Application Note
No. 783/2021

Citrus essential oils determination in juice

Dist Line:
Citrus Essential Oils (Limonene) Determination in Juice

1. Introduction

The concentration of essential oils in citrus juice is a quality measure with maximum limits that are set by authorities such as the European Fruit Juice Association (AIJN) and the United States Department of Agriculture (USDA). The essential oils consist to a majority of approximately 90 % of D-limonene [1].
An easy and reliable method for the determination of essential oils in juice and juice concentrates of citrus fruits, according to IFU Method No. 45 (2005) and AOAC 968.20, is introduced below. The procedure follows the bromate method by Scott and Veldhuis [2]. Here, the essential oils are separated by steam distillation on the BasicDist and trapped in an acidic solution with red color indicator (methyl orange). A mix of bromide and bromate solution is titrated until the solution becomes colorless to determine the content of D-limonene.

2. Equipment

- BasicDist
- Metrohm Eco Titrator (11072748)
- Optical sensor (Optrode) (11066601)

3. Chemicals and Materials

Chemicals:

- Isopropanol, technical (VWR, >97%)
- Methyl orange indicator aqueous solution (0.1 % methyl orange),
- (made from > 85%, Sigma-Aldrich)
- Hydrochloric acid solution 12 %
- Potassum bromate (VWR, >99.8%)
- Potassium bromide (VWR, >99.5%)
- D-limonene (Alfa Aesar, 96%)

For a safe handling please pay attention to all corresponding MSDS!

Samples were purchased at a local supermarket:

- Orange juice
- Orange juice, organic
- Grapefruit juice
- Lemon juice

4. Procedure

The determination of D-Limonene as a measure of essential oils in citrus juice products includes the following steps:

- Distillation of the sample, using BasicDist
- Colorimetric titration of the distillate, using Eco Titrator with Optrode

4.1 Distillation

1. Add 10 ml of 0.1 % methyl orange solution to 500 mL 12 % HCl solution. Add 25 mL via a volumetric pipette of this solution to the receiving vessel before distillation. Please use a suitable receiving vessel which has a diameter of about 5 cm (Receiving vessel (Optrode) - 11068263 or alternatively a 100 mL beaker).
2. Stock solution: Prepare 1 L of a KBr / KBrO3 solution (16.5 mmol, 2.7557 g KBrO3 / 100 mmol, 11.880 g KBr equals 0.1 N bromine solution)

3. Titrant solution: Dilute 250 mL of stock solution with 750 mL of distilled water (equals 0.025 N bromine solution).
4. Complete a priming and preheating step.
5. Measure blanks of distilled water (25 mL added via volumetric pipette) together with 25 mL isopropyl alcohol according to the parameters listed in Table 1 (approximately 0.1 – 0.3 mL of titrant should be used).
6. Measure samples: Distill the 25 mL (added via volumetric pipette) of juice sample (or 5 g of juice concentrate sample with 20 ml of water) together with 25 mL isopropyl alcohol according to the parameters listed in Table 1.

Table 1: Parameters for distillation with the BasicDist.

Method parameters			
Acid volume (Isopropyl alcohol)	25 mL	Level Detection	Off
Reaction time	1 s	Distillation Time	60 s
Steam steps	No	Sample tube aspiration	20 s
Steam power	60 %	Stirrer speed titration	1
Settings parameters			
AutoDist mode	On	MaxAccuracy mode	On

4.2 Colorimetric Titration

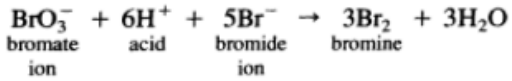
1. After the distillation is finished, wait another 20 – 30 s before you transfer the sample to the titrator to make sure enough distillate is flowing into the receiving vessel / beaker after the distillation. If you are using a MultiDist connected to a titrator, you can set the titration start time to 60 s, as it stays in the same position to collect the distillate.
2. Titrate the distillate in the receiving vessel according to the parameters listed in Table 2 and make sure there are no bubbles during stirring. The wavelength of the Optrode should be adjusted to 520 nm before titration start and the Optrode must be turned so that the opening slit of the light beam points to the middle of the vessel to make sure air bubbles are removed by the stirring.

Table 2: Parameters for colorimetric titration with the Eco Titrator with Optrode.

