Method OIV-MA-AS311-06 Type IV method

Determination of polyols derived from sugars and residual sugars found in dry wines by means of gas chromatography

(Resolution Oeno 9/2006)

1. Scope

Simultaneous determination of the erythritol, arabitol, mannitol, sorbitol and meso-inositol content of wines.
Because the determination of sugars by gas chromatography (GC) is long and complicated, it is reserved for the determination of traces of sugars and, especially, of sugars for which no other routine enzyme method exists – (Arabinose, Rhamnose, Mannose and Galactose) although it is also applicable to glucose and fructose, the advantage being that it is possible to simultaneously determine all sugar monomers, dimers and even trimers.

Comment 1 - It is not possible to determine sugars once they have been reduced to alditol form because of the presence of corresponding polyols.
Comment 2 - In the form of trimethylsilylated derivatives (TMS), sugars give 2 α and β forms and occasionally 3 or 4 (Gamma…) corresponding to the different anomers present in wines.
Comment 3 - Without prior dilution, it is difficult to determine glucose and fructose content using this method when it exceeds 5 g/l.

2. Principle

Residual sugars in dry wines can be determined by gas chromatography after the formation of their trimethylsilylated derivatives.

The internal standard is pentaerythritol.

3. Reagents

Silane mixture for example purposes:
3.1 Pure hexamethyldesilazane (HMDS)
3.2 Pure trifluoroacetic anhydride (TFA)
3.3 Pure pyridine

3.4 Pure pentaerythritol
3.5. Distilled water
3.6 10 g/l pentaerythritol (internal standard solution): dissolve 0.15 g of pentaerythritol (3.4) in 100 ml of water (3.5)
3.7 Pure products that may be used to prepare control solutions, notably glucose, fructose, arabinose, mannitol and sorbitol (non-exhaustive list)
3.8 Control solutions of pure products at 200 mg/l: dissolve 20 mg of each of the products to be determined (3.6) in 100 ml of water.
Comment – Sugar solutions should be prepared immediately prior to use.

4. Apparatus and Equipment

4.1 1-ml pipettes, with $1/10^{th}$ ml graduations
4.2 Propipette™ bulbs
4.3 100-µl syringe
4.4 5-ml tubes with screw stoppers fitted with a Teflon-coated sealing cap.
4.5 Rotary vacuum evaporator capable of housing screw-cap test tubes (4.4) in order to evaporate samples to dryness
4.6 Gas chromatograph fitted with a flame ionisation detector x g, and an injector operating in "split" mode - $1/30^{th}$ to $1/50^{th}$ division of the injected volume (1 µl)
4.7 Non-polar capillary column (SE-30, CPSil-5, HP-1, etc.) 50 m x 0.25 mm, 15 mµ stationary phase film thickness (as an example).
4.8 10-µl injection syringe
4.9 Data acquisition system
4.10 Ultra-sonic bath
4.11 Laboratory fume cupboard

5. Preparation of samples

5.1 Addition of the internal standard: 1 ml of wine (pipette, 4.1) or of 200 mg/l control solution (3.6) is placed in the screw-cap test tube (4.4)
Note: It is possible to operate with lower volumes of wine especially in high content sugar environments.

50 µl of the 10 g/l pentaerythritol solution (3.5) is added by means of the syringe (4.3)

5.2 Obtaining dry solid matter:

The screw-cap test tube is placed on the rotary evaporator, with a water bath kept below 40°C. Evaporation continues until all traces of liquid have disappeared.

5.3 Addition of reagents

5.3.1 Place the tubes containing the dry solid matter and reagents 3.1, 3.2 and 3.3 in the fume cupboard (4.11) and switch on the ventilation.

5.3.2 Using the pipettes (4.1) and Propipette™ bulbs (4.2), add 0.20 ml of pyridine (3.3), 0.7 ml of HMDS (3.1) and 0.1 ml of TFA (3.2) to the test tube one after the other.

5.3.3 Seal the test tube with its stopper.

5.3.4 Put the test tube in the ultra-sonic bath (4.10) for 5 minutes until the dry solid matter has completely dispersed.

5.3.5 Place the test tube in a laboratory kiln at 60°C for two hours in order to obtain the total substitution of the hydroxyl or acid hydrogen by the trimethylsilyl groups (TMS).

Comment: a single phase only should remain after heating (if not, water would be left in the test tube). Likewise, there should be no brownish deposit, which would indicate an excess of non-derived sugar.

