SARTORIUS

Application Note

July 8, 2021

Keywords or phrases:
Standard Solutions, Calibration, Gas Chromatography, Volatile Liquid, Ethanol, Pipetting

How to Prepare GC and HPLC Standards

Sandra Söderholm,[1] Paulus Artimo[2]

[1] Lab Essentials Applications Development, Sartorius Biohit Liquid Handling, Helsinki, Finland

[2] Product Management, Sartorius Biohit Liquid Handling, Helsinki, Finland

Correspondence
Email: LHinfo.Finland@Sartorius.com

Abstract

The preparation of ethanol standard solutions is a routine step in quantifying the alcohol content of beverages by gas chromatography (GC), however, accurate pipetting of volatile liquids, such as ethanol, can be challenging. Preparing standards by pipetting offers cost and time benefits compared to protocols that involve weighing. You can save precious time and money, and avoid washing excessive amounts of glassware. Accurate pipetting can also speed up the preparation process. You can skip the separate internal standard dilution and reduce the volumes to < 1 mL when diluting the final GC samples with the internal standard. Here, we show that applying the right pipetting technique when preparing ethanol standards generates reproducible results with GC.

Find out more: www.sartorius.com/en/products/pipetting

Introduction

Standard solutions are the backbone for accurate analyses by high performance liquid chromatography (HPLC) and gas chromatography (GC). Standard solutions, which contain known concentrations of an analyte, are used to quantify an analyte of unknown concentration, and must be prepared in a precise manner to ensure successful measurement.

GC is commonly used to quantify the ethanol content in alcoholic beverages, such as wine and beer. Ethanol quantification with GC requires a calibration curve that is generated using ethanol standard solutions, as well as an internal standard (ISTD), such as 1-butanol. Both ethanol and 1-butanol are volatile liquids, which are difficult to pipette accurately. This can be addressed with air-displacement pipettes by following good pipetting practices and accommodating for the specific characteristics of volatile liquids.

Volatile liquids evaporate until an equilibrium is reached with the environment. Thus, pre-wetting the pipette tip with the liquid by pipetting up and down before dispensing equilibrates the environment inside the tip and improves pipetting accuracy. Reverse pipetting is also beneficial when working with volatile liquids. In reverse pipetting, an excess of liquid is aspirated into the tip, but only the target volume is dispensed. If further evaporation occurs after pre-wetting the tip, it occurs from the excess volume, allowing reproducible dispensing of the target volume.

In this application note, we compared the accuracy of preparing standard solutions and samples for GC using the Sartorius Tacta® pipettes and Sartorius Optifit pipette tips versus the reference method by weighing.

Materials and Methods

Pipettes and Pipette Tips

Sartorius Tacta® pipettes (0.1–10 µL, 10–100 µL, 100–1,000 µL, 500–5,000 µL, 1,000–10,000 µL) and Sartorius Optifit pipette tips (10, 200, 1,000, 5,000 and 10,000 µL) were used for the preparation of standard ethanol solutions, ISTD, and samples.

Water

Type 2 water from a Sartorius Arium® Advance equipped with an Arium® Bagtank 20 was used.

Preparation of Standard Ethanol Solutions

Absolute ethanol (Altia ETAX Aa, 99.5% V/V) was used for the preparation of standard ethanol solutions. Five standard solutions were prepared to cover the expected ethanol concentration range in the samples (approximately 0.5–7% V/V). The concentrations of the standard solutions were: 0.53%, 0.95%, 2.00%, 5.03%, and 7.17%. To achieve these respective concentrations, 53 µL, 95 µL, 201 µL, 506 µL, and 721 µL was pipetted with Tacta® 10-100 µL or Tacta® 100–1,000 µL pipettes into 10 mL volumetric flasks (Table 1). When preparing the standard solutions according to the reference method (Table 1), we weighed ethanol in the amounts 0.042 g, 0.0752 g, 0.159 g, 0.399 g, and 0.569 g with a Sartorius Cubis® balance. The reference method is described in Analytica-European Brewery Convention (EBC) Method 9.2.4.

Table 1

Methods for Preparing the Ethanol Standard Solutions

Method	Description
Reference (described in Analytica-EBC, Method 9.2.4)	Weigh the appropriate amount of absolute ethanol into a 10 mL volumetric flask and make to the mark with water. Make a note of the exact weight of ethanol taken for the preparation of each standard. The ethanol standards should be stored in sealed glass bottles at 0–4° C.
A	Pipette 5 mL of water into a 10 mL volumetric flask. Use a Tacta® 10–100 µL and 100–1,000 µL pipette to pipette the ethanol. Without pre-wetting the tip, forward pipette ethanol into the flask and make to the mark with water.
B	Pipette 5 mL of water into a 10 mL volumetric flask. Use a Tacta® 10–100 µL and 100–1,000 µL pipette to pipette the ethanol. Pre-wet tip 10 times, reverse pipette ethanol into the flask, and make to the mark with water.
C	Pipette 5 mL of water into a 10 mL volumetric flask. Pre-wet tip 10 times, reverse pipette ethanol into the flask, and make to the mark with water. In comparison to Method B, use only a Tacta® 100–1,000 µL pipette for pipetting ethanol when preparing all five standard solutions.

