (n= 9). This reached the required precision of a few per mil for the irm- ^{13}C -NMR measurement for a given compound at natural abundance.
- The expanded combined uncertainty based on the carbon isotope values, homogeneity and stabilities is being evaluated.
- This work contributes to certify the two first sets of vanillin-based carbon site-specific isotope CRMs.