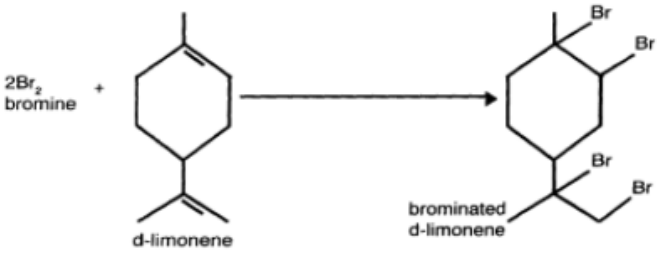
Method parameters Eco Titrator			
Start conditions		-	
Activation pulse	On	Everything else	0 / Off
Titration parameters		-	
Vol increment	0.05	Temp	25 °C
Dosing rate	max	Sensor	Optrode
Signal drift	50 mV/min	Solution	Bromide bromate
Min wait time	0 s	Stirrer	Off
Max wait time	26 s	Stirring rate [1]	8 [2]
Stop conditions		-	
Stop vol	30	Vol after EP	0.5 mL
Stop meas. Value	Off	Stop time	Off
Stop EP	1	Filling rate	Max
Evaluation		-	
Window	Off	EP recognition	Greatest
Fixed EP1 at	Off	Fixed EP2 at	Off
EP criterion	15 mV	-	

[1] depends on the size of the stirring bar, if the stirring bar is larger (>3.5 cm), a stirring rate of 6 is more suitable
[2] if a MultiDist with stirrer is used, the titration stirring rate can be set to 8 as well

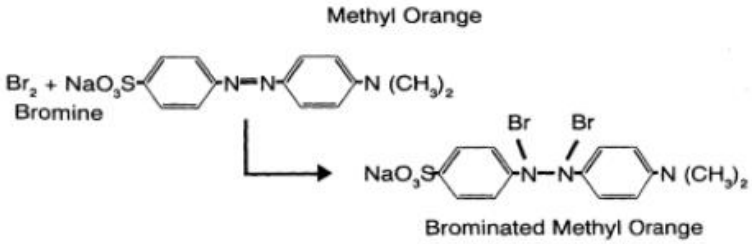
The distillate is collected in the receiving vessel in an acidic solution of methyl orange and titrated with a KBr / $KBrO_3$ solution. Once the titrant is dosed into the acidic receiver solution, the comproportionation yields elemental bromine *in-situ* in the solution:



The bromine reacts with the C-C double bonds of the limonene molecules.



As soon as all limonene molecules are saturated with bromine, the bromine molecules react with the N-N double bonds of the methyl orange molecules. This results in a loss of red color in the solution which is detected by the Optrode at 520 nm wavelength.



4.3 Calculation

The results are calculated as mL/L or mL/kg of essential oil in the sample according to [1]. In order to calculate the essential oil content of the sample, the concentration of titrant is adjusted to make the calculation formula as simple as possible. The following equations (1) and (2) are used to calculate the results. For the reference substance, the purity of the D-limonene is considered in equation (4).

4.3.1 Juices

Essential oils in mL/L:

$$v(Oil) = \frac{(V_1 - V_2)}{V_{Sample}} \quad (1)$$

$v_{(Oil)}$: volume fraction of essential oil in juice sample [mL/L]
V_1: amount of titrant for the sample [mL]
V_2: mean amount of titrant for the blank [mL]
V_{Sample}: sample volume [mL]

4.3.2 Calculation in mg/L

If the result is required in mg/L, the result should be multiplied with the density of limonene: 841 mg/mL

F : calculation factor (841 for mL/L to mL/kg)

4.3.3 Detailed calculation:

Stock solution: 0.0165 mol/L $KBrO_3$ = 0.0495 mol/L Br_2 in stock solution (*0.25) = 0.01237 mol/L Br_2 titrant solution (1 to 4 dilution)

Titrant solution: 0.01237 mol/L Br_2 solution, 1 mL contains 0.01237 mmol Br_2

Each D-Limonene molecule reacts with 2 Br_2 molecules → 1 mL of used titrant solution equals (*0.5) = 0.006188 mmol D-Limonene

M_W(D-Limonene) = 136.23 g/mol = 136.23 mg/mmol

0.006188 mmol * 136.23 mg/mmol = 0.843 mg (D-Limonene)

Density (D-Limonene) = 0.841 g/mL → 0.843 mg / 0.841 mg/µL = 1.00236 µL (D-Limonene)

Summary:

- 1 mL of titrant equals to 1.00 µL of absolute D-Limonene in the sample volume.
- 1 mL of used titrant solution equals to a D-Limonene amount of 0.040 mL / L (at a sample volume of 25 mL)

5. Results

5.1 Measurement of citrus juices

The titration curve of an orange juice sample can be seen in Figure 1. Measured contents of D-Limonene in different kinds of citrus juices are shown in Table 3-6.