Note. The pipetting steps were carried out with Tacta® 10–100 µL, 100–1,000 µL, or 500–5,000 µL pipettes.

Preparation of Beverage Samples

Excess carbon dioxide was removed from 200 mL of carbonated beverage samples (beer and sparkling wine) by incubation for 10 min in an ultrasonic bath (Branson 2510). The beer samples were filtrated as described in Analytica-EBC, Method 9.2.4. Alcoholic beverages were diluted by pipetting with Tacta® 500–5,000 µL and 1,000–10,000 µL pipettes as described in Table 2.

Table 2

Beverages That Were Analyzed

Sample No.	Beverage Type	Ethanol Content Reported on Bottle, % (V/V)	Dilution in Type 2 Water by Sartorius Arium®
1	Non-alcoholic beer	0.5	No dilution
2	Wheat beer	6.6	1:1 (5 mL sample + 5 mL water)
3	Wheat beer	7	1:1 (5 mL sample + 5 mL water)
4	Sparkling white wine	11	1:1 (5 mL sample + 5 mL water)
5	Non-alcoholic red wine	0.5	No dilution
6	Red wine	14	1:3 (3 mL sample + 6 mL water)
7	Spirit	40	1:10 (1 mL sample + 9 mL water)

Preparation of Internal Standard

1-butanol (Analytical Reagent, Riedel-de Haën) was used as ISTD. Similar to ethanol, 1-butanol is a volatile liquid. The ISTD was prepared in two different ways: as described in Analytica-EBC, Method 9.2.4 (preparing a 0.5% 1-butanol solution), or by pipetting 1-butanol directly into the GC vial with a Tacta® 0.1–10 µL pipette (Table 3, Method 2). The Analytica-EBC, Method 9.2.4 was performed as follows: pipette 10 mL of 1-butanol at 20 ± 0.1° C into a two-liter volumetric flask and dilute to volume with water at 20 ± 0.1° C. Add stopper and shake well.

GC Instrument and Method

The final samples were prepared as described in Table 3. The final concentration of the ISTD in the samples was 0.45% V/V. Samples (1 µL) were injected into the GC (6890N, Agilent Technologies) with an Agilent J&W DB-1701 (Cat. No. 122-0732, 30 m x 0.25 mm, 0.25 µm) column. Duplicate samples were prepared, and each sample was analyzed twice.

Table 3

Preparation of the Final GC Samples

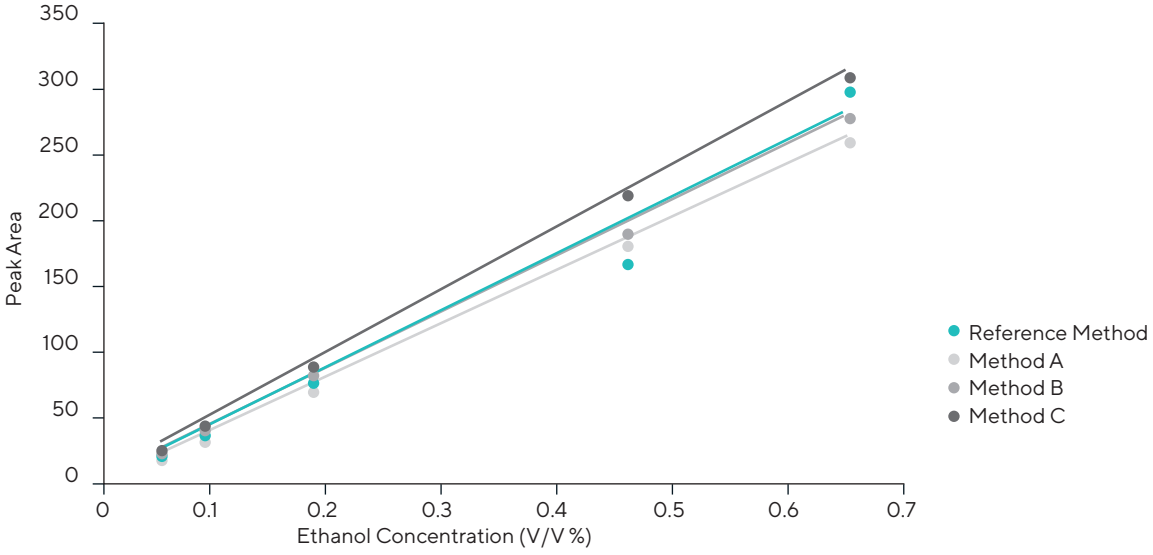
Method	Description
Reference (described in Analytica-EBC, Method 9.2.4)	Dilute 2 mL of each ethanol standard solution or sample with 20 mL of the 0.5% ISTD by pipetting into a clean, dry 50 mL conical flask. Ensure that both solutions are maintained at 20 ± 0.1° C before diluting. Temperature accuracy at this stage is critical to the accuracy of the method. Add stopper and mix well. Pipette 1 mL into GC vials.
1	Pipette 909 µL 0.5% ISTD (prepared according to Analytica-EBC, Method 9.2.4) and 91 µL sample or ethanol standard solution directly into GC vial.
2	Pipette 904.5 µL water, 91 µL sample or ethanol standard solution, and 4.5 µL 1-butanol directly into GC vial. Pre-wet tip 10 times and reverse pipette when pipetting the 1-butanol.

