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ANALYTICAL METHODS FOR METHANOL DETECTION IN ALCOHOLIC BEVERAGES: A COMPARATIVE REVIEW OF CLASSICAL, COLORIMETRIC, AND CHROMATOGRAPHIC APPROACHES

MÉTODOS ANALÍTICOS PARA DETECÇÃO DE METANOL EM BEBIDAS ALCOÓLICAS: UMA REVISÃO COMPARATIVA DE ABORDAGENS CLÁSSICAS, COLORIMÉTRICAS E CROMATOGRÁFICAS

DE BONI, Luis Alcides Brandini[1*]; FERNANDES, Rochele da Silva[2];

[1] Southern Journal of Sciences. Brazil. ORCID: 0009-0000-8102-6197

[2] Universidade Federal do Rio Grande do Sul, Instituto de Química. Brazil. ORCID: 0009-0008-5454-0287

*Corresponding author: labdeboni@gmail.com

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ABSTRACT

Introduction: The detection of methanol in alcoholic beverages represents a critical public health issue, particularly in light of the recent outbreak of poisonings in Brazil, which registered 58 confirmed cases and 15 deaths through October 2025. Methanol's toxicity, with an estimated lethal dose ranging from 0.5 to 1.5 g/kg, requires reliable analytical methods for health surveillance. Brazilian legislation establishes a maximum limit of 20 mg/100 mL of anhydrous alcohol; however, the need for accessible screening methods in field settings remains an important challenge. **Objective:** To critically compare three analytical methods for methanol determination: classical qualitative methods (Lucas Test and dichromate/Schiff), Brazilian colorimetric method, and gas chromatography with flame ionization detector (GC-FID), evaluating their performance and applicability in resource-limited contexts. **Methods:** Theoretical-comparative approach through critical analysis of specialized literature and normative technical documentation. Methods were evaluated according to: operational principle, sensitivity (LOD/LOQ), selectivity, operational complexity, analysis time, and practical applicability. **Results:** The Lucas Test is not applicable for methanol detection. Colorimetric methods showed moderate sensitivity (LOD ~20-160 mg/100 mL), a 10-30-minute execution time, low operational complexity, and excellent portability. The Brazilian method presented chemical equivalence with international standards, differing only in the type of reading performed. GC-FID has shown superior sensitivity (LOD ≤ 1 mg/100 mL) and high specificity, but it requires extended time (~45-60 minutes), complex laboratory infrastructure, and specialized operators. Sugars interfere with colorimetric methods. **Conclusions:** The methods are complementary within a hierarchical system. Colorimetric methods enable rapid field screening, while GC-FID serves as the confirmatory method for forensic analyses. We recommend implementing integrated protocols that combine in situ colorimetric screening with GC-FID confirmation in accredited laboratories for effective health surveillance.

Keywords: *Methanol, Alcoholic beverages, Analytical methods, Health surveillance, Public health.*

RESUMO

Introdução: A detecção de metanol em bebidas alcoólicas representa uma questão crítica de saúde pública, particularmente à luz do recente surto de intoxicações no Brasil, que registrou 58 casos confirmados e 15 mortes até outubro de 2025. A toxicidade do metanol, com dose letal estimada entre 0,5 e 1,5 g/kg, exige métodos analíticos confiáveis para vigilância sanitária. A legislação brasileira estabelece limite máximo de 20 mg/100 mL de álcool anidro; entretanto, a necessidade de métodos de triagem acessíveis em condições de campo permanece um desafio importante. **Objetivo**: Comparar criticamente três métodos analíticos para determinação de metanol: métodos qualitativos clássicos (Teste de Lucas e dicromato/Schiff), método colorimétrico brasileiro

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e cromatografia gasosa com detector de ionização em chama (CG-DIC), avaliando seu desempenho e aplicabilidade em contextos com recursos limitados. **Métodos**: Abordagem teórico-comparativa através de análise crítica de literatura especializada e documentação técnica normativa. Os métodos foram avaliados segundo: princípio operacional, sensibilidade (LD/LQ), seletividade, complexidade operacional, tempo de análise e aplicabilidade prática. **Resultados**: O Teste de Lucas não é aplicável para detecção de metanol. Métodos colorimétricos apresentaram sensibilidade moderada (LD ~20-160 mg/100 mL), tempo de execução de 10-30 minutos, baixa complexidade operacional e excelente portabilidade. O método brasileiro apresentou equivalência química com padrões internacionais, diferindo apenas no tipo de leitura realizada. CG-DIC demonstrou sensibilidade superior (LD ≤ 1 mg/100 mL) e alta especificidade, porém requer tempo prolongado (~45-60 minutos), infraestrutura laboratorial complexa e operadores especializados. Açúcares interferem nos métodos colorimétricos. **Conclusões**: Os métodos são complementares dentro de um sistema hierárquico. Métodos colorimétricos possibilitam triagem rápida em campo, enquanto CG-DIC serve como método confirmatório para análises forenses. Recomenda-se implementar protocolos integrados que combinem triagem colorimétrica in situ com confirmação por CG-DIC em laboratórios credenciados para vigilância sanitária efetiva.

Palavras-chave: *Metanol, Bebidas alcoólicas, Métodos analíticos, Vigilância sanitária, Saúde pública*.

1. INTRODUCTION

1.1. The Problem of Methanol Intoxication

Accurate detection of methanol in alcoholic beverages is a critical public health issue, given the high toxicity of this compound. As demonstrated by Gosselin *et al*. (1984), the estimated lethal dose for a 70 kg human is only 0.5 to 1.5 g/kg, highlighting the danger even at relatively small quantities.

The urgency of this monitoring was tragically illustrated recently when Brazil's Ministry of Health reported an outbreak of intoxications, with 108 notifications registered up to October 24, 2025. Of these, 58 cases were confirmed, 50 remain under investigation, and 635 notifications were ruled out. São Paulo state has the highest number of confirmed cases (44), followed by Pernambuco (5), Paraná (6), Rio Grande do Sul (1), Mato Grosso (1), and Tocantins (1). To date, 15 deaths have been confirmed, distributed among São Paulo (9), Paraná (3), and Pernambuco (3), with another 9 deaths still under investigation (Ministry of Health, 2025). This situation clearly reveals the pressing need for effective health surveillance.

1.2. Characteristics of Methanol

To understand the risks involved and the importance of accurate detection, it's important to know the physicochemical and toxicological characteristics of methanol. Methanol (CH_3OH), also known as methyl alcohol, carbinol, or wood alcohol, is an organic chemical compound with a molecular mass of 32.04 g/mol, characterized as a colorless, polar, volatile, and highly flammable liquid with a mild alcoholic odor when pure (World Health Organization [WHO], 1997). It is the simplest alcohol in the homologous series of aliphatic alcohols, being completely miscible in water and most organic solvents (International Labour Organization [ILO] & World Health Organization [WHO], 2018).

From a physicochemical perspective, methanol has a boiling point of 64.7 °C, melting point of -97.8 °C, relative density of 0.79 (water = 1), and vapor pressure of 12.3 kPa at 20 °C (ILO & WHO, 2018). Its flammability characteristics include a flash point of 12.2 °C (closed cup) and autoignition temperature of 470 °C, with explosive limits in air ranging from 5.5% to 44% by volume (WHO, 1997). Its vapors mix easily with air, forming explosive mixtures (limits between 6-50% by volume). Methanol has a low flash point (9 °C c.c.) and is dangerous due to its violent reactivity with strong oxidizers, acids, and reducing agents, posing a risk of fire and explosion (ILO, 2018).

Modern industrial production of methanol is based exclusively on the catalytic conversion of pressurized synthesis gas (hydrogen, carbon monoxide, and carbon dioxide) in the presence of heterogeneous metallic catalysts (WHO, 1997). The chemical sector accounted for 66% of global methanol consumption in 2024 (Mordor Intelligence, 2025).

In terms of occupational safety and human health, methanol is an important risk. The substance is classified by the UN GHS as a highly flammable liquid and vapor, and is toxic if swallowed and harmful if inhaled, causing damage to the central nervous system (CNS). Absorption can occur through inhalation, ingestion, or dermal contact, and evaporation at 20 °C can quickly lead to harmful atmospheric contamination (ILO, 2018).

Acute exposure through inhalation, ingestion, or percutaneous absorption can result in central nervous system depression, metabolic acidosis, visual disturbances, blindness, coma,

and death, with symptoms potentially delayed by 12 to 24 hours (WHO, 1997). Short-term exposure irritates the eyes, skin, and respiratory tract, and may cause loss of consciousness and, possibly blindness and death. Symptoms include dizziness, headache, visual disturbances, and seizures, and effects may be delayed, indicating the need for medical observation. Methanol is readily absorbed through respiratory, oral, and dermal routes, being slowly metabolized to formate, which is considered primarily responsible for the characteristic visual damage of intoxication (WHO, 1997).

Repeated or prolonged exposure can result in dermatitis and cause chronic effects on the CNS, such as recurring headaches and impaired vision. Specific treatment is necessary in case of poisoning, and periodic medical examination is suggested, depending on the degree of exposure (ILO, 2018).

1.3. Regulatory Framework

This risk scenario is acknowledged by Brazilian legislation, which establishes specific limits for methanol in distilled beverages. Normative Instruction No. 13, dated June 29, 2005, from the Ministry of Agriculture, Livestock and Food Supply (MAPA) approves the Technical Regulation for Establishing Identity and Quality Standards for Sugarcane Spirit and Cachaça, setting the limit at 20 mg of methanol per 100 mL of anhydrous alcohol. In 2009, the National Institute of Metrology, Quality and Technology (INMETRO) approved Ordinance No. 276, dated September 24, 2009, which instituted the Conformity Assessment Regulation for cachaça. In 2022, MAPA published Ordinance No. 539, dated December 26, 2022, establishing new identity and quality standards for sugarcane spirit and cachaça, maintaining the limit of 20 mg of methanol per 100 mL of anhydrous alcohol.

1.4. Context of Cachaça Production

Within the context of national production of distilled beverages, the efficiency of the distillation process in cachaça production is a critical factor for the quality and safety of the final product. During distillation, methanol, due to its high volatility, is mostly removed in the initial phase, known as the head fraction. Studies have shown that discarding 1-2% of the total distillate volume is sufficient to eliminate most of this unwanted compound (SCANAVINI *et al*., 2012). As a result of this control practice, commercial cachaça presents a low methanol content.

A benchmark analysis in the sector identified an average value of approximately 6 mg per 100 mL of anhydrous alcohol in the final product, a level lower than that found in other spirits, such as wine-based spirits, confirming the effectiveness of separation during distillation (BOSCOLO *et al*., 2000).

