Benchtop NMR analysis of piperazine-based drugs hyperpolarised by SABRE

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Abstract

Piperazine-based drugs, such as *N*-benzylpiperazine (BZP), became attractive in the 2000s due to possessing effects similar to amphetamines. Herein, BZP, in addition to its pyridyl analogues, 2-, 3- and 4-pyridylmethylpiperazine (2-PMP, 3-PMP and 4-PMP respectively) were subjected to the hyperpolarisation technique SABRE (Signal Amplification By Reversible Exchange) in order to demonstrate the use of this technique to detect these piperazine-based drugs. Although BZP was not hyperpolarised *via* SABRE, 2-PMP, 3-PMP and 4-PMP were, with the *ortho*- and *meta*-pyridyl protons of 4-PMP showing the largest enhancement of 313-fold and 267-fold respectively in a 1.4 T detection field, following polarisation transfer at earth's magnetic field. In addition to the freebase, 4-PMP.3HCl was also appraised by SABRE and was found not to polarise, however, the addition of increasing equivalents of triethylamine (TEA) produced the freebase, with a maximum enhancement observed upon the addition of three equivalents of TEA. Further addition of TEA led to a reduction in the observed enhancement. SABRE was also employed to polarise 4-PMP.3HCl (*ca.* 20% w/w) in a simulated tablet to demonstrate the forensic application of the technique (138-fold enhancement for the *ortho*-pyridyl protons). The amount of 4-PMP.3HCl present in the simulated tablet was quantified *via* NMR using D_2O as a solvent and compared well to complimentary GC-MS data. Exchanging D_2O for CD_3OD as the solvent utilised for analysis resulted in a significantly lower amount of 4-PMP.3HCl being determined, thus highlighting safeguarding issues linked to drug abuse in relation to determining the amount of active pharmaceutical ingredient present.

Introduction

Utilisation of hyperpolarization techniques to overcome the inherent sensitivity issue that is associated with Nuclear Magnetic Resonance (NMR), and by extension, Magnetic Resonance Imaging (MRI), is well documented.[1] The inherent insensitivity associated with both of these techniques arises due to the small population differences of the energy states that it probes. *Para*hydrogen induced polarization (PHIP)[2] is one of many hyperpolarisation techniques that has been employed to circumvent the inherent insensitivity of NMR and MRI through the creation of non-Boltzmann distributions of nuclear spins in the molecule(s) of interest.

PHIP utilises *para*hydrogen, a spin-isomer of hydrogen, as the source of polarisation.[3] As *para*hydrogen is a nuclear singlet, it is NMR silent and to gain access to the polarised state, its symmetry must be broken.[4] To achieve this, PHIP makes use of a metal centred catalyst, which catalyses the hydrogenation of a target ligand. Thus, polarisation is transferred to the target ligand resulting in dramatically increased signals in the NMR spectrum or MR image. However, this process

does necessitate the chemical transformation of the target ligand, and as such, they must be chemically synthesised to possess functional groups that can undergo hydrogenation.

An established non-hydrogenative *para*hydrogen-based technique is Signal Amplification By Reversible Exchange (SABRE), first reported in 2009.[5] Target ligands for this technique are largely limited to *N*-heterocycles, although there is increasing evidence of alcohols,[6] amines[7] and phosphates[7b] being able to be polarised by this technique. ^{1}H,[5, 7-8] ^{13}C,[9] ^{15}N,[10] ^{19}F,[11] ^{31}P,[7b, 12] ^{29}Si and ^{119}Sn[13] are all nuclei that have been polarised using the technique. An iridium-centred catalyst is typically employed to propagate polarisation, although examples of cobalt complexes have also been reported.[14] Polarisation is transferred from *para*hydrogen-derived hydrides and target ligand spin ½ nuclei *via* J-coupling,[15] with polarisation transfer occurring optimally at a level anti-crossing.[16] Polarisation transfer can be facilitated at low-magnetic field or at high-magnetic field, the latter being possible only through the use of specialised sequences.[17] The pre-catalyst, [Ir(IMes)(COD)Cl] (IMes = 1,3-*bis*(2,4,6-trimethylphenyl)imidazole-2-ylidene, COD = cyclooctadiene) has been shown to be an excellent catalyst, following activation, for facilitating polarisation transfer. Methyl-4,6-d_2-nicotinate has been polarised to a level of 50% using [Ir(IMes-d_{22})(COD)Cl] as the pre-catalyst.[18]

A large number of studies have focused on pyridine,[5, 8c, 9b, 9c, 17, 19] which is not biologically relevant. Thus, a number of drug molecules have been polarised by SABRE to not only study polarisation transfer but also to collect MR images of phantoms. Nicotinamide, a form of vitamin B_3, has been investigated using SABRE to probe the physical constraints and the population of spin order terms through the use of a flow system,[20] to relay polarisation to a second metal complex in solution[21] and to obtain MR images.[17, 22] Other examples of drugs that have been polarised and studied using SABRE include the tuberculosis drugs isoniazid and pyrazinamide,[8b, 23] the anti-fungal drug voriconazole[11c], the antibiotic metronidazole[10c, 24] and dalfampridine (4-aminopyridine),[25] which is used to study potassium channels and manage symptoms associated with multiple sclerosis. The use of SABRE in detecting drugs, such as harmine and morphine, down to the micromolar regime has been reported, as has the use of SABRE to detect selectively pyridyl fentalogues against a heroin background.[26]

