Full Length Research Paper

Assessment of the composition of gins by nuclear magnetic resonance spectroscopy

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A limited number of samples of gin and related alcoholic beverages have been analysed using high-resolution ^{1}H NMR Spectroscopy to investigate the effectiveness of the technique as a means of gaining insight into their chemical composition. Various types of gin were investigated, including London Dry, Compound, Old Tom, and fruit gins. The study utilized some advanced NMR experiments, including multiple solvent suppression and total correlation spectroscopy, allowing for the identification and partial quantification of constituents, including terpenes, carbohydrates, organic acids, and phenolic compounds. Overall, based on the evidence of this focused study, NMR shows great promise for the chemical analysis of gins.

Key words: Nuclear magnetic resonance spectroscopy, gin, complex mixture analysis, quantification, solvent suppression.

INTRODUCTION

Gin is a popular alcoholic beverage defined as a clear alcoholic spirit distilled from grain or malt and flavored with juniper berries (Pearsall, 2002). However, this broad definition does not fully encompass all spirits currently sold as 'gin.' The term 'gin' is used relatively loosely, with a simple online search revealing a variety of products from different locations, flavored with a range of compounds. This is in sharp contrast to Scotch Whisky production, which is extremely tightly regulated.

Gin drinks have experienced a surge in popularity in recent years, partly due to the spread of flavored gins, sweetened beverages with lower alcoholic content (Thompson, 2021). The status of these so-called "gin liqueurs" is a point of debate within the gin community, questioning whether they should be considered true gins. According to EU regulations, for an alcoholic beverage to be considered gin, it must have at least 37.5% alcohol content by volume (ABV, defined as the number of mL of ethanol in 100 mL of solution) (European Parliament, 2008). Despite such regulations, products containing the word 'gin' in their name are available for purchase, even with ABV as low as 20%, potentially misleading the consumer. Several categories of gins are known, defined in terms of origin, sugar content, and method of

production. However, these classifications have no legal basis. Unambiguous identification and distinction of gins are further made difficult due to limited available information on their chemical composition.

The most "traditional" gin group is London Dry, characterized by the absence of any additives, aside from flavoring substances from botanicals used during redistillation, with the predominant flavor being juniper. In this category, the allowed amount of sugar is strictly regulated and cannot exceed 0.1 g/L of the product. A second category is Compound Gin, in which botanicals and other flavoring compounds are added without redistillation. There are several classes of gins with added sugar, such as Old Tom Gins, utilizing traditional recipes, or fruit gins flavored with various compounds. These drinks are often distinguishable from the aforementioned gin liqueurs by their higher alcohol content.

Previous research on gins has utilized gas chromatography combined with mass spectrometry (GC/MS) and has focused on terpenes and their derivatives (Vichi et al., 2005). Terpenes have the generic chemical formula $(C_5H_8)n$ and include flavor compounds such as α-pinene, limonene, β-pinene, sabinene, and myrcene. Several oxygenated terpenes have also been detected, including linalool, verbenyl ethyl ether, and α-terpineol. Some of the oxygenated monoterpenes were found in juniper berry essential oil, though in significantly smaller quantities relative to the monoterpenes (Höferl et al., 2014).

Nuclear Magnetic Resonance (NMR) spectroscopy is an analytical technique commonly used in organic and synthetic chemistry for the structural identification of molecules, both as pure materials and within complex mixtures. It utilizes the polarization of spin populations within the nuclear (Zeeman) energy manifold, which undergo transitions when excited by electromagnetic radiation of the appropriate frequency. The magnitude of absorbed frequencies is directly related to the nature of the chemical environment, providing information on molecular structure.

NMR is a promising alternative to GC/MS methods for the detection of a variety of compounds in a single experiment for several reasons. It can identify all components of a mixture in a single experiment without prior separation (unlike GC/MS), and experiments are generally rapid for ^{1}H and, in principle, quantitative (though see below). It possesses the additional advantage of swift sample preparation, usually requiring only the addition of deuterated solvent and/or buffer solution. NMR spectroscopy has already been successfully applied to the analysis of several alcoholic products, including beer (Duarte et al., 2002) and wine (Brescia et al., 2002), as well as beverages with higher alcoholic content such as rum (Belmonte-Sanchez et al., 2020) and Scotch Whisky (Kew et al., 2019; Stockwell et al., 2020).

