Annex D

First Month Project Report (Updated October 2024)

Study of Neutral Spirits for Alcoholic Drinks for Their Quality and Safety Parameters

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Literature Review

Neutral spirits (NS) are intended for manufacturing alcoholic drinks, such as Vodka, Gin, Absinthe, Liqueurs, Brandies, Whisky, Rum, and Baiju (Qian et al., 2019). It has been employed as a solvent for flavoring in the food sector and vinegar and apple cider manufacturing. These are also used as the base for cocktails. These can provide high-proof alcohol without imparting any flavor that enhances the desired taste of the cocktail, making it a popular base for many different types of cocktails. Another application of the NS is that fruits, herbs, or spices are soaked in them to impart their flavors, and this is how it's commonly employed as a base for infused spirits. Since neutral spirits have no taste, they can create a wide range of flavored spirits, such as gin or vodka infused with fruit.

A study focused on determining the concentrations of methanol and ethanol in alcoholic beverages and food products was conducted by Tulashie et al., (2017) Using gas chromatography, this study measured the concentrations of these chemicals in several fermented food products from Ghana as well as in alcoholic beverages from both domestic and international sources. Contrary to popular belief, the results showed that the amounts of methanol in these alcoholic beverages do not present a health concern when ingested. However, if ingested excessively or carelessly, the amounts of ethanol in particular beverages could pose serious health hazards. In addition, the study made policy proposals to control alcohol production and consumption.

Debebe et al. (2017) conducted a comprehensive study on the use of Fourier-transform mid-infrared (FT-MIR) and near-infrared (NIR) spectrophotometry for the direct determination of ethanol and methanol in alcoholic beverages, providing a non-invasive and efficient approach. The study employed both FT-MIR and NIR spectrophotometry, which complemented each other in analyzing ethanol and methanol content in alcoholic beverages. FT-MIR was used to confirm the presence or absence of methanol, while NIR was utilized to assess alcoholic strength without the need for sample dilution. Calibration curves were

developed by correlating peak areas or heights with the percentages (%, w/w) of ethanol and methanol. The linearity range for ethanol was 0-15% using FT-MIR and up to 50% with NIR spectrophotometry. The results obtained from FT-MIR and NIR spectrophotometry were highly consistent with those from gas chromatography. These methods demonstrated simplicity, speed, precision, and accuracy, eliminating the need for sample preparation. Notably, methanol was not detected in any of the samples analyzed using these spectrophotometric techniques, which aligned with the results from gas chromatography. Overall, the study highlights the effectiveness of FT-MIR and NIR spectrophotometry in determining ethanol and methanol in alcoholic beverages. These methods offer several advantages, including simplicity, rapidity, precision, and accuracy, making them valuable tools for quality control and regulatory compliance in the beverage industry. Additionally, the confirmation of methanol absence in the samples underscores the reliability of these spectrophotometric techniques for safety assessments.

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A comprehensive overview was given to analyze spirit-based alcoholic beverages using gas chromatography. The article covers various sample preparation methods for GC analysis, including liquid-liquid extraction (LLE), solid-phase extraction (SPE), solid-phase microextraction (SPME), stirring bar sorbent extraction (SBSE), and supercritical fluid extraction (SFE). It also details the principles and benefits of different chromatographic techniques, such as one-dimensional and two-dimensional gas chromatography. The authors discuss the types of injectors, columns, and detectors employed in the analysis of spirit-based beverages and the role of modulators in a GC x GC system, which trap and release analytes from the first column to the second. Detectors, which generate an electrical signal when analytes exit the second column, must possess high-frequency data acquisition and sensitivity. Examples include flame ionization detectors (FID), electron capture detectors (ECD), atomic emission detectors (AED), and time-of-flight mass spectrometers (TOF-MS). Additionally, the article explores the use of gas chromatography for identifying and quantifying volatile compounds in alcoholic beverages, such as esters, alcohols, acids, aldehydes, and terpenes. Overall, the study provides valuable insights into the application of gas chromatography in analyzing spirit-based alcoholic beverages (Wiśniewska et al., 2015).

