Identification and quantification of isolated medicinal compounds by PSYCHE NMR and DOSY NMR

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Introduction

NMR spectroscopy is a powerful tool for natural product research, as it can be used to probe many structural features as well as quantify. Primary and secondary metabolites contribute to the complex nature of plant mixtures and make a ^{1}H NMR spectrum very complicated. In order to alleviate the problems associated with multiplet peak overlap, various pure shift methods have been developed, the most recent being **P**ure **S**hift **Y**ielded by **C**hirp **E**xcitation (PSYCHE).

The NMR approach used in this study aims to improve the quantitative and qualitative analysis of mixtures without the need for preliminary separation or fractionation therefore offering gains in time efficiency.

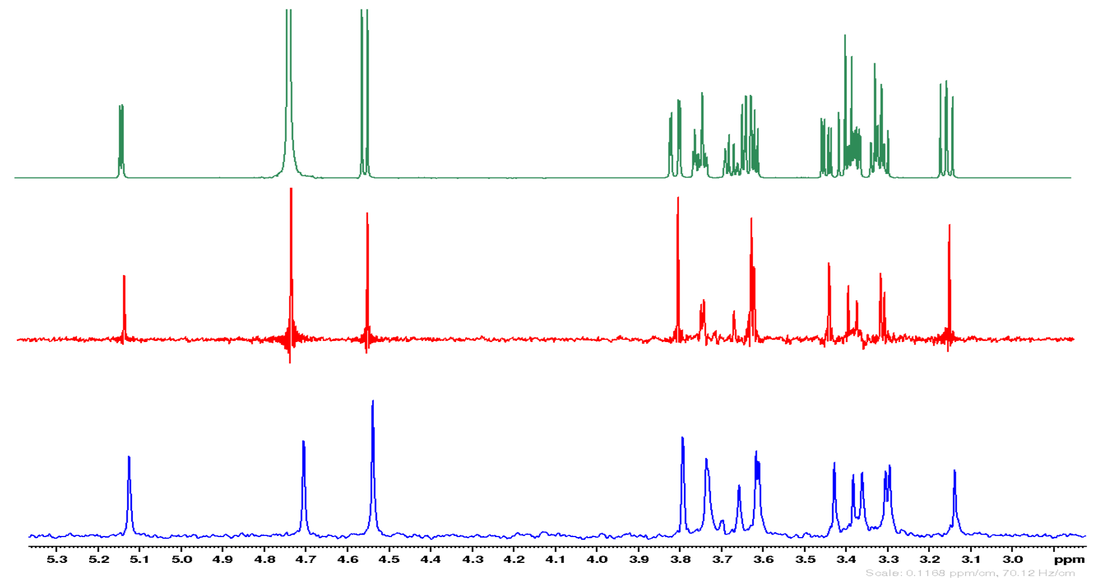

Figure 1: Comparison of ^{1}H NMR spectrum (green) vs. PSYCHE NMR spectra without (red) and with the implementation of saltire chirp pulse (blue)

Methodology

- Approximately 10 mg of the analyte and reference standard was accurately weighed and recorded three times.
- Both crucibles were added to a clear glass vial and 1mL of the respective solvent was added.
- The solution was vortexed for 30 seconds and 600 µL of the solution mixture was transferred to a 5 mm NMR tube for analysis.
- All samples were prepared in triplicate.
- The NMR spectra were recorded on a Bruker Avance III 600 MHz NMR spectrometer using a 5 mm PATXI ^{1}H/D{^{13}C,^{15}N} Z-GRD, triple-resonance, autotune probehead (Bruker BioSpin GmbH, Switzerland), and controlled with TopSpin 3.5.7 and Icon NMR 5.0.7 © 2017 Bruker Biospin GmbH.
- All measurements were performed with thermostatic control at 298ºK and data processing was caried out using TopSpin 3.6.3.

Results and Discussion

qNMR analysis of the ^{1}H spectrum and the ^{1}H PSYCHE spectrum was conducted using maleic acid as an internal standard. The purity values respectively calculated from the qNMR and the PSYCHE spectra were compared to assess the efficiency of the PSYCHE-based method and establish if it could be used in an accurate quantitative capacity. This is illustrated in **Table 1.** A difference of 1.11 % purity between ^{1}H qNMR and the PSYCHE NMR was reported.

Table 1: qNMR comparisons of 1H and PSYCHE NMR spectra of glucose

	^{1}H purity value	PSYCHE purity value
Replicate 1	99.03 %	101.10 %
Replicate 2	99.01 %	100.85 %
Replicate 3	99.03 %	101.54 %
	Standard Deviation = 0.01	Standard Deviation= 0.35

Further PSYCHE NMR experiments were conducted implementing parameters to investigate the impact on spectral quality in addition to the aim of achieving accurate quantitation.