The technique is not, however, without challenges, as difficulties with the analysis of complex mixtures can arise due to signal overlap, and, in the case of alcoholic beverages, by the presence of intense alcohol/OH resonances. The latter problem may be mitigated through the application of solvent suppression sequences, which can be specifically optimized for the suppression of ethanol and water signals (Kew et al., 2017; Monakhova et al., 2011).

The aim of this study is to explore the potential of ^{1}H liquid-state NMR spectroscopy to act as an analytical tool for the compositional analysis of a selected range of gins.

MATERIALS AND METHODS

Sample preparation

Sixteen samples in total were studied. Seven samples were alcoholic beverages of known brands, purchased at a local store in Edinburgh, UK. These included: CG1 hop gin, GL1 gin liqueur, CG2 barley gin, LD1 dry gin, CG3 compound gin, OT1 gin, SG1 sloe gin (CG = Compound Gin, GL = Gin Liqueur, LD = London Dry, OT = Old Tom, SG = Sloe Gin). A sample of LD2 dry gin was purchased in a store in Poland. Eight samples were provided by Dr. Matthew Pauley from The HWU International Centre for Brewing and Distilling (UG145, 307, 477, 552, 638, 790, 863, and 999). These samples served as unknowns (no information about them was provided prior to analysis) to assess the ability of NMR-based methodologies to identify and categorize gins. 3-(Trimethylsilyl)-1-propanesulfonic acid (DSS), D_2O, acetic acid-d4, sodium acetate-d3, and methanol-d4 were acquired from Cambridge Isotope laboratories. Maleic acid was acquired from Sigma-Aldrich. α-pinene, β-myrcene, linalool, DL-limonene, α-terpinene, sabinene, and p-cymene were acquired from Acros Organics. Samples were analyzed neat (0.6 mL of analyte), as solutions in D2O (0.1 mL of analyte, total sample volume unchanged), and with added acetic acid/sodium acetate buffer solution, with a formulation based on that previously used by Kew et al. (2017) for Scotch Whisky analysis (0.5 mL of sample and 0.1 mL of buffer solution). The solution used for this study was prepared by mixing sodium acetate-d3 (0.0277 g, 0.204 mmol), acetic acid-d4 (0.0820 g, 1.28 mmol), DSS (0.0131 g, 0.0600 mmol), and filling up to the mark with D_2O in a 10 mL volumetric flask. The resulting mixture had a pH of 3.95, and the final concentration of DSS in the standard 5mm NMR tube was 1.00 mM. Assignment of signals was done through comparison with online databases (Saito et al., 2022), literature (Fotakis et al., 2013; Jeong et al., 2017; Kew et al., 2019; Mannina et al., 2016; Monakhova et al., 2012; Nord et al., 2004; Teipel et al., 2020), or by comparison with spectra of the pure compounds through spiking experiments.

NMR spectroscopy

^{1}H NMR experiments were performed on the following instruments: Bruker AVIIIHD Spectrometer at 400.03 MHz, T=298.1K at Heriot-Watt University, Bruker AVIIIHD Spectrometer at 600.75 MHz equipped with a TCI cryoprobe, T=300.0K at the School of Chemistry, University of Edinburgh. For further details see ESI.

RESULTS AND DISCUSSION

Initial screening revealed the presence of compounds

Table 1. Quantification of selected compounds in known gin samples, obtained from ^{1}H NMR Spectroscopy.

Sample (Notable compounds, mg/L)	LD1	CG1	CG3	OT1	CG2	GL1	LD2	Reference[a]
α-pinene	-	2.0	2.4	3.7	NQ	-	7.6	1.95-6.12
Linalool	5.9	-	-	10.4	15.7	-	-	1.93-36.99
Limonene	1.6	18.9	6.9	6.8	NQ	-	2.2	1.22-17.21
Acetic acid	1.2	3.8	2.5	3.1	6.8	1.4	3.1	-
Ethyl acetate	-	-	-	-	87.4	144	0.8	-
Methanol	NQ	2.2	2.8	5.1	NQ	NQ	3.4	-
Formic acid	-	1.2	0.6	1.0	0.6	0.7	1.4	-
Glucose	-	-	-	NQ	-	45500	-	-
Sucrose	-	-	-	NQ	-	48900	-	-
Acetaldehyde	-	3.4	0.4	1.0	-	-	0.6	-
Lactic acid	-	-	-	-	-	81.0	-	-
Citric acid	-	-	-	-	-	1280	118	-
3-methylbutanol	-	-	-	-	30.0	-	-	-
2-methylbutanol	-	-	-	-	6.4	-	-	-
Acetone	2.3	5.1	0.8	0.8	2.0	32.5	1.5	-