Aylott (2013) addresses the issues and challenges associated with protected designations of origin (PDO) and geographical indications (GI) for alcoholic beverages, which are based on the product's country or region of origin and its manufacturing processes. The paper provides an overview of common themes and variations in the fermentation, distillation, and maturation processes across different categories of alcoholic beverages, including rum, whisky, brandy, vodka, gin, tequila, and liqueurs. It also reviews the legal definitions and standards for flavored spirits in the European Union, the USA, Canada, and Australia, detailing the minimum alcohol content, permitted sources of alcohol and flavorings, and labeling requirements for various flavored spirits. Additionally, the study presents statistics and examples of leading brands and markets for flavored spirits worldwide, highlighting consumer diversity and regional preferences. It also discusses the challenges and opportunities these factors present for the industry.

Analysis and characterization of alcoholic products (ACAP) to provide scientific support for classifying alcoholic beverages for excise duties based on analytical methods was done. The study addressed key challenges in classifying alcoholic beverages, including (i) the extent to which a fermented product can undergo a "cleaning-up" process while still being

classified as a fermented beverage, intermediate product, or ethyl alcohol; and (ii) the amount of ethyl alcohol that can be added to a fermented beverage without it being reclassified as a spirit drink. The research explored two complementary approaches: NMR fingerprinting and classical analysis, combined with multivariate data analysis. The team analyzed 114 samples of various alcoholic beverages, including beers, wines, distillates, and alcopops. The study found that it is impossible to determine the exact amounts of added alcohol based solely on the scientific analysis of the finished product. Consequently, the study concluded that a case-by-case approach remains necessary for classification under current legislation. The research also discussed analytical methods for detecting the addition of ethanol from different sources to wine and other fermented beverages using ethanol's deuterium/hydrogen ratio. It explained that while the deuterium/hydrogen ratio can identify the botanical origin of ethanol, it cannot determine the fermented character of the beverage. Additionally, the study noted that some vermouths are produced using a second fermentation of sugars, resulting in deuterium/hydrogen values outside the typical range. The research also described a statistical analysis using SIMCA (soft independent modeling of class analogy) to investigate the addition of distilled alcohol to beer and wine, concluding that the added alcohol can only be analyzed if it exceeds 100% (i.e., when the final mixture consists of 50% fermented alcohol and 50% distilled alcohol) (Segebarth et al., 2008).

Curyło and Wardencki (2006) studied the Application of the Single Drop Extraction (SDE) Gas Chromatography Method for determiningcarbonyl compounds in Vodkas and Spirits. The study describes a new procedure for analyzing low molecular weight aldehydes in alcoholic beverages coupled with electron capture detection (ECD). The study claims the proposed method is reliable, sensitive, cost-effective, and simple. It also compares the method with other existing techniques, such as solid phase microextraction (SPME), liquid-liquid extraction (LLE), and static headspace analysis (HS). The paper reports the results of optimizing various parameters of the SDE-GC-ECD method, such as extraction solvent, drop size, alcohol concentration, sample volume, temperature, and extraction time. It also provides the validation parameters of the method, such as linearity, limit of detection, limit of quantification, and relative standard deviation. The document applies the SDE-GC-ECD method to commercially available spirits and vodkas and determines the content of different aldehydes in them. It also discusses the correlation between the aldehyde content, the organoleptic properties, and the technological process of alcoholic products. The study demonstrates that the PFBHA derivatization protocol, combined with SDME-GC ECD, is

reliable for quantifying trace concentrations of carbonyl compounds in vodka and spirits. It requires minimal analysis time and organic solvent consumption and can determine low molecular weight aldehydes in alcoholic drinks. This simple and cost-effective method isused for routine analysis in research labs and the industry.

