

# Courtesy Copy

SSD:TM:103	Rev. 4
Issue Date: 08/06/2020	Page 1 of 6
Implementation Date: 08/20/2020	

The colored ink stamp indicates this is a controlled document. Absence of color indicates this copy is not controlled and will not receive revision updates.

## Ethanol, Specific Gravity, Density, and Refractive Index Determination in Nonbeverage Products

### Scope and Application

This method follows from SSD:TM:102. The purpose of this method is to apply the ethanol and specific gravity determination methodologies from SSD:TM:102 to nonbeverage products, as well as to determine refractive index of nonbeverage products.

This method applies to the determination of the specific gravity, density and refractive index of liquid products (flavors, medicines, dietary supplements, perfumes, and products found fit for beverage purposes). The specific gravity is reported as a dimensionless quantity. Density is reported as g/mL or lb/gal. The Refractive Index is reported as nD, the ratio of the velocity of light (sodium D line) in air to the velocity of light in the sample (at 20°C, unless otherwise indicated).

This method applies to the determination of ethanol in neat samples or standards. It does not apply if distillation is required to determine the ethanol content of a sample or standard. (Please refer to SSD:TM:102 for additional information if distillation is required.)

NOTE: The regulatory tolerances for alcohol beverages are more strict than what is required for nonbeverage products. For example, the Tolerances for Item #10 (Appendix 1) shows the acceptable alcohol ranges for nonbeverage products (Item #10 refers to Item 10 on TTB F 5154.1 (10/2008), Alcohol Content by Volume of Finished Product). For this reason, a less accurate and precise DMA may be used for nonbeverage applications.

### Levels and Limitations

Analyte	Detection Limit	Quantitation Limit	Linear Range	Interferences
Ethanol (Neat)	0.02% by vol.	0.05% by vol	0.05 – 100% by vol	<u>Solids, carbonation</u>

The range of the Abbemat 300 Refractometer is 1.26 to 1.72 nD at 20.00°C.

### Supplemental Documents

1. SSD:TM:102, Ethanol Determination by Specific Gravity
2. NPL:WG:105 DMA 4500M and Abbemat 300 Startup and Shutdown Procedure
3. NPL:WG:106 NPL DMA/Refractometer Potentially Useful Information for Coprincipal Analysts and Other Responsible Parties
4. NPL:WG:107 How to Analyze Samples using NLC\_1\_001 DMA and RI
5. NPL:Form:103-2 Densitometer Performance Checks

# Courtesy Copy

SSD:TM:103	Rev. 4
Issue Date: 08/06/2020	Page 2 of 6
Implementation Date: 08/20/2020	

The colored ink stamp indicates this is a controlled document. Absence of color indicates this copy is not controlled and will not receive revision updates.

## Equipment

Density Meter (Anton—Paar, DMA-4500M or equivalent), reporting specific gravity to 4 decimal places, temperature controlled 20.00±0.01°C

Refractometer (Anton—Paar, Abbemat-300 or equivalent), reporting specific gravity to 4 decimal places, temperature controlled 20.00±0.01°C

Automatic Sample Changer (Anton—Paar XSample 452 or equivalent)

Fisherbrand VersaClosure Tube Closures, Fisher Scientific part number 02-707-17, or equivalent

Kimble Disposable Culture Tubes (16 x 100 mm), Fisher Scientific part number 03-341-4, or equivalent

## Reagent and Sample Preparation and Handling

### Reagents:

Deionized water, 18 MΩ/cm or better

Ethyl Alcohol, 200 Proof, (CAS#64-17-5)

Any other solvents deemed appropriate for cleaning and drying the instrument, as needed

## Procedures

1. Verify that the 18 MΩ/cm water and 200 proof ethanol (or other appropriate cleaning and drying solvents) vessels attached to the DMA contain sufficient quantity to analyze (ask co-principal analyst, if needed).
2. Confirm that the density meter and refractometer (as needed) have been properly calibrated. Before running any samples, first run a tube containing DI water to prime the instrument. Next, obtain the density and refractive index of a water blank (DI water) and verify that the values fall within the established range allowed, as stated in the Performance Checks (NPL:Form:103-2). If either value is outside the allowed range, refer to the Quality Control section of this method.
3. Opened LCS bottles are stored at 1-6°C and have a shelf life of 3 weeks:
  - a. Before using an opened LCS bottle, verify that the opened date is within 3 weeks (21 days or less) of the current date. If the opened date is more than 3 weeks (more than 21 days) prior to the current date, properly dispose of that LCS and obtain an unopened bottle of LCS.
  - b. When a new, unopened bottle of LCS is used, write the opened date on the LCS bottle and store at 1-6°C after use.

# Courtesy Copy

SSD:TM:103	Rev. 4
Issue Date: 08/06/2020	Page 3 of 6
Implementation Date: 08/20/2020	

The colored ink stamp indicates this is a controlled document. Absence of color indicates this copy is not controlled and will not receive revision updates.

- Care should be taken to minimize evaporation of the sample prior to instrument sampling. This is accomplished by capping each tube before placing them in the autosampler.

**NOTE:** If LCS or sample is refrigerated, allow it to warm closer to 20 °C before running on the DMA.

**NOTE:** Leave approximately 1” headspace when filling the DMA vials when the DMA is set to operate in pressure mode. Overfilling the vials may lead to sample leakage resulting in possible QC failures.