Herein, the focus is to polarise *N*-benzylpiperazine (BZP) and its pyridyl derivatives, 2-, 3- or 4-pyridylmethylpiperazine (2-PMP, 3-PMP or 4-PMP respectively, Figure 1). BZP is a recreational drug that rose to prominence due to the growing popularity of 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy), which influenced an influx of similarly structured compounds known as piperazines.[27] BZP became the most popular MDMA/amphetamine substitute, with most of its distribution found in New Zealand (under the name BZP party pills), however, BZP has also been detected in the UK, Brazil and Japan.[27b, 28] The attractive euphoric effects saw them become prominent on the club scene as a safer alternative to MDMA, with an estimated 8 million doses sold between 2000 and 2005 in New Zealand.[27b]

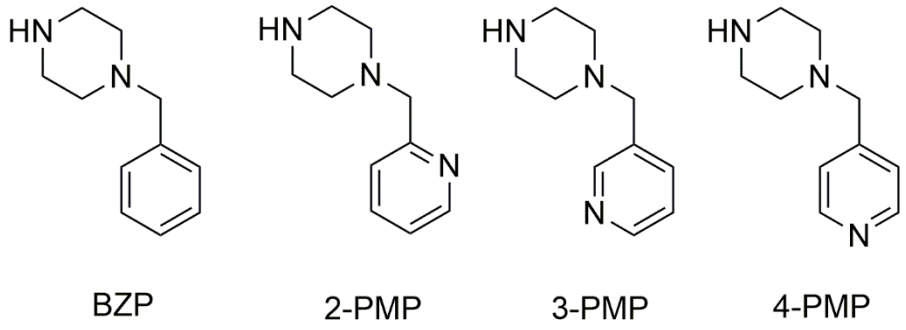

Figure 1. Chemical structures of BZP, 2-, 3- and 4-PMP

This paper details the employment of SABRE to hyperpolarise BZP and the PMP derivatives in both their hydrochloride and freebase forms utilising benchtop NMR (60 MHz) for detection. The PMP derivatives are pyridyl analogues of BZP and as such, could be synthesised by clandestine laboratories to circumvent current legislation. Thus, although 2-, 3- and 4-PMP have not been reported as being abused recreationally, they represent future potential forensic targets. An emphasis is placed on the detection of ^{1}H polarisation only. The effect of the addition of base (triethylamine (TEA)), on the enhancement observed for both freebase and hydrochloride forms of 4-PMP.3HCl is examined. Lastly, the extraction, and subsequent hyperpolarisation and quantification, of 4-PMP.3HCl from a simulated pill is detailed, in order to highlight how the approach could be applied to forensic casework.

Experimental

2, 3 and 4-PMP freebase were purchased from Fluorochem Ltd. (Hadfield, UK) and were used without further purification. BZP.2HCl was purchased from Sigma-Aldrich. Solvents (Sigma-Aldrich, Gillingham, UK and Fisher Scientific, Loughborough, UK) were used as received. ^{1}H NMR and ^{13}C NMR spectra utilised for the characterisation of 4-PMP.3HCl were acquired on a JEOL ECS-400 (JEOL, Tokyo, Japan) NMR spectrometer operating at a ^{1}H resonance frequency of 400 MHz and referenced to the residual OH peak of D_2O (δ = 4.79). Hyperpolarisation studies were conducted on an bench-top Pulsar NMR spectrometer (Oxford Instruments, Oxford, UK) operating at a ^{1}H resonance frequency of 60 MHz and referenced to the residual methyl solvent peak of CD_3OD (δ = 3.33).

1-(4-Pyridylmethyl)piperazine hydrochloride (4-PMP.3HCl) was prepared by treating a solution of 1-(4-pyridylmethyl)piperazine (0.5 g, 2.82 mmol) in diethyl ether (10 mL) with 4 M HCl in dioxane (5.6 mL, 11.2 mmol, 4 eq.). The resulting powder was collected by filtration and washed with diethyl ether (2 x 5 mL). The crude product was purified by recrystallisation from acetone to give the target compound as a beige powder (0.81 g, 100%); ^{1}H NMR (400 MHz, D_2O) δ 8.91 (d, 2H, J = 6.02 Hz, *ortho*-pyridyl protons), 8.26 (d, 2H, J = 6.02 Hz, *meta*-pyridyl protons), 4.58 (s, 2H, CH_2), 3.57 (t, 4H, J = 5.02 Hz, piperazine CH_2), 3.46 (t, 4H, J = 5.02, piperazine CH_2); $^{13}C\{^{1}H\}$ NMR (100 MHz, D_2O) δ 154.7 (C_{quat} pyridyl), 144.85 (C_{ortho} pyridyl), 131.70 (C_{meta} pyridyl), 61.75 (CH_2), 51.48, 44.48 (CH_2 piperazine).