Results and Discussion

The ethanol calibration curves are shown in Figure 1. The calibration curve generated from standards prepared according to Method B (follows best pipetting practice, including pre-wetting and using reverse pipetting technique) was comparable to the calibration curve generated from the standards prepared according to the reference method. The curve generated from standards prepared according to Method A (no pre-wetting of pipette tip and using the forward pipetting technique) showed low similarity to the reference method. Method C was the same as Method B, except that only one pipette (Tacta® 100–1,000 µL pipette) was used to pipette ethanol (53 µL, 95 µL, 201 µL, 506 µL, and 721 µL) when preparing the standard solutions. This calibration curve also showed low similarity to the reference method. We recommend using a pipette that has a nominal volume as close as possible to the volume that you intend to pipette. Additionally, the pipette volume range should cover the volume you intend to pipette.

Figure 1

Calibration Curves of Standard Solutions Prepared Using the Reference Method and Methods A–C

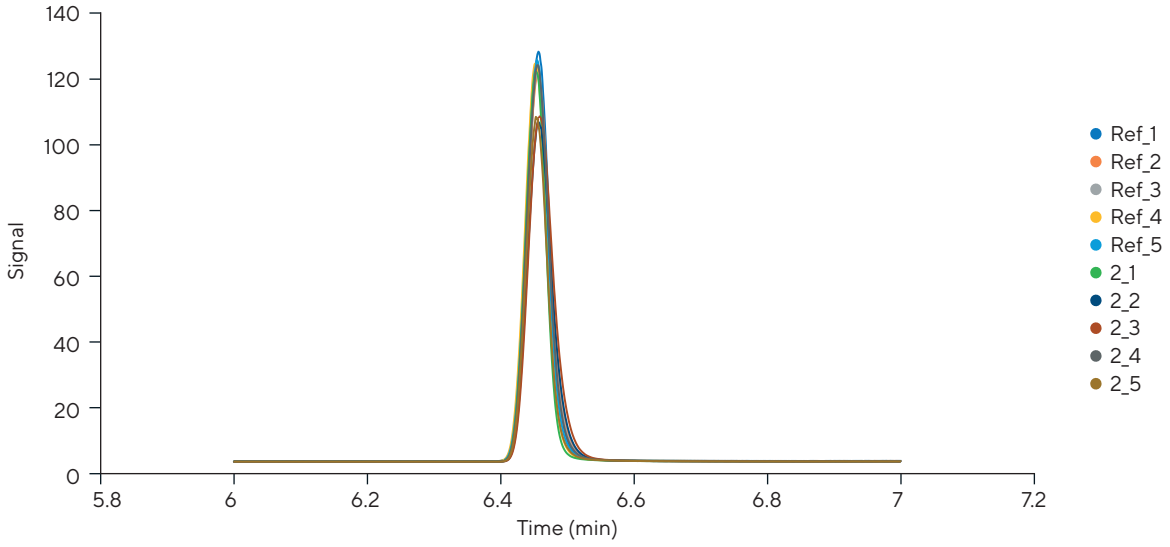


Note. The standard curves for the reference method and Method B are overlapping, suggesting that the preparation of ethanol standard solutions by pipetting, as described for Method B (see Table 1), generates very similar calibration curves to the reference method (Table 1). The standard curves prepared according to Methods A and C are not similar to the curve based on the reference method, suggesting that choosing the correct pipette and applying the right pipetting technique are crucial for preparing accurate standard ethanol solutions.

Pipetting the ISTD, 1-butanol, directly into the GC vial (Table 3, Method 2) gave comparable results to the reference method (Figure 2). All ten chromatographic peaks co-eluted and exhibited similar areas under the curve. Five of these samples were prepared according to the reference method (Table 3), and the other five samples were prepared according to Method 2 (Table 3). The average peak areas of the two groups differ by only 2.45%. The coefficient of variance (CV%) between the 1-butanol peak areas within the groups was 7.40% for the reference method and 5.95% for Method 2. This suggests that you can omit the preparation of a separate ISTD dilution, and instead pipette the 1-butanol directly into the vial, even though the volume is very low, in this case 4.5 µL.

