ALCOHOL IN BEVERAGES AND INTERMEDIATES

Principle
The actual content of alcohol is determined with the help of the density of the distillate assuming the same amount of volume for the sample and distillate.
Using a water steam distillation alcohol (ethanol) is distilled from the matrix, which has to be examined.
The density can be determined using the pycnometer method, the araeometer method (spindle), or the oscillating U-tube method.

This application is divided into the following chapters acc. to the sample matrices to be examined:
1. Beer
2. Wine
3. Sparkling wines
4. Liqueurs
5. Essences and denaturates
6. Spirit
7. Production cooler sludge, wine cooler sludge, fruit mash, red wine mash

The procedure is the same for all distillations. However, there are different methods for the sample preparation or the parameters of the distillation.

Area of usage

Type of Sample	Alcohol Content [%vol]	Amount of Sample	Amount of Distillate
Beer	3 - 10	50 ml	100 ml
Wine	8 - 15	50 ml	50 ml
Sparkling wine	9 - 14	50 ml	50 ml
Liqueurs	15 - 25	50 ml	100 ml
Spirits	Up to 40	50 ml	100 ml
Apple/Fruit mash	Up to 10	50 g	50 ml or g
Wine cooler sludge		100 g	100 ml or g
Production cooler sludge		100 g	100 ml or g
Essences	Up to 60	50 g	100 ml
Acetators	Up to 3	50 g	50 ml
Denaturants	Up to 60	50 g	100 ml

1. BEER

1.1. Chemicals
Silicone antifoam
Aquadest

1.2. Instruments
VAPODEST 10s – 45s, modified for the alcohol determination
Kjeldahl flask with wide neck opening 500 or 750 ml, cat. no. 6465 or cat. no. 6467
Pycnometer, oscillating U-tube, spindle.
Folded filter and funnel
Erlenmeyer flask 500 ml
Glass beaker 800 ml
Volumetric flask 100 ml
Thermostat

1.3. Procedure

1.3.1. Sample Preparation

In order to decarbonise the sample it is filtered using a folded filter. Putting the sample in an ultrasound bath afterwards can support this process. The thus defoamed sample is then put into a 100 ml volumetric flask and brought to 20 °C in a water bath.
After the thermo stability has been achieved the sample is put into the distillation flask for the VAPODEST while thoroughly rinsing the sample with distilled water.
The following program settings are recommended for the various VAPODEST models. Please note however, that these can only be regarded as guidelines for the use of C. Gerhardt equipment.
Users may have to adapt this method to meet their own analytical requirements.

1.3.2. Distillation and Programming of the VAPODEST

	VAP 10s	VAP 20s	VAP 30s	VAP 45s
H_2O Addition	-	-	0 ml	0 ml
NaOH Addition	0 ml	0 ml	0 ml	0 ml
Reaction Time	0 s	0 s	0 s	0 s
Distillation Time	160 s	160 s	160 s	160 s
Steam Power	90 %	90 %	90 %	90 %
Suction Sample	manual	manual	30 s	30 s

Use the VAPODEST following the instruction manual. As a start run a blank distillation in order to heat up and clean the instrument. Check whether all chemicals are present in the required quantities!

After 3 drops of silicon anti foam have been added the distillation flask with the sample is put into the VAPODEST. The 100 ml volumetric flask containing 20 ml Aquadest is used as a receiver. The outlet tubing of the distillate has to be immerged in the Aquadest. Then the program is started and the distillation is run. The sample is distilled until the calibration mark on the volumetric flask is nearly reached.

The first distillation should be watched closely to optimize the amount of distillate and the distillation time. If needed, the parameters chosen for the VAPODEST can be corrected. After the distillation is terminated, the receiver is taken out and the outlet tubing is rinsed with distilled water.

1.4. Evaluation

The measuring flask containing the distillate is tempered and filled with distilled water up to the calibration mark. After intensive mixing, the relative density 20 °C / 20 °C is detected using an oscillating U-tube. When over distilling into a pycnometer, the density is detected using a weighing comparison. The alcohol content is then determined using the alcohol table.

The following pages 3 and 4 give a sample of a result and the comparison to the traditional method.