SABRE measurements were conducted as follows: the substrate to be polarised (5 equivalents, $1.5x10^{-5}$ mol) and the pre-catalyst ([Ir(IMes)(COD)Cl], 0.0031 mmol) were dissolved in d_4-methanol (600 µL). If TEA was required, then this was added to the substrate prior to the addition of the pre-catalyst. The amount of d_4-methanol was also reduced to maintain a volume of 600 µL. The resulting solutions were then taken up by syringe and transferred into a NMR tube equipped with a Young's tap. The sample was then degassed on a high-vacuum line *via* three 'cool'-pump-thaw cycles (using a acetone/CO_2 slush bath). *Para*hydrogen, at a pressure of 3.0 atmospheres was then admitted to the NMR tube. *Para*hydrogen was produced by cooling hydrogen gas to 77 K over charcoal.[29] The

sample was then shaken to form the catalytically active species in solution prior to collecting a thermal ^{1}H NMR spectrum. Complete activation occurred within ~5 min and was evidenced by a single hydride signal being observable at *ca.* δ –23. For the collection of SABRE measurements, the hydrogen in the head-space of the tube was replenished with *para*hydrogen prior to shaking for 10 s in earth's magnetic field (*ca.* 0.05 mT). The sample was then rapidly inserted in to the NMR spectrometer and a ^{1}H NMR spectrum acquired using a π/2 pulse.

SABRE-relay measurements were conducted as follows: the substrate to be polarised (4 equivalents, 1.2x10^{-5} mol) was solvated in D_2O (500 µL) and ammonia (100 µL, 35% in H_2O) to which was added the pre-catalyst ([Ir(IMes)(COD)Cl], 0.0031 mmol). The sample was shaken to ensure full ligand and pre-catalyst solvation before transfer into a Young's capped NMR tube. The sample was then degassed as described above, with the exception that the sample was immersed in liquid nitrogen instead of an acetone/CO_2 slush bath.

The simulated tablet (522.5 mg) consisted of 4-PMP.3HCl (106.9 mg) and hydroxypropyl methylcellulose (HPMC, 415.6 mg). The tablets were made using an adaptation of the procedure reported by Hamad *et al*.[30] For the analysis of the tablet *via* SABRE, 30 mg of the tablet (containing *ca.* 6 mg of active pharmaceutical ingredient) was added to 900 μL of CD_3OD prior to filtering through a through a 0.45 µm polyvinylidene difluoride syringe filter (Whatman). 300 μL of the resulting filtered solution was added to a vial prior to the addition of 3 equivalents of TEA (3 μL), 150 μL catalyst stock solution (6.66 mg mL^{-1}) and 147 μL CD_3OD. The sample was then subjected to SABRE, as detailed in the paragraph above. For the quantification of the tablet by NMR, a weighed amount of the tablet (*ca.* 5 mg) was dissolved in CD_3OD (1 mL) and TEA (12 μL) or D_2O (1 mL) and then analysed.

Results and discussion

Initially, BZP was selected for polarisation using SABRE given its reported use as a recreational drug. BZP was available commercially as the di-hydrochloride salt, and it was in this form that it was subjected to SABRE. Following exposure of *para*hydrogen to a methanolic solution containing BZP.2HCl and the pre-catalyst [Ir(IMes)(COD)Cl], **1**, no polarisation transfer was observed. Furthermore, the lack of a hydride signal in the ^{1}H NMR spectrum and retention of the yellow colour of the [Ir(IMes)(COD)Cl] inferred that activation of the catalyst had not occurred. This was expected, as the lack of suitable atoms for ligation to the hydrochloride form meant the required J-coupling network could not be established. Thus, BZP.2HCl was further examined in two further experiments in which 1.2 equivalents or 2 equivalents of TEA was utilised in order to either partially or completely freebase the ligand respectively. Resultant SABRE-based experiments again yielded no hyperpolarisation. It is likely that in order to hyperpolarise this molecule, especially as it possesses a piperazine ring rather than an *N*-heterocycle, SABRE-relay[6-7, 21] may need to be employed in order to facilitate polarisation.

Our attention then turned to the 2-, 3- and 4-PMP ligands in their freebase forms. The applicability of SABRE to polarise 4-PMP was assessed using **1**. A sample consisting of 4-PMP and **1** in a ratio of 5:1 was constituted in CD_3OD. Upon the addition of hydrogen, the sample became colourless and a single hydride was evident at δ –22.80. Similar chemical shifts have been reported for the hydride ligand for a range of different [Ir(NHC)(COD)Cl] complexes when pyridine is the ligand due to the formation of $[Ir(NHC)(pyridine)_3(H)_2]^+$.[31] In this instance, the presence of a single hydride results from symmetry in the complex, due to the formation of $[Ir(IMes)(4\text{-}PMP)_3(H)_2]^+$ (Figure 2). There was no evidence for the formation of $[Ir(IMes)(4\text{-}PMP)_2(H)_2(CD_3OD)]^+$ or $[Ir(IMes)(4\text{-}PMP)_2(H)_2(Cl)]$ unlike previous reports concerned with pyridine.[32]