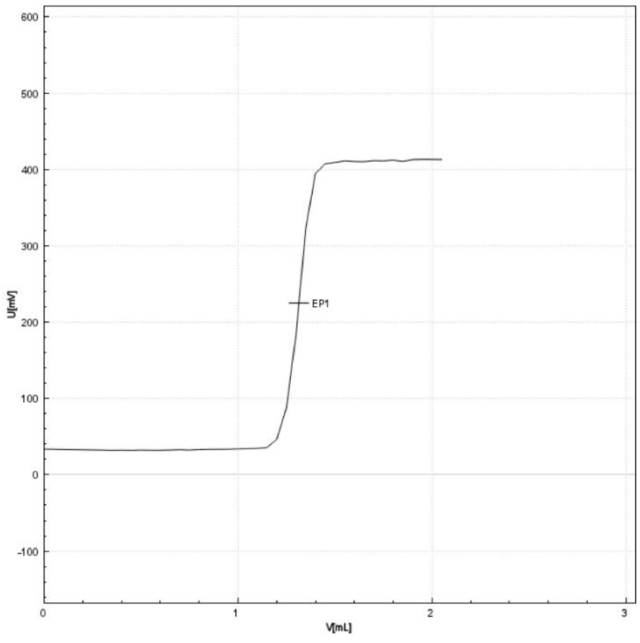


Figure 1: Titration curve and endpoint (EP) determination for an orange juice sample (Table 3, sample 2).

Table 3: Results for orange juice samples (25 mL used as sample volume).

Orange juice	$V_{titrant}$ [mL]	D-Limonene measured [mL/L]	Average Blank [mL]
Sample 1	1.3493	0.0478	0.1542
Sample 2	1.3148	0.0464	
Sample 3	1.3386	0.0474	
Sample 4	1.2786	0.0450	
Sample 5	1.3003	0.0459	
Average	1.3163	0.0465	
SD	0.0256	0.0010	
Rsd [%]	1.94	2.20	

Table 4: Results for organic orange juice samples (25 mL used as sample volume).

Orange juice (organic)	$V_{titrant}$ [mL]	D-Limonene measured [mL/L]	Average Blank [mL]
Sample 1	4.2655	0.1645	0.1542
Sample 2	4.1207	0.1587	
Sample 3	3.8979	0.1498	
Sample 4	4.0497	0.1559	
Sample 5	4.3083	0.1662	
Average	4.1284	0.1590	
SD	0.1487	0.0059	
Rsd [%]	3.60	3.74	

Table 5: Results for grapefruit juice samples (25 mL used as sample volume).

Grapefruit juice	$V_{titrant}$ [mL]	D-Limonene measured [mL/L]	Average Blank [mL]
Sample 1	1.1734	0.0408	0.1542
Sample 2	1.2393	0.0434	
Sample 3	1.2448	0.0436	
Sample 4	1.1037	0.0380	
Sample 5	1.1533	0.0400	
Average	1.1829	0.0412	
SD	0.0534	0.0021	
Rsd [%]	4.51	5.19	

Table 6: Results for lemon juice samples (25 mL used as sample volume).

Lemon juice	$V_{titrant}$ [mL]	D-Limonene measured [mL/L]	Average Blank [mL]
Sample 1	4.047	0.1558	0.1542
Sample 2	4.0555	0.1561	
Sample 3	3.9925	0.1536	
Sample 4	3.9502	0.1367	
Sample 5	3.5700	0.1519	
Average	3.9230	0.1508	
SD	0.1806	0.0072	
Rsd [%]	4.60	4.79	

5.2 Measurement of spiked aliquots

Aqueous samples were spiked with a specific amount of D-Limonene (total sample volume = 25 mL) and measured according to the previously mentioned protocol. Measurements were done with contents of 0.025, 0.1 and 0.3 mL of Limonene per Liter of sample. Concentrations of 0.05 mL/L, 0.2 mL/L, 0.4 mL/L and 0.5 mL/L can be found in the appendix.

Table 7: Results for spiked aqueous samples (25 mL used as sample volume).

Measurement	D-Limonene [mL/L]	$V_{titrant}$ [mL]	D-Limonene measured [mL/L]	Average Blank [mL]	Recovery Purity Corrected [%]	Average Recovery	Relative Standard Deviation
Blank-1	-	0.2498	-	0.252		-	0.9 %
Blank-2	-	0.2531	-				
Blank-3	-	0.2561	-				
Blank-4	-	0.2511	-				
Blank-5	-	0.2503	-				
Ref-1	0.025	0.8444	0.025		99.35%	98.61 %	1.7 %
Ref-2	0.025	0.8244	0.024		96.07 %		
Ref-3	0.025	0.8503	0.025		100.32 %		
Ref-4	0.025	0.8308	0.024		97.12 %		
Ref-5	0.025	0.8495	0.025		100.18 %		
Ref-1	0.100	2.7584	0.103		102.65 %	102.22 %	1.2 %
Ref-2	0.100	2.7717	0.103		103.19 %		
Ref-3	0.100	2.7673	0.103		103.01 %		
Ref-4	0.100	2.6876	0.100		99.75 %		
Ref-5	0.100	2.7548	0.102		102.50 %		
Ref-1	0.300	7.7063	0.305		101.76 %	102.14 %	1.9 %
Ref-2	0.300	7.7199	0.306		101.95 %		
Ref-3	0.300	7.5217	0.298		99.24 %		
Ref-4	0.300	7.7512	0.307		102.37 %		
Ref-5	0.300	7.9717	0.316		105.38 %		

6. Comparison to Standard Methods

This application note is based on the standard methods IFU 45 and AOAC 968.20 with minor differences. These differences are shown in the following table.