NQ = Not quantifiable due to signal overlap, suppression or distortions. - = not present or concentration too low to be observed. [a]Range of reference values for gins analysed using GC/MS, only α-pinene, linalool and limonene were quantified, the values for which are taken from work of Vichi et al. (2005).
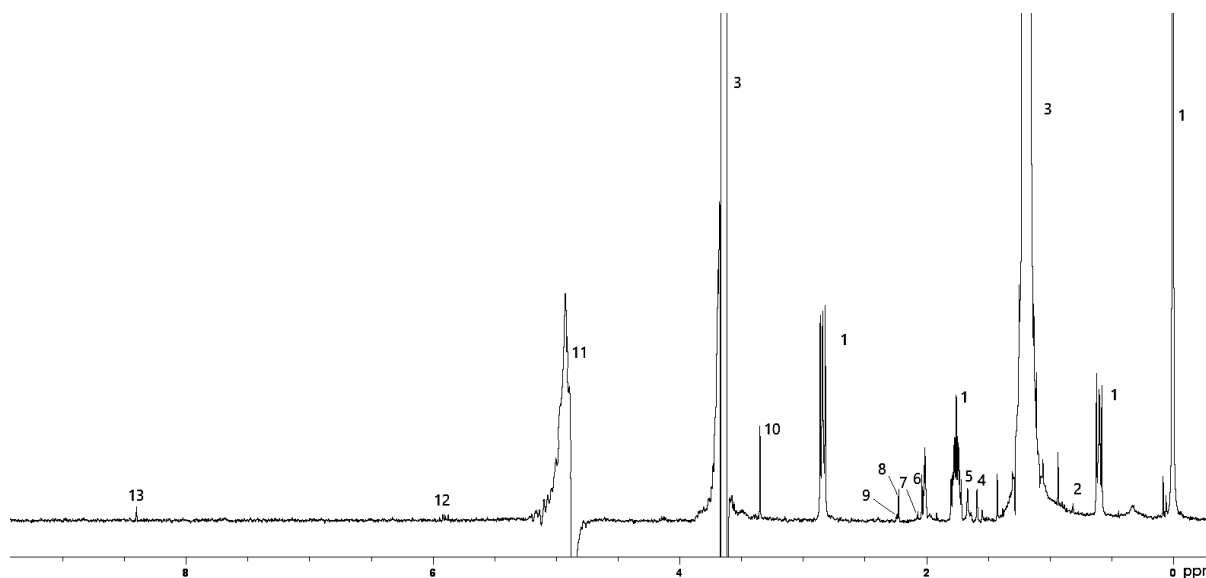

Figure 1. Spectrum of unknown gin sample UG 999 with buffer solution, recorded on a Bruker 400MHz AVIII HD spectrometer, with solvent suppression sequence. Signal assignment: 1: DSS, 2: α-pinene, 3: ethanol, 4: limonene, 5: limonene, overlap with α-pinene, 6: acetic acid, 7: Ethyl acetate, 8: acetone, 9: acetaldehyde, 10: methanol, 11: water, 12: linalool, 13: formic acid.

common across most of the samples (Figure 1). These included signals associated with α-pinene, limonene, and methanol plus unidentified resonances, possibly resulting from other terpenes, previously detected in juniper essential oil and gin (Höferl et al., 2014; Vichi et al., 2005). Several samples showed a characteristic doublet of doublets at 5.91 ppm, assigned to linalool and confirmed by comparison with the spectrum of a pure sample. The same method was used to confirm the identity of α-pinene and limonene resonances. Most samples contained a signal at 2.22 ppm, characteristic of acetone (Gottlieb et al., 1997), possibly an impurity. The

addition of a buffer solution with a known concentration of DSS allowed for quantification of compounds through a comparison of integrals of the DSS signal at 0 ppm, which was also used to confirm chemical shift calibration.