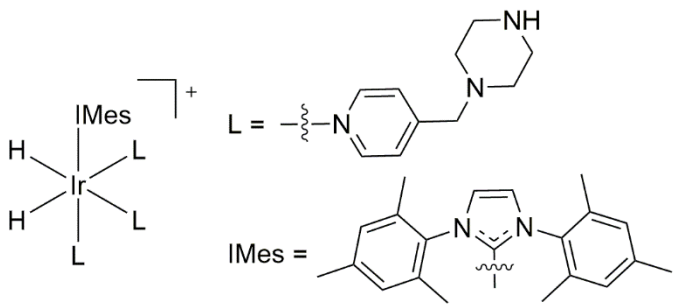

Figure 2. Chemical structure of $[Ir(IMes)(4\text{-}PMP)_3(H)_2]^+$

Charging this sample with *para*hydrogen followed by polarisation transfer in earth's magnetic field (0.5×10^{-4} T) provided evidence for SABRE-based hyperpolarisation having occurred (Figure 3). As a result of polarisation transfer, the *ortho*-pyridyl protons were enhanced by 313-fold, and provided an emission signal. Similarly, the *meta*-pyridyl protons were enhanced by 267-fold, but were observed as an absorption signal. The 4-PMP ligands bound to the catalyst (*trans*- to hydride) were also clearly evidenced following polarisation transfer at earth's magnetic field. Although we note the observed enhancement could be improved by using different magnetic field strengths, earth's magnetic field was chosen to provide a common magnetic field strength for polarisation transfer for all BZP and PMP derivatives tested herein.

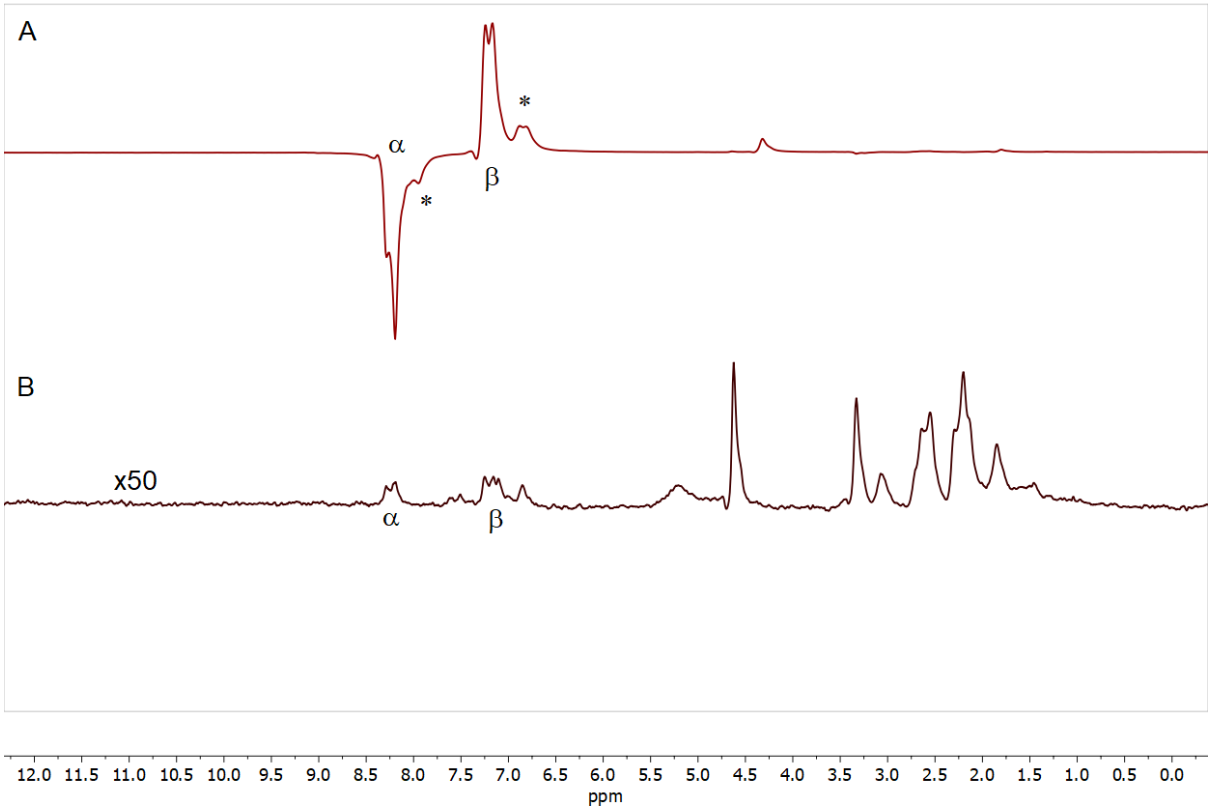

Figure 3. ^{1}H NMR spectra collected at 1.4 T of 4-PMP (0.025 M) after hyperpolarisation transfer at earth's magnetic field in the presence of **1** and *para*hydrogen (spectrum A) and the same sample that is fully relaxed sample (spectrum B). The thermal trace has been enlarged by a factor of 50 relative to the hyperpolarised trace. The peaks marked α and β refer to the *ortho*- and *meta*-pyridyl ^{1}H nuclei of 4-PMP respectively. Signals marked with an * indicate *ortho*- and *meta*-pyridyl ^{1}H signals for bound 4-PMP ligands *trans*- to hydride.