Table 8: Differences to IFU 45 and AOAC 928.20

	Application note	IFU 45 / AOAC 968.20	Notes / Impact
Sample tube : Isopropyl alcohol volume	25 mL	25 mL	-
Receiving vessel: Volume of 12% HCl / methylorange solution	30 mL	10 mL	The volume needs to be higher than in the norm because otherwise the stirrer cannot reach the solution in the colorimetric receiving vessel (~ 70 mL total distillate volume) and the solution will not fully cover the optical electrode.

7. Conclusion

The determination of essential oils in juice products using the BasicDist with Eco Titrator provides reliable and reproducible results. These results correspond well to the values of the spiked reference samples. The results of this automated method comply with the norms of IFU 45 and AOAC 968.20 with low relative standard deviations (rsd) of < 2% for the spiked aliquots.
Together with the Eco Titrator, the time to result is significantly reduced and it offers a time-saving, automated solution.

8. References

[1] IFU method 45
[2] Scott, W. C. and Veldhuis, M. K. 1966. Rapid estimation of recoverable oil in citrus juices by bromate titration. *J. of the A.O.A.C.*, *49*, 628–633.

Appendix:
Detailed results of spiked aliquots
Titer Determination

9. Appendix

9.1 Measurement of spiked aliquots

Table 9: Results for spiked aqueous samples (25 mL used as sample volume) as addition to Table 7.

Measurement	D-Limonene [mL/L]	$V_{titrant}$ [mL]	D-Limonene measured [mL/L]	Average Blank [mL]	Recovery Purity Corrected [%]	Average Recovery	Relative Standard Deviation
Ref-1	0.200	5.202	0.200		99.84 %		
Ref-2	0.200	5.1436	0.197		98.65 %		
Ref-3	0.200	5.3003	0.204		101.82 %	100.13 %	1.2 %
Ref-4	0.200	5.2687	0.202		101.18 %		
Ref-5	0.200	5.1678	0.198		99.14 %		
Ref-1	0.400	10.0518	0.399		99.86 %		
Ref-2	0.400	10.0000	0.395		98.69 %		
Ref-3	0.400	10.1136	0.402		100.49 %	100.44 %	1.1 %
Ref-4	0.400	10.1920	0.405		101.29 %		
Ref-5	0.400	10.2511	0.407		101.89 %		
Ref-1	0.500	12.0300	0.480		96.03 %		
Ref-2	0.500	12.4545	0.497		99.50 %		
Ref-3	0.500	12.2224	0.488		97.60 %	97.51 %	1.3 %
Ref-4	0.500	12.2960	0.491		98.20 %		
Ref-5	0.500	12.0554	0.481		96.24 %		

9.2 Titer Determination

The titer can be determined according to the following protocol (AOAC 943.17):

1. Dry As_2O_3 at 105°C for 1 h
2. Weigh in 2.475g As_2O_3 precisely in a beaker (250ml)
3. Add 25 mL of 1N NaOH and dissolve in boiling waterbath
4. Add carefully 25 mL of 1N H_2SO_4 and let it cool down
5. Transfer quantitatively into a 500 mL volumetric flask, wait for temperature equilibration and fill it up. The solution should be neutral.
6. Pipet 5 mL / 20 mL of As_2O_3 solution in a 300 mL Erlenmeyer flask
   (5 mL for titer determination of titrant solution / 20 mL for the titer determination of the stock solution)
7. Add 10 mL of HCl / Methyl orange solution (4.1) and titrate titrant solution / stock solution from purple to colorless to determine the titer.

Normality stock solution (S1) = $\frac{\text{V As2O3[ml]} * \text{Normality As2O3}}{\text{V S1 [ml]}}$ ($=\frac{20\text{ml} * 0.1003}{20.5\text{ml}} = 0.0978$)

Normality titrant solution (S2) = $\frac{\text{V As2O3[ml]} * \text{Normality As2O3} * 4}{\text{V S2 [ml]}}$ ($=\frac{5\text{ml} * 0.1003 * 4}{20.5\text{ml}} = 0.0978$)

Expected value = 0.099

Titer= Expected value / Determined value (= 0.099 / 0.0978 = 1.012)

The Titer should be in the limits of ±0.03.