# OIV-MA-AS315-07B Examination of artificial sweeteners

**Type IV method**

## 1. Principle of the method

Examination of saccharine, Dulcin and cyclamate.

These sweeteners are extracted from wine using a liquid ion exchanger, then re-extracted with dilute ammonia hydroxide, and are separated by thin layer chromatography using a mixture of cellulose powder and polyamide powder (solvent: xylene; *n*-propanol; glacial acetic acid; formic acid). These sweeteners have a blue fluorescence on a yellow background under ultraviolet light after spraying with a 2,7-dichlorofluorescein solution.

Subsequent spraying with 1,4-dimethylaminobenzaldehyde solution allows differentiation of Dulcin, which gives only one orange spot, from vanillin and the esters of *p*hydroxybenzoic acid which migrate with the same Rf.

## 2. Method

Examination of saccharine, cyclamate and Dulcin.

2.1. Apparatus

2.1.1. Apparatus for expression by thin layer

2.1.2. Glass plate 20 x 20 cm

Preparation of the plates: mix thoroughly 9 g of dry cellulose powder and 6 g of polyamide powder. Add, while stirring, 60 mL methanol. Spread on the plates to a thickness of 0.25 mm. Dry for 10 minutes at 70°C. The quantities prepared are sufficient for the preparation of 5 plates.

2.1.3. Water bath with a temperature regulator or a rotary evaporator,

2.1.4. UV lamp for examination of the chromatography plates.

2.2. Reagents

2.2.1. Petroleum ether (40□60°)

2.2.2. Ion exchange resin, for example: Amberlite LA□2

2.2.3. Acetic acid diluted to 20% (*v/v*)

2.2.4. Ion exchange solution: 5 mL of ion exchanger is vigorously agitated with 95 mL petroleum ether and 20 mL of 20% acetic acid. Use the upper phase.

2.2.5. Nitric acid in solution, 1 M

2.2.6. Sulfuric acid, 10 % (*v/v*)

2.2.7. Ammonium hydroxide diluted to 25% (*v/v*)

2.2.8. Polyamide powder, for example: Macherey-Nagel or Merck

2.2.9. Cellulose powder, for example: Macherey-Nagel MN 300 AC

2.2.10. Solvent for chromatography:

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| Xylene | 45 parts |
| *n*-Propanol | 6 parts |
| Glacial acetic acid ($\rho_{20}$= 1.05 g/mL) | 7 parts |
| Formic acid 98□100% | 2 parts |

2.2.11. Developers:

- solution of 2,7-dichlorofluorescein, 0.2 % (*m/v*), in ethanol,
- solution of 1,4-dimethylaminobenzaldehyde: dissolve 1 g of dimethylamino-benzaldehyde placed in a 100 mL volumetric flask with about 50 mL ethanol. Add 10 mL of nitric acid, 25% (*v/v*), and bring to volume with ethanol.

12. Standard solution:

- solution of Dulcin, 0.1 % (*m/v*), in methanol,
- solution of saccharine at 0.1 g per 100 mL in a mixture of equal parts methanol and water,
- cyclamate solution: solution containing 1 g of the sodium or calcium salt of cyclohexylsulfamic acid in 100 mL of a mixture of equal parts methanol and water,
- solution of vanillin at 1 g /100 mL in a mixture of equal parts methanol and water,
- solution of the ester of *p*-hydroxybenzoic acid at 1 g /100 mL in methanol.

2.3 Procedure*:*

50 mL of wine is placed in a separatory funnel, acidified with 10 mL dilute sulfuric acid (2.2.6) and extracted with two aliquots of the ion exchange solution using 25 mL each time. The 50 mL of ion exchange solution is washed three times using 50 mL of distilled water each time, which is discarded, then three times with 15 mL of dilute ammonium hydroxide (2.2.7). The ammonia solutions recovered are then carefully

evaporated at 50°C until dry on a water bath or in a rotary evaporator. The residue is recovered with 5 mL of acetone and 2 drops 1 M nitric acid solution, filtered, and again evaporated dry at 70°C on a water bath. It is necessary to avoid heating for too long and above 70°C. The residue is recovered with 1 mL of methanol.

5 to 10 µL of this solution and 2 µL of the standard solutions are spotted on the plate. Let the solvent migrate (xylene: *n*-propanol: acetic acid: formic acid) (2.2.10) to a height of about 15 cm, which takes about 1 hour.

After air-drying, the dichlorofluorescein solution is thoroughly sprayed on the plate. The saccharine and the cyclamate appear immediately as light spots on a salmon colored background. Under examination in ultraviolet light (254 or 360 nm), the three sweeteners appear as a fluorescent blue on a yellow background.

The sweeteners separate, from the bottom to the top of the plate, in the following order: cyclamate, saccharine, Dulcin.

The vanillin and the esters of *p*-hydroxybenzoic acid migrate with the same Rf as the Dulcin. To identify Dulcin in the presence of these substances, the plate then must be sprayed with a solution of dimethylaminobenzaldehyde. The Dulcin appears as an orange spot, whereas the other substances do not react.

*Sensitivity* - The quantity limitation shown on the chromatography plate is 5 µg for the three substances.

This method permits detection of:

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| Saccharin | 10 mg/L |
| Cyclamate | 50 mg/L |
| Dulcin | 10 mg/L |

**BIBLIOGRAPHY**


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