Exchanging 4-PMP for 3-PMP and 2-PMP did not yield the same levels of enhancement. 3-PMP produced an enhancement that was two orders of magnitude lower than 4-PMP (ε = 8-fold for the *ortho*-pyridyl protons) whereas 2-PMP yielded negligible enhancement (the signal for the *ortho*-pyridyl ^{1}H was observed to be in emission). Steric hindrance surrounding the pyridyl binding site could be a major contributing factor as to why these compounds possessed limited enhancement *via* SABRE. Thus, the focus of future studies was directed towards 4-PMP.

As the polarisation of 4-PMP via SABRE was successful, attention then turned to the polarisation of 4-PMP.3HCl. The reason for wanting to study this hydrochloride salt is because many drugs are formulated in their salt form, rather than as the freebase, to facilitate pharmaceutical processing and aqueous solubility. Attempts to polarise this salt using the same methodology as 4-PMP did not result in polarisation transfer. The reason for this is that there are no donors available to ligate to the metal centre (due to the nitrogen atoms not being in their freebase form) and hence polarisation cannot be transferred via scalar coupling as none are established. Thus, 4-PMP.3HCl was partially free-based using triethylamine (TEA) *in situ*, prior to the addition of the pre-catalyst. TEA was selected because of the solubility of its hydrochloride salt in methanol. Only 1.2 equivalents of TEA was added because it was desired, initially, to only partially freebase the ligand. As the pyridine ring nitrogen is the least basic in that pK_b for pyridine is 8.8 whereas the pK_b of piperazine is 4.19, it was envisaged that this approach would enable ligation to the SABRE catalyst through the pyridyl ring primarily in order to facilitate polarisation transfer. Polarisation transfer in earth's magnetic field resulted in an enhancement of ~8 fold for the *ortho*-pyridyl protons, which is a significant decrease compared to the free-base form of 4-PMP.

To investigate further the effect of TEA on hyperpolarisation, 1.2 equivalents of TEA was added to a methanolic solution of freebased 4-PMP, prior to the addition of **1**. Resultant polarisation transfer in earth's magnetic field yielded only a 161-fold enhancement – roughly half the enhancement observed for the same experiment conducted in the absence of TEA. The presence of TEA in this experiment is clearly detrimental to SABRE. In a separate experiment, *para*hydrogen was introduced to a methanolic solution containing 4-PMP freebase and **1**. The sample demonstrated SABRE as previously with the *ortho*-pyridyl protons being enhanced to a similar enhancement as before (300-fold obtained compared to 313-fold previously). The 13-fold difference in enhancement is due to the manual shaking method employed here; a ±20% variation in enhancement values has been reported for the same sample when analysed by different experimenters.[20] Five equivalents of TEA was then added, and after the sample was degassed and placed under a *para*hydrogen atmosphere, the sample now displayed no SABRE activity at all – polarisation transfer had ceased. The basicity of TEA (pK_b = 3.38) compared to the pyridyl moiety of 4-PMP means that TEA is by far the more competitive ligand for the SABRE catalyst employed. The lack of access to the metal centre means that polarisation transfer can no longer be transferred to 4-PMP and hence the resulting NMR spectrum shows no hyperpolarisation of this substrate. This also coincides with the loss of the hydride signal observed at δ –22.80 due to lack of exchange with *para*hydrogen.

Given this observation, the number of equivalents of TEA utilized to free-base 4-PMP.3HCl was then probed. A series of solutions were prepared to produce an equivalence range of TEA, relative to 4-PMP.3HCl, of 0-10 relative equivalents. The normalised results from this study are shown graphically in Figure 4. In the absence of TEA, there is no evidence of hyperpolarisation *via* SABRE following polarisation transfer in earth's magnetic field. As the number of equivalents of TEA is increased, the enhancement of the *ortho*-pyridyl protons increases, reaching a maximum at three equivalents. This is to be expected given that 4-PMP.3HCl has been completely converted to 4-PMP at this point. A signal enhancement of 363-fold is observed at this equivalence, and this is comparable to the value

obtained when 4-PMP freebase was examined, in the absence of TEA, taking in to account the experimental error associated with the measurement. Further addition of TEA, however, leads to a decrease in signal enhancement. This is again due to competition between TEA and 4-PMP for metal binding sites, of which the former is significantly more favoured. After the addition of four equivalents of TEA, the signal enhancement fell to 200-fold. Again, this is comparable to the drop in signal enhancement when 1.2 equivalents of TEA was added to 4-PMP freebase. Further addition of TEA causes the enhancement to drop to 20-fold when 10 equivalents have been added. It is noteworthy that the addition of TEA does not seem to change the species responsible for polarisation transfer as the main hydride present at δ −22.80 persists regardless of the concentration of TEA added.