6 Chromatographic assay

6.1 Place the cooled test tube in the ventilated fume cupboard (4.11), remove 1 µl with the syringe (4.8) and inject into the chromatograph in "split" mode (permanent split).

Treat the wine-derived and control sample in the same way.

6.2 Programme the kiln temperature, for example from 60°C to 240°C at a rate of 3°C per minute, such that the complete assay lasts, for example, one hour for complete mannitol and sorbitol separation (resolution higher than 1.5).

7. Calculations

Example: calculation of sorbitol concentration

If

s	= the peak area of the sorbitol in the wine
S	= the peak area of the sorbitol in the control solution
i	= the peak area of the internal standard in the wine
I	= the peak area of the internal standard in the control solution

The sorbitol content of the wine (ts) will be

$$ts = 200 \times \frac{s}{S} \times \frac{I}{i} \quad \text{in mg per litre}$$

The same logic makes it possible to calculate the glucose content (tg)

$$tg = 200 \times \frac{g}{G} \times \frac{I}{i} \quad \text{in mg per litre}$$

when g is the sum of the areas of the two peaks of glucose in the wine and G is the sum of the areas of the two peaks of glucose in the control solution.

8. Characteristics of the method

Detection threshold approximately 5 mg/l for a polyol (a single chromatographic peak). Average repeatability in the region of 10% for a sugar or polyol concentration in the region of 100 mg/l.

Table 1 Repeatability of the determination of a number of substances found in the dry solid matter of wine after TMS derivatization.

	Tartaric acid	Fructose	Glucose	Mannitol	Sorbitol	Dulcitol	Meso-inositol
Average (mg/l)	2013	1238	255	164	58	31	456
Typical variance(mg/l)	184	118	27	8	2	2	28
CV (%)	9	10	11	5	3	8	6

REFERENCES

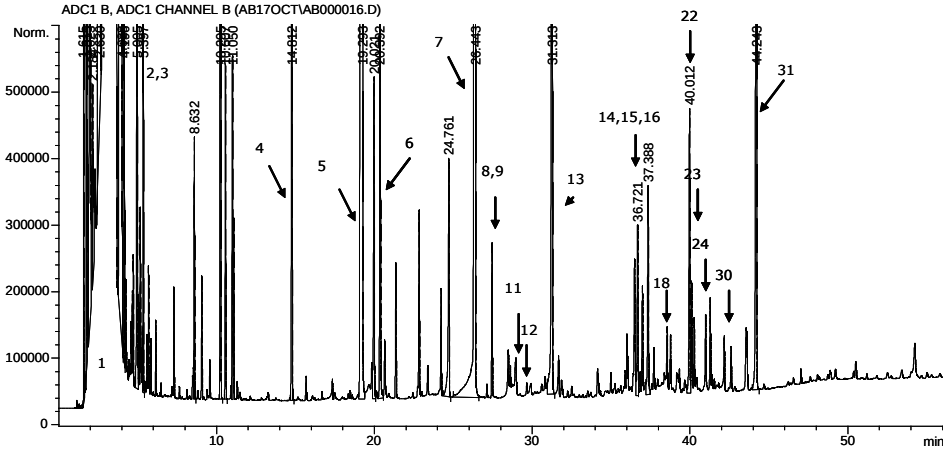
RIBEREAU-GAYON P. and BERTRAND A. 1972, Nouvelles applications de la chromatographie en phase gazeuse à l'analyse des vins *et* au contrôle de leur qualité, Vitis, 10, 318-322.

BERTRAND A**.** (1974), Dosage des principaux acides du vin par chromatographie en phase gazeuse. FV OIV 717—718, 253—274.

DUBERNET M.0. (1974), Application de la chromatographie en phase gazeuse à l'étude des sucres et polyols du vin: thèse 3° Cycle, Bordeaux.

Figure 1

Chromatogram of a white wine following silylation. CPSil-5CB 50 m x 0.25 mm x 0.15 µm column. Split injection, 60°C, 3°C/min, 240°C. Magnification below.



Identification of peaks: 1 : reactive mixture; 2 and 3: unknown acids; 4: pentaerythriol; 5 and 6: unknown; 7: tartaric acid and arabinose; 8, 10 and 11: rhamnose; 9: arabinose; 12: xylitol; 13: arabitol; 14, 15 and 16: fructose; 17: galactose and unknown; 18: glucose α; 19: galactose and galacturonic acid; 20 and 21: unknown; 22: mannitol; 23: sorbitol; 24: glucose β; 25 and 27: unknown; 26: galacturonic acid; 28 and 30: galactonolactone; 29: mucic acid; 31: meso-inositol.

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