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Trace analysis in water-alcohol mixtures by continuous p-H_2 hyperpolarization at high magnetic field

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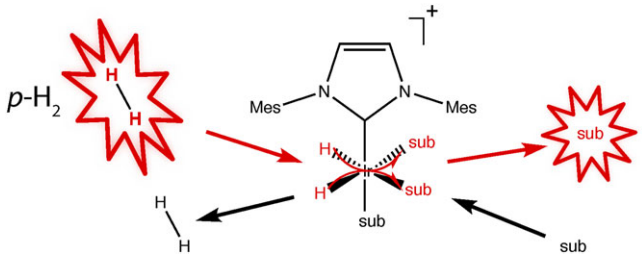
Nuclear magnetic resonance (NMR) studies of complex mixtures are often limited by the low sensitivity of the technique and by spectral overlap. We have recently reported on an NMR chemosensor on the basis of para-Hydrogen Induced Polarization that potentially addresses both these issues, albeit for specific classes of compounds. This approach makes use of Signal Amplification By Reversible Exchange (SABRE) catalysts in methanol and allows selective detection and quantification of dilute analytes in complex mixtures. Herein, we demonstrate that, despite a large decrease in attained hyperpolarization, this method can be extended to water–alcohol mixtures. Our approach was tested on whisky, where nitrogenous heterocyclic flavor components at low-micromolar concentration could be detected and quantified.

KEYWORDS
hyperpolarization, NMR, para-hydrogen, PHIP, pyrazines, pyridines, SABRE, whisky

1 | INTRODUCTION

Nuclear magnetic resonance (NMR) has proved over the last decades to be an invaluable tool for chemical analysis, with applications in chemistry, biology, and medicine. Thanks to the sensitivity of NMR resonances to molecular structures, this technique is routinely applied in the investigation and characterization of complex mixtures. The intrinsic low sensitivity of NMR spectroscopy has so far limited its analytical applications to relatively concentrated systems (i.e., high micromolar concentrations). However, such limitation can be effectively lifted by using nuclear spin hyperpolarization in solution[1–8] to enhance NMR signals. Particularly, SABRE[9–15] is a hyperpolarization technique, albeit for specific classes of compounds (nitrogenous heteroaromatics,[9] sulfur heteroaromatics,[16] nitriles,[17] Schiff bases,[18] and diazirines[19]), that depends on the reversible association of small ligands and para-hydrogen (p-H_2) to an iridium catalyst in solution (see Scheme 1). When such complex is formed at low magnetic field, a transient scalar couplings network determines the spontaneous conversion of p-H_2 spin order to enhanced magnetization of the nuclear spins of the other ligands. Upon complex dissociation, free hyperpolarized ligands are released in solution and can be detected, after sample transfer to high magnetic field, as enhanced NMR signals. The same reversible interactions with a SABRE catalyst can be exploited at high magnetic field in a PHIP (p-H_2 induced hyperpolarization) experiment to produce enhanced (up to 1,000-fold) NMR hydride signals.[20] The advantage of this high-field PHIP approach is that hyperpolarization can be realized in a continuous fashion at the beginning of each transient, by shortly (typically 1–3 s) bubbling p-H_2 in the sample inside the NMR spectrometer.[21] This allows the combination of PHIP with signal averaging as well as with the standard set of NMR tools (phase cycling, multidimensional experiments, etc.), as recently demonstrated for quantitative investigations of methanol-d_4 extracts of coffee[22] and urine[23] at low micromolar



SCHEME 1 Schematic representation of SABRE hyperpolarization. Parahydrogen (p-H_2), Ir-catalyst, and substrate are involved in a reversible binding equilibrium. Spin order transfer from p-H_2 to the substrate (sub) at the iridium catalyst occurs spontaneously at low magnetic field, resulting in nuclear spin hyperpolarization of the substrate both bound and free in solution

concentrations. Extending these studies to aqueous mixtures is not straightforward, as SABRE signal enhancements in the presence of water are substantially lower,[24–27] due to slower ligand exchange and, presumably, lower p-H_2 solubility than in methanol.

Here, we report on the application of a high-field PHIP experiment to the investigation of a distilled alcoholic beverage with water as a co-solvent, namely, an Islay cask strength (58% ethanol/vol) single malt Scotch whisky. Maillard reactions that occur within the malt during kilning are responsible for the formation of aroma components such as pyridines and pyrazines,[28] typically at concentrations well below the detection limit of standard NMR.[28–30] As previously demonstrated, such compounds are capable to weakly associate to SABRE catalysts and could, therefore, be detected and quantified via the high-field PHIP approach, despite the presence of 30% water in the NMR sample.

2 | MATERIALS AND METHODS

2.1 | Chemicals and materials

Complex precursor [Ir(COD)(IMes)Cl] (IMes = 1,3-bis(2,4,6-trimethylphenyl)imidazole-2-ylidene; COD = cyclooctadiene) and co-substrate 1-methyl-1,2,3-triazole (*mtz*) were synthesized according to published methods.[31,32] Methanol-d_4 was purchased from Cambridge Isotope Laboratories. All other chemicals were purchased from Sigma-Aldrich (pyridine, pyrazine, 2-methylpyrazine, and 3-methylpyridine). Para-hydrogen (p-H_2) was produced with an in-house designed 2 L vessel embedded in a liquid nitrogen bath. Normal hydrogen (purity 5.0) was cooled down to 77 K in the presence of 100 ml of 4–8 MESH charcoal (Sigma-Aldrich). The resulting 51% p-H_2 was transported to an aluminum cylinder (Nitrous Oxides Systems, Holley Performance Products, Bowling Green, KY, USA),[33] with an adjustable output-pressure valve. Cask strength (58% ethanol/vol) single malt Scotch whisky was used for this investigation.