The quantification data for terpenes α-pinene, limonene, and linalool are in general agreement with the range of values for gins obtained from GC/MS and published by Vichi et al. (2005), with 0.6-3.7 mg/L concentration of α-pinene, 0.9-19 mg/L of limonene, and 4.0-18 mg/L of linalool. It may be noted that the characteristic methyl-proton resonance of methanol at 3.34 ppm was detected in all samples and was successfully quantified in most of them. In all cases, the concentration is below the generally accepted limit for London Dry Gins of 5 g/hl of 100% v ethanol (European Parliament, 2008). This equates to 21.5 mg/L for a 43% ABV drink. The highest methanol content in the analysed samples were 5.1 mg/L for the OT1 gin, roughly four times less than the limit specified above. Detailed quantification and comparison to GC/MS data are shown in Table 1. Several other compounds were quantified in the same experiment, not possible using chromatographic methods, highlighting the advantage of the NMR analysis. It must be noted, however, that quantification was not possible in all cases due to the proximity of key signals to those of (suppressed) ethanol or water, overlap with other resonances, or baseline distortions.

In the case of the first issue, active suppression of undesirable intense resonances from the solvent inevitably leads to impacts on adjacent signals of interest, irrespective of the type of suppression utilized. Baseline distortions resulting from the suppression element within the sequence also reduce the reliability of quantitation in those regions of the spectra directly affected, but qualitative interpretation of the data is not compromised, with much useful compositional information being revealed. Quantification based on the Electronic reference to access *In-vivo* Concentrations (ERETIC) method produced results similar to those obtained using an internal standard for most samples, but the method was ultimately deemed too unreliable, as three of the analysed samples showed deviations too large to be explained by measurement errors (up to 50% difference between ERETIC and internal standard measurements).

ERETIC acts through using an electronic 'spike' in an arbitrary reference sample against which to calibrate concentrations in genuine samples and has the advantage of not requiring adulteration of samples with reference compounds. In this study, it was deemed insufficiently robust in some cases when compared with the traditional method of quantifying through the addition of a standard to the sample, and so the method was not pursued further.

The analytes, which displayed commonalities outlined and no other signals associated with additional flavorings were classified as London Dry Gins and included: LD1, LD2, UG145, UG307, UG477, UG790, UG863, and UG999. In addition to the signals commonly found in all samples, several analytes showed unique spectral profiles with resonances corresponding to substances not found in other samples, which are especially useful for the purpose of authentication. CG2 gin displayed several resonances associated with fusel alcohols, such as 2-methylbutanol, 3-methylbutanol, and 1-propanol (Figure 2). Additionally, the ethyl acetate content was significantly greater than for other gins. This observation can be rationalized through consideration of the process of gin production, namely the addition of barley, as in the production of beer (The Borders Distillery) (Oliver, 2012). The aforementioned substances, along with several others, are synthesized during fermentation, which leads to their appearances in the final product. Such clear distinction from other tested products validates how NMR can be utilized to identify and "protect" specific brands of gin, though similar inferences can be made based on more subtle differences.

Compound Gins analyzed (CG1 and CG3) showed no significant differences in concentrations of terpenes compared to other beverages tested, though neither of them showed any linalool. The CG1 sample showed the largest concentration of limonene out of all the analytes and was the only one to have signals correlating to malic acid (2.52, 3.15, and 4.26 ppm (dd)). These observations may reflect the presence of flavoring compounds added to the gin. Both compound gins had distinct colors: yellow for CG1 and pink for CG3. This suggests that some of the unidentified signals may arise from the colorants. Though most of the studied samples showed no traces of carbohydrates, two did.

Unknown gins 145 and 477 contained sugars in sufficiently low concentration (22 mg/L combined sucrose and glucose in UG145 and 18.1 mg/L of glucose in UG477) to still be classified as London Dry, according to European regulations (European Parliament, 2008). Interestingly, only glucose was present in the unknown sample 477, which may signify that it was added artificially; otherwise, sucrose and glucose, as well as potentially other carbohydrates, would be expected. A similar situation was observed for sample UG638. In this case, however, only sucrose was detected and in much greater amounts (1.8 g/L).

Again, this suggests that additional sugar was added to sweeten the gin, and as a result, we classify it as a sweetened gin. Unknown sample 552 contained around 0.5 g/L of both sucrose and glucose, as well as a significant concentration of vanillin (40 mg/L). These observations, combined with the clear presence of α-pinene and limonene (which distinguishes it from gin liqueurs), suggest that this sample may also be classified as a sweetened gin.