1.5. Global Overview of Methanol Poisonings

The recent case of methanol poisoning in Brazil (MS, 2025) is not an isolated incident globally. Table 1 presents a compilation of similar incidents across more than 20 countries. Typically, these methanol poisoning incidents are related to the consumption of adulterated alcoholic beverages; however, a case with distinct characteristics occurred in the USA during the pandemic, where hand sanitizer containing ethanol mixed with methanol was ingested (Konkel *et al*., 2024).

1.6. Study Objective and Approach

Given the context of toxicological risks, regulatory frameworks, production-related technical challenges, and the global occurrence of outbreaks, this article adopts a theoretical-comparative approach to analyze three representative analytical methods for methanol detection. The goal is to provide a critical foundation for selecting control measure methodologies, including in resource-limited settings.

1.6.1. General Objective

To critically compare the three analytical methods for determining methanol in alcoholic beverages - classical qualitative teaching methods, national colorimetric method, and the gold standard (gas chromatography) - evaluating their performance and applicability in resource-limited contexts.

1.6.2. Specific Objectives

To describe the operational principles, advantages, and limitations of the three selected paradigmatic methods.

To characterize the performance of the methods based on key analytical parameters: sensitivity (LOD/LOQ), selectivity against ethanol, cost, operational complexity, and analysis time.

To evaluate the feasibility of the classical and national colorimetric methods for applications in rapid screening and health surveillance, contrasting their results with the gold standard method.

To synthesize the trade-offs identified in the comparison, highlighting technological gaps and opportunities for developing accessible methodologies for the Brazilian scenario.

2. METHODOLOGICAL APPROACH

To address the proposed objectives, particularly the need to compare methods applicable in resource-limited contexts with the gold-standard method, this article adopts a theoretical and comparative research strategy. The critical analysis focuses on three representative analytical approaches, selected for their relevance across different application scenarios: combined classical organic chemistry methods, a validated national colorimetric method, and the chromatographic method considered the gold standard.

2.1. Method Selection and Rationale

The selection was intentional, aiming to cover a significant spectrum of complexity, cost, and applicability:

2.1.1. Combined Classical Methods (Lucas Test followed by Dichromate/Schiff Oxidation)

These correspond to the traditional didactic approach, commonly used in teaching organic chemistry at the university level. Although they consist of two distinct tests, they are performed sequentially within the same analysis:

- Lucas Test: a screening method by exclusion that classifies alcohols by structure (primary, secondary, tertiary). Since methanol does not react in this test (a negative result is expected for simple primary alcohols), it serves to rule out the presence of secondary and tertiary alcohols that could interfere.
- Dichromate Oxidation + Schiff's Reagent: a confirmatory method that specifically identifies methanol through the formation of formaldehyde, which reacts with Schiff's reagent, producing a characteristic pink-violet coloration.

Inclusion rationale: To assess whether established didactic protocols can be adapted for low-cost health surveillance screening.

2.1.2. Brazilian Colorimetric Method (Modesto, 2020)

A colorimetric technique developed and validated in Brazil specifically for alcoholic beverage analysis. It is based on the oxidation of methanol to formaldehyde, followed by reaction with chromotropic acid, allowing visual or instrumental detection.

Inclusion rationale: It is an effort to develop an accessible methodology, with potential for field application or laboratories with limited infrastructure.

2.1.3. Gold Standard Method (Gas Chromatography with Flame Ionization Detector - GC-FID)

An analytical technique described in international standards (ASTM E681, AOAC 972.11, IAL and OIV methods) and recognized by MAPA as the official method for forensic and regulatory analyses.

Inclusion rationale: It is a reference for sensitivity, specificity, and accuracy against which simpler methods will be evaluated.

2.2. Information Sources

Method characterization was based on a comprehensive review of specialized literature in analytical chemistry and method validation, complemented by national and international normative technical documentation. The analysis also incorporated peer-reviewed scientific publications and official beverage analysis manuals from recognized institutions such as MAPA (Ministry of Agriculture, Livestock and Food Supply), IAL (Adolfo Lutz Institute), and OIV (International Organisation of Vine and Wine).

To survey documented cases of methanol poisoning worldwide, a systematic search was conducted in the Science.gov database (https://www.science.gov/scigov/desktop/en/results.html) using the search term "methanol outbreak". The search yielded 462 records.

The document selection criteria were: a) relevance according to the database's own classification, b) contribution to presenting a broad overview of methanol poisoning cases in different geographical and temporal contexts. And the

exclusion of duplicate or too-similar records.

2.3. Parameters for Comparative Analysis

For systematic comparison, the methods were evaluated according to criteria extracted from specialized literature on analytical method validation and aligned with the study's specific objectives:

1. Operating principle (chemical mechanism)
2. Sensitivity (LOD/LOQ relative to the regulatory limit of 20 mg/100 mL)
3. Selectivity against interferents (ethanol, sugars, other compounds)
4. Operational complexity (equipment, reagents, training)
5. Cost per analysis and initial investment
6. Total analysis time (preparation + execution + reading)
7. Practical applicability and recommended use context (laboratory vs. field)

2.4. Analysis Structure

The results of this comparison will be organized in three stages:

a. Detailed description of each method (Section 3), including procedures, reagents, and chemical fundamentals

b. Comparative synthesis in tabular format (Section 4), presenting side-by-side performance of each method according to established criteria

c. Critical discussion (Section 5) that will highlight the trade-offs inherent in choosing each methodology, especially considering Brazilian health surveillance scenarios with different levels of available resources

This structure will allow us to identify technological gaps and opportunities to develop or adapt accessible methodologies without compromising the analytical reliability required for public health protection.

3. Analytical Methods

In this section, the three methods selected for comparison are described in detail: the combined classical organic chemistry methods, the colorimetric method developed in Brazil, and gas chromatography with flame ionization detector. For each method, the chemical fundamentals, experimental procedures, required reagents, and criteria for interpreting results are presented.

3.1. Combined Classical Methods

The classical method combines two sequential analyses commonly performed in organic chemistry practical classes: the Lucas Test followed by dichromate oxidation, with confirmation using Schiff's reagent. This didactic approach, extensively documented in organic chemistry experimental compendia (Furniss *et al*., 1989), establishes a screening protocol followed by confirmation, based on the following analytical reasoning:

Protocol Logic:

1. The Lucas Test (Lucas, 1905) classifies alcohols by structure (primary, secondary, or tertiary)
2. Since methanol is a primary alcohol that does not react in the Lucas Test, a negative result in this test rules out the presence of secondary and tertiary alcohols that could cause false-positive results in subsequent tests
3. Once the presence of a primary alcohol (or mixture containing primary alcohols) is confirmed, oxidation with dichromate proceeds
4. The formaldehyde formed specifically from methanol is then identified by Schiff's reagent (Schiff, 1866)

Why perform the Lucas Test if it doesn't detect methanol?

The Lucas Test functions as a "gatekeeper" for the analytical protocol. Although it does not directly detect methanol, it plays a crucial role as an exclusion screening step:

NEGATIVE Result (absence of turbidity): Confirms that there are NO secondary or tertiary alcohols in the sample. This increases the reliability of subsequent tests by excluding potential interferents.

POSITIVE Result (presence of turbidity): Alerts that secondary or tertiary alcohols are present, requiring caution in interpreting the tests.

This prior exclusion of potential interferents

reduces the likelihood of false-positive or ambiguous results in the confirmatory stage with Schiff's reagent, serving as a quality-control measure integrated into the analytical protocol.

Figure 1 presents the complete flowchart of this combined analytical protocol.

3.1.1. Step 1 – Lucas Test (Exclusion Screening)

Purpose and Principle: The Lucas Test (Lucas, 1905) is used for classifying primary, secondary, and tertiary alcohols, based on the difference in reactivity of these compounds toward hydrochloric acid (HCl) in the presence of zinc chloride ($ZnCl_2$) as a Lewis acid catalyst. In this protocol, the test functions as an exclusion screening step: a negative result (absence of turbidity) indicates that the sample does not contain secondary or tertiary alcohols, allowing safe progression to the confirmatory steps specific for methanol.

The reaction occurs according to Equation 1:

$$R\text{-}OH + HCl + ZnCl_2 \rightarrow R\text{-}Cl + H_2O \quad \text{(Eq. 1)}$$

The reaction rate depends on the alcohol structure:

- Tertiary alcohols: immediate reaction (instantaneous turbidity)
- Secondary alcohols: 5-10 minutes (gradual turbidity)
- Primary alcohols: do not react at room temperature
- Methanol: does not react (simple primary alcohol) – expected result to proceed

Reagents

Lucas Reagent composition anhydrous $ZnCl_2$ (136 g), concentrated HCl (105 mL). Preparation: dissolve in an ice bath; store in an amber bottle.

Procedure

The procedure requires basic laboratory glassware, including clean, dry test tubes with a 10 mL capacity, Pasteur pipettes for precise liquid transfer, and a stopwatch for time control.

The analytical procedure begins by adding 2 mL of Lucas reagent to a test tube, followed by the addition of 3-4 drops (approximately 0.2 mL) of the alcoholic sample to be tested. The mixture is then shaken vigorously for 10 seconds to ensure proper contact between the reagent and the sample. After shaking, the test tube is allowed to stand at room temperature and observed for up to 60 minutes for any signs of turbidity or phase separation.

Expected Results and Interpretation

Table 2 presents the typical results obtained with the Lucas Test for different alcohols.

Decision criterion: If turbidity occurs (positive result), the sample contains secondary or tertiary alcohols, which may interfere with subsequent stages. If there is no turbidity after 60 minutes (negative result), proceed to Stage 2.

3.1.2. Stage 2 – Dichromate Oxidation and Confirmation with Schiff's Reagent

Purpose and Principle: This stage specifically identifies methanol by forming formaldehyde as an oxidation product. The technique is based on the selective oxidation of primary alcohols with potassium dichromate in an acidic medium, followed by confirmation with Schiff's reagent (Schiff, 1866). Schiff's reagent reacts specifically with aldehydes, being highly sensitive to formaldehyde (the oxidation product of methanol) and much less reactive to acetaldehyde (the oxidation product of ethanol), thus allowing differentiation between the two primary alcohols.