To identify biogenic amines (BAs) in alcoholic beverages, a novel high-performance liquid chromatography (HPLC) approach with potentiometric detection is presented in this work. By attaining much lower detection limits and improving the efficiency and reliability of BA determination, this novel strategy addresses the shortcomings of earlier techniques. The invention of an HPLC-potentiometric technique that greatly raises the biogenic amine detection limits is described in the paper. The exceptional sensitivity of this approach, which can identify all BAs at detection limits as low as 3.0 x 10-7 mol/L, sets it apart. This is a significant increase compared to previously published methods like HPLC-MS/MS and older HPLC-potentiometric approaches, which had detection limits ranging from 9.3 to 60.7 mol/L and 0.023 to 83 mol/L, respectively. The research emphasizes how background ions affect the potentiometric response and how important it is to have such low detection limits. This feature plays a major role in the method's increased sensitivity and accuracy. A miniature amine-selective electrode is used in the research, which is essential to the accuracy and sensitivity of the technique. The method's overall performance and lower detection limits are attributed to its miniaturization. The methodology was confirmed in compliance with Eurachem and the International Conference on Harmonisation (ICH) requirements, guaranteeing dependable and accurate findings. The thorough validation procedure emphasises how applicable the approach is to real-world food quality control applications. The performance of the approach was improved significantly by adding multi-walled carbon nanotubes (MWCNTs) to the sensing membrane. Lower detection limits can be attributed to MWCNTs' enhancement of the sensor's sensitivity and selectivity. The authors optimized the mobile phase composition to improve separation efficiency, essential for the accurate and reliable detection of biogenic amines. This optimization further establishes the method as a competitive tool for food quality control. The study demonstrates the method's applicability to various alcoholic beverages, making it a valuable tool for ensuring food safety and quality. This broad applicability highlights the versatility and potential of the method for use in the beverage industry. The paper presents a ground breaking HPLC-potentiometric method for determining biogenic amines in alcoholic beverages (Gil et al., 2021).

There are many challenges faced by beverage industries related to the safety and quality of the products being manufactured, viz., protein-, lipid- and photo-oxidation, non-enzymatic or enzymatic browning, contaminations due to microbes, nutritional losses, physiochemical changes, shelf life stability, packaging material, bottle leakage, production of acrylamide and furfural compounds, along with water-related problems. Thus, this project was conceptualized to monitor the safety and quality parameters of neutral spirits for the preparation of alcoholic beverages.

Objectives of the project

i) Collection & physicochemical analysis of neutral spirit as per BIS specifications

ii) Data preparation for customer-based feedback on intended applications, product quality, and performance satisfaction.

Details of Laboratory and Work Progress

Testing of the neutral spirit samples is being conducted in the Food Safety & Analytical Quality Control Laboratory (FS&AQCL). This laboratory is a NABL accredited laboratory as per ISO/IEC 17025:2017, situated in the CSIR-CFTRI, Mysuru. This laboratory has state-of-the-art facilities for analyzing neutral spirits (alcoholic beverages).

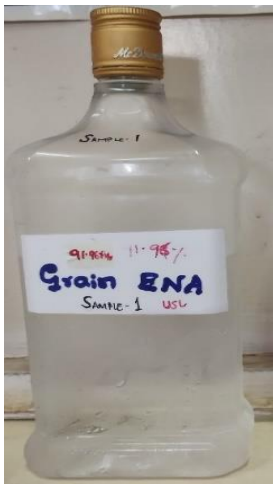
Details of Manufacturers and Sample Providers

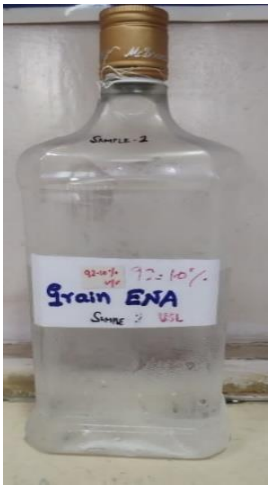
1) Bio Liqua Research, Pvt. Ltd. Bangalore, Karnataka