2.2 | Preparation of the NMR sample for PHIP experiments

A stock solution of 4.8 mM [Ir(COD)(IMes)Cl] complex precursor and 72 mM 1-methyl-1,2,3-triazole (*mtz*) as co-substrate was prepared in methanol-d_4. Prior to the

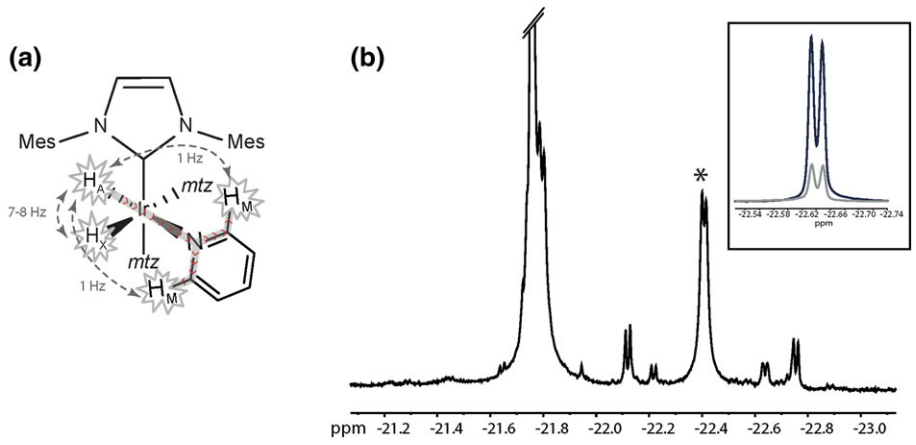


FIGURE 1 (a) Schematic representation of the asymmetric [Ir(IMes)$(H)_2$$(mtz)_2$(py)]Cl complex for a pyridine-like substrate, formed in the presence of a large excess of 1-methyl-1,2,3-triazole (*mtz*) as co-substrate. Scalar couplings between hydrides and from hydride "A" to substrate protons are indicated. (b) p-H_2 enhanced nuclear magnetic resonance hydride signals of whisky in the presence of 1.2 mM metal complex, 18 mM *mtz*, and 5 bar 51% enriched p-H_2. The large signal at approximately−21.8 ppm originates from the symmetrical complex in which two units of co-substrate (*mtz*) bind in the equatorial plane. The signal marked by an asterisk corresponds to the complex formed by methanol binding. Insert: p-H_2 enhanced nuclear magnetic resonance hydride signal of [Ir(IMes)$(H)_2$$(mtz)_2$(2-methylpyrazine)]Cl complex in methanol-d_4 (black) and in a mixture methanol-d_4(25%)-D_2O(31.5%)-ethanol(43.5%; grey)

NMR measurements, 130 mg of stock solution, corresponding to ¼ of the final NMR sample volume, was transferred into a 5 mm Wilmad quick pressure valve NMR tube. The tube was pressurized under 5 bar of H_2 to convert the complex precursor into the activated symmetric complex $[Ir(IMes)(H)_2(mtz)_3]Cl$. Complete activation required approximately 120 min. Immediately before the NMR measurements, 400 mg whisky (¾ of the total NMR sample volume) was added to the activated catalyst solution in the quick pressure valve NMR tube. Final concentrations of the stock solution components were 1.2 mM iridium complex and 18 mM *mtz*, whereas the whisky components were diluted to 75.3%.

2.3 | Standard addition

For the standard addition experiments, stock solutions (100 and 1 mM) of the compound under investigation were prepared using whisky as a solvent. A typical standard addition series consisted of the original whisky and four additional samples at increasing concentration of the analyte. Typically, the highest analyte concentration in the series corresponded to approximately 4 times the estimated original concentration. All solutions were prepared by gravimetric mixing of solvents and analytes.

2.4 | NMR experiments

All NMR spectra were acquired at 298 K at 499.91 MHz ^1H resonance frequency using a Varian UnityInova 500 spectrometer equipped with a triple-resonance HCN cryo-cooled probe with a shielded *z*-gradient coil. In all PHIP experiments, p-H_2 was bubbled at the beginning of each transient for 0.75 s (1 s for the 2D Selective Excitation of Polarization using PASADENA - Homonuclear Single Quantum Correlation (Sepp-HoSQC)[22] experiments).

The hydride PHIP 1D spectrum was acquired with 16 transients in ca. 1 min. For the 1D correlation spectroscopy (COSY) traces used in the standard addition series 32 or 64 scans were acquired, for a total acquisition time of 1.5 or 2.5 min.

The 2D PHIP DQF-COSY data matrix consisted of $32(t_1) \times 1600(t_2)$ complex points, acquired with eight scans per increment in ca. 20 min. The 2D data sets were processed using 72° shifted squared sine-bell apodization in both dimensions, prior to zero filling to $256(t_1) \times 8192(t_2)$ complex points, and Fourier transformation.