The spectrum of the gin liqueur showed the most obvious differences, as expected due to the addition of sugars and flavor compounds (Figure 3). Several substances commonly used as food additives were
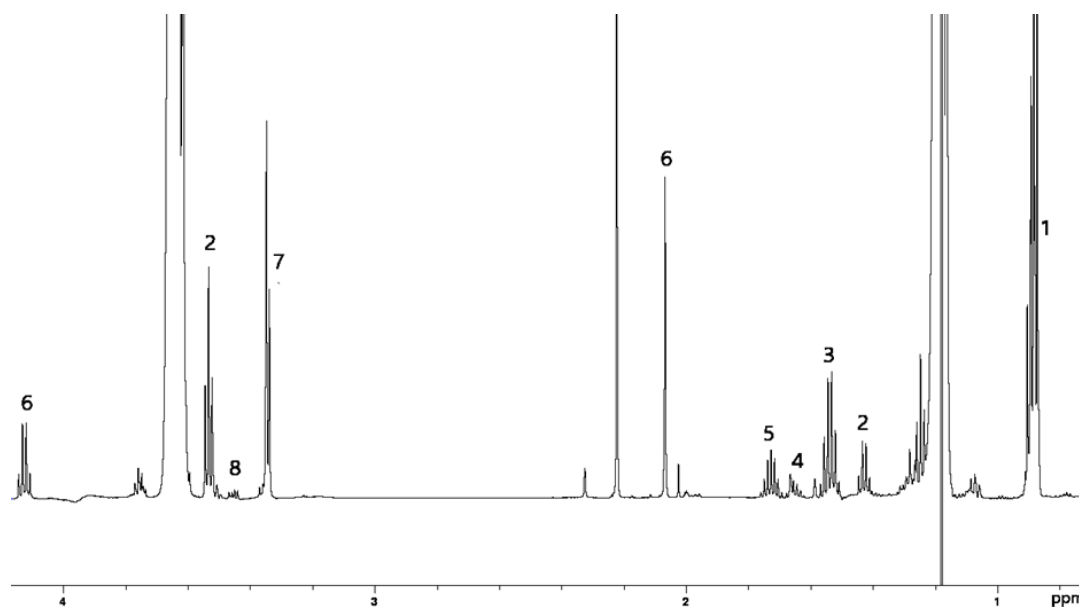
Figure 2. Partial ^{1}H NMR spectrum of CG2 gin sample, recorded on a Bruker 600MHz AVIII spectrometer. Signal assignment: 1: Overlapping signals of higher alcohols, 2: 3-methylbutanol, 3: 1-propanol, overlap with linalool, 4: 2-methylbutanol, overlap with linalool, 5: 2-methylpropanol, 3-methylbutanol, 6: ethyl acetate, 7: 2-methylpropanol, overlap with methanol, 8: 2-methylbutanol.
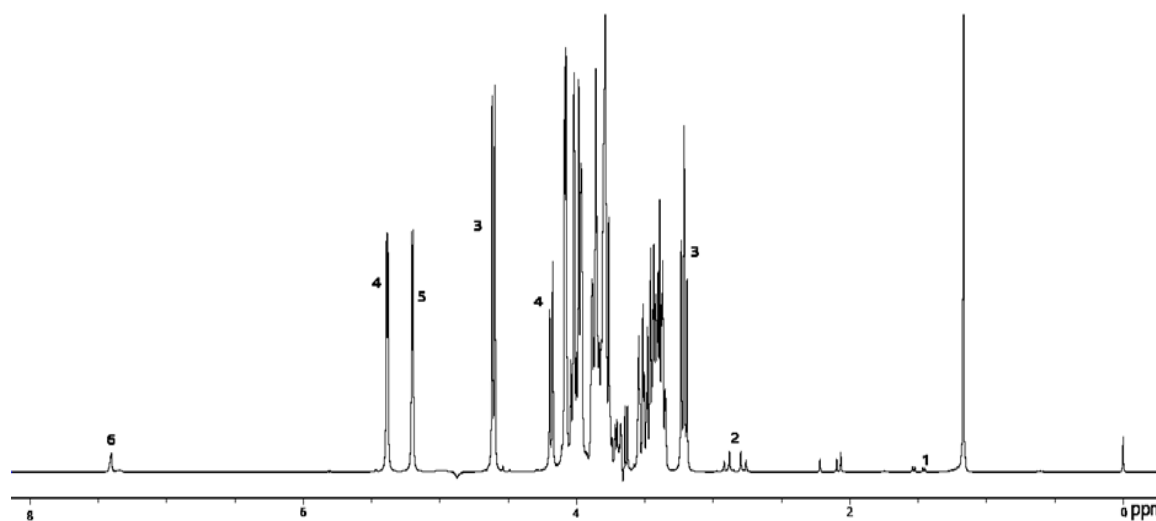