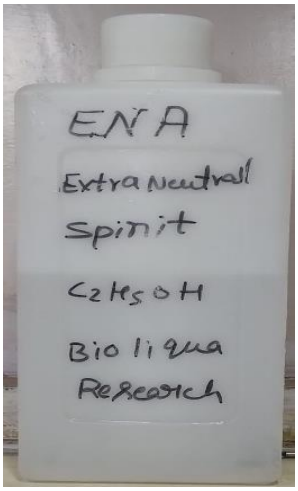
2) Nirani Sugars, Bagalkot, Karnataka

3) United Spirits, Bangalore, Karnataka

Sample Collection







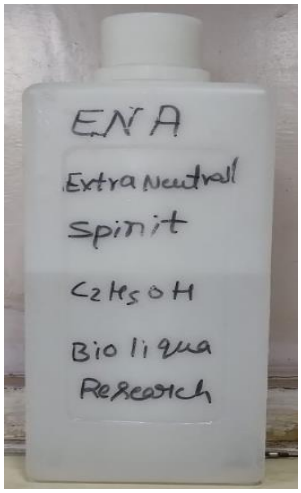


Figure 1: Initial samples of Neutral Spirits collected from different sources

Initially, a total of 4 samples have been collected from the suppliers, as mentioned above, of Neutral Spirits from the Karnataka region. The pictorial depiction of the collected samples is given in Figure 1. Furthermore, a total 8 new samples were collected from United Spirits Ltd. Bangalore. The pictorial depiction of the collected samples is given in Figure 2. These samples are under analysis.

Figure 2: Sample collection of recently acquired Neutral Spirits from United Spirits Ltd.

Analysis of Neutral Spirit

Standardization and validation of the methods performed for the analysis of NS has been initiated in FS&AQCL. Four samples of NS were analyzed according to IS 3752: 2005 & IS 6613: 2002. The results of the tested parameters for four samples of NS are depicted in Table 1. The NS samples were tested for parameters like total acidity, volatile acidity, ester content, residue after evaporation, furfural content, aldehyde content, higher alcohol content, oxidative impurities, and methanol content. The results obtained during the analysis of NS revealed that the acidity, ester, and aldehyde content were higher than the specified limits. The possible reason for this deviation could be various factors that affect the production process. In three of the samples, ethanol content was found lower than the minimum specification. Meanwhile, the methanol content fell within the expected range. The potassium permanganate reaction time test met the 30-minute standard, furfural content was absent in all the samples analyzed, and all the samples were miscible in water. Further analysis with more numbersof neutral spirit samples is in progress.

Table 1:Results of the analyzed neutral spirit samples regarding the standard values (IS 6613: 2002)

Sl. No.	Test parameters	Standard Values	ENA-1	ENA-2	ENA-3	Rectified Spirit
1.	Relative density at 20/20°C, Max	0.80692	0.93664	0.94195	0.93780	0.93640
2.	Ethanol per cent, (v/v at 20°C) Min	96%	95%	96.3%	93.8%	95%
3.	Miscibility with water	Miscible	Miscible	Miscible	Miscible	Miscible
4.	Acidity as acetic acid, g/100 l, absolute alcohol Max	1.5	4.42105	3.86004	3.8379	2.5263
5.	Residue on evaporation, g/100 l, absolute alcohol Max	1.5	0.0012	0.0024	BDL of 0.001	0.0016
6.	Total tartaric acid, g/100L	1.3	3.1578	5.0790	6.3965	6.3157
7.	Esters as CH3COOC2H8, g/100 l, absolute alcohol Max	0.1	3.7052	9.9322	7.5053	14.82105
8.	Methyl alcohol, g/100 l, absolute alcohol, Max	50	0.1971	0.14036	0.1361	0.13259
9.	Furfural content, Max	Not detectable	Absent	Absent	Absent	Absent
10.	Aldehyde as acetaldehyde, g/100 l, absolute alcohol, Max	0.0005	25.9368	0.9932	13.1343	9.26315
11.	Permanganate reaction time, in minutes, Min	30	Pink colour persist till 30 minutes	Pink colour persist till 30 minutes	Pink colour persist till 30 minutes	Pink colour persist till 30 minutes
12.	Higher alcohol as iso-amyl alcohol, Max	30	Only traces	Only traces	About 0.01%	0.02 – 0.03%