The 2D PHIP Sepp-HoSQC data matrix consisted of $64(t_1) \times 750(t_2)$ complex points, acquired with eight and 24 scans per increment for the spiked (approximately 10 nmoles) and the original whisky sample, respectively. The 2D data set were processed using 72° shifted squared sine-bell apodization in both dimensions, prior to zero filling to $1024(t_1) \times 4096(t_2)$ complex points, and Fourier transformation. For NMR data processing, NMRPipe[34] was used while spectra analysis was performed with iNMR.[35]

The 1D thermal ^1H spectra on the whisky samples (original and spiked with approximately 10 nmoles of analytes) were acquired with a recovery delay of 60 s and a 30-degree excitation pulse. Suppression of the solvents (water and ethanol) signals was achieved by applying a DPFGSE scheme[36] using a *reburp*[37] shaped pulse centered at 7.6 ppm with a bandwidth of 1500 Hz as a refocusing element. Acquisition time were approximately 95 and 17 hr for the original and the spiked whisky sample, respectively.

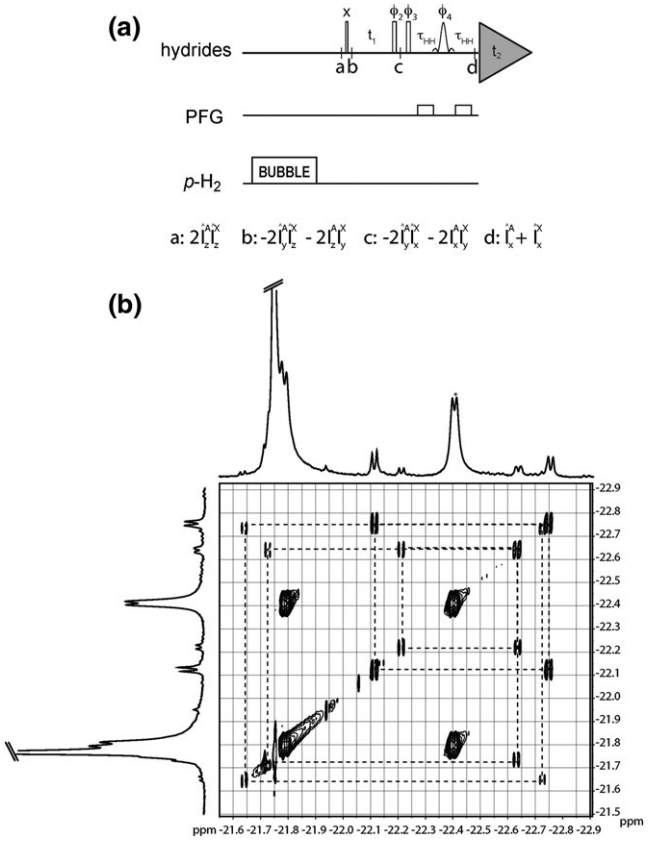


FIGURE 2 (a) Pulse scheme to acquire a p-H_2 induced hyperpolarization 2D DQF-COSY spectrum of the hydrides in the iridium complex $[Ir(IMes)(H)_2(mtz)_2(sub)]Cl$. At the beginning of every transient for 0.75 s, p-H_2 is bubbled. Rectangular pulses indicate low-power (RF field approximately 2.5 kHz) 45- and 90-degree pulses, whereas the shape represents a selective *reburp*[37] pulse with a bandwidth of 2000 Hz. Delay durations: $\tau_{HH} = 29.4$ ms. Phase cycling: ϕ_2: x, −x; ϕ_3: 2(x), 2(y), 2(−x), 2(−y); ϕ_4: 2(y), 2(−x), 2(−y), 2(x); receiver: x,-x,-y,y,-x,x,y,-y. (b) 2D DQF-COSY spectrum of the hydride region acquired on a whisky sample in the presence of 1.2 mM metal complex, 18 mM *mtz* as co-substrate, and 5 bar 51% enriched p-H_2. Final solvent composition: methanol-d_4(25%)-water(31.5%)-ethanol(43.5%)

3 | RESULTS AND DISCUSSION

In the following, the species that can bind to the chemosensing receptor will be referred to as "analytes" or "substrates," interchangeably. We have previously demonstrated that, in order to hyperpolarize dilute substrates, a large excess of a co-substrate (1-methyl-1,2,3-triazole [*mtz*] in the present case) must be added to the solution to stabilize the active SABRE catalyst.[38] Binding of a pyridine-like analyte to the catalyst, together with p-H_2 and *mtz*, results in the asymmetric complex displayed in Figure 1a. Because of the chemical inequivalence of the two hydrides, only longitudinal spin order survives from the p-H_2 derived singlet state.[4,39] A SEPP[40,41] NMR pulse scheme can be used to convert such longitudinal spin order into hydrides enhanced magnetization, which can be detected as an NMR signal increased up to three orders of magnitude.[20] Therefore, for each compound associating to the iridium center, the chemosensor response consists of a pair of hyperpolarized hydride resonances in the NMR spectrum. The PHIP-enhanced NMR spectrum displaying the hydrides NMR signals in whisky is shown in Figure 1b: Five major peaks can be distinctly recognized, together with several minor components above the noise level. The signal marked with an asterisk originates from the asymmetric complex involving the binding of methanol and will not be considered any further.