Oxidation of primary alcohols:

Methanol: $CH_3OH \rightarrow HCHO$ (formaldehyde) $\rightarrow HCOOH$ (formic acid)

Ethanol: $CH_3CH_2OH \rightarrow CH_3CHO$ (acetaldehyde) $\rightarrow CH_3COOH$ (acetic acid)

Dichromate reaction (Eq. 2):

$$3\ CH_3OH + Cr_2O_7^{2-} + 8\ H^+ \rightarrow 3\ HCHO + 2\ Cr^{3+} + 7\ H_2O \quad \text{(Eq. 2)}$$ (orange → green)

Reagents

The required reagents include an oxidizing solution and Schiff's reagent. The oxidizing

solution is prepared by first dissolving 5 g of $K_2Cr_2O_7$ in 90 mL of distilled water, then carefully adding 10 mL of concentrated H_2SO_4 while stirring continuously to dissipate heat. Schiff's reagent is prepared by dissolving 0.1 g of basic fuchsin in 100 mL of distilled water and treating with SO_2 or bisulfite until the solution becomes colorless; it should be stored in an amber bottle for stability.

Procedure

Part A – Alcohol oxidation:

1. In a clean, dry test tube, add 1 mL of dichromate/H_2SO_4 solution
2. Add 3-5 drops of the sample (previously tested in Stage 1)
3. Gently shake
4. Observe color change (orange → green) and development of characteristic odor
5. Wait 2-3 minutes

Table 3 presents the expected oxidation results for different alcohols.

Part B – Confirmation with Schiff's Reagent:

1. Transfer 0.5 mL of the oxidized solution to a new tube
2. Add 1 mL of Schiff's reagent
3. Shake and wait 5-10 minutes
4. Observe color development

Table 4 presents the results of the confirmation test with Schiff's reagent.

Result Interpretation

Methanol identification relies on a characteristic analytical profile across multiple tests. A positive result presents as negative turbidity in the Lucas test (confirming the primary alcohol structure), an efficient orange-to-green color transition during dichromate oxidation, development of formaldehyde's distinctive pungent odor, and, most critically, an intense pink-violet coloration in Schiff's test, which serves as confirmatory evidence. Ethanol, conversely, shares some features with methanol—both show no Lucas test turbidity and similar oxidation color changes—but differs critically in producing acetaldehyde's sweet odor and yielding either a very faint or absent pink coloration in Schiff's test, thus excluding methanol presence.

Quality Control

Analytical validation requires systematic inclusion of control samples in each batch. Known methanol standards serve as positive controls to verify reagent functionality, while ethanol or water serves as negative controls to exclude false positives. Reagent blanks assess potential contamination, and parallel analysis of both methanol and ethanol reference solutions provides direct comparative standards for interpreting unknown samples and ensuring accurate alcohol differentiation.

Standard solutions should yield expected results, as outlined in Tables 2, 3, and 4, to validate reagent quality and correct procedure execution, following the practices documented in experimental organic chemistry protocols (Furniss *et al.*, 1989).

3.2. Brazilian Colorimetric Method

This colorimetric method was developed and validated in Brazil specifically for the determination of methanol in various alcoholic matrices, including beverages (Modesto, 2020). The technique indicates an effort to develop a low-cost, highly portable analytical methodology suitable for both laboratory analyses and field screening.

A distinctive feature of this method is its chemical equivalence with recognized international protocols, such as the OIV-MA-AS315-27 standard from the International Organisation of Vine and Wine. Both employ oxidation followed by reaction with chromotropic acid, differing essentially in the type of reading: while international standards prescribe spectrophotometry at 575 nm, the Brazilian method allows visual reading or RGB analysis with low-cost portable devices (smartphone or portable photometers), making it especially suitable for resource-limited contexts.

3.2.1. Objective

To detect and/or quantify the presence of methanol in alcoholic beverages (vodka, whisky, cachaça) through a colorimetric method based on the oxidation of methanol to formaldehyde, followed by reaction with chromotropic acid, with

the possibility of field execution.

3.2.2. Method Principle

Methanol in the sample is oxidized to formaldehyde in an acidic medium using an oxidizing agent (potassium dichromate or potassium permanganate). The generated formaldehyde reacts with chromotropic acid in a strongly acidic medium (H_2SO_4 16.5-18 mol/L), forming a violet/purple complex whose intensity is proportional to the methanol concentration (Modesto, 2020). The reading can be performed visually or with the aid of color analysis devices. Figure 2 presents the complete flowchart of both protocols.

Selectivity: Ethanol is also oxidized, but its products (acetaldehyde and acetic acid) do not react meaningfully with chromotropic acid, ensuring selectivity for methanol.

Method variants: Two oxidizing routes can be used:

- Method A (Dichromate): $K_2Cr_2O_7$ as oxidizing agent
- Method B (Permanganate): $KMnO_4$ as oxidizing agent (more sensitive, better visual contrast)

3.2.3. Reagents and Materials

3.2.3.1. Reagents

The analytical procedure requires concentrated sulfuric acid at different molarities: 18 mol/L for the dichromate-based approach (method A) and 16.5 mol/L for the permanganate-based approach (method B). The oxidizing agent varies according to the selected method. Method A employs a potassium dichromate solution prepared by dissolving 1 g of $K_2Cr_2O_7$ in 40 mL of water, followed by the addition of 10 mL of concentrated H_2SO_4. Method B utilizes a potassium permanganate solution consisting of 0.75 g of $KMnO_4$ dissolved in water with 2 mL of orthophosphoric acid (H_3PO_4), adjusted to a final volume of 50 mL. This second method additionally requires a 0.05 mol/L sodium sulfite solution, prepared by dissolving 0.315 g of Na_2SO_3 in 50 mL of distilled water. Both methods use chromotropic acid as the colorimetric reagent, prepared at 0.5% (w/w) by dissolving 0.5 g of the disodium salt dihydrate ($C_{10}H_8O_8S_2$) in 49.5 g of sulfuric acid at the molarity specified for the chosen method. Distilled water is used throughout for solution preparation and dilution.

3.2.3.2. Materials

- Test tubes (10–15 mL)
- Graduated pipettes or micropipettes
- Stopwatch
- Optional: smartphone with camera, portable photometer, or spectrophotometer

Note: A water bath is not required in the optimized versions of the method (Modesto, 2020).

3.2.4. Experimental Procedure

Method A – Oxidation with Potassium Dichromate ($K_2Cr_2O_7$)

Step 1 – Oxidation

1. In a clean, dry test tube, add 5 mL of the alcoholic beverage (vodka, whiskey, or non-sweetened cachaça)
2. Add 4 mL of potassium dichromate oxidizing solution
3. Gently shake and let stand for 5 minutes at room temperature

Note: The solution should turn green (reduction of Cr^{6+} to Cr^{3+}), indicating alcohol oxidation.

Step 2 – Colorimetric Reaction

1. Transfer 1 mL of the oxidized solution to a new test tube
2. Add 3 mL of chromotropic acid solution in 18 mol/L H_2SO_4
3. Wait 10 minutes at room temperature
4. Observe color formation

Method B – Oxidation with Potassium Permanganate ($KMnO_4$) [Recommended]

Step 1 – Oxidation

1. In a test tube, add 1 mL of the alcoholic beverage
2. Add 1.6 mL of $KMnO_4$ oxidizing solution (0.75 g in 50 mL with H_3PO_4)
3. Wait 5 minutes at room temperature

Note: Formation of MnO_2 (brown precipitate).

Step 2 – Decolorization

1. Add 4 mL of sodium sulfite solution (0.05 mol/L)
2. Shake until the solution becomes colorless (reduction of excess MnO_4^- and MnO_2)

Step 3 – Colorimetric Reaction

1. Transfer 1 mL of the decolorized solution to a new tube
2. Add 3 mL of chromotropic acid solution in 16.5 mol/L H_2SO_4
3. Wait 10 minutes at room temperature
4. Observe the developed color

3.2.5. Result Interpretation

Regulatory limit (MAPA): ≤ 20 mg/100 mL of methanol in vodka, whiskey, and cachaça. Table 5 presents the visual interpretation of results for both methods.

Comparison between methods:

- Method B ($KMnO_4$): More sensitive with better color contrast (yellow → clean violet), recommended for visual screening
- Method A ($K_2Cr_2O_7$): Resulting color is a mixture (green + violet = brown), requiring more careful reading or use of auxiliary devices

Important limitation: Do not apply to samples containing sugar (cocktails, sweetened cachaças, liqueurs) – sugar interferes by generating dark brown coloration that prevents accurate reading.

Recommendation for quantification: Use digital RGB analysis (smartphone camera and software) or a portable photometer for greater objectivity at concentrations near the regulatory limit (20 mg/100 mL). Portable devices allow the construction of calibration curves and quantification with reasonable precision (Modesto, 2020).

3.2.6. Controls and Validation

Quality assurance protocols require systematic inclusion of control samples in each analytical batch. Positive controls are prepared by spiking beverages with methanol to achieve 20 mg/100 mL, the regulatory threshold set by MAPA, thereby confirming the method's sensitivity at the critical concentration. Negative controls use either GC-FID-verified methanol-free beverages or analytical-grade ethanol to exclude false positives. Reagent blanks assess baseline interference and contamination potential. For quantitative applications employing instrumental detection, calibration standards at known methanol concentrations enable construction of analytical curves for accurate quantification.

Performance characteristics established by Modesto (2020) demonstrate the method's fitness for purpose. Selectivity validation encompassed multiple beverage matrices including vodka, whiskey, and non-sweetened cachaça, confirming applicability across typical distilled spirit compositions. Detection capabilities vary with the measurement approach: visual observation achieves approximately 160 mg/100 mL (0.2% v/v) detection limits, whereas instrumental measurements with RGB colorimeters or spectrophotometers achieve approximately 20 mg/100 mL, aligning with regulatory requirements. The complete analytical procedure, from sample introduction through final measurement, requires 20 to 30 minutes, facilitating rapid screening applications.

3.2.7. Applicability and Context of Use

This method offers advantages for health surveillance through its portability, low cost, operational simplicity, rapid 20-30 minute analysis time, and versatility in providing both visual and instrumental reading options. All reagents and materials are transportable and accessible, requiring no complex equipment for basic screening applications. However, limitations include moderate visual sensitivity, unsuitable for precise quantification; susceptibility to sugar interference, which restricts use in sweetened beverages; and inherent subjectivity in visual readings, though auxiliary colorimetric devices can mitigate this concern.

The method finds optimal application in three key contexts: field screening during inspections, events, and at retail locations where rapid results support immediate public safety decisions; routine analyses in resource-limited laboratories seeking

cost-effective alternatives to chromatographic methods; and educational settings where the technique's simplicity and visual nature facilitate demonstrations and training in analytical chemistry and methanol detection principles.

Important Note on Confirmation: These colorimetric methods are qualitative/semi-quantitative and intended for rapid screening in the field or teaching laboratory. For official, forensic analyses or precise quantification, confirmation by gas chromatography (GC-FID) is recommended according to MAPA/ABNT NBR 16041 standards (Modesto, 2020).