Table 2:Results of the analyzed freshly acquired neutral spirit samples regarding the standard values (IS 6613: 2002)

Sl. No.	Test parameters	Standard Values	Sample 1	Sample 2	Sample 3	Sample 4
1.	Relative density at 20/20°C, Max	0.80692	0.80935	0.80717	0.80490	0.80814
2.	Ethanol per cent, (v/v at 20°C) Min	96%	95.88%	96.41%	96.93%	96.07%
3.	Miscibility with water	Miscible	Miscible	Miscible	Miscible	Miscible
4.	Residue on evaporation, g/100 l, absolute alcohol Max	1.5	0.004	BDL of 0.001	BDL of 0.001	0.004
5.	Total tartaric acid, g/100L	1.3	1.88	1.53	1.54	1.56
6.	Esters as CH3COOC2H8, g/100 l, absolute alcohol Max	0.1	Nd	Nd	Nd	Nd
7.	Methyl alcohol, g/100 l, absolute alcohol, Max	50	Nd	Nd	Nd	Nd
8.	Furfural content, Max	Not detectable	Not detected	Not detected	Not detected	Not detected
9.	Aldehyde as acetaldehyde, g/100 l, absolute alcohol, Max	0.0005	Nd	Nd	Nd	Nd
10.	Permanganate reaction time, in minutes, Min	30	Pink colour persist till 30 minutes	Pink colour persist till 30 minutes	Pink colour persist till 30 minutes	Pink colour persist till 30 minutes
11.	Higher alcohol as iso-amyl alcohol, Max	30	Nd	Nd	Nd	Nd

Nd – Not determined

Changes and alternative tests that have been made during the analysis

1. **Total acidity and volatile Acidity:** A solution of 0.01N NaOH instead of 0.05 N NaOH was more suitable for analyzing Acidity. However the readings were then converted to 0.05N NaOH equivalent to work with the previous formula in IS 3752: 2005.
2. **For total acidity,** a validation of the method was performed by spiking of ENA-3 with acetic acid (20, 40, and 80 µL in 100 ml neutral spirits, spike 1, 2, and 3, respectively) and examined the recovery to verify this change in protocol. The recovery is within the acceptable range of 80-110% (Table 3).

   Table 3: Recovery of volatile acidity content

Sl. No.	Samples	Volume (NaOH)	Total acidity (g tartaric acid/100 L abs. alcohol)	Recovery, %
1	ENA-3	0.18	3.91	-
2	ENA-3 – spike1	1.25	35.19	**87.3%**
3	ENA-3 – spike2	1.83	71.68	**94.6%**
4	ENA-3- spike3	3.31	129.69	**87.8%**

3. **For ester content,** a validation of the method was performed by spiking of ENA-2 with ethyl acetate (40, 80, 160 and 320 µL in 100 ml neutral spirits, spike 1, 2, 3, and 4, respectively) and examined the recovery to verify this change in protocol. The recovery of ester content is within the acceptable range of 80-110% (Table 3).

   Table 4: Recovery of ester content

SR NO.	SAMPLEs	ESTER CONTENT (g ethyl ester/100 L abs. alcohol)	RECOVERY
1	ENA-2	3.67	
2	ENA-2 – spike1	14.68	114 %
3	ENA-2 – spike2	22.02	95.7%
4	ENA-2- spike3	44.04	105.2%
5	ENA-2- spike4	66.06	81.3%

4. **New Test for Methanol Content:** Methanol is oxidized to formaldehyde using potassium permanganate. The formaldehyde reacts with pentane-2,4-dione and ammonium acetate to form a yellow compound (3,5-diacetyl-1,4-dihydrolutidine), which can then be detected spectrophotometrically at 412 nm (Wood and Siddiqui, 1971). Here is the standard graph (Fig. 3). It require further optimization and validation, which is under way.