In the case of resolved resonances, identification (and quantification) of the species bound to the iridium complex can be obtained by titrating a given compound and following the signal increase of the corresponding hyperpolarized hydride(s). In complex mixtures, in which spectral overlap is to be expected, a different NMR approach must be taken to resolve individual hydride signals. A possible solution consists in acquiring 2D Sepp-HoSQC[22] correlation spectra of hyperpolarized hydrides with the protons of the substrate via long-range scalar couplings, as previously demonstrated in a quantitative investigation of coffee extracts in methanol. However, because of the relatively weak signals obtained in preliminary PHIP NMR experiments on whisky, presumably due to the presence of water as co-solvent and to the low analytes concentrations, the 2D Sepp-HoSQC was considered unpractical in the present study for its low sensitivity. The insert in Figure 1a clearly illustrates the

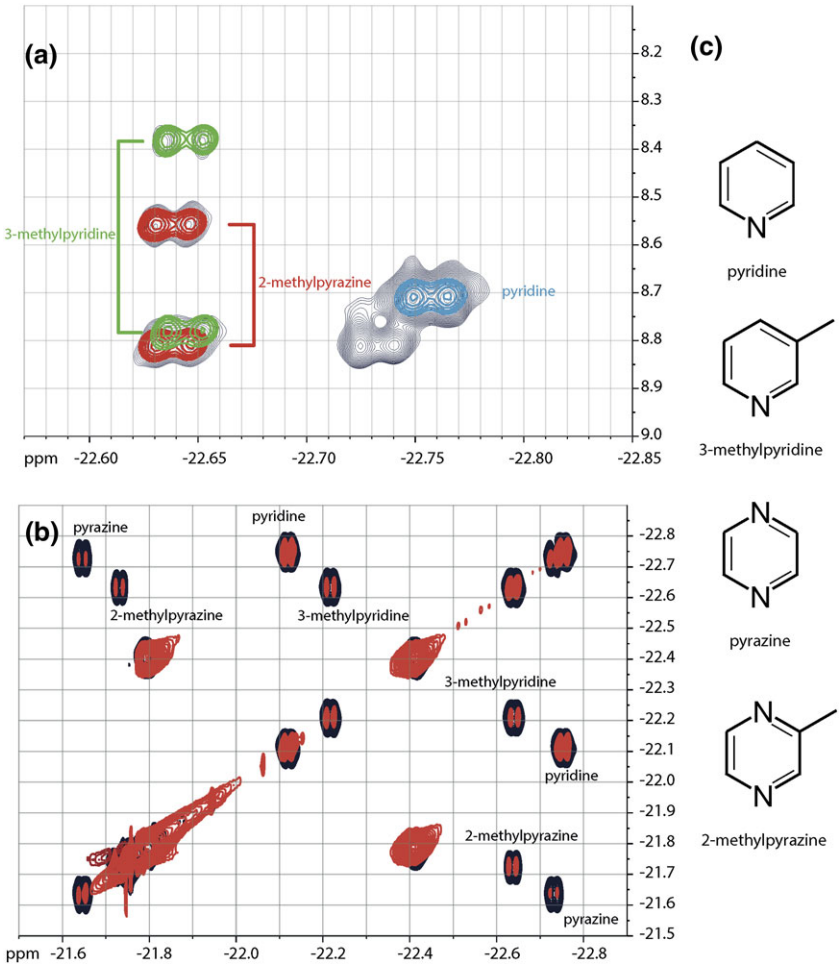


FIGURE 3 (a) Overlay of the p-H_2 induced hyperpolarization 2D Sepp-HoSQC[22] correlation spectra between hydrides "A" and *ortho* aromatic protons in the iridium complex measured on whisky (thin line, black) and on whisky spiked with pyridine (cinder, thick line), 3-methylpyridine (green, thick line), and 2-methylpyrazine (red, thick line). The experiment was performed in the presence of 1.2 mM metal complex, 18 mM *mtz* as co-substrate, and 5 bar 51% enriched p-H_2. The spectra are plotted with different threshold. (b) Overlay of the p-H_2 induced hyperpolarization DQF-COSY spectrum between hydrides "A" and "X" measured on whisky (red line) and on whisky spiked with approximately 25 nmoles of pyridine, pyrazine, 3-methylpyridine, and 2-methylpyrazine (black line). The spectra are plotted with different threshold. (c) Chemical formula of the four analytes determined in the present study

decrease in sensitivity (approximately 4-fold) in a water–alcohol mixture compared to a methanol solution.

Therefore, in order to maximize sensitivity, resolution of individual hydride signals was achieved via a correlation experiment between the two hydrides in the SABRE complexes; this approach relies on the inter-hydride couplings (typically 7–8 Hz) rather than the long-range couplings (at most ~1 Hz[42]) between hydrides and corresponding substrate protons (see Figure 1a) and it is, therefore, far more efficient. The scheme of the 2D NMR experiment here used is sketched in Figure 2a; a 45-degree pulse is used to convert the hydride longitudinal spin order into antiphase coherence. The rest of the pulse scheme, including the phase cycle, corresponds to the standard DQF-COSY experiment, with the final refocusing period allowing for the acquisition of in-phase NMR signals. In Figure 2b a DQF-COSY spectrum, recorded in ca. 20 min for the hydride region of a whisky sample is displayed. Note that the inter-hydrides cross-peaks provide well resolved signals that allow analytes' identification and quantification. On the basis of the reported presence of pyridines and pyrazines in whisky,[28,29] we could identify the hydride signals in the corresponding SABRE complexes by spiking the NMR sample. In Figure 3, the assignment of the four main signals on the basis of 2D Sepp-HoSQC as well as on PHIP 2D DQF-COSY spectra of the original and the spiked whisky samples is illustrated.