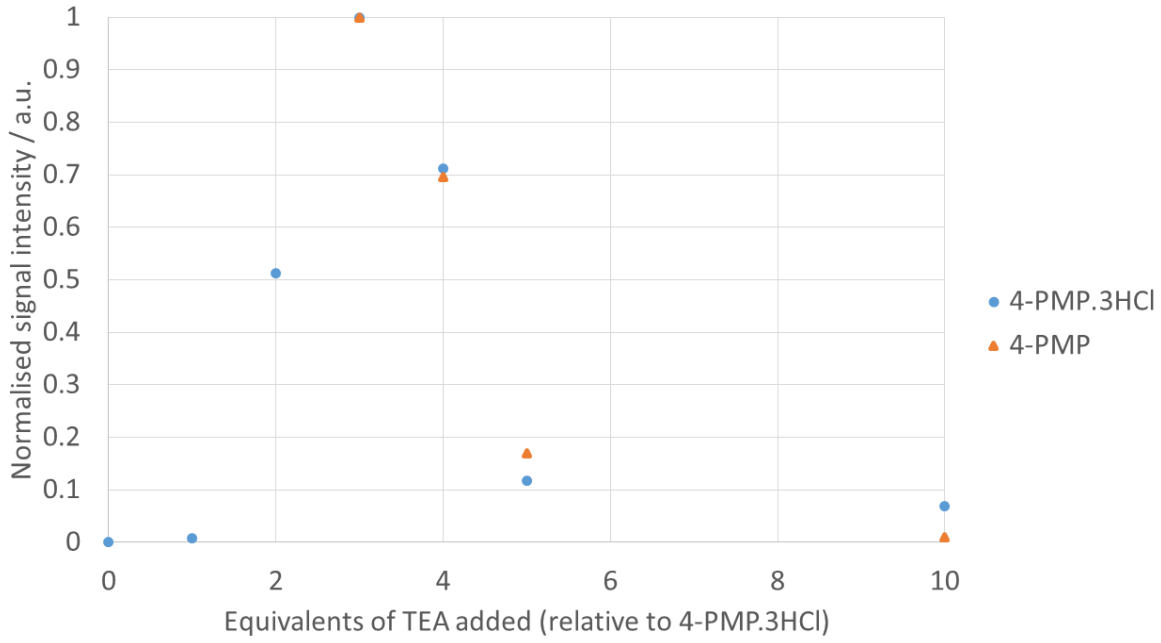

Figure 4. Normalised signal enhancement plotted against the equivalents of TEA added (relative to 4-PMP.3HCl) for 4-PMP.3HCl and 4-PMP.

In a further investigation, in which the enhancement of 4-PMP was monitored against increasing concentration of TEA, an expected decrease in signal was observed. When this data is normalised and contrasted with that of the analogous data for 4-PMP.3HCl, the slopes are in very good agreement. However, it should be noted that the level of signal enhancement after the addition of 7 equivalents of TEA (equivalent to 10 equivalents of TEA added to 4-PMP.3HCl) results in effectively negligible signal enhancement (ca. ≤ −1-fold). This difference is significant and may be due to the large difference in the concentration of chloride. Iali and co-workers have reported that for the hyperpolarisation of pyrazine by SABRE, large differences in the signal intensity can be obtained when not only varying the ratio of $CDCl_3$:D_2O but also the identity of the salt additive used to increase phase transfer.[33] It is reported that for a sample consisting of pyrazine dissolved in 200 μL:400 μL $CDCl_3$:D_2O that, after polarisation transfer at 3 mT, an enhancement of 690-fold is obtained. The addition of 0.0170 mmol NaCl leads to an increase in enhancement to 780-fold. It should be noted, however, that for other solution of differing ratios of $CDCl_3$:D_2O, but the same concentration of NaCl, the presence of NaCl had either no effect or was detrimental in terms of the enhancement observed. Knecht and co-workers have identified chloride as being able to re-bind to the main complex responsible for the polarisation of pyridine, $[Ir(IMes)(pyridine)(H)_2]^+$, through the

implementation of chemical exchange saturation transfer (CEST).[32] This chloride complex, [Ir(IMes)(Cl)(d_5-pyridine)$_2$($H)_2$], was shown to be largely responsible for shortening the nuclear singlet lifetime of the *para*hydrogen-derived hydride protons; removal of chloride from solution, and thus [Ir(IMes)(Cl)(d_5-pyridine)$_2$($H)_2$] also, caused the nuclear singlet lifetime to increase by an order of magnitude. A similar effect could be occurring here, and may account for the difference in enhancements observed between samples of 4-PMP.3HCl and 4-PMP.