3.3. Gas Chromatography with Flame Ionization Detection (GC-FID)

Gas chromatography with flame ionization detection is the gold standard method for the quantitative determination of methanol in alcoholic beverages, recognized by national and international regulatory bodies as the reference technique for forensic and fiscal analyses.

The analytical superiority of GC-FID is based on three fundamental characteristics: (1) exceptional specificity provided by the physical separation of volatile compounds in the chromatographic column, eliminating interferences from other alcohols and matrix components; (2) high sensitivity with detection limits on the order of 1 mg/100 mL, well below the regulatory limit of 20 mg/100 mL; and (3) accuracy and precision that allow exact and reproducible quantification, which are necessary for technical reports with legal value.

Several standardized methods employ GC-FID for methanol determination, all based on the same chromatographic principles but with variations in instrumental parameters. The AOAC 972.11 method (Methanol in Distilled Liquors) is widely used internationally (AOAC, 1972). In Brazil, the IAL 228/IV method (Instituto Adolfo Lutz, 2008) is particularly suitable for national laboratories, as it provides specific validation for the characteristics of Brazilian beverages. The International Organisation of Vine and Wine provides methods OIV-MA-AS315-27 (OIV, 2016a) and OIV-MA-BS-14 (OIV, 2016b) for analysis of volatile compounds including methanol. The Ministry of Agriculture and Livestock recognizes these methods in its Manual of Official Methods for Analysis of Wines, Beverages and Acetic Fermented Products (MAPA, 2025).

The detailed description that follows is based on the IAL 228/IV method (IAL, 2008), chosen for its suitability to the Brazilian context and the availability of complete technical documentation.

3.3.1. Objective

To quantitatively determine the concentration of methanol in distilled alcoholic beverages (aguardente, cachaça, whiskey, vodka, rum) by gas phase chromatography with flame ionization detection, using internal standardization with 3-pentanol (IAL, 2008).

3.3.2. Method Principle

The method is based on the chromatographic separation of the sample's volatile components on a capillary column with a polar stationary phase (polyethylene glycol, commercially known as Carbowax), followed by flame ionization detection (FID).

Separation principle: Volatile compounds are vaporized in the heated injector and transported by carrier gas (hydrogen) through the capillary column. The polar stationary phase differentially retains compounds based on their polarity and volatility, thereby promoting physical separation. Methanol, being highly polar, has a characteristic retention time that allows its unequivocal identification.

Detection principle: In the FID detector, organic compounds are burned in a hydrogen/air flame, generating ions that produce an electric current proportional to the amount of carbon present. The chromatographic peak area is directly proportional to the compound concentration.

Quantification by internal standardization: 3-pentanol is used as internal standard, added at a known concentration to both the sample and calibration standards. This procedure eliminates variations in injection volume, volatile losses, and instrumental fluctuations, ensuring precision superior to simple external calibration. Figure 3 presents the complete flowchart of the analytical procedure.

3.3.3. Equipment and Materials

3.3.3.1. Equipment

- Gas chromatograph equipped with: ○ Flame ionization detector (FID) ○ Split/splitless injector with precise

temperature control ○ Data acquisition system and integration software

- Capillary column: Carbowax (polyethylene glycol) – 30 m × 0.53 mm i.d. × 1 µm film thickness (or similar)
- Analytical balance: 0.1 mg resolution
- Microsyringe: 10 µL (compatible with GC injector)

3.3.3.2. Gases (high purity)

The chromatographic analysis requires three high-purity gases at 99.999% minimum purity. Hydrogen serves dual purposes as both carrier gas and flame supply for the detector. Synthetic air functions as the oxidant in the flame ionization detector (FID). Nitrogen is employed as auxiliary gas (make-up) to optimize detector performance. Due to hydrogen's highly flammable nature, special safety precautions are mandatory, including the use of leak detectors and ensuring adequate laboratory ventilation.

3.3.3.3. Glassware

Precise volumetric glassware is needed for accurate solution preparation. Class A volumetric flasks in 10 mL and 100 mL capacities are required for standard preparation. Class A volumetric pipettes of 1 mL and 10 mL sizes ensure accurate volume transfers during analytical procedures.

3.3.4. Reagents

All chemical reagents must meet chromatographic-grade specifications with a minimum purity of 99.5%. The required materials include methanol as the calibration reference standard, 3-pentanol as the internal standard, absolute ethyl alcohol at ≥99.5% purity as the primary solvent, and distilled or ultrapure water for dilutions and auxiliary preparations.

3.3.5. Solution preparation

All analytical solutions are prepared using gravimetric (weight-based) methodology rather than volumetric methods to achieve maximum precision, following protocols established by IAL (2008). This approach eliminates volumetric errors associated with temperature variations and meniscus reading.

3.3.5.1. Methanol Stock Standard Solution (A)

A 100 mL volumetric flask containing approximately 40 mL of absolute alcohol is first tared on an analytical balance. Approximately 2.0 mL of methanol is then added and precisely weighed. The flask is brought to volume with absolute alcohol, weighed again, and the final mass is recorded. The methanol concentration in mg/100 g is calculated from the mass difference between the initial and final weighings.

3.3.5.2. Internal Standard Stock Solution (B)

A 100 mL volumetric flask containing approximately 40 mL of absolute alcohol is tared on an analytical balance. Approximately 2.5 mL of 3-pentanol is added and precisely weighed. After diluting to volume with absolute alcohol, the flask is weighed again to record the final mass. The 3-pentanol concentration, in mg/100 g, is determined from the mass difference.

3.3.5.3. 40% v/v Alcohol Solution Dilute 40 mL of absolute alcohol to 100 mL with distilled water (to simulate beverage matrix).

3.3.5.4. Working Standard Solution (C) – for calibration

1. In a tared 100 mL flask with ~40 mL of 40% alcohol, add: ○ 1 mL of solution A (methanol) – weigh ○ 1 mL of solution B (internal standard) – weigh
2. Make up to volume with 40% alcohol
3. Weigh and record masses for exact concentration calculation

3.3.5.5. Working Internal Standard Solution (E) – for samples

1. In a tared 100 mL flask with ~40 mL of 40% alcohol: ○ Pipette 10 mL of solution B
2. Make up to volume with 40% alcohol
3. Weigh and record masses

Storage:

- All solutions: 4-8°C in well-sealed amber bottles
- Stability: Solutions A and B (stocks) – 6 months; Solutions C and E (working) – 1 month

3.3.6. Procedure

3.3.6.1. Sample Preparation

1. Tare a 10 mL volumetric flask
2. Add 9 mL of sample (weigh precisely)
3. Add 1 mL of solution E (internal standard) – weigh
4. Homogenize by gentle shaking
5. Record all masses for calculations

Critical note: Beverages with dry residue > 0.4 g/100 mL (sweetened drinks, liqueurs) must be distilled beforehand to avoid contamination of the chromatographic column.

3.3.6.2. Chromatographic Conditions

Table 6 presents the optimized instrumental parameters for methanol separation.

3.3.6.3. Analysis

1. Calibration: ○ Inject standard solution C at least 4 times (replicates) ○ Determine retention time of methanol and internal standard ○ Calculate correction factor (see section 3.3.7.1)
2. Sample analysis: ○ Inject each prepared sample at least 4 times (replicates) ○ Identify methanol peak by comparison with the standard retention time ○ Record peak areas of methanol and internal standard

3.3.7. Calculations

3.3.7.1. Methanol Correction Factor (CF)

The correction factor relates the detector response to methanol versus the internal standard:

$$CF_{methanol} = (C_{methanol} \times A_{IS}) / (C_{IS} \times A_{methanol})$$

Where:

- $C_{methanol}$ = Methanol concentration in solution C (mg/100 g)
- C_{IS} = Internal standard concentration in solution C (mg/100 g)
- $A_{methanol}$ = Methanol peak area in solution C
- A_{IS} = Internal standard peak area in solution C

3.3.7.2. Methanol Concentration in Sample (mg/100 g)

Using the correction factor, the concentration in the sample is calculated:

$$C_{methanol,sample} = (A_{methanol,sam} \times CF_{methanol} \times C_{IS,sam}) / A_{IS,sam}$$

Where:

- $A_{methanol,sam}$ = Methanol peak area in the sample
- $A_{IS,sam}$ = Internal standard peak area in the sample
- $C_{IS,sam}$ = Internal standard concentration in the sample (mg/100 g)

3.3.7.3. Result in mg/100 mL of Anhydrous Alcohol (Regulatory format)

To express according to MAPA requirements:

$$C = (C_{methanol,sample} \times d_{abs} \times 100) / G$$

Where:

- C = Final methanol concentration (mg/100 mL of anhydrous alcohol)
- d_{abs} = Absolute density of the sample (g/mL)
- G = Alcoholic strength of the sample (% v/v)

3.3.8. Expression of Results

Express the result in mg/100 mL of anhydrous alcohol, with one decimal place (eg. 8.5 mg/100 mL).

Regulatory limit (MAPA): Maximum of 20.0 mg/100 mL of anhydrous alcohol for aguardente, cachaça, vodka, and whiskey.

3.3.9. Quality Control

3.3.9.1. Acceptance Criteria

To ensure result reliability, these criteria must be met:

- Coefficient of variation between injections: < 5%
- Chromatographic resolution: between methanol and adjacent peaks > 1.5
- Peak asymmetry factor: 0.8 - 1.5 (indicator of column efficiency)
- Spike recovery: 95-105%

3.3.10. Advantages and Limitations of the Method

Table 7 summarizes the distinctive characteristics of GC-FID.

3.3.11. Applicability and Context of Use

Ideal application context:

- Forensic and fiscal analyses (legal value)
- Confirmation of screening method results
- Precise quantification for industrial quality control
- Research and development of new products
- Audits and certifications

Not recommended for:

- Rapid field screening
- High-volume routine analyses (low throughput)
- Laboratories without adequate infrastructure
- Situations requiring immediate results

Role in the Health Surveillance System

GC-FID does not replace screening methods; rather, it complements the analytical system. Its function is to confirm, with legal authority, the suspicious or positive results obtained by simpler methods (e.g., colorimetric), ensuring legal certainty for regulatory actions such as seizures, fines, and criminal proceedings.

4. RESULTS

4.1. Contextualization: The Reference Method

Gas Chromatography with Flame Ionization Detection (GC-FID) is the reference method for the determination of methanol in alcoholic beverages. This prominent position is recognized by the Ministry of Agriculture, Livestock and Food Supply (MAPA), which establishes it as the official technique for fiscal and forensic analyses. The technical superiority of GC-FID is founded on three analytical pillars: exceptional specificity provided by chromatographic separation, superior sensitivity with detection limits below 1 mg/100 mL, and accuracy that confers legal value to the results.