Because of the large excess of co-substrate in solution, the integrals of signals hyperpolarized via SABRE or PHIP depend linearly on the concentration of dilute substrates.[21,38] We have previously exploited this linear dependence to quantify the concentration of analytes in artificial[21] as well as natural mixtures[22,23] via standard addition. The same approach was followed in this study, by spiking whisky with known amounts of substrate and following the increase of the integrals of the hydride–hydride cross-peaks. For the purpose of time optimization, the actual quantification was performed on 1D NMR spectra, recording selective COSY traces of individual "AX" hydrides pair with the pulse scheme sketched in Figure 4a. The signals of the "A" hydrides in the original whisky sample (before spiking) are displayed in Figure 4b. The standard addition curves for the main pyridines and pyrazines flavor components are shown in Figure 5: Analytic concentrations were obtained from the abscissa intercept of the standard-addition curve and are summarized in Table 1, after correction for the 75.3% dilution in the NMR sample. All these values, in the low-micromolar range, appear to largely exceed the concentrations reported in the literature (nM range, see Table 1). Note, however, that large differences (up to five orders of magnitude in the case of 2-methylpyrazine) are found for such

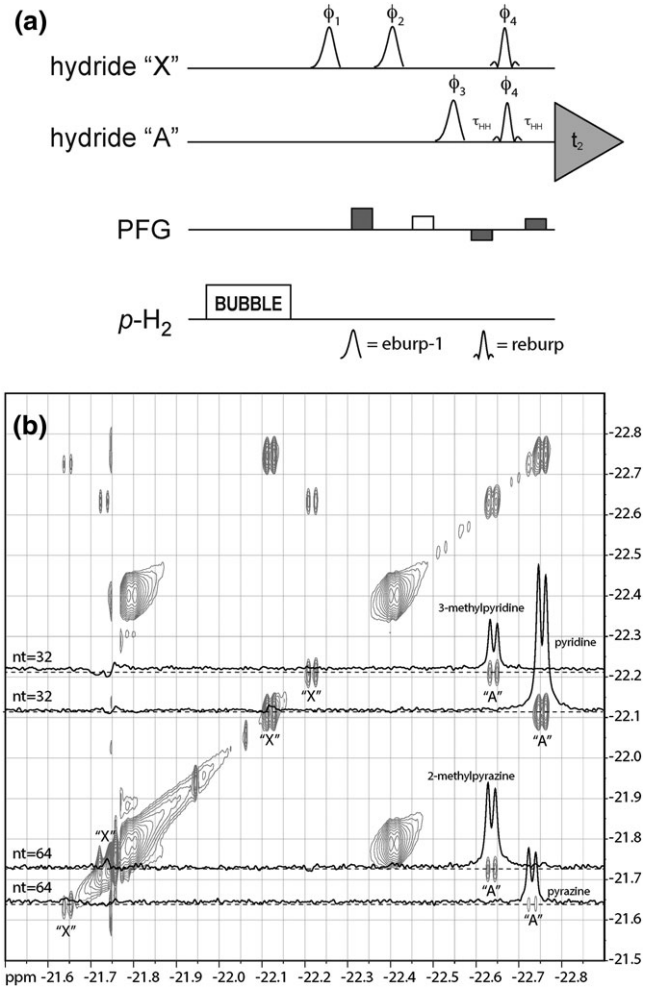


FIGURE 4 (a) Pulse scheme to acquire the p-H_2 induced hyperpolarization 1D COSY signals of the "A" hydrides in the iridium complex [Ir(IMes)$(H)_2$$(mtz)_2$(sub)]Cl. At the beginning of every transient for 0.75 s, p-H_2 is bubbled. A bandwidth of 30 Hz was used for the *eburp-1*[37] shaped pulses. For the refocusing *reburp*[37] pulse a bandwidth of 2000 Hz was employed. Delay durations: $\tau_{HH} = 29.4$ ms. Phase cycling: ϕ_1: x, −x; ϕ_2: 2(x), 2(−x); ϕ_3: 4(x), 4(y), 4(−x), 4(−y) ϕ_4: 4(y), 4(−x), 4(−y), 4(x); receiver: x,-x,-x,x,y,-y,-y,y,-x, x,x,-x,-y,y,y,-y. Pulsed field gradients (filled square boxes) can be optionally used for gradient coherence selection.(b) p-H_2 induced hyperpolarization 1D COSY traces acquired on the whisky sample in the presence of 1.2 mM metal complex, 18 mM *mtz* as co-substrate, and 5 bar 51% enriched p-H_2

aroma components in whiskies, probably determined by the influence of the production process. In order to confirm our results, we have acquired standard 1D NMR ^{1}H spectra under thermal equilibrium conditions, both for the whisky sample and after spiking with approximately 10 nmoles of the four analytes under investigation (see Figure 5e). For three out of four analytes (i.e., pyridine, pyrazine, and 2-methylpyrazine) well-resolved signals could be observed and integrated for an approximate estimate of the concentration. The values obtained from the