Sample of a result / Comparison to traditional method: Alcohol standard

Table 1: Expected standard deviation

Traditional distillation + Pycnometer [%vol]	VAPODEST + Pycnometer [%vol]	Difference
0,94	0,93	0,01
1,04	1,04	0,00
3,24	3,24	0,00
3,05	3,04	0,01
3,21	3,21	0,00
3,58	3,59	0,01
3,19	3,21	0,02
3,64	3,67	0,03
3,62	3,63	0,01
4,23	4,21	0,02
4,27	4,28	0,01
4,13	4,14	0,01
4,18	4,14	0,04
4,83	4,87	0,04
4,76	4,80	0,04
4,71	4,80	0,09
5,61	5,70	0,09
5,40	5,40	0,00
9,31	9,31	0,00
9,06	9,15	0,09
10,93	11,00	0,07
4,93	5,02	0,09
3,49	3,43	0,06
5,68	5,72	0,04

Mean value traditional distillation:	4,64
Mean value VAPODEST distillation:	4,63
Bias (methodic deviation of the result):	0,017916
SEP	0,0467
SEP (probability 95 %)	0,0935
Number of samples	24

Sample of a result /Comparison to traditional method:

Table 2: Precision - repeatability

Sample with average alcohol content:

[%vol]
3,51
3,51
3,53
3,48
3,51
3,51
3,53
3,51
3,51
3,48

Repeatability (probability 95 %):	0,047
Average	3,508
Range:	0,050
No. of samples:	10

Sample with high alcohol content:

[%vol]
8,61
8,67
8,70
8,64
8,63
8,63
8,66
8,63
8,59
8,67

Repeatability (probability 95 %):	0,0905
Average :	8,64
Range:	0,11
No. of samples:	10

2.WINE

2.1. Chemicals

Silicone antifoam
Aquadest

2.2. Instruments

VAPODEST 10s – 45s, modified for alcohol determination
Kjeldahl flask with wide neck opening 500 or 750 ml cat. no. 6465 or cat. no. 6467
Pycnometer, oscillating U-tube, spindle
Volumetric flask 100 ml
Thermostat

2.3. Procedure

After the thermo stability has been achieved the sample is put into the distillation flask for the VAPODEST while thoroughly rinsing the sample with distilled water.
The following program settings are recommended for the various VAPODEST models. Please note however, that these can only be regarded as guidelines for the use of C. Gerhardt equipment.
Users may have to adapt this method to suit their own analytical requirements.

Distillation and Programming of the VAPODEST:

	VAP 10s	VAP 20s	VAP 30s	VAP 45s
H_2O Addition	-	-	0 ml	0 ml
NaOH Addition	0 ml	0 ml	0 ml	0 ml
Reaction Time	0 s	0 s	0 s	0 s
Distillation Time	~120 s	~120 s	~120 s	~120 s
Steam Power	~95 %	~95 %	~95 %	~95 %
Suction Sample	manual	manual	30 s	30 s

Use the VAPODEST following the instruction manual. As a start run a blank distillation in order to heat up and clean the instrument. Check whether all chemicals are present in the required quantities!

The distillation flask with the sample is put into the VAPODEST after 3 drops of silicon anti foam have been added.
The 100 ml volumetric flask containing approx. 20 ml Aquadest is used as a receiver. The outlet tubing has to be immerged in the Aquadest. The program is started, the distillation is run. The sample is distilled until the calibration mark on the volumetric flask is nearly reached.
The first distillation should be watched closely to optimize the amount of distillate and the distillation time.
If needed, the parameters chosen for the VAPODEST can be corrected. After the distillation is terminated, the receiver is taken out and the outlet tubing is rinsed with distilled water.

2.4. Evaluation

The measuring flask containing the distillate is tempered and filled with distilled water up to the calibration mark. After intensive mixing, the relative density 20 °C / 20 °C is detected using an oscillating U-tube method. When over distilling into a pycnometer, the density is detected using a weighing comparison. The alcohol content is then determined using the alcohol table.

A sample of a result and comparison to the traditional method: see page 6

2.5. Comments

Samples known for their heavy foaming can be treated with a little bit of bentonite to avoid foaming.

Add. to 2. Wine: Sample of a result /Comparison to traditional method:

	Trad. distillating unit + density measurement [%vol]	VAPODEST + density measurement [%vol]	Difference	Mean deviation [%vol]	Deviation in percent [%]
Acetatores and compounds:	1,01	1,01	0	**0,021**	**1,3**
	1,35	1,28	0,07		
	1,76	1,69	0,07		
	3,17	3,17	0		
	1,82	1,76	0,06		
	0,54	0,61	0,07		
	1,01	1,01	0		
	2,31	2,24	0,07		
	1,69	1,69	0		
	1,01	1,01	0		
	1,82	1,82	0		
	1,48	1,48	0		
	1,35	1,35	0		
	2,18	2,18	0		
	1,82	1,82	0		
	2,24	2,24	0		
Cidre / fruit mash:	6,42	6,46	0,04	**0,039**	**0,6**
	6,27	6,12	0,15		
	6,27	6,27	0		
	6,34	6,34	0		
	6,42	6,34	0,08		
	6,34	6,34	0		
	6,34	6,34	0		
Red wine / white wine / wine mash:	11,05	11,1	0,05	**0,021**	**0,2**
	11,05	11,02	0,03		
	10,61	10,61	0		
	10,95	10,87	0,08		
	10,55	10,55	0		
	11,38	11,38	0		
	10,61	10,52	0,09		
	11,05	11,05	0		
	11,38	11,38	0		
	10,87	10,87	0		
	10,61	10,61	0		
	11,05	11,05	0		

3. SPARKLING WINES
Sparkling wines are treated the same way as regular wine. However, they have to be decarbonized prior to the analysis.

