OIV-MA-AS313-16 Determination of organic acids and mineral anions in wines by ionic chromatography

Type IV method

Preamble

The development of high performance ionic chromatography in laboratories has enabled the study the determination of organic acids and mineral anions in alcoholic and non alcoholic beverages by this technique.

Particularly concerning the analysis of wines, the results of intercomparison test trials and the measurements of recovery rates have enabled us to validate an analytical methodology.

The major interest of this method is that the ion exchange columns allow the separation of most organic acids and anions, and the detection by conductimetry frees the analysis from interferences due to the presence of phenolic compounds. This type of interference is very notable in chromatographic methods that include detection in ultra-violet radiation at 210 nm.

1. Object and field of application

This method for mineral anions and organic acids by ionic chromatography is applicable to alcoholic beverages (wines, wine spirits and liqueurs). It enables the determination of organic acids in the ranges of concentration listed in table 1; these concentrations are obtained by diluting samples.

Table 1: range of concentration of anions for their analysis by ionic chromatography

Sulfate: 0.1 to 10 mg/l

Ortho-phosphate: 0.2 to 10 mg/l

Malic acid: 1 to 20 mg/l

Tartaric acid: 1 to 20 mg/l

Citric acid: 1 to 20 mg/l

Isocitric acid: 0.5 to 5 mg/l

The ranges of the above-mentioned work are given as an example. They include the methods of calibration commonly practiced and are therefore adaptable according to the type of apparatus used (nature of column, sensitivity of the detector, etc.) and procedure (volume of sample injected, dilution, etc.).

2. Principle

Separation of mineral and organic anions on an ion exchanger resin.

Detection by conductimetry.

Identification after the retention time and quantification using the calibration curve.

3. Reagents

All the reagents used during the analysis must be of analytical quality. The water used for the preparation of solutions must be distilled or deionised water of a conductivity lower than 0.06 µS, free from anions determined at thresholds compatible with the detection limits of the apparatus used.

3.1. Eluant

The composition of the eluant depends on the nature of the separation column and the nature of the sample to be analysed. Nevertheless it is always prepared using aqueous solutions of sodium hydroxide.

The performances of the chromatographic analysis are alternated by carbonation of the sodium hydroxide solution; consequently, the mobile phase flasks are swept with helium before adding sodium hydroxide and all precautions should be taken in order to avoid contaminating them with room air.

Lastly, commercial concentrated sodium hydroxide solutions will be used.

Remark

The table in chapter 9 mentions the main interferents susceptible of being present in the samples.

It is therefore necessary to know beforehand if they coelute with the ions to be determined and if they are present at such a concentration that the analysis is disrupted.

Fermented drinks contain succinic acid which can interfere with the malic acid determination. To this effect, it is necessary to add methanol to the eluant in order to improve the resolution of the column for these two substances (20% of methanol).

3.2. Calibration reference solutions

Prepare calibration reference solutions of precise concentrations close to those indicated in the following table. Dissolve in water, quantities of salts or corresponding acids in 1000 ml volumetric flasks. (Table 2)

Table 2: Concentration of anions determined in calibration reference solutions

Anions and acids	Compounds weighed	Concentration final (mg/l)	Quantity weighed (mg)
Sulphate	Na2SO4	500	739.5

Orthophosphate	KH2PO4	700	1003.1
Malic acid	Malic acid	1000	1000.0
Tartaric acid	Tartaric acid	1000	1000.0
Citric acid	Citric acid, H_2O	1000	1093.8
Isocitric acid	Isocitrate 3Na, $2H_2O$	400	612.4

Remark

The laboratory must take the necessary precautions regarding the hygroscopic character of certain salts.

3.3. Calibration solutions

The calibration solutions are obtained by diluting the reference solutions of each ion or acid in water.

These solutions should contain all the ions or acids determined in a range of concentrations covering those corresponding to the samples to be analysed. They must be prepared the day of their use.

At least two calibration solutions and a blank must be analysed so as to establish, for each substance, the calibration curves using three points (0, maximum semi-concentration, maximum concentration).

Remark

Table 1 gives indications on the maximum concentrations of anions and acids in calibration solutions but the performances of the chromatographic columns are better with very diluted solutions.

So the best adequation possible between the performances of the column and the level of dilution of the samples should be looked for.