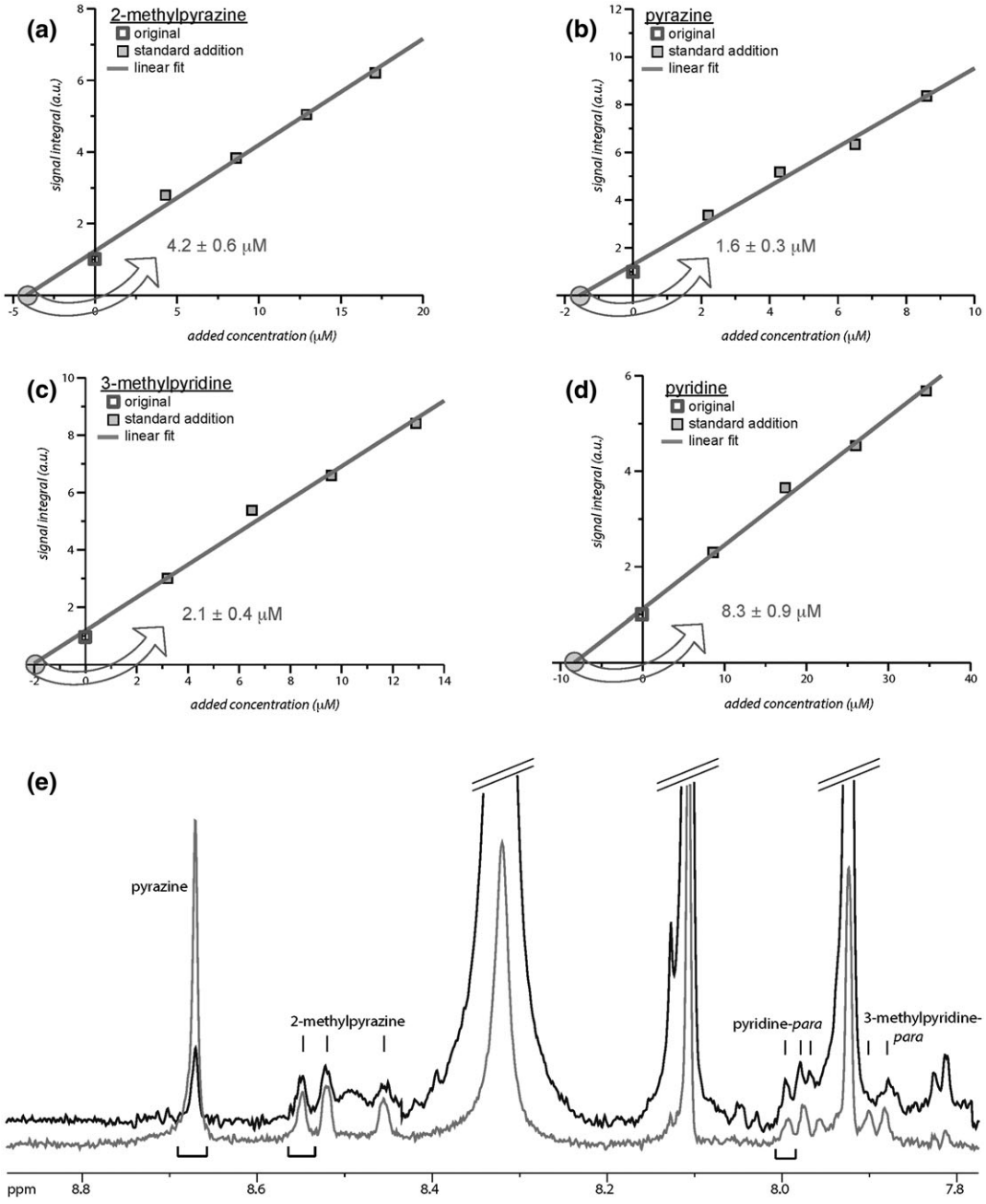


FIGURE 5 (a–d) standard-addition curves for the hydride "A" resonance of 2-methylpyrazine (a), pyrazine (b), 3-methylpyridine (c), and pyridine (d). Concentrations are estimated from the abscissa intercept (circled) of the standard-addition curves (grey lines). Experimental uncertainties were derived by error propagation. (e) Overlay of the thermal spectra of whisky (5,632 transients, black) and whisky spiked with approximately 10 nmoles of the four analytes (1,024 transients, grey). A vertical offset has been added to facilitate the comparison. The assignment of the resonances of the four analytes is indicated. In both samples methanol-d_4 was added (20% [vol]) for deuterium-lock

TABLE 1 Concentration of the main pyridines and pyrazines flavor components in cask strength (58% ethanol) single-malt Scotch whisky as derived from standard addition. A value of 0.91353 g ml^{-1} was used for the density of the whisky sample under investigation (58% vol at 20 °C)[43]

Analyte	Conc. (μM)	Conc. (ppm)	Conc. Literature[28–30] (μM)
Pyridine	11.0 ± 1.2	0.95 ± 0.10	1.6×10^{-5}–4.2×10^{-1}
Pyrazine	2.1 ± 0.4	0.18 ± 0.03	Not reported
2-methylpyrazine	5.6 ± 0.8	0.58 ± 0.08	2.9×10^{-6}–8.5×10^{-1}
3-methylpyridine	2.8 ± 0.5	0.29 ± 0.05	6.1×10^{-7}–4.6×10^{-3}

conventional 1D NMR approach are in good agreement with the results of the PHIP experiments (see Supporting Information) and are certainly not consistent with analytes concentrations in the nanomolare range.