The optimised parameters were excitation sculpting, receiver gain, swept-pulse flip angle, pure shift tau-delay, and the implementation of the saltire chirp pulse. In **Figure 1**, the implementation of the saltire chirp pulse (blue PSYCHE NMR spectrum) produced better spectral results with less recoupling artefacts and chunking sidebands presents.

A range of compounds, some illustrated in **Figure 2**, were analysed to establish whether the PSYCHE technique was compound specific to glucose only or if it was an effective tool for numerous compounds.

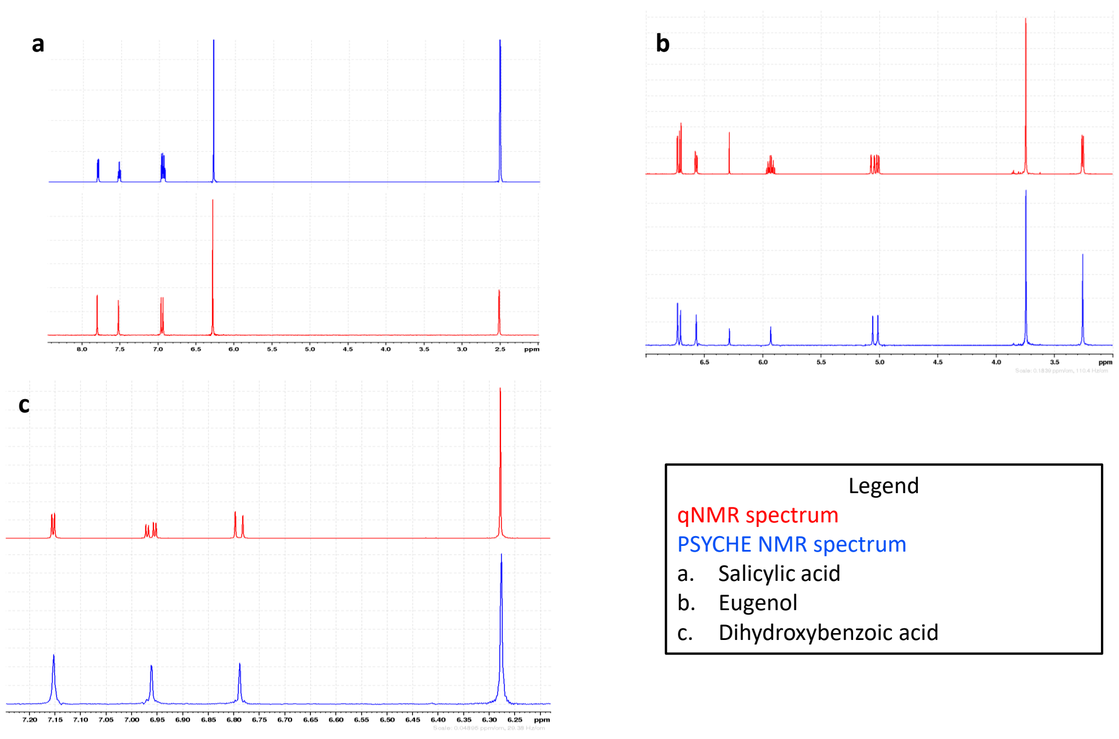

Figure 2: Comparison of ^{1}H NMR spectrum vs. PSYCHE NMR spectra of varying compounds

Phenolic, aromatic and aliphatic compounds were analysed, comparing the ^{1}H NMR spectrum of each compound with its corresponding PSYCHE NMR spectrum **(Table 2)**. Across all the spectra, it was observed that the PSYCHE analysis consisting of the newly implemented parameters is an efficient method in suppressing homonuclear coupling and is not compound selective to glucose.

Table 2: Comparison of ^{1}H qNMR and PSYCHE NMR spectra for various compounds

Compound	True Value	qNMR purity	PSYCHE NMR purity
Glucose	99.90 %	99.02 ± 0.01 %	100.13 ± 0.35 %
Eugenol*	99.94 %	99.83 ± 0.04 %	98.99 ± 0.02 %
Dihydroxybenzoic acid*	99.99 %	99.32 ± 0.25 %	100.51 ± 0.41 %
Salicylic acid	99.99 %	99.83 ± 0.02 %	82.36 ± 0.33 %

Conclusion

Earlier research on pure shift NMR techniques invariably suffered from the presence of undesirable artefacts. These could lead to baseline distortion, inaccurate purity assessment and misidentification of sample constituents in the case of mixtures. The new approach introduced here, allows acquisition of considerably cleaner high resolution PSYCHE NMR spectra, where artefacts are reduced while simultaneously producing efficient and accurate quantitative results.

References

Zangger (2015). Elsevier B.V. 86-87, 1-20.

Foroozandeh *et al.* (2014). Angewandte Chemie - International Edition. 53, 6990-6992.

Stark *et al.* (2020). Molecules. 25, 21.