Even so, this analytical robustness imposes substantial practical limitations. The operational complexity, the need for specialized laboratory infrastructure, and the impossibility of field execution restrict its application to accredited laboratories. This scenario reveals a critical gap: the lack of analytical methods that reconcile reliability with portability and accessibility for on-site health surveillance. In contrast, colorimetric methods emerge as viable alternatives for field screening, enabling analyses at the collection point without the need for sample transport or complex laboratory infrastructure.

4.2. Comparative Analysis of Colorimetric Methods

The systematic investigation of colorimetric methods revealed fundamental differences in their chemical principles, sensitivity, applicability, and limitations. The Brazilian Method (Modesto, 2020) encompasses two oxidizing routes: Method A employs potassium dichromate ($K_2Cr_2O_7$) as oxidant, while Method B uses potassium permanganate ($KMnO_4$). Both routes converge on the same chromogenic detection step using chromotropic acid in strongly acidic medium, resulting in chemically equivalent analytical performance. The critical difference lies in visual interpretability: Method A generates a violet color superimposed on residual green from chromium(III) species, producing a brownish- tan appearance that complicates visual assessment. In contrast, Method B exhibits a clean yellow-to-violet color transition with minimal background interference. This superior visual contrast makes Method B the preferred choice for field screening applications where instrumental reading is

unavailable, while Method A remains viable for laboratory use with colorimetric devices.

Table 8 summarizes the analytical characteristics of all evaluated colorimetric methods, including both Brazilian Method variants, enabling systematic comparison of their capabilities and limitations.

4.3. Functional Classification of Methods

The analysis of data presented in Table 8 allowed classification of the methods into two distinct functional categories. The Lucas Test proved inapplicable for methanol determination, as it is intended solely for structural classification of alcohols (primary, secondary, and tertiary). This method does not detect methanol but functions as an exclusion screening step in the combined protocol, eliminating potential interferents before confirmatory stages.

The three remaining methods share the same analytical strategy: oxidation of methanol to formaldehyde, followed by colorimetric detection of the formed aldehyde. However, they diverge significantly in the oxidant/detector reagent pairs employed. The method with dichromate and Schiff's reagent uses a distinct chemical system from those based on permanganate and chromotropic acid, resulting in differences in sensitivity and in the color pattern developed.

4.4. Chemical Equivalence between Brazilian Method and OIV Standard

A relevant finding from this analysis was the verification that the Brazilian Method (Modesto, 2020) and the OIV-MA-AS315-27 Standard are chemically identical in their oxidation and chromogenic detection steps. Both employ potassium permanganate as oxidizing agent and chromotropic acid as a detection reagent, and follow essentially equivalent protocols.

The fundamental difference between them lies exclusively in the mode of reading the final result: the Brazilian Method allows visual analysis or with the aid of portable devices (smartphones with RGB analysis or benchtop photometers), conferring greater flexibility and portability; the OIV Standard, in turn, rigorously prescribes the use of spectrophotometry at 575 nm for instrumental quantification, ensuring maximum precision and interlaboratory reproducibility. This chemical equivalence validates the national method as a technically sound alternative to international protocols, with the additional advantage of adaptability to resource-limited contexts.

4.5. Limitations from Analytical Interferences

Investigation of interferences revealed a critical restriction common to methods based on chromotropic acid (the Brazilian Method and the OIV Standard). The presence of sugars and reducing compounds in the sample matrix promotes parallel reactions that generate dark brown coloration, masking or distorting the characteristic violet analytical signal of the methanol-chromotropic complex. This interference notably limits the applicability of these methods to non-sweetened beverages, such as vodka, whiskey, and pure cachaça. Consequently, cocktails, liqueurs, sweetened cachaças, and other beverages containing added sugar cannot be analyzed by these protocols without prior sample treatment (e.g., distillation or extensive dilution).

4.6. Comparative Synthesis: Analytical Performance and Applicability

To contextualize colorimetric methods relative to the gold standard, Table 9 presents a comprehensive comparison of critical analytical parameters, operational aspects, costs, and application contexts.

4.7. Integrated Interpretation of Results

The comparative analysis shows that the investigated methods do not compete with one another but rather occupy complementary niches within a hierarchical analytical system. The classical colorimetric methods and the Brazilian Method stand out for their portability, low cost, and speed of execution—characteristics essential for field screening during inspections, health surveillance actions, and response to public health emergencies. Their moderate sensitivity (LOD ~20-160 mg/100 mL) is sufficient to detect significant contamination above the regulatory limit of 20 mg/100 mL, enabling rapid decision-making regarding the recall of suspect products.

On the other hand, GC-FID maintains its unquestionable superiority in specificity (LOD ≤ 1 mg/100 mL) and accuracy, characteristics indispensable for confirmatory analyses, technical expertise, and precise quantification in legal reports. Its operational complexity and high cost are justified when absolute precision is mandatory, but make it impractical for routine high-volume screening or in locations without laboratory

infrastructure.

Finally, the finding that sugars interfere with methods based on chromotropic acid clearly delimits the scope of application: these protocols are suitable for non-sweetened distilled beverages (vodka, whiskey, pure cachaça) but require prior treatment for complex matrices such as liqueurs and cocktails. This limitation does not compromise their utility in health surveillance, since outbreaks of methanol poisoning frequently involve adulterated distilled beverages—precisely the type of matrix in which these methods perform best.

5. DISCUSSION

5.1. Analytical Hierarchy: Complementarity between Methods

The classification of GC-FID as a reference method derives from its undeniable analytical advantages. The specificity allows physical separation of methanol from other matrix components, such as ethanol and acetaldehyde, eliminating interferences that can lead to false-positive or false-negative results in colorimetric methods. The technique's sensitivity, capable of detecting concentrations on the order of 1 mg/100 mL, is vital for identifying contamination well below the regulatory limit of 20 mg/100 mL, providing a robust analytical safety margin. Finally, the accuracy and precision of instrumental analysis remove the subjectivity of visual interpretation, ensuring reproducible, reliable results that are required for forensic reports with legal value.

The complexity of GC-FID—which includes high equipment costs (R$ 100-300 thousand), the need for high-purity gases, specialized chromatographic columns, and highly qualified operators—makes it a confirmatory and quantitative method. In contrast, colorimetric methods are more appropriately classified as screening and semi-quantitative methods. The comparison between them, therefore, is not one of absolute quality but rather of function within a hierarchical control system.

The limitation of GC-FID regarding field use is, in practice, what creates the need and defines the role of simpler methods. A fiscal agent cannot transport a chromatograph to an inspection at a party, artisanal distillery, or border inspection post. In this scenario, colorimetric methods are valuable tools, enabling rapid, immediate assessment. A positive or suspicious field result justifies sample collection, which is then sealed and sent to an accredited laboratory for confirmatory analysis by GC-FID, ensuring both operational agility and regulatory compliance.

5.2. Chemical Equivalence and Cross-Validation: Brazilian Method versus International Standard

One of the most relevant findings of this study was the verification that the Brazilian Method developed by Modesto (2020) is chemically identical to the OIV-MA-AS315-27 Standard in its oxidation and chromogenic detection steps. Both employ potassium permanganate as the oxidizing agent and chromotropic acid as the detection reagent, following protocols that are nearly identical in reagent concentrations, reaction times, and experimental conditions.

This chemical equivalence has important practical implications. First, it technically validates the national method as a solid alternative to international protocols, conferring scientific credibility supported by standards recognized by the International Organisation of Vine and Wine. Second, it shows that the fundamental difference between the methods lies exclusively in the reading mode: while the OIV standard rigorously prescribes spectrophotometry at 575 nm, the Brazilian method offers flexibility, allowing visual reading or portable devices (smartphones with RGB analysis or benchtop photometers).

This instrumental flexibility is a strategic advantage in resource-limited contexts, enabling protocol adaptation to available infrastructure without compromising the chemical foundation of the analysis. Equipped laboratories can opt for spectrophotometric reading for maximum precision and interlaboratory comparability; field agents can employ visual analysis or portable devices for rapid screening; small distilleries can implement quality control with minimal investment in equipment.

5.3. Analytical Limitations: Sugar Interferences and Practical Implications

The interference of sugars and reducing compounds in methods based on chromotropic acid is a relevant but not critical technical limitation when properly contextualized. This interference restricts the direct application of these methods to non-sweetened distilled beverages (vodka, whiskey, pure cachaça), requiring prior treatment (distillation or extensive dilution) for complex matrices such as liqueurs, cocktails, and

sweetened cachaças.

While this limitation does not substantially compromise the utility of these methods in health surveillance for two reasons. First, the epidemiology of methanol poisoning outbreaks shows that most cases are associated with consumption of adulterated distilled beverages (artisanal cachaças, clandestine vodkas, counterfeit whiskeys)—precisely the type of matrix where colorimetric methods show best performance. Table 1 documents outbreaks in 22 countries, predominantly involving non-sweetened distilled beverages.

Second, for situations where analysis of sweetened beverages is necessary, the protocol can be adapted through prior sample distillation. This relatively simple procedure removes sugars and other volatile interferents, allowing subsequent colorimetric analysis of the distillate. This additional step, while reducing portability, keeps the method more accessible and faster than GC-FID.

5.4. Analytical Trade-offs: Sensitivity versus Portability

The comparative analysis revealed a fundamental trade-off between analytical sensitivity and portability/accessibility. Colorimetric methods exhibit moderate sensitivity (LOD ~20-160 mg/100 mL) compared with GC-FID (LOD ≤1 mg/100 mL), but this difference should be interpreted in light of the application context and regulatory limits.

The Brazilian regulatory limit established by MAPA is 20 mg/100 mL of anhydrous alcohol. The Brazilian Method, when aided by RGB reading devices or portable photometers, achieves an LOD of approximately 20 mg/100 mL, which matches the regulatory limit exactly. This sensitivity is analytically adequate for screening, as it detects contaminations at the critical level that separates compliant from non-compliant products.

The lower sensitivity of colorimetric methods implies a smaller safety margin for detecting discrete contaminations below the regulatory limit. Samples with 5-15 mg/100 mL of methanol, although compliant, could go unnoticed in visual screening. This is precisely the scenario where the hierarchical approach is justified: colorimetric methods identify gross contaminations (outbreaks, adulterations), while GC-FID ensures fine control for quality certification and detailed forensic investigations.

5.5. Well-Produced Cachaça: Safety Context and Real Risk

Methanol toxicity is a potential public health risk, with a lethal dose estimated between 0.5 and 1.5 g/kg body weight for adult humans (Gosselin *et al.*, 1984). Studies demonstrate that well-produced commercial cachaça has an average methanol content of approximately 6 mg per 100 mL of anhydrous alcohol (Boscolo *et al.*, 2000), a value much lower than the regulatory limit of 20 mg/100 mL. To contextualize this value in terms of real risk, we calculated the amount of beverage necessary to reach the lethal dose.