4 | CONCLUSIONS

We have applied a p-H_2 enhanced NMR chemosensing approach to the quantitative determination of specific flavor components in cask strength (58% ethanol/vol) single-malt Scotch whisky. As previously demonstrated, the PHIP NMR chemosensor allows the selective detection of pyridines and pyrazines while removing the large background contributions from the complex matrix. Please note that this method does not require any preliminary extraction, fractionation, or target functionalization and that sample preparation simply consists in mixing whisky to a methanol solution of the activated iridium catalyst. This is a clear advantage when compared to alternative approaches that require extensive sample manipulation, in view of possible analytes' loss. As previously reported, the presence of 30% water as co-solvent strongly reduces the NMR signal enhancement provided by PHIP. However, we have partly compensated this signal loss with an NMR approach on the basis of an inter-hydride COSY transfer to resolve individual resonances. The attained sensitivity was sufficient to quantitatively determine target analytes at low micromolar concentrations, a range that was confirmed by standard NMR measurements. Interestingly, the concentrations determined in the present study greatly exceed the values reported in the literature by different techniques. Further studies on different whisky samples will be necessary to generalize this result.

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REFERENCES

[1] J. H. Ardenkjær-Larsen, B. Fridlund, A. Gram, G. Hansson, L. Hansson, M. H. Lerche, R. Servin, M. Thaning, K. Golman, *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 10158.

[2] J. Wolber, F. Ellner, B. Fridlund, A. Gram, H. Jóhannesson, G. Hansson, L. H. Hansson, M. H. Lerche, S. Månsson, R. Servin, M. Thaning, K. Golman, J. H. Ardenkjær-Larsen, *Nucl. Instrum. Methods Phys. Res., Sect. A* **2004**, *526*, 173.

[3] L. Frydman, D. Blazina, *Nat. Phys.* **2007**, *3*, 415.

[4] C. R. Bowers, D. P. Weitekamp, *Phys. Rev. Lett.* **1986**, *57*, 2645.

[5] M. G. Pravica, D. P. Weitekamp, *Chem. Phys. Lett.* **1988**, *145*, 255.

[6] K. V. Kovtunov, I. E. Beck, V. I. Bukhtiyarov, I. V. Koptyug, *Angew. Chem. Int. Ed.* **2008**, *47*, 1492.

[7] M. Roth, P. Kindervater, H.-P. Raich, J. Bargon, H. W. Spiess, K. Münnemann, *Angew. Chem. Int. Ed.* **2010**, *49*, 8358.

[8] T. C. Eisenschmid, R. U. Kirss, P. P. Deutsch, S. I. Hommeltoft, R. Eisenberg, J. Bargon, R. G. Lawler, A. L. Balch, *J. Am. Chem. Soc.* **1987**, *109*, 8089.

[9] R. W. Adams, J. A. Aguilar, K. D. Atkinson, M. J. Cowley, P. I. P. Elliott, S. B. Duckett, G. G. R. Green, I. G. Khazal, J. Lopez-Serrano, D. C. Williamson, *Science* **2009**, *323*, 1708.

[10] L. S. Lloyd, R. W. Adams, M. Bernstein, S. Coombes, S. B. Duckett, G. G. R. Green, R. J. Lewis, R. E. Mewis, C. J. Sleigh, *J. Am. Chem. Soc.* **2012**, *134*, 12904.

[11] J. B. Hövener, N. Schwaderlapp, T. Lickert, S. B. Duckett, R. E. Mewis, L. A. R. Highton, S. M. Kenny, G. G. R. Green, D. Leibfritz, J. G. Korvink, J. Hennig, D. von Elverfeldt, *Nat. Commun.* **2013**, *4*, 2946.

[12] F. Shi, A. M. Coffey, K. W. Waddell, E. Y. Chekmenev, B. M. Goodson, *Angew. Chem. Int. Ed.* **2014**, *53*, 7495.

[13] V. Daniele, F. X. Legrand, P. Berthault, J. N. Dumez, G. Huber, *ChemPhysChem* **2015**, *16*, 3413.

[14] T. Theis, M. L. Truong, A. M. Coffey, R. V. Shchepin, K. W. Waddell, F. Shi, B. M. Goodson, W. S. Warren, E. Y. Chekmenev, *J. Am. Chem. Soc.* **2015**, *137*, 1404.

[15] R. V. Shchepin, B. M. Goodson, T. Theis, W. S. Warren, E. Y. Chekmenev, *ChemPhysChem* **2017**, *18*, 1961.

[16] R. V. Shchepin, D. A. Barskiy, A. M. Coffey, B. M. Goodson, E. Y. Chekmenev, *ChemistrySelect* **2016**, *1*, 2552.

[17] R. E. Mewis, R. A. Green, M. C. R. Cockett, M. J. Cowley, S. B. Duckett, G. G. R. Green, R. O. John, P. J. Rayner, D. C. Williamson, *J. Phys. Chem. B* **2015**, *119*, 1416.

[18] A. W. J. Logan, T. Theis, J. F. P. Colell, W. S. Warren, S. J. Malcolmson, *Chem. - A Eur. J.* **2016**, *22*, 10777.