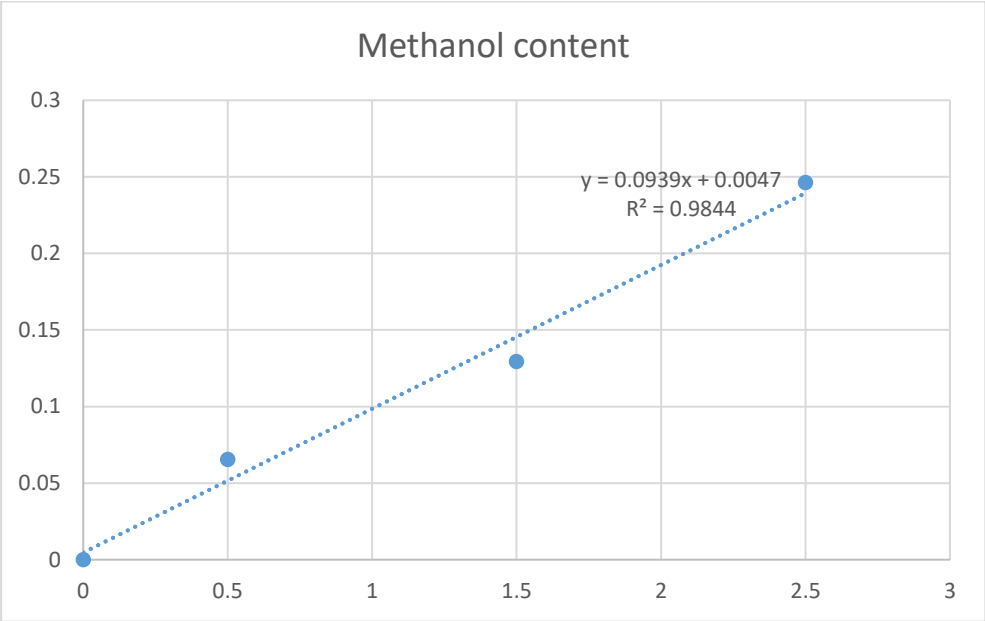


Figure 3: Graph depicting the relation between the absorbance and methanol content

The protocol for the test requires optimizations however seems promising in providing an alternative test for methanol content determination in ethanol.

Questionnaires from the Manufacturer of Neutral Spirit

i. What are the requirements for the preparation of neutral spirits?

ii. What is the source of raw material for neutral spirits preparation?

iii. What are the mechanical requirements for the production of neutral spirit?

iv. What are the fermenter and distillation unit requirements for the preparation of neutral spirits?

v. What are the sources of contaminations in neutral spirit?

vi. What type of congeners/ impurities are present in neutral spirits?

vii. What are the common denaturing agents used in neutral spirits?

Recommendations by the FAD 0015 Committee Member

i. Identification of another testing laboratory for the analysis of neutral spirit

Ans: We will identify another NABL accredited food testing laboratory to cross-check data.

ii. Identification of manufacturers of neutral spirit as small, medium, and large

Ans: We have identified few manufacturers of neutral spirits.

iii. Which test methods are being followed other than BIS standards and their inclusion in the analysis.

Ans: So far, we followed BIS methods of analysis. Other simple and accurate methods willbe validated first, before the actual use for analysis.

iv. Identification of user base of neutral spirits for alcoholic drinks

Ans: We do this by visiting the industries, and following questionnaire analysis.

v. Preparation of a sampling plan for the collection of neutral spirits samples from various manufacturers/ distilleries for approval by the FAD committee.

Ans: We have already made the sampling plan, and is presented in the proposed programme of work for the next month

vi. Collection of information regarding the use of packaging material

Ans: We are collecting information regarding the good packaging material for storage of neutral spirit.

vii. Fund utilization report to be submitted for the further release of the second instalment

Ans: We have utilized or committed for utilization, more than 75% of the money released, and we are submiting the UC and SoE, along with the from PMC regarding the committed funds, for the release of 2nd instalment.

viii. Testing infrastructure is not mentioned?

Ans: We will take care in future.