Figure 3. Fragment of the ^{1}H NMR spectrum of GL1 gin liqueur sample, recorded on a Bruker 400MHz AVIII HD spectrometer. Signal assignment: 1: lactic acid, 2: citric acid, 3: β-glucose, 4: sucrose, 5: α-glucose, 6: phenyl ethanol.

detected in significant quantities, including lactic and citric acids. Another clear distinction from the true gin samples was the lack of signals due to terpenes. Assignment of carbohydrate signals proved relatively straightforward in this sample, as the concentrations of both glucose and sucrose were relatively high (45.5 and 45.8 g/L, respectively), and so this spectrum was able to be used as a reference. The inferences made using the following

methodology were then used to assign carbohydrate signals in all other samples where appropriate.

Initial analysis of the reference ^{1}H spectrum revealed signals characteristic of the anomeric CH of α-glucose (5.20 ppm d, JHH = 3.7 Hz), anomeric CH of β-glucose (4.60 ppm, JHH = 7.9 Hz), CH of sucrose glycosidic linkage (5.38 ppm, JHH = 3.8 Hz), and sucrose CH in the fructose ring (4.18 ppm, JHH = 8.7 Hz). These signals
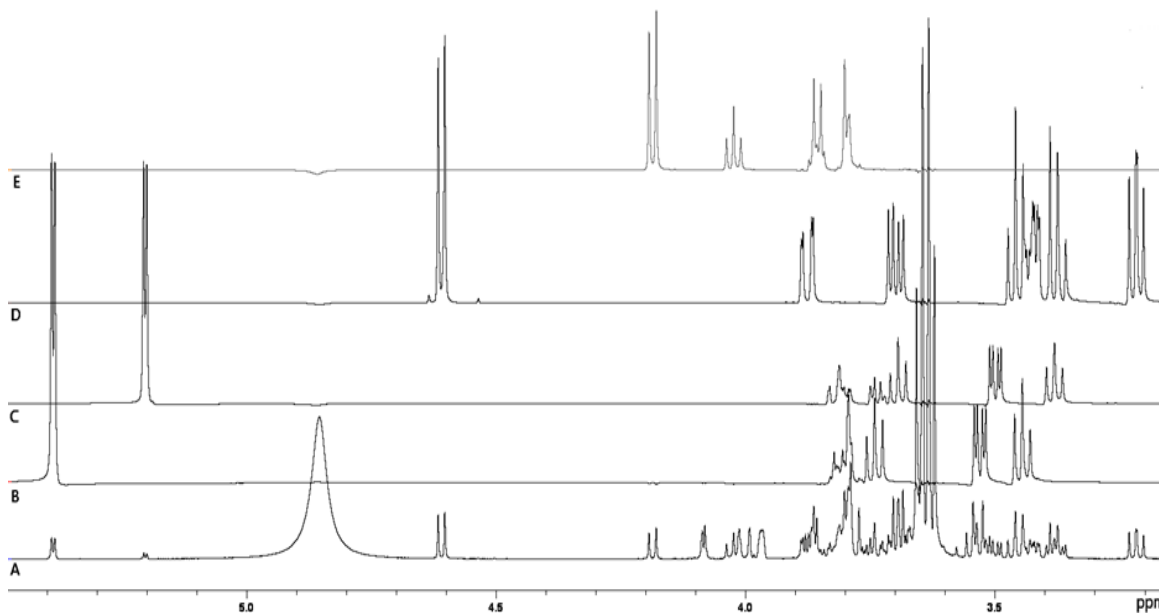
Figure 4. Series of one dimensional TOCSY spectra recorded on a Bruker 600MHz AVIII spectrometer. A: Partial ^{1}H NMR spectrum of GL1. Resonances correlating to the signal at B: 5.38 ppm (sucrose: glucose ring), C: 5.20 ppm (α-glucose), D: 4.60 ppm (β-glucose), E: 4.18 ppm (sucrose: fructose ring).

are clearly separated from other carbohydrate signals located in the 3.4 to 4.0 range and far enough away from the suppressed water and ethanol signals to be used for quantification. Differentiation between α and β anomers of glucose was possible because the positions of hydrogens in the ring are fixed, resulting in different J-coupling values, depending on whether they are in axial or equatorial positions (Bubb, 2003).