[19] T. Theis, G. X. Ortiz, A. W. J. Logan, K. E. Claytor, Y. Feng, W. P. Huhn, V. Blum, S. J. Malcolmson, E. Y. Chekmenev, Q. Wang, W. S. Warren, *Sci. Adv.* **2016**, *2*, e1501438, e1501438.

[20] N. J. Wood, J. A. Brannigan, S. B. Duckett, S. L. Heath, J. Wagstaff, *J. Am. Chem. Soc.* **2007**, *129*, 11012.

[21] N. Eshuis, R. L. E. G. Aspers, B. J. A. van Weerdenburg, M. C. Feiters, F. P. J. T. Rutjes, S. S. Wijmenga, M. Tessari, *Angew. Chem. Int. Ed.* **2015**, *54*, 14527.

[22] N. K. J. Hermkens, N. Eshuis, B. J. A. van Weerdenburg, M. C. Feiters, F. P. J. T. Rutjes, S. S. Wijmenga, M. Tessari, *Anal. Chem.* **2016**, *88*, 3406.

[23] I. Reile, N. Eshuis, N. K. J. Hermkens, B. J. A. van Weerdenburg, M. C. Feiters, F. P. J. T. Rutjes, M. Tessari, *Analyst* **2016**, *141*, 4001.

[24] J. B. Hovener, N. Schwaderlapp, R. Borowiak, T. Lickert, S. B. Duckett, R. E. Mewis, R. W. Adams, M. J. Burns, L. A. R. Highton, G. G. R. Green, A. Olaru, J. Hennig, D. von Elverfeldtt, *Anal. Chem.* **2014**, *86*, 1767.

[25] H. Zeng, J. Xu, M. T. McMahon, J. A. B. Lohman, P. C. M. van Zijl, *J. Magn. Reson.* **2014**, *246*, 119.

[26] P. Spannring, I. Reile, M. Emondts, P. P. M. Schleker, N. K. J. Hermkens, N. G. J. van der Zwaluw, B. J. A. van Weerdenburg, P. Tinnemans, M. Tessari, B. Blümich, F. P. J. T. Rutjes, M. C. Feiters, *Chem. – Eur. J.* **2016**, *22*, 9277.

[27] F. Shi, P. He, Q. A. Best, K. Groome, M. L. Truong, A. M. Coffey, G. Zimay, R. V. Shchepin, K. W. Waddell, E. Y. Chekmenev, B. M. Goodson, *J. Phys. Chem. C* **2016**, *120*, 12149.

[28] K.-Y. M. Lee, A. Paterson, J. R. Piggott, *J. Inst. Brew.* **2001**, *107*, 287.

[29] G. Charalambous, *Food flavors, ingredients and composition; developments in food science*, Vol. 32, Elsevier, Amsterdam **1993**.

[30] M. Viro, *Chromatographia* **1984**, *19*, 448.

[31] R. A. Kelly III, H. Clavier, S. Giudice, N. M. Scott, E. D. Stevens, J. Bordner, I. Samardjiev, C. D. Hoff, L. Cavallo, S. P. Nolan, *Organometallics* **2008**, *27*, 202.

[32] M. B. Seefeld, D. A. Heerding, S. Peace, D. Yamashita, K. C. McNulty, *"Inhibitors of AKT activity", WO2008/098104 A1, August 14*, Smithkline Beecham Corporation **2008**.

[33] B. Feng, A. M. Coffey, R. D. Colon, E. Y. Chekmenev, K. W. Waddell, *J. Magn. Reson.* **2012**, *214*, 258.

[34] F. Delaglio, S. Grzesiek, G. W. Vuister, G. Zhu, J. Pfeifer, A. Bax, J. Biomol, *NMR* **1995**, *6*, 277.

[35] G. Balacco, C. Marino http://www.inmr.net/, **2005**.

[36] K. Stott, J. Keeler, Q. N. Van, A. J. Shaka, *J. Magn. Reson.* **1997**, *125*, 302.

[37] H. Geen, R. Freeman, *J. Magn. Reson.* **1991**, *93*, 93.

[38] N. Eshuis, N. Hermkens, B. J. A. van Weerdenburg, M. C. Feiters, F. P. J. T. Rutjes, S. S. Wijmenga, M. Tessari, *J. Am. Chem. Soc.* **2014**, *136*, 2695.

[39] O. Torres, B. Procacci, M. E. Halse, R. W. Adams, D. Blazina, S. B. Duckett, B. Eguillor, R. A. Green, R. N. Perutz, D. C. Williamson, *J. Am. Chem. Soc.* **2014**, *136*, 10124.

[40] H. Sengstschmid, R. Freeman, J. Barkemeyer, J. Bargon, *Magn. Reson., Ser. A* **1996**, *120*, 249.

[41] J. Barkemeyer, J. Bargon, H. Sengstschmid, R. Freeman, *J. Magn. Reson., Ser. A* **1996**, *120*, 129.

[42] N. Eshuis, R. L. E. G. Aspers, B. J. A. van Weerdenburg, M. C. Feiters, F. P. J. T. Rutjes, S. S. Wijmenga, M. Tessari, *J. Magn. Reson.* **2016**, *265*, 59.

[43] International Bureau of Legal Metrology, International Organisation of Legal Metrology, *International alcoholimetric tables*, Paris, **1975**, Vol. 22.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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