To estimate the lethal methanol dose for a 70 kg adult, we start from the lower end of the toxicity range (0.5 $g \cdot kg^{-1}$). This gives 35 g — i.e. 35 000 mg — of methanol, an amount that could theoretically be fatal. The next question is how much cachaça would have to be consumed to reach this figure. First, we consider the concentration in anhydrous alcohol (6 mg per 100 mL, or 60 mg L^{-1}). Dividing the lethal dose by this concentration shows that 583 L of pure alcohol would be required. Yet cachaça is only about 40 % ABV, so every 100 mL of spirit contains 40 mL of anhydrous alcohol. The methanol content therefore, drops to 2.4 mg per 100 mL (24 $mg \cdot L^{-1}$) and the volume needed rises to 1.458 L.

This implausible volume becomes even more remote when we compare it with ethanol toxicity. The human lethal dose of ethanol is reported to be 5–8 $g \cdot kg^{-1}$ (Vonghia et al., 2008), corresponding to 350–560 g for a 70 kg person. With ethanol density at 789 $g \cdot L^{-1}$, a litre of 40 % ABV cachaça delivers 316 g of pure ethanol. Consequently, death from ethanol would occur after 1.1–1.8 L of cachaça — orders of magnitude below the 1.458 L required for methanol.

Water toxicity provides a second biological ceiling. Roughly 60 % of cachaça is water, so the hypothetical 1.458 L would include about 875 L of water. The acute lethal volume of water is approximately 6 L for an adult (Farrell & Bower, 2003), meaning water intoxication would occur long before methanol reached dangerous levels.

These back-of-the-envelope calculations ignore pharmacokinetic nuances such as absorption and metabolic rate, but they clearly show that the naturally low methanol content of well-produced cachaça poses no practical threat when the beverage is consumed in moderation. Real danger arises only from criminal adulteration or sloppy distillation in which the “heads” fraction is not discarded, pushing methanol concentrations tens or hundreds of times above normal levels.

5.6. Recent Outbreaks and the Urgency of Rapid Detection

The Brazilian outbreak of October 2025, with 58 confirmed cases and 15 deaths reported by the Ministry of Health (2025), reinforces the urgency of accessible analytical methods for rapid response in public health emergencies. Table 1 documents outbreaks in more than 22 countries, with alarming fatality rates—80% in Uganda (2017), 44% in Estonia (2001), 37% in Czech Republic (2012-2014)—demonstrating that the time window for intervention is critical.

In these emergency scenarios, the portability and speed of colorimetric methods become decisive attributes. The ability to perform on-site analyses in distributors, bars, events, and households allows:

1. Rapid identification of contamination source, reducing time between first cases and product recall;
2. Mass screening of multiple suspect samples within hours, impossible with GC-FID due to analysis time (~45-60 min/sample);
3. Immediate mobilization of resources for recall of contaminated batches before additional consumption;
4. Effective risk communication to the population, based on quickly obtained analytical data.

The Iranian experience during the COVID-19 pandemic is emblematic: between February and May 2020, Iran recorded 534-800 deaths from methanol poisoning associated with consumption of adulterated alcoholic beverages supposedly "protective" against the virus (Hassanian-Moghaddam *et al.*, 2020). Rapid detection and risk communication could have significantly mitigated the number of victims.

5.7. Integrated Hierarchical System: Protocol Proposal for Health Surveillance

Based on the findings of this study, we propose implementing an integrated hierarchical system that combines colorimetric screening methods with GC-FID for confirmation, optimizing inspection resources, and balancing operational agility with analytical reliability. The suggested protocol consists of three levels:

Level 1 – Field Screening (Colorimetric Methods):

- Objective: Rapid identification of suspect samples
- Method: Brazilian Method ($KMnO_4$ + Chromotropic) with visual reading or portable device
- Decision criterion: Positive result (violet coloration) → Suspect sample
- Action: Immediate collection, sealing, and forwarding to Level 2
- Time: 20-30 minutes

Level 2 – Laboratory Confirmation (GC-FID):

- Objective: Precise quantification for forensic report
- Method: GC-FID according to IAL 228/IV or AOAC 972.11 method
- Decision criterion: Concentration > 20 mg/100 mL → Confirmed non-compliance
- Action: Issuance of technical report, definitive seizure, administrative/criminal sanctions
- Time: 45-60 minutes/sample

Level 3 – Counterproof (Reference Laboratory):

- Objective: Resolution of challenges and litigation
- Method: GC-FID or GC-MS in a certified laboratory (INMETRO, MAPA)
- Action: Final judicial decision

This hierarchical protocol offers concrete operational advantages:

- Resource efficiency: Expensive analyses (GC-FID) are reserved for confirmation of suspect cases, not for mass screening;
- Operational agility: Field decisions based on analytical evidence within minutes;
- Legal security: Forensic reports by GC-FID ensure legal value for administrative and criminal proceedings;

- Scalability: Multiple teams can perform simultaneous screening at different locations;
- Cost-effectiveness: Reduction of operational costs without compromising reliability.

5.8. Technological Gaps and Development Opportunities

Despite the advances introduced by the Brazilian Method (Modesto, 2022), some technological gaps persist and are opportunities for research and development:

5.8.1. Development of Integrated Portable Devices

The integration of RGB analysis systems, whether on smartphones or dedicated portable photometers, can enhance the sensitivity of colorimetric methods, bringing them closer to the regulatory limit of 20 mg/100 mL while maintaining objectivity and reproducibility superior to visual reading. Devices with automated calibration and an intuitive interface would further reduce subjectivity and training requirements, democratizing access to reliable semi-quantitative analyses.

5.8.2. Treatment of Complex Samples

The development of simplified pre-treatment protocols for sweetened beverages (flash distillation, solid-phase extraction, or controlled dilution) would expand the scope of application of colorimetric methods without excessively compromising portability. Portable micro-distillers or disposable purification cartridges could enable analysis of liqueurs and cocktails in the field.

5.8.3. Ready-to-Use Kits

The commercialization of standardized colorimetric kits containing pre-dosed reagents, positive/negative controls, and visual instructions would facilitate large-scale adoption by small producers, health inspectors, and laboratories with limited resources. These kits, validated and certified, would ensure reproducibility among different users and contexts.

5.8.4. Interlaboratory Validation

Collaborative interlaboratory validation studies of the Brazilian Method in accordance with ISO/IEC 17025 standards would strengthen its official acceptance and international comparability, thereby consolidating it as a recognized alternative for screening in resource-limited contexts.

5.9. Brazilian Context: Implications for Public Policies

Brazil has unique characteristics that make the implementation of hierarchical protocols especially relevant. With more than 40,000 cachaça-producing establishments (including small artisanal distilleries), a continental territory with remote, difficult-to-access regions, and limited inspection resources, the country faces meaningful challenges in ensuring food safety for distilled alcoholic beverages.

The adoption of colorimetric methods for field screening would enable:

- Decentralized inspection in municipalities without accredited laboratories;
- Training of artisanal producers for quality self-control;
- Rapid response to outbreaks in regions far from urban centers;
- Resource optimization of surveillance agencies (ANVISA, State Surveillance, MAPA).

The experience of the October 2025 outbreak, concentrated in São Paulo but with cases in six states, shows the need for analytical capillarity that simple methods can provide, complementing the network of certified laboratories.

6. CONCLUSIONS

This study critically compared analytical methods for methanol determination in alcoholic beverages, evaluating the combined classical methods, the Brazilian colorimetric method, and gas chromatography (GC-FID) with respect to performance and applicability in resource-limited contexts.

The results confirm that the methods are not competitors but rather complementary within a hierarchical system. GC-FID maintains its position as the reference method for confirmatory analyses (LOD ≤1 mg/100 mL, accuracy and legal value),

while colorimetric methods stand out as field screening tools (portability, low operational cost, execution in 20-30 minutes).

It was verified that the Brazilian Method is chemically equivalent to the OIV Standard, differing only in the type of reading, which technically validates the national protocol as an alternative to international methods. The Lucas Test proved inapplicable for methanol detection, being restricted to structural classification of alcohols. Sugar interference limits the use of chromotropic methods to non-sweetened distilled beverages, which is consistent with the epidemiological profile of outbreaks (adulterated distilled beverages).

The implementation of integrated protocols—colorimetric field screening followed by GC-FID confirmation in the laboratory— is an efficient operational strategy that optimizes inspection resources, ensuring operational agility and legal security. This approach is especially relevant in the Brazilian context, where the October 2025 outbreak (58 cases, 15 deaths) underscores the need for accessible, rapid-response methods in health surveillance.

As a limitation, this study adopted a theoretical-comparative approach without its own experimental validation. Future studies should include interlaboratory validation of the Brazilian Method, the development of portable devices with automated reading, and the development of simplified protocols for complex beverages.

We conclude that public health protection requires not only precise methods but also accessible ones that can be implemented at scale, making colorimetric methods a strategic tool for effective health surveillance in countries with limited resources.

7. DECLARATIONS

7.1. Study Limitations

This study adopted a theoretical-comparative approach based on a critical review of specialized literature and normative documentation, without its own experimental validation of the methods. The main limitations include:

- Absence of experimental validation directly comparing the three methods with real contaminated beverage samples;
- Lack of field studies evaluating the practical applicability of colorimetric methods under real inspection conditions;
- Limited scope to non-sweetened distilled beverages, with a superficial approach to complex matrices (liqueurs, cocktails);
- Absence of primary data on interlaboratory precision, robustness, and measurement uncertainty of colorimetric methods.

Despite these limitations, the study provided a solid, critical, and comparative foundation for analytical methods for methanol detection, clearly identifying the advantages, limitations, and applicability contexts of each technique, contributing to informed selection of methodologies for health surveillance and quality control in the Brazilian context.

7.2. Future Perspectives

Future studies could strengthen the conclusions through: (i) experimental validation with real fortified samples; (ii) field studies during inspections; (iii) interlaboratory validation of the Brazilian Method according to ISO/IEC 17025; (iv) development of portable devices with automated reading; (v) protocols for beverages not covered in this study. Additionally, emerging technologies (electrochemical sensors, portable Raman spectroscopy, microfluidic devices) may expand the arsenal of tools for health surveillance in resource-limited contexts.

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guidelines of the Southern Journal of Sciences, which do not allow donations from authors with manuscripts under evaluation (even when research funds are available) or in cases of authors' financial constraints, publication costs were fully absorbed by the journal under our Platinum Open Access policy, through the support of the Araucária Scientific Association (https://acaria.org/). This policy aims to ensure complete independence between the editorial process and any financial aspects, reinforcing our commitment to scientific integrity and equity in knowledge dissemination.