4. LIQUEURS
The special advantage of the water steam distillation used for those samples compared to the traditional method is the stirring effect of the incoming water steam. The sample does not burn and as an additional benefit is stirred well. The settings for the program depend very much on the content that is expected. It is recommended to use a steam output of 100 %. The distillation time and amount of sample have to be set on a trial basis. Samples with excessive foaming should be decarbonized or mixed with antifoam prior to the analysis.

Enclosed you find an example on how to test the linearity of the method.

5. ESSENCES and DENATURANTS
Depending on the structure of the essences 4 different approaches have to be distinguished:

5.1. Method 1: Sample consisting of distillate and alcohol
Up to 50 vol% 100 g weighted in quantity and 20 g water
More than 50 vol% 50 g weighted in quantity and 70 g water

5.1.1. Instruments
VAPODEST 10s – 45s, modified for alcohol determination
Kjeldahl flask with wide neck opening 500 or 750 ml cat. no. 6465 or cat. no. 6467
oscillating U-tube, spindle
Volumetric flask
Thermostat

5.1.2. Procedure
Prepare the samples according to standard procedure and put them into the distillation flask.
The following program settings are recommended for the various VAPODEST models.
Please note however, that they can only be regarded as guidelines for the use of C. Gerhardt equipment.
Users may have to adapt this method to suit their own analytical requirements.

Distillation and Programming of the VAPODEST:

	VAP 10s	VAP 20s	VAP 30s	VAP 45s
H_2O Addition	-	-	0 ml	0 ml
NaOH Addition	0 ml	0 ml	0 ml	0 ml
Reaction Time	0 s	0 s	0 s	0 s
Distillation Time	~240 – 300 s	~240 – 300 s	~240 – 300 s	~240 – 300 s
Steam Power	100 %	100 %	100 %	100 %
Suction Sample	manual	manual	30 s	30 s

Use the VAPODEST following the instruction manual. As a start run a blank distillation in order to heat up and clean the instrument. Check whether all chemicals are present in the required quantities!

The distillation flask with the sample is put into the VAPODEST. A 200 ml volumetric flask filled with about 30 ml distilled water is put under the outlet tubing. The outlet tubing has to be immerged in the water.
Then the program is started and the distillation is run. The sample is distilled until the calibration mark on the volumetric flask is nearly reached.
The first distillation should be watched closely to optimize the amount of distillate and the distillation time. If needed, the parameters chosen for the VAPODEST can be corrected. After the distillation is terminated, the receiver is taken out and the outlet tubing is rinsed with distilled water.

5.1.3. Evaluation
The volumetric flask filled with distillate is moderated and distilled water is added to reach the desired volume. Now, the measurement of the density can be done by using various methods.

5.2. Method 2: Samples consisting of distillate, alcohol and extract
Follow procedures for method 1

5.3. Method 3: Samples consisting of distillate, alcohol and essential oil
5.3.1. Chemicals
Silicone anti foam
Aquadest
Common salt
Ether

5.3.2. Instruments
VAPODEST 10s – 45s, modified for alcohol distillation
Kjeldahl flask with wide neck opening 500 or 750 ml cat. no.6465 or cat. no.6467
oscillating U-tube, spindle
Volumetric flask
Separating funnel 250 ml
Thermostat

5.3.3. Procedure
The samples are weighted in a separating funnel; 50 g ether and 10 g salt are added, shaken out and left to stand over night in a closed container. The next day, the ether phase is separated from the water phase and rejected. For the further procedure see Method 1.

Up to 50 vol% 100 g weighted in quantity and 20 g water
More than 50 vol% 50 g weighted in quantity and 20 g water

5.4. Method 4: Samples consisting of distillate, alcohol, extract and essential oil
This kind of structure requires the distillation into a separating funnel, which contains 50 ml ether and 40 g of a 4 % salt solution. Then continue following method 3.

6. SPIRIT
The distillation of spirits is similar to method 1 used for essences. The amount of distillate used is vital for the accuracy. It is recommended to work with a bigger amount of sample than in the wine analysis and thus, a bigger amount of distillate in order to obtain an adequate accuracy. 200 ml sample measured in a volumetric flask have been proven successful in such a case.