Following these assignments, a series of one-dimensional TOCSY (TOtal Correlation SpectroscopY) spectra were acquired, revealing correlations between the aforementioned signals and all other resonances arising from the same spin system. This procedure allowed for the discrimination between signals of α-glucose, β-glucose, and sucrose rings (Figure 4).

The correlations were further validated through the mapping of ^{1}H signals to ^{13}C signals using Heteronuclear Single-Quantum Coherence (HSQC) spectroscopy. The investigation of Sloe Gin faced challenges in quantifying compounds due to significant baseline distortions. Despite this issue, several compounds were identified, including sucrose, both anomers of glucose, and limonene. The 6 to 10 ppm region (aromatics) exhibited numerous signals absent from other samples, attributed to phenolic compounds found in sloe, such as myricetin, ferulic acid, caffeic acid, and gallic acid (For full assignment of aromatic resonances, see Figure 5) (Aliyazicioglu et al., 2015; Najgebauer-Lejko et al., 2021).

The spectrum of SG1 was compared to that of the sloe extract. Several resonances matched, although almost none of the aromatic signals found in the gin were present in the extract. A possible explanation for this observation is the poor transfer of this particular class of compounds during extraction (Alara et al., 2018).

Conclusions

Several samples of gins and related alcoholic beverages were analyzed using high-resolution ^{1}H NMR Spectroscopy. The employed methods, including a custom solvent suppression sequence, enabled the detection, identification, and quantification of various compounds, including those previously identified in GC/MS studies and some newly identified in gin samples (Vichi et al., 2005). This study represents a focused and preliminary examination of the potential use of NMR spectroscopy in gin analysis. The results suggest its viability, complementing more established techniques like GC/MS. Further investigation is needed to determine the full scope, involving statistical analysis of a truly representative sample set. This work serves as a 'proof-of-concept' contribution, validating the approach and laying the groundwork for more comprehensive studies and statistical evaluations in the future.
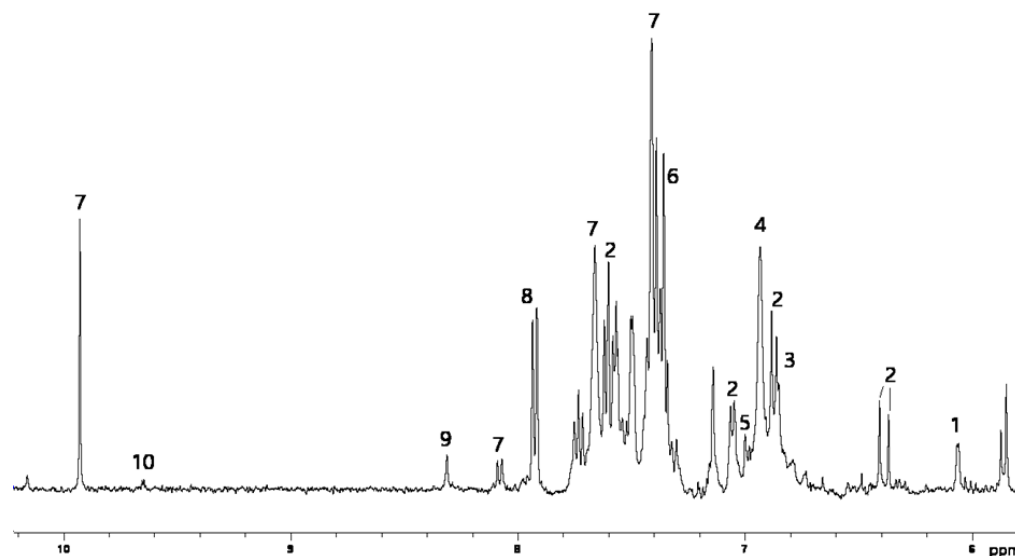
Figure 5. Aromatic region of the SG1 ^{1}H NMR spectrum (with no buffer solution) recorded using Bruker 400MHz AVIII HD spectrometer. Signal assignment: 1: caffeic acid overlapping with myricetin, 2: ferulic acid, 3: caffeic acid, 4: myricetin, 5: caffeic acid, 6: gallic acid, 7: benzaldehyde, 8: benzoic acid, 9: formic acid, 10: acetaldehyde.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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