The following full text is a publisher’s version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/192300

Please be advised that this information was generated on 2019-07-24 and may be subject to change.
**Article 25fa pilot End User Agreement**

This publication is distributed under the terms of Article 25fa of the Dutch Copyright Act (Auteurswet) with explicit consent by the author. Dutch law entitles the maker of a short scientific work funded either wholly or partially by Dutch public funds to make that work publicly available for no consideration following a reasonable period of time after the work was first published, provided that clear reference is made to the source of the first publication of the work.

This publication is distributed under The Association of Universities in the Netherlands (VSNU) ‘Article 25fa implementation’ pilot project. In this pilot research outputs of researchers employed by Dutch Universities that comply with the legal requirements of Article 25fa of the Dutch Copyright Act are distributed online and free of cost or other barriers in institutional repositories. Research outputs are distributed six months after their first online publication in the original published version and with proper attribution to the source of the original publication.

You are permitted to download and use the publication for personal purposes. All rights remain with the author(s) and/or copyrights owner(s) of this work. Any use of the publication other than authorised under this licence or copyright law is prohibited.

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please contact the Library through email: copyright@ubn.ru.nl, or send a letter to:

University Library
Radboud University
Copyright Information Point
PO Box 9100
6500 HA Nijmegen

You will be contacted as soon as possible.
Trace analysis in water-alcohol mixtures by continuous p-H₂ hyperpolarization at high magnetic field

Niels K.J. Hermkens | Ruud L.E.G. Aspers | Martin C. Feiters | Floris P.J.T. Rutjes | Marco Tessari

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 to enhance NMR signals. Particularly, SABRE is a hyperpolarization technique, albeit for specific classes of compounds (nitrogenous heteroaromatics, sulfur heteroaromatics, nitriles, Schiff bases, and diazirines), that depends on the reversible association of small ligands and para-hydrogen (p-H₂) 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-H₂ 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-H₂ induced hyperpolarization) experiment to produce enhanced (up to 1,000-fold) NMR hydride signals. 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-H₂ in the sample inside the NMR spectrometer. 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-d₄ extracts of coffee and urine at low micromolar concentrations.
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-H2 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-d4 was purchased from Cambridge Isotope Laboratories. All other chemicals were purchased from Sigma-Aldrich (pyridine, pyrazine, 2-methylpyrazine, and 3-methylpyridine). Para-hydrogen (p-H2) 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-H2 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-d4. Prior to the
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 H₂ 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-H₂ 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) 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(t₁) × 1600(t₂) 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(t₁) × 8192(t₂) complex points, and Fourier transformation. For NMR data processing, NMRPipe was used while spectra analysis was performed with iNMR.

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 using a rebump 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.

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(t₁) × 1600(t₂) 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(t₁) × 8192(t₂) complex points, and Fourier transformation. For NMR data processing, NMRPipe was used while spectra analysis was performed with iNMR.

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 using a rebump 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.

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-H₂ 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) 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(t₁) × 1600(t₂) 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(t₁) × 8192(t₂) complex points, and Fourier transformation. For NMR data processing, NMRPipe was used while spectra analysis was performed with iNMR.

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 using a rebump 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.

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 using a rebump 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.

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 using a rebump 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.
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-H₂ 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-H₂ 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-H₂ 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-H₂. The spectra are plotted with different threshold. (b) Overlay of the p-H₂ 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) 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, 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. We have previously exploited this linear dependence to quantify the concentration of analytes in artificial as well as natural mixtures 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 hydrides by spiking whisky with known amounts of substrate and addition. The same approach was followed in this study, recording selective COSY traces of individual hydrides in whisky, we could identify the hydride signals in the corresponding SABRE complexes by spiking the NMR sample. In Figure 4a, the signals of the ‘‘A’’ 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 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 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-d₄ 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]

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Conc. (μM)</th>
<th>Conc. (ppm)</th>
<th>Conc. Literature [28–30] (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyridine</td>
<td>11.0 ± 1.2</td>
<td>0.95 ± 0.10</td>
<td>1.6 × 10⁻⁵–4.2 × 10⁻¹</td>
</tr>
<tr>
<td>Pyrazine</td>
<td>2.1 ± 0.4</td>
<td>0.18 ± 0.03</td>
<td>Not reported</td>
</tr>
<tr>
<td>2-methylpyrazine</td>
<td>5.6 ± 0.8</td>
<td>0.58 ± 0.08</td>
<td>2.9 × 10⁻⁶–8.5 × 10⁻¹</td>
</tr>
<tr>
<td>3-methylpyridine</td>
<td>2.8 ± 0.5</td>
<td>0.29 ± 0.05</td>
<td>6.1 × 10⁻⁷–4.6 × 10⁻¹</td>
</tr>
</tbody>
</table>
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-hydrate 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.

ACKNOWLEDGEMENT

We acknowledge financial support from the European Union and the provinces of Gelderland and Overijssel through the EFRO Ultrasense NMR project.

ORCID

Marco Tessari  
http://orcid.org/0000-0001-8793-0072

REFERENCES


SUPPORTING INFORMATION

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

**How to cite this article:** Hermkens NKJ, Aspers RLEG, Feiters MC, Rutjes FPJT, Tessari M. Trace analysis in water-alcohol mixtures by continuous p-H2 hyperpolarization at high magnetic field. *Magn Reson Chem*. 2018;56:633–640. [https://doi.org/10.1002/mrc.4692](https://doi.org/10.1002/mrc.4692)