7. PRODUCTION COOLER SLUDGE, WINE COOLER SLUDGE, FRUIT MASH, RED WINE MASH
The special advantage of the water steam distillation used for those samples compared to the traditional method is the stirring effect of the incoming water steam. The sample does not burn and as an additional benefit is stirred well. The settings for the program depend very much on the content that is expected. It is recommended to use a steam output of 100 %. The distillation time and amount of sample have to be set on a trial basis. Samples with excessive foaming should be decarbonized or mixed with antifoam prior to the analysis.

APPENDIX

VERIFICATION OF THE LINEARITY

Linearity is the capability of a method to present results within a given concentration range which are directly proportional to the concentrations of the analytes.
In order to verify the linearity of method 3[*)], ethanolic solutions of various concentrations have been made and distilled. A total of 6 concentrations have been selected, all in the range of about 5 %vol to about 50 %vol according to the samples to be examined. Each of these solutions was used seven times for the '50 ml to 100 ml' distillation: 50 ml solution, measured with the help of a pycnometer, is distilled over into a 100 ml measuring flask; the alcohol content is then measured using a flexural resonator after tempering to 20 °C. Table 5.6. shows the results.

Table 5.6. Verification of the Linearity - Measured Values

	Point 1	Point 2	Point 3	Point 4	Point 5	Point 6
Alcohol content, %vol	5,08	13,35	19,76	29,66	39,91	49,73
Single values %vol	5,09	13,24	19,69	29,63	39,85	49,63
	5,06	13,30	19,76	29,66	39,94	49,52
	5,10	13,33	19,75	29,67	39,88	49,62
	5,12	13,34	19,76	29,65	39,87	49,56
	5,08	13,32	19,74	29,62	39,83	49,84*
	5,10	13,39	19,83	29,75*	40,06*	49,54
	5,10	13,32	19,77	29,65	39,84	
Mean value %vol	5,09	13,32	19,76	29,66	39,90	49,62
Standard deviation %vol	0,02	0,04	0,04	0,04	0,08	0,11
Variation coefficient R, %	0,39	0,30	0,20	0,13	0,20	0,22
Recovery %	100,20	99,78	100,00	100,00	99,98	99,78
* Values identified as outliers						

The single values which are then obtained for the determination of any alcoholic solution have been analyzed using the statistic program 'Ebel' (also see enclosures).
'Ebel' contains statistical tests, like e.g. the analysis of the mean value, the median, the standard deviation, the variation coefficient, a test of the normal distribution (test acc. to David, test acc. to Kolmogoroff-Smirnov), the test of a trend (test acc. to Grubbs, test acc. to Dixon), as well as the determination of the regression of the straight line of the entire measurement. Some values were identified as outliers (are marked with an asterisk in the table). These values should be eliminated for a correct analysis.

In order to set up the calibration line, the 'true' alcohol contents (prior to the distillation) are plotted against the alcohol contents measured after the distillation and with taking the dilution factor 2 into consideration as well. The illustration below shows the calibration line of measuring points and the corresponding regression line.

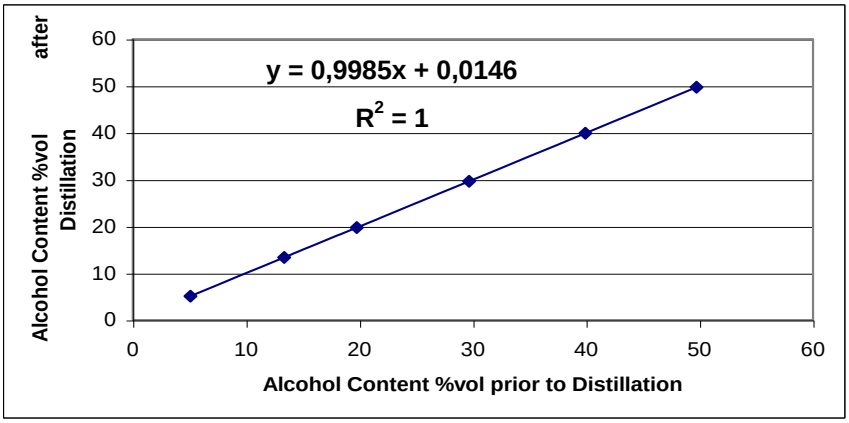

Illustration 5.1. Calibration line of the linearity verification

A line is described by the equation y=ax+b. In the procedure described above, the coefficients should be ideally a=1 and b=0. The deviations from the ideal case coefficient are [0.0015] for a and [0.0146] for b. The values obtained make it possible to say, that there is linearity in the verified range.

[*)]Method 3 – Water steam distillation: Distill 50 ml on 100 ml (dilution factor 2) at 85 % steam pressure for 250 s