7.5. Conflicts of Interest

The authors declare the absence of financial or commercial conflicts of interest related to this study. The analytical methods described are in the public domain or widely documented in scientific literature. There is no commercial relationship with manufacturers of equipment, reagents, or analysis kits. This work is strictly academic in nature and does not promote specific products or services. The author did not receive any form of compensation from organizations that might have interest in the results presented.

7.6. Open Access

7.7. Author Contributions

Luis Alcides Brandini De Boni (LABD) conceived the study design, conducted the literature review, critically analyzed the analytical methods, developed the theoretical comparisons, prepared the tables and figures, and wrote the manuscript. Rochele da Silva Fernandes (RSF) critically reviewed the manuscript, identified inconsistencies in data presentation, verified technical accuracy of analytical method descriptions, and provided substantive suggestions for improving methodological clarity and standardization. Both authors reviewed and approved the final version of the manuscript and are accountable for all aspects of the work.

7.8. AI Use declaration

This manuscript was prepared with assistance from artificial intelligence tools. ChatGPT (OpenAI), Claude AI (Anthropic), and Grammarly were used to support literature organization, improve text clarity, and refine grammar and style. All technical content, data interpretation, comparative analysis, and scientific conclusions are the sole responsibility of the author. The AI tools served exclusively as writing assistants and did not generate original scientific content or analysis. The author critically reviewed, verified, and validated all information presented.

8. HUMAN AND ANIMAL-RELATED STUDIES

8.1. Ethical Approval

Not plicable.

8.2. Informed Consent

Not plicable.

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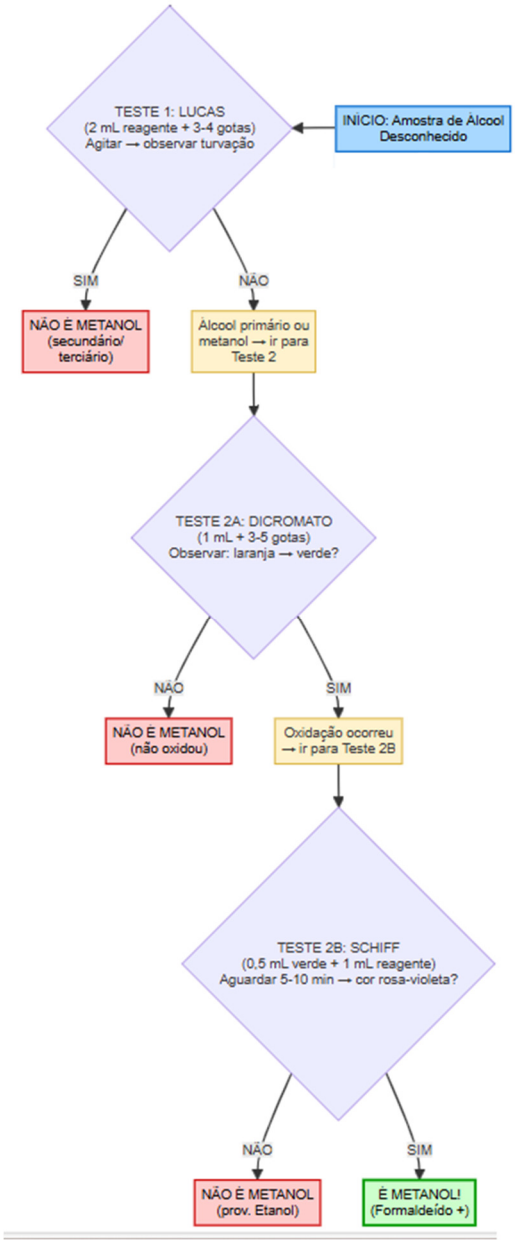

Figure 1. *Flowchart of the combined analysis protocol for methanol detection in alcoholic beverages using classical methods. The process consists of initial screening with Lucas Test (elimination of secondary and tertiary alcohols), followed by dichromate oxidation and specific confirmation with Schiff's reagent.*

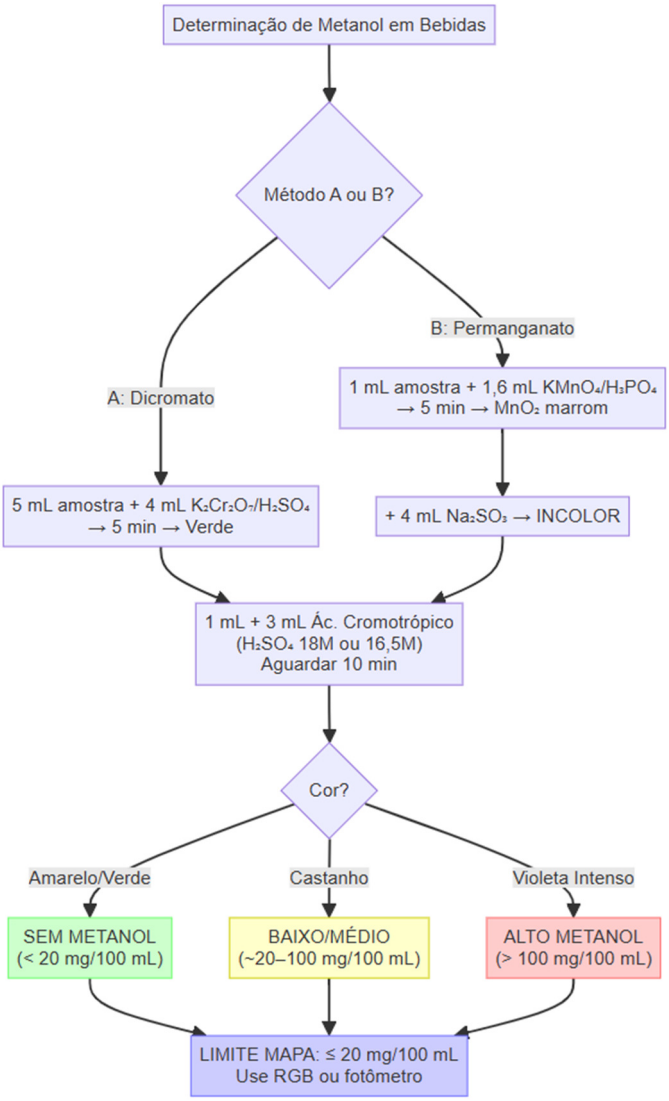

Figure 2. *Flowchart of Brazilian colorimetric methods for methanol determination in alcoholic beverages. The method offers two oxidizing routes (A: dichromate or B: permanganate), both followed by a chromogenic reaction with chromotropic acid. The violet color intensity is proportional to methanol concentration, allowing visual screening or quantification with portable devices.*

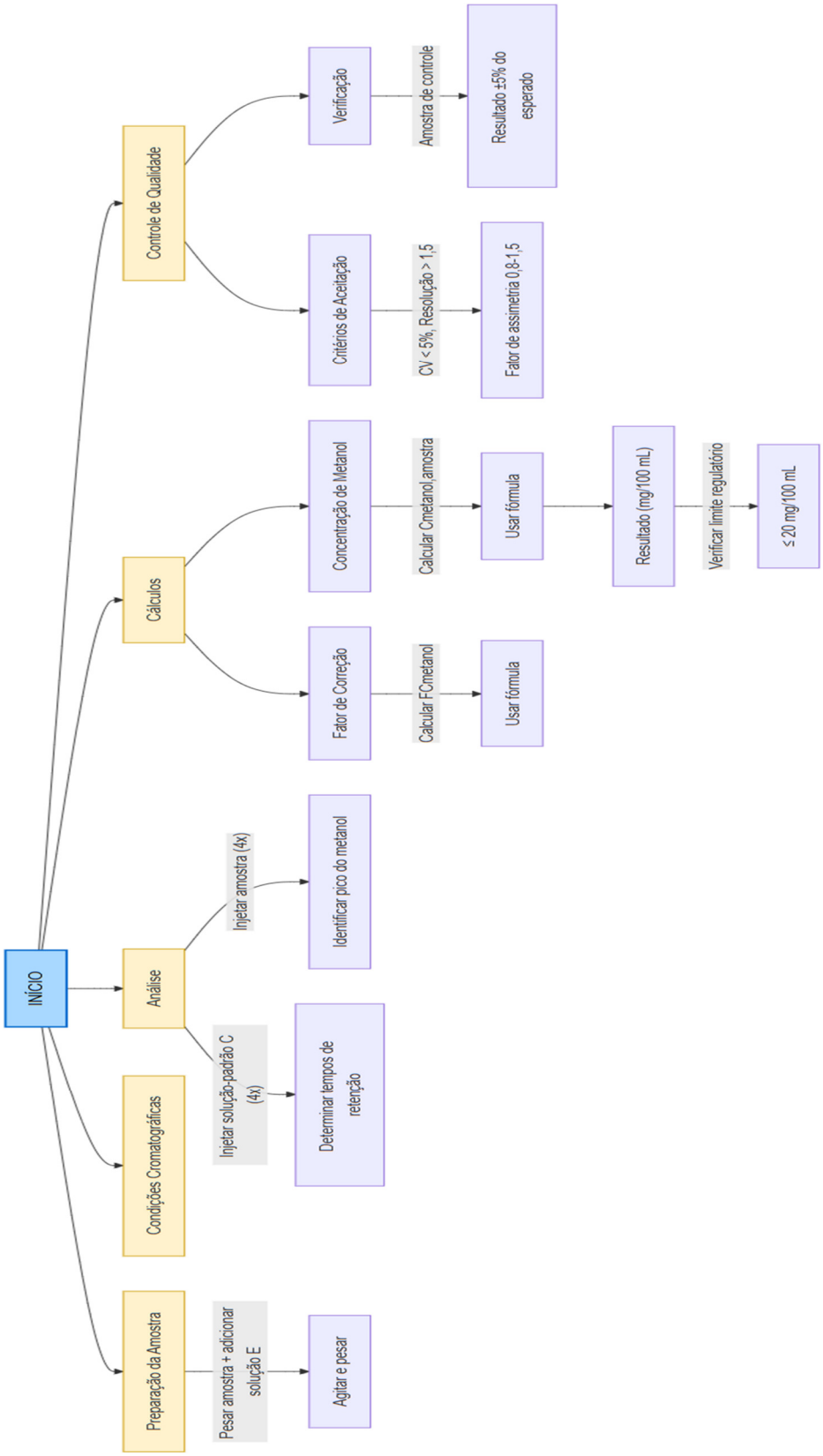

Figure 3. *Flowchart of the method for methanol determination by gas chromatography with flame ionization detection (GC-FID). The process includes sample preparation with internal standard addition, conditioning of chromatographic conditions, instrumental analysis, quantification calculations, and rigorous quality control.*