In general, the sample is diluted between 50 and 200 times maximum except for particular cases.

For prolonging the life span of the dilution solutions, it is preferable to prepare them in a water/methanol solution (80/20).

4. Apparatus

4.1. Instrument system for ionic chromatography including:

4.1.1. Eluant reservoir(s)

4.1.2. Constant-stroke pump, without pulsing action

4.1.3. Injector, either manual or automatic with a loop sampling valve (for example 25 or 50 µl).

4.1.4. Separation columns

System made up of an anion exchanger column of controlled performance, possibly a precolumn of the same type as the main column. For example, it is possible to use the AS11 columns and DIONEX AG11 precolumn.

4.1.5. Detection system

Circulation conductivity cell of very low volume connected to a conductivity meter with several ranges of sensitivity.

In order to lower the conductivity of the eluant, a chemical suppression mechanism, a cation exchanger is installed in front of the conductivity cell.

4.1.6. Recorder, integrator or other device for the treatment of signals.

4.2. Precise balance to 1 mg

4.3. Volumetric flasks from 10 to 1000 ml

4.4. Calibrated pipettes from 1 to 50 ml

4.5. Filtrating membranes with an average pore diameter of 0.45 µm.

5. Sampling

The samples are diluted while taking into account the mineral anions and organic acids that are to be determined.

If their concentration is very variable in the sample, two levels of dilution will be necessary in order to respect the ranges of concentration covered by the calibration solutions.

6. Procedure

Turn on the apparatus by following the manufacturer's instructions.

Adjust the pumping (eluant flux) and detection conditions so as to obtain good separations of the peaks in the range of concentrations of ions to be analysed.

Allow the system to balance until a stable base line is obtained.

6.1. Calibration

Prepare the calibration solutions as indicated in 3.3.

Inject the calibration solutions so that the volume injected is at least 5 times that of

the sampling loop to allow the rinsing of the system.

Trace the calibration curves for each ion. These must normally be straight.

6.2. Blank trial

Inject the water used for the preparation of the calibration solutions and samples.

Control the absence of parasite peaks and quantify the mineral anions present (chloride, sulphate, etc.).

6.3. Analysis

Dilute the sample possibly at two different levels as indicated in 5, so that the anions and acids to be determined are present in the range of concentrations of the calibration solutions.

Filter the diluted sample on a filtrating membrane (4.5) before injection.

Then proceed as for the calibration (6.1).

7. Repetability, reproducibility

An interlaboratory circuit tested this method, but this does not constitute a formal validation according to The OIV protocol (Oeno 6/99).

A repeatability limit and a reproducibility limit for the determination of each ion in wine were calculated according to the ISO 5725 standard.

Each analysis was repeated 3 times.

Number of participating laboratories: 11; the results were as follows:

White wine

	No labs	Average (mg/l)	Repeatability (mg/l)	Reproducibility (mg/l)
Malic acid	11/11	2745	11à	559
Citric acid	9/11	124	13	37
Tartaric acid	10/11	2001	96	527
Sulphate	10/11	253	15	43
O.phosphate	9/11	57	5	18

Red wine

	No labs (mg/l)	Average	Repeatability (mg/l)	Reproducibility (mg/l)
Malic acid	8/11	128	16	99
Citric acid	8/10	117	8	44
Tartaric acid	9/11	2154	48	393
Sulphate	10/11	324	17	85
O.phosphate	10/11	269	38	46

8. Calculation of recovery rate

The supplemented sample is a white wine.

Determination	No labs	Concentration initial (mg/l)	Real addition (mg/l)	Measured addition (mg/l)	Recovery rate (%)
Citric acid	11/11	122	25.8	24.2	93.8
Malic acid	11/11	2746	600	577	96.2
Tartaric acid	11/11	2018	401	366	91.3

9. Risks of interferences

Any substance whose retention time coincides with that of one of the ions analysed can constitute an interference.

The most common interference include the following:

Anions	Or interferents acids
Nitrate	Bromide
Sulphate	Oxalate, maleate Ortophophatephtalate
Malic acid	Succinic acid, citramalic acid
Tartric acid	Malonic acid

Citric acid	-
Isocitric acid	-

Remark: The addition of methanol in the mobile phase can resolve certain analytical problems.