- De-carbonate any carbonated samples, as per laboratory policy.
- Determine density in Anton Paar DMA model 4500M using the pre-set methods.
- Determine  $SG_{app}^{20/20}$  in Anton Paar DMA model 4500M using the pre-set methods.
- Determine nD in Anton Paar Abbemat 300 using the pre-set methods.

**NOTE:** The system will display a Filling Warning when gas bubbles are detected in the density measuring cell when sampling viscous samples. It has been determined that this status does not affect the quality of the data from the DMA or RI for NPL purposes up to and including glycerol with a viscosity of 1.2 PA\*s.

## Quality Control

- DI water is run at the beginning and end of every sample set, and at least every 12<sup>th</sup> sample, to check the system calibration. The density and refractive index value for water should fall within the allowed range.
- Samples bracketed by passing water checks are reportable. If the density or refractive index value of a water fall outside the allowed range, re-run at least 3 DI waters and record their values in the logbook. If the 3 DI waters are within the allowed range, the data is reportable. If these values fall outside the allowed range, stop using the instrument, place a “Do Not Operate” sign on the instrument, and inform one of the co-principal analysts.
- Duplicate LCS is run with each sample set for accuracy and count as samples for determining when to run DI water.
- An LCS is run in duplicate (non-sequentially) for precision control charting. The duplicate values for density and refractive index need to fall within the allowed ranges posted in the instrument log book (NPL:Form:103-2).
- If the density or refractive index of the LCS fails, re-run the same bottle of LCS at least three times and record the values in the logbook. If all of the values fall within the allowed range then the data is reportable. Initiate a CAR (incident report). If any of the values fall outside the allowed range, stop using the instrument, place an “Out

# Courtesy Copy

SSD:TM:103	Rev. 4
Issue Date: 08/06/2020	Page 4 of 6
Implementation Date: 08/20/2020	

The colored ink stamp indicates this is a controlled document. Absence of color indicates this copy is not controlled and will not receive revision updates.

of Service" tag on the instrument, inform one of the co-principal analysts and initiate a CAR.

## Sources of Uncertainty

1. Improper cleaning or drying of instrument
2. Temperature of the sample cell (DMA) or prism (RI)
3. Overfilling DMA tubes
4. Evaporation of ethanol due to delay in capping tubes or improperly handled samples

## Calculations

Calculations are done by the instrument software. If % w/w needs to be reported, please refer to SSD:TM:102 Ethanol Determination by Specific Gravity.

## Reporting Results

Report density to four decimal places, x.XXXX.

Report specific gravity to four decimal places, x.XXXX.

Report alcohol by volume of nonbeverage products to one decimal place x.X.

Report refractive index to four decimal places, x.XXXX nD.

Report the temperature at which the Refractive Index value was obtained to two decimal places, xx.XX °C.

## Safety Notes

Normal laboratory safety protocol should be followed. High proof alcoholic products are flammable. Ethanol burns with an almost invisible blue flame.

## References

1. Anton Paar Instruction Manual for DMA 4100/4500/5000. Document # C76IB003EN-I. Rev: 04/09/2014. Issue Date: 07/01/2014.

2. Anton Paar Instruction Manual for Abbemat 300/500. Document # D22IB002EN-D Rev: 03/25/2013. Issue Date: 07/01/2014.

## Required Training, Certification and Re-certification

1. In-house training by a certified chemist in distillation and DMA operation.

# Courtesy Copy

SSD:TM:103	Rev. 4
Issue Date: 08/06/2020	Page 5 of 6
Implementation Date: 08/20/2020	

The colored ink stamp indicates this is a controlled document. Absence of color indicates this copy is not controlled and will not receive revision updates.

2. Initial certification is achieved by running blind or LCS samples with results of precision and reproducibility in agreement with the logbook guidelines.
3. Proficiency testing or re-demonstration of competency every 5 years.

## Revision History

Rev. 1 – Initial revision

Rev. 2 – Added new procedures for LCS storage and LCS shelf life. Made minor spelling corrections.

Rev. 3 – Updated LCS shelf life requirements.

Rev. 4 - Updated control charting from specific gravity to density. Also clarified the QC procedures for QC/LCS issues.

Courtesy Copy

# Courtesy Copy

SSD:TM:103	Rev. 4
Issue Date: 08/06/2020	Page 6 of 6
Implementation Date: 08/20/2020	

The colored ink stamp indicates this is a controlled document. Absence of color indicates this copy is not controlled and will not receive revision updates.

## Appendix 1

Acceptable alcohol ranges for nonbeverage products. (Item #10 on TTB F 5154.1 (10/2008), Alcohol Content by Volume of Finished Product).

### Tolerances for Item #10

Alcohol Percentage	Simple Mixtures	Processes Other Than Simple Mixtures *
> 95-100	93-100	91-100
>90-95	88-95	86-95
>80-90	+/- 3.5	+/- 4.5
>70-80	+/-3.0	+/- 4.0
>60-70	+/- 2.5	+/- 3.5
>40-60	+/- 2.0	+/- 3.0
>20-40	+/- 1.5	+/- 2.5
>1-20	+/-1.0	+/- 2.0
0.5-1.0	+/-0.5	+/- 0.5

Please note that the percentages are absolute. For example, at 18% alcohol by volume, the value in #10 for a simple mixture should be 18 +/- 1.0. In addition, the value in box # 10 cannot be less than zero for any range.

\* Due to the inherent variability associated with processing botanical materials, we allow a range of +/- 5 for dietary supplements. However, the value in box #10 cannot exceed the amount of alcohol theoretically present, and cannot be less than zero.