CARE AND USE MANUAL

Welchrom HON SPE Cartridge Care and Use Manual

Welch

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Welch Materials

Welchrom HON Solid-Phase Extraction Cartridge Care and Use Manual

1.Scope

Applicable to the identification of oligosaccharide in honey in accordance with Chinese Pharmacopoeia 2015 Edition, Volume 1.

2. Principles

High-molecular-weight saccharides in the sample are enriched and concentrated by an activated carbon–diatomaceous earth column, then separated and detected by thin-layer chromatography (TLC). The presence of oligosaccharides in honey is determined by comparison with pure honey spiked with a known amount of maltopentaose.

3. Materials and Reagents

Standards: pure honey; maltopentaose.

Sample: adulterated honey.

Reagents: ethanol (≥99.7%); 1-butanol (≥99.7%); acetic acid (≥99.7%); diphenylamine (CAS: 537-67-7); acetone (≥99.7%); aniline; phosphoric acid (≥99.8%); ultrapure water.

TLC plates: Silica G plates, 200 mm × 200 mm, film thickness 0.25 mm. Prior to use, mark the plate as follows: leave 2 cm from the lower edge; the application width is 2.5 cm. From the lower edge measure up 3.5 cm and draw a line — this marks the Rf reference line. From the position 2 cm from the lower edge measure up 10 cm and draw a line — this is the development end line; any migration beyond this line is considered the end of development. After marking, activate the plates in an oven at 110 °C for 1 hour, then cool them in a desiccator to room temperature and set aside for use.

SPE cartridge: Welchrom HON SPE cartridge (Welch Materials), specification: 500 mg / 12 mL.

4. Procedure

4.1 SPE Pre-treatment Steps

Activation: pass 25 mL water through the cartridge at a flow rate of ~1 drop/second.

Sample loading: weigh 2.000 g of sample (precision to 1 mg) into a 50 mL beaker, dissolve in 10 mL water, then slowly apply the solution to the Welchrom HON cartridge.

Washing: wash with 25 mL of 7% ethanol in water.

Elution: elute with 10 mL of 50% ethanol in water and collect the eluate.

Concentration and reconstitution: evaporate the collected eluate to dryness under reduced pressure in a 65 °C water bath. Dissolve the residue in 1 mL of 30% ethanol to prepare the test solution. (Ensure the residue is completely redissolved during reconstitution.)

Preparation of the maltopentaose standard solution: dissolve the maltopentaose standard in 30% ethanol to a concentration of 1 mg/mL; use this as the standard solution.

Note:

1) A solid-phase extraction vacuum apparatus must be used throughout the cartridge pre-treatment to maintain sufficient vacuum so that liquid flows through the cartridge dropwise.

2) During rotary-evaporation concentration, use the smallest practicable pear flask (25 mL recommended) to minimize loss during reconstitution due to large contact area; if necessary, add reconstitution solvent in small portions multiple times.

3) When activating the cartridge with ultrapure water, begin applying the sample as soon as the liquid level has descended to the top of the cartridge packing; do not allow the cartridge to run dry during activation, as this will adversely affect retention of oligosaccharides.

4.2 Plate and Development Conditions

TLC plate: silica gel plate for thin-layer chromatography (coating thickness 0.20–0.25 mm).

Developer: n-propanol : water : triethylamine = 60 : 30 : 0.7 (v/v/v).

Visualization stain: weigh 1.0 g diphenylamine hydrochloride and place it into a beaker containing 50.0 mL acetone; then add 1 mL aniline and mix with stirring, then add 5 mL of 85% phosphoric acid. A white flocculent may appear; once fully dissolved the reagent is ready for use. Prepare this reagent fresh immediately before use and add reagents in the sequence given above.
Note: The order of addition for the stain reagent must be strictly followed and must not be altered.

4.3 Development Procedure

Using a 10 µL microsyringe, spot 3.0 µL of the test solution at the position 2 cm from the lower edge of the TLC plate. Prepare a separate plate and spot the same volume of processed pure honey and the maltopentaose standard solution as controls. Smaller, more concentrated spots yield better development. Place the plates in the development chamber (use a 250 mL wide-mouth jar as the development chamber and add 10 mL of the developer). After development (one run requires approximately 3.5 hours), dry the plate with a hair dryer, uniformly spray the visualization stain, dry again, then bake in an oven at 110 °C for 20–30 minutes (bake until color develops). Observe the developed TLC plate.

5. Results and Acceptance Criteria

Remove the TLC plate from the oven and observe the color development. Pure honey shows only 2–3 blue spots with grayish

or coffee-colored centers located in the region corresponding to Rf > 0.35. In contrast, maltopentaose or honey adulterated with maltopentaose exhibits blue, band-like spot groups beginning from the origin.

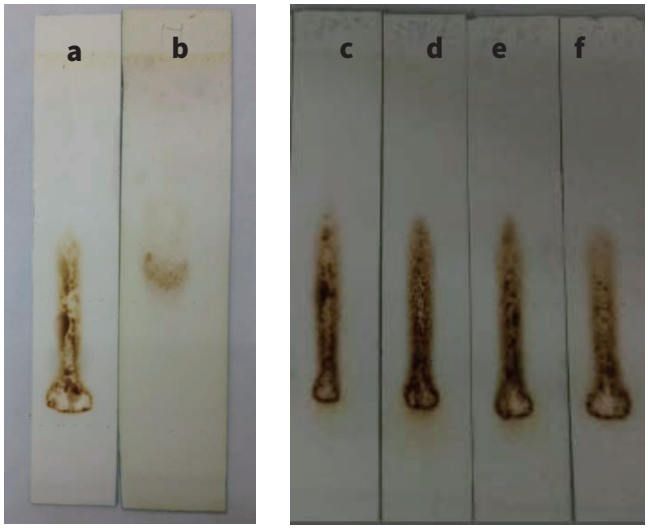


Figure 1. a: maltopentaose TLC; b: pure honey TLC; c–f: adulterated honey TLC.

For any inquiries, feel free to contact Welch Materials.

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