Extrapolation of the technique to a simulated pill sample

As BZP.2HCl could not be hyperpolarised by utilising the method employed herein, a simulated street sample was prepared and analysed, in which the API was 4-PMP.3HCl. There is no current data on 4-PMP.3HCl being abused, and as such, this approach merely sought to demonstrate the applicability of applying SABRE to detect the API in a tablet. The procedure for hyperpolarisation needed to be adapted to include a filtration step prior to the addition of TEA. This was required to remove the insoluble binder (hydroxypropyl methylcellulose, HPMC) used in the formation of the tablet. The amount of TEA added was based on that fact that the tablet comprised of ~100 mg of 4-PMP.3HCl. Following polarisation transfer in earths' field, an enhancement of 138-fold was obtained for the *ortho*-pyridyl protons of 4-PMP (Figure 5). The enhancement is lower than previously stated and this is because the amount of 4-PMP present in the sample is only *ca*. 2 mg, instead of *ca.* 4.5 mg for the other 4-PMP.3HCl samples investigated herein. This process highlights the ability of the method to detect a low amount of API, following an extraction process to remove binder / filler material of the tablet. The downside of this approach is that the amount of API in the tablet must be known, so that the appropriate amount of TEA can be added; excess TEA would decrease the enhancement observed, whereas too little TEA would result in no enhancement at all. GC-MS (gas chromatography-mass spectrometry) was employed to also analyse the tablet prepared, which identified 4-PMP.3HCl as being the sole component present (R_t = 5.913 mins) from the chromatogram, with the eicosane internal standard possessing a R_t of 7.223 mins. Quantitative GC-MS analysis of the tablet revealed that the tablet consisted of either 111.8 mg or 107.5 mg of 4-PMP.3HCl after being analysed with or without an internal standard (eicosane). This compares well with the measured amount (106.9 mg).

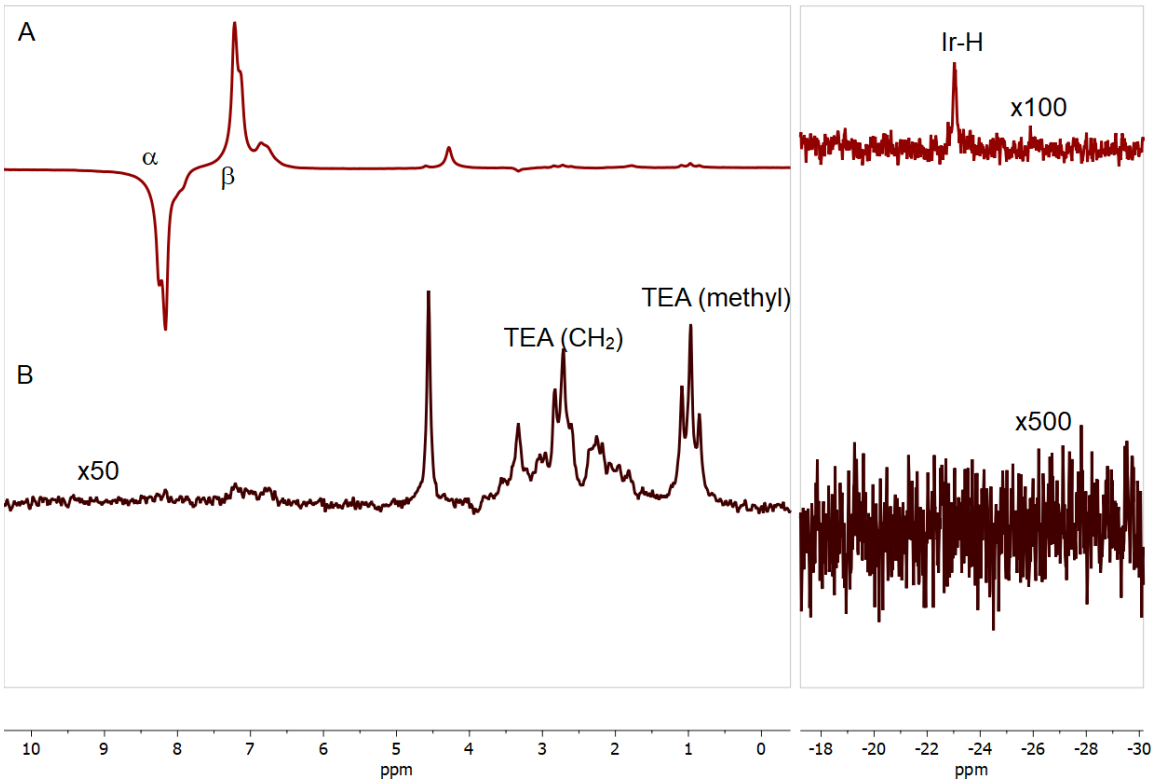

Figure 5. ^{1}H NMR spectra collected at 1.4 T of 4-PMP.3HCl following extraction from a simulated pill. Spectrum A is after hyperpolarisation transfer at earth's magnetic field in the presence of **1** and *para*hydrogen whereas spectrum B is the sample at thermal equilibrium. The values indicated above the baseline indicate the enlargement factor of that part of the spectrum. The peaks marked α and β refer to the *ortho*- and *meta*-pyridyl ^{1}H nuclei of 4-PMP respectively.