Figure 2

Reproducibility in GC with Reference Method and Method 2

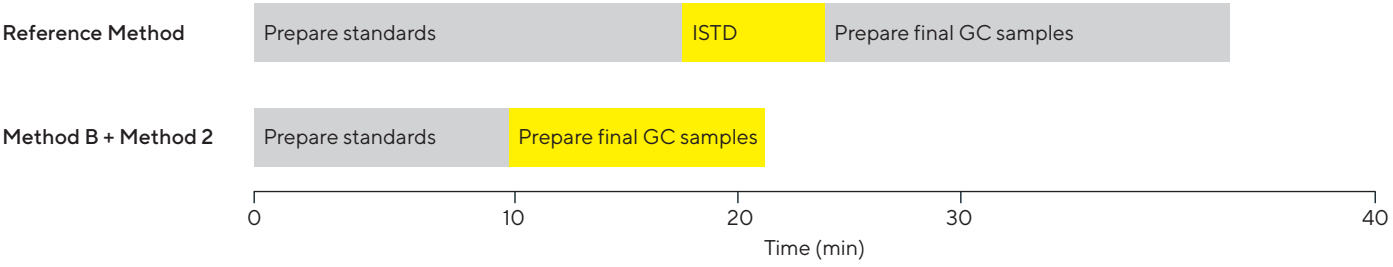


Note. Pipetting the internal standard (1-butanol) directly into the GC sample vial produced reproducible peaks in GC. The overlapping peaks from ten different samples are shown. Five of these samples were prepared according to the reference method, and the other five samples were prepared according to Method 2 (Table 3). All ten peaks are co-eluting and shows similar areas under the curve.

In summary, applying Method B for preparing standard ethanol solutions and Method 2 for preparing the final GC sample decreases the sample preparation time from approximately 35 minutes to 18 minutes (Figure 3). Additionally, the combination of Method B and Method 2 reduces the amount of required glassware to a minimum, as there is no need to prepare a separate 0.5% ISTD solution and dilute the samples and standards with this ISTD solution before preparing the actual GC samples. Moreover, the working volumes are also lower in Method 2 compared to the reference method.

Figure 3

Comparison of sample preparation time



Note. Applying Method B for preparing ethanol standards in combination with Method 2 for final preparation of GC samples decreased the sample prep time by 50% compared to the reference method.

Beverage samples were analyzed with GC and their ethanol contents were determined (Table 4). The experimentally determined ethanol contents were close to the ethanol contents reported on the bottles. The standard ethanol solution with the lowest ethanol concentration was 0.53% V/V, which is higher than the reported ethanol concentration of the non-alcoholic beverages (Samples 1 and 5). Therefore, the results of these samples are likely not very accurate, as they are outside the calibration range. We recommend preparing standard solutions spanning the whole concentration range; including standard solutions that are below the sample with the lowest expected ethanol concentration and above the sample with the highest expected ethanol concentration. In this work, we prepared five standard solutions. You can also increase the number of standard solutions in order to increase accuracy.

Table 4: *Reported and Determined Ethanol Content of Beverages*

Sample No.	Beverage Type	Ethanol Content Reported on Bottle, % (V/V)	Experimentally Determined Ethanol Content % (V/V)	Relative Difference (%)
1	Non-alcoholic beer	0.5	0.21	58
2	Wheat beer	6.6	6.52	1.2
3	Wheat beer	7	6.00	14
4	Sparkling white wine	11	10.58	4
5	Non-alcoholic red wine	0.5	0.21	58
6	Red wine	14	13.68	2.3
7	Spirit	40	38.65	3.4

Note. Alcoholic and non-alcoholic beverages were analyzed with GC and the ethanol content was determined using the calibration curve prepared according to the reference method (Table 1). The samples were prepared according to Method 1 (Table 3).

Conclusions

In this work, we applied pipetting best practices for simple and accurate preparation of standard ethanol solutions and the internal standard for semi-quantitation of ethanol in beverages by GC. Reverse pipetting, which is recommended for pipetting volatile liquids, allowed for reliable pipetting of ethanol and butanol in this study. Importantly, pipetting helps improve workflows by saving time during sample prep and cleanup.

References

European Brewery Convention, Analytica-EBC, Issued by the EBC Analysis Committee, 2010, Fachverlag Hans Carl, Nürnberg. ISBN 978-3-418-00759-5

Germany
Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 631 254 4249
Toll-free +1 800 635 2906

For additional information,
visit www.sartorius.com