Table 1. *Methanol Poisoning Outbreaks*

Location (Country)	Outbreak Year(s)	Victims / Reported Fatalities	APA Reference(s)
Brazil (Oct 2025)	2025	108 records; 15 reported deaths	Ministério da Saúde, 2025
Iran	Feb-May 2020 (COVID-19 Peak)	534-800 deaths (13.6% mortality)	Hassanian-Moghaddam *et al.*, 2020
Czech Republic	2012-2014 (Mass outbreaks)	137 confirmed cases; 51 deaths (37% mortality)	(Rulisek *et al.*, 2017; Šejvl *et al.*, 2019; Zakharov *et al.*, 2014)
Malaysia (Klang Valley)	Sep-Oct 2018	64 fatalities; 16 fatal cases autopsied	(Rahimi *et al.*, 2021)
Uganda (Wakiso District)	June 2017	15 cases; 12 deaths (80% fatality)	(Birungi Doreen *et al.*, 2020)
Estonia	2001	154 patients; 68 deaths (44% mortality)	(Paasma *et al.*, 2007; Šejvl *et al.*, 2019)
Libya	2013	1,066 cases; ~90 deaths (10% fatality)	(Rostrup *et al.*, 2016)
Kenya	2014	Documented outbreaks	(Rostrup *et al.*, 2016)
Norway (Kristiansand)	October 1979	11 hemodialyzed patients; 3 deaths (non-dialyzed)	(Jacobsen *et al.*, 1982)
Norway (Prolonged epidemic)	2002-2004	53 cases	(Hovda *et al.*, 2005; Šejvl *et al.*, 2019)
Turkey	1993-2002	113 cases	(Šejvl *et al.*, 2019)
Turkey (Istanbul, Ankara)	Nov 2024 - Feb 2025	>235 cases (Istanbul); >94 cases (Ankara)	(Sarıbaş *et al.*, 2025)
Saudi Arabia (Riyadh)	Not specified	Approximately 25 individuals affected	(Eskandrani *et al.*, 2022)
Morocco	Recent (Published 2025)	22 cases treated with hemodialysis	(Zniber *et al.*, 2025)
Poland	2009-2013	49 cases (peak of 15 in 2013)	(Šejvl *et al.*, 2019)
Russia	Dec 2016	88 deaths	(Šejvl *et al.*, 2019)
Brazil	1999	35 deaths	González, C. (1999, March 12).
Dominican Republic	Dec 2020	199 deaths	World Health Organization (WHO). (2020, July 29).
Peru	2018-2022	9 deaths	Venegas-Justiniano *et al.*, 2024
South Africa	2022	21 adolescents killed	Voice of America. (2022, July 19).
India (Assam)	Feb 2019	150+ deaths	Schwartz, M. (2019, February 23).
Mexico	May and June 2020	100 deaths	World Health Organization (WHO). (2020, July 29).
Cambodia	June 2020	43	World Health Organization (WHO). (2020, July 29).
USA (Hand Sanitizer)	May 2020 - Nov 2021	58 cases; 23 fatalities (40%)	(Konkel *et al.*, 2024)

Table 2. Lucas Test results for different types of alcohols.

Alcohol	Time	Observation	Interpretation for the protocol
Methanol	>60 min	No reaction, solution remains clear	Proceed to Stage 2
Ethanol	>60 min	No reaction	Proceed to Stage 2
n-Propanol	>60 min	No reaction	Proceed to Stage 2
Isopropanol	5-10 min	Gradual turbidity	Secondary alcohol present
tert-Butanol	Immediate	Immediate turbidity, phase separation	Tertiary alcohol present

Table 3. Dichromate oxidation results for different alcohols.

Alcohol	Color change	Odor	Product
Methanol	Orange → green	Pungent, irritating	Formaldehyde
Ethanol	Orange → green	Sweet, fruity	Acetaldehyde
Isopropanol	Orange → green	Acetone-like	Acetone
tert-Butanol	No change	-	Does not oxidize

Table 4. Schiff's test results for formaldehyde confirmation.

Product formed	Color developed	Diagnosis
Formaldehyde (methanol)	Intense pink-violet	POSITIVE
Acetaldehyde (ethanol)	Very faint pink or none	Negative
Acetone (isopropanol)	None (not an aldehyde)	Negative

Table 5. Visual interpretation of results from Brazilian colorimetric methods.

Method	Observed color	Color intensity	Diagnosis	Estimated concentration
A (Dichromate)	Yellow/Green	-	Negative	< 20 mg/100 mL
A (Dichromate)	Brown-tan	Weak to moderate	Positive	~20-100 mg/100 mL
A (Dichromate)	Violet + green	Intense	Positive	> 100 mg/100 mL
B (Permanganate)	Light yellow	-	Negative	< 20 mg/100 mL
B (Permanganate)	Light violet	Weak	Positive	~20-50 mg/100 mL
B (Permanganate)	Intense violet	Strong	Positive	> 100 mg/100 mL

Table 6. Chromatographic conditions for methanol determination by GC-FID (IAL 228/IV method).

Parameter	**Condition**
Injector temperature	200°C
Detector temperature	250°C
Oven programming:	
- Initial temperature	40°C (hold 4 min)
- Ramp 1	15°C/min to 60°C (hold 5 min)
- Ramp 2	30°C/min to 170°C (hold 10 min)
Carrier gas (H_2)	1 mL/min (constant flow)
H_2 for flame (FID)	20 mL/min
Synthetic air (FID)	175 mL/min
N_2 make-up (FID)	25-30 mL/min
Injection mode	Split 1:100
Injection volume	1.0 µL
Total run time	~45-60 minutes

Table 7. Advantages and limitations of the GC-FID method for methanol determination.

Advantages	**Limitations**
Exceptional specificity: Physical separation eliminates interferences	High cost: Equipment (R$ 100-300 thousand)
Superior sensitivity: LOD ≤ 1 mg/100 mL	Complex infrastructure: Gases, ventilation, space
Precision and accuracy: CV < 5%, recovery 95-105%	Specialized operator: Extensive training required
Exact quantification: Results with legal value	Analysis time: 45-60 min per sample
Official recognition: Accepted by MAPA, AOAC, OIV	Not portable: Impossible to use in the field
Multicomponent analysis: Detects other volatiles simultaneously	Operating cost: Gases, maintenance, standards

Table 8. Comparative analytical characteristics of colorimetric methods for methanol detection.

Analytical Criterion	Lucas Test	Dichromate + Schiff	Brazilian A ($K_2Cr_2O_7$)	Brazilian B ($KMnO_4$)	OIV Standard
Fundamental Principle	Nucleophilic substitution (SN1)	Oxidation to formaldehyde + colorimetric reaction	Oxidation to formaldehyde + chromotropic reaction	Oxidation to formaldehyde + chromotropic reaction	Oxidation to formaldehyde + chromotropic reaction
Oxidizing Agent	$ZnCl_2$ (catalyst)	$K_2Cr_2O_7$	$K_2Cr_2O_7$	$KMnO_4$	$KMnO_4$
Detection Agent	Visual turbidity	Schiff's Reagent	Chromotropic Acid	Chromotropic Acid	Chromotropic Acid
Primary Objective	Alcohol classification	Methanol identification	Semi-quantitative screening	Semi-quantitative screening	Precise quantification
Reading Type	Visual (turbidity time)	Visual (color intensity)	Visual or RGB/photometer	Visual or RGB/photometer	Spectrophotometer (575 nm)
Observed Sensitivity	N/A (not for methanol)	Moderate (~160 mg/100 mL visual)	~20-160 mg/100 mL	~20-160 mg/100 mL	~20 mg/100 mL
Color Developed	Turbidity (cloudy)	Pink-violet	Violet + green (brownish)	Clean violet	Violet
Visual Contrast	Good (clear→cloudy)	Moderate	Poor (mixed colors)	Excellent (yellow→violet)	N/A (instrumental)
Main Interferences	2° and 3° alcohols	Other aldehydes (weak)	Sugars, reducing compounds	Sugars, reducing compounds	Sugars, reducing compounds
Execution Complexity	Low	Moderate	Moderate	Moderate	High
Analysis Time	5-60 min	15-30 min	20-30 min	20-30 min	20-30 min
Portability	Excellent	Excellent	Excellent	Excellent	Poor (lab only)
Typical Applicability	Exclusion screening	Laboratory screening	Field/lab screening	**Field screening (recommended)**	Laboratory confirmation

LOD = Limit of Detection; RGB = Red-Green-Blue color analysis via digital imaging; N/A = Not applicable. *Method B (permanganate-based) is recommended for field screening due to superior visual contrast. Method A (dichromate-based) is suitable for laboratory settings with colorimetric reading devices. **All methods based on chromotropic acid are not applicable to sugar-containing beverages without prior sample treatment (distillation or dilution).

Table 9. Comparison of analytical performance and applicability of classical colorimetric methods, national method, and gold standard (GC-FID).

Criterion	**Combined Classical Methods**	**National Method (Modesto, 2020)**	**Gold Standard Method (GC-FID)**
Principle	Oxidation with dichromate + reaction with Schiff or chromotropic acid	Colorimetry with chromotropic acid after oxidation	Chromatographic separation with flame ionization detection
LOD (mg/100 mL)	~160 (without optical aid)	~20 (with RGB reading or photometer)	≤1
LOQ (mg/100 mL)	~200	~30	~5
Selectivity	Medium – ethanol also oxidizes but does not react with dye	Good – ethanol does not interfere in final reading	High–efficiency chromatographic separation
Analysis time	15–30 min	20–30 min	~45–60 min (including preparation and run)
Initial investment	Minimal (tubes, basic reagents)	Low (photometer or smartphone)	High (chromatograph, gases, column)
Training required	Basic – technical level	Basic to intermediate	Advanced – specialized analyst
Field applicability	Excellent – portable, no electricity	Excellent – can use a smartphone	Poor – requires laboratory
Suitability for screening	Yes	Yes	Not recommended
Suitability for confirmation	Not recommended	Possible, with validation	Ideal
MAPA compliance	Not officially quantitative	Semi-quantitative – accepted with validation	Fully compliant
Main limitations	Low sensitivity, subjective interpretation	Sugar interference, calibration necessary	High cost, time, and requires infrastructure

Legend: LOD = Limit of Detection; LOQ = Limit of Quantification; RGB = Color analysis via digital image; MAPA = Ministry of Agriculture, Livestock and Food Supply (regulatory limit: 20 mg/100 mL of anhydrous alcohol); ✓ = Suitable; ✗ = Unsuitable; △ = Partially suitable.