The amount of 4-PMP.3HCl present in the tablet was also quantified by NMR *via* the use of a calibration plot (see SI). Although it has been reported by Eshuis and co-workers that a standard addition approach coupled with SABRE can be used to quantify the amount of nicotinamide, pyrazine, isoxazole and quinazoline present in a 16-component mixture,[8a] the same approach could not be utilised here. This is due to the requirement of needing to use an excess of TEA to compensate for the HPMC binder that was used in tablet manufacture. The amount of 4-PMP.3HCl determined to be in the tablet was only 23 mg (~ 22% of the weighed amount), which is a significant departure from the amount determined by GC. However, it must be noted that the analysis of the tablet by NMR required a higher concentration (~5 mg mL^{-1}) whereas analysis by GC, which is significantly more sensitive, operated in the μg mL^{-1} regime, following dilution. Solubility was, therefore, suspected of being the underlying factor as to why the deduced amount of 4-PMP was *ca.* 20% of the correct value. Thus, a further calibration plot was obtained that used D_2O as the solvent. 4-PMP.3HCl is very soluble in D_2O and as such, it was found that TEA was not required. Resultantly, the amount of 4-PMP.3HCl was deduced as being 113.7 mg in the parent tablet; this value is not only comparable to that obtained by GC-MS but also reflects the amount that was weighed out originally. The quantification of NMR of 4-PMP.3HCl present in the simulated tablet highlights the significance of the solubility when determining the amount of active pharmaceutical ingredient (API) present in a sample. Evidently, the difference between the amount of 4-PMP.3HCl determined, using either

methanol or D_2O as a solvent, could have serious ramifications regarding safeguarding issues linked to drug abuse.

Conclusions

This paper describes the employment of SABRE to hyperpolarise BZP, 2-, 3- and 4-PMP, as well as the hydrochloride salts of BZP and 4-PMP. These studies revealed that BZP.HCl could not be polarised under the conditions employed, due to inability of the ligand to chelate to the metal centre of the SABRE catalyst employed, [Ir(IMes)(COD)Cl]. Addition of either 1.2 or 2 equivalents to either partially or completely freebase BZP still resulted in no enhancement of the signals in the ^{1}H NMR spectrum due to hyperpolarisation transfer.

2-, 3- and 4-PMP were also subjected to SABRE, and the resultant ^{1}H NMR spectra highlighted that polarisation transfer had occurred with varying degrees of success. 2-PMP showed negligible enhancement (*ortho*-pyridyl protons observed to be emission, ε = ~1) whereas the *ortho*- and *meta*-pyridyl protons of 4-PMP were enhanced by 313-fold and 267-fold respectively, following polarisation transfer at earth's magnetic field. The *ortho*-pyridyl protons of 3-PMP possessed an 8-fold enhancement. Optimisation of the magnetic field utilised for polarisation transfer could improve these enhancements further.

The hyperpolarisation of the hydrochloride salt of 4-PMP was also explored. In the absence of TEA, 4-PMP.3HCl showed no enhancement. However, the addition of increasing equivalents of TEA produced an observable enhancement, with a maximum of 300-fold being observed for 3 equivalent of TEA. The addition of further equivalents of TEA caused the enhancement to fall, with only a 20-fold enhancement being observed for 10 equivalents of TEA.

The effect of TEA on the enhancement observed for 4-PMP was probed similarly and the results from this study again showed a decrease drop in enhancement upon the addition of TEA, which mirrored that of 4-PMP.3HCl. After the addition of 7 equivalents of TEA, which is comparable to the 4-PMP.3HCl in the presence of 10 equivalents of TEA, the enhancement of the *ortho*-pyridyl protons was only ~1-fold. This significant difference in enhancement (approximately 20-fold) is believed to be due to the difference in the chloride concentrations, with 4-PMP.3HCl possessing the higher chloride concentration.

The approach developed herein was extended to polarise 4-PMP.3HCl within a simulated tablet sample. Although this approach requires the amount of 4-PMP.3HCl to be known, in order to add the corresponding amount of TEA to the sample to form the (polarisable) freebase, following polarisation transfer in earths' field, an enhancement of 138-fold was obtained for the *ortho*-pyridyl protons of 4-PMP. The enhancement is lower than the freebase on its own, and this was because the amount of 4-PMP present was *ca*. 2 mg, instead of *ca.* 4.5 mg for the other 4-PMP.3HCl samples investigated herein.

The amount of 4-PMP.3HCl in the simulated was quantified by GC-MS and NMR. The amount of 4-PMP.3HCl determined by GC-MS matched well with the weighed value, whereas the amount determined by NMR when samples were solvated in CD_3OD were considerably lower. This is believed to be due to issues surrounding solubility of 4-PMP.3HCl, despite being free-based due to the presence of TEA. Solubility is far improved in D_2O, and as such a value of 113.7 mg was determined. The quantification of 4-PMP.3HCl by NMR highlights a potential safeguarding issue linked to drug abuse when determining the potency of a tablet based on the amount of API present.

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