

# As sweet as? Detecting aspartame in a table-top sweetener

## Time

**Stage 1** Hydrolysis of CandereI®: 1–1.5 h (includes 0.5–1 h refluxing).

**Stage 2** Paper chromatography: 2.5 h (includes 1–2 h for running chromatogram).

## Curriculum links

Amino acids, peptide linkage. Paper chromatography.

## Group size

**Stage 1** Hydrolysis of CandereI®: If time is limited, one batch could be hydrolysed to provide material for all the groups to use in preparing chromatograms.

**Stage 2** Paper chromatography: 2–3.

## Materials and equipment

### Materials per group

**Stage 1** Hydrolysis of CandereI®

- 12 g CandereI® sweetener
- 200 cm<sup>3</sup> of 6 mol dm<sup>-3</sup> hydrochloric acid. (Irritant to the eyes and skin)

**Stage 2** Paper chromatography

- about 5 cm<sup>3</sup> of the solution produced in Stage 1 (acid hydrolysis of CandereI®)
- 100 mg activated charcoal
- about 50 cm<sup>3</sup> of solvent mixture for chromatography (ethanol:water:880 ammonia in the ratio 80:10:10) (880 (concentrated) ammonia is corrosive. It causes burns, is dangerous to eyes, causes severe internal damage if swallowed. Gas will be present and pressure can increase on hot days. It is dangerous for the environment. Ethanol solution is highly flammable)
- ninhydrin, 0.2% solution in propanone, stored in a spray bottle (Ninhydrin is also available from Merck in a spray can as a 0.5% solution in butanol) (Ninhydrin is harmful if swallowed and causes skin, eye and respiratory irritation. Skin contact produces a violet stain that may persist for several days: wear nitrile gloves when spraying ninhydrin)

Reference amino acids

- 1 cm<sup>3</sup> of a DL-aspartic acid 0.01 mol dm<sup>-3</sup> solution dissolved in 10% v/v propan-2-ol/water
- 1 cm<sup>3</sup> of a DL-phenylalanine 0.01 mol dm<sup>-3</sup> solution dissolved in 10% v/v propan-2-ol/water.

### Equipment per group

**Stage 1** Hydrolysis of CandereI®

- 500 cm<sup>3</sup> round-bottomed flask
- condenser
- Bunsen burner or heating mantle
- safety glasses.

**Stage 2** Paper chromatography

- pasteur pipette

- 5 cm<sup>3</sup> measuring cylinder
- test-tubes
- small funnel and filter paper
- chromatography tank or 1 dm<sup>3</sup> beaker and cling film to cover
- chromatography paper (Whatman No. 1)
- 25 or 50 cm<sup>3</sup> measuring cylinder depending on size of tank
- clips for paper, pencil

Access to:

- fume cupboard
- oven at 110 °C
- spray bottle containing 0.2% ninhydrin solution in propanone.

## Safety

Eye protection (splash proof goggles to BS EN 166 3) must be worn.

880 (concentrated) ammonia is corrosive. It causes burns, is dangerous to eyes, causes severe internal damage if swallowed. Gas will be present and pressure can increase on hot days. It is dangerous for the environment.

Ethanol solution is highly flammable

Ninhydrin is harmful if swallowed and causes skin, eye and respiratory irritation. Skin contact produces a violet stain that may persist for several days: wear nitrile gloves when spraying ninhydrin. The ninhydrin spray should be used only in a fume cupboard. The chromatogram must be hung up inside the fume cupboard to be sprayed.

**Disposal:** any remaining chromatography solvent (if not being kept for further use) should be neutralised with weak acid before being washed to waste.

## Risk assessment

It is the responsibility of the teacher to carry out a suitable risk assessment.

This is an open-ended problem solving activity, so the guidance given here is necessarily incomplete. Teachers need to be particularly vigilant, and a higher degree of supervision is needed than in activities which have more closed outcomes. Students must be encouraged to take a responsible attitude towards safety, both their own and that of others. In planning an activity students should always include safety as a factor to be considered. Plans should be checked by the teacher before implementing them.

You must always comply with your employer's procedures and in some cases may decide that a particular activity is inappropriate in your situation. Further information on Health and Safety should be obtained from reputable sources such as CLEAPSS [<http://science.cleapss.org.uk/>] in England, Wales and Northern Ireland and, in Scotland, SSERC [<https://www.sserc.org.uk/>].

## Commentary

The idea of applying the techniques of chromatography to the analysis of Candere<sup>®</sup> is based on an experiment described by A. D. Heaton.<sup>1</sup> If the students follow the approach suggested below they should obtain clear results as only two amino acids are involved.

### Aspartame

The sweet taste of aspartame was discovered accidentally in 1965 by James Schatter who was synthesising a product for treating ulcers.<sup>2</sup> He was heating aspartame in a flask with methanol when the mixture bumped onto the outside of the flask. He later detected a strong sweet taste on his fingers

which he traced back to powdered aspartame on the flask. This method of discovery is not an example of good laboratory practice!

Aspartame is valued because it has a clean sweet taste similar to that of sucrose. The aspartame in Candere!® is bulked out with carbohydrate so that one teaspoonful is perceived to be as sweet as one teaspoonful of sugar.

Aspartame is one of the most thoroughly tested food additives.<sup>3</sup> Aspartate, phenylalanine and methanol are produced when it is metabolised. The safety of aspartame has been called into question because high blood levels of each of these compounds is associated with toxicity. Because aspartame is approximately 200 times sweeter than sugar very little is needed to provide the equivalent sweetness. The amounts of the amino acids and methanol provided by a normal diet are much larger than those likely to be ingested as aspartame. A teaspoonful of Candere!® contains 20 mg phenylalanine while an 8 oz glass of milk provides 542 mg.

An infant suffering from the genetic disease phenylketonurea(PKU) is likely to be on a phenylalanine-restricted diet as this can prevent the onset of mental retardation that is associated with the disease. In such cases aspartame should be avoided.

## Procedure

The procedure for paper chromatography is adapted from a manual by Smith and Feinberg.<sup>4</sup> An experiment on the chromatographic analysis of amino acids forms part of the Nuffield chemistry course.<sup>5</sup>

### Stage 1 Hydrolysis of Candere!®

12 g of Candere!® is placed in a round-bottomed flask and 200 cm<sup>3</sup> of hydrochloric acid (6 mol dm<sup>-3</sup>) is added. The mixture is then refluxed for ½ –1 h. After a short time the mixture will begin to turn brown, by the time this stage is finished it will be black.

### Stage 2 Paper chromatography

Care should be taken to touch the chromatography sheets only at the top corners as fingerprints contain traces of amino acids.

A small sample of the black mixture is first decolorised by using activated charcoal. A pasteur pipette is used to transfer ca 5 cm<sup>3</sup> of the hydrolysate to a clean test-tube. This is decolorised with ca 100 mg activated charcoal and filtered to give a clear solution for spotting onto the chromatogram.

The solvent mixture (ethanol:water:880 ammonia) is placed in the tank which is covered to produce a saturated atmosphere.

The paper is prepared and spots of each of the reference amino acids and also the sample are placed on the paper. Pencil identification marks are made at the top of the paper.



The paper is formed into a cylinder and secured with clips. It is then placed, with the spotted end down, in the tank taking care not to let the paper touch the glass walls. The tank is closed. No observations can be made while the chromatogram is running because the compounds used are colourless. The chromatogram is run for a minimum of 1 h and longer if possible. It is then removed from the tank, the solvent front is marked with a pencil, and the paper is allowed to dry.

The paper is then hung up in a fume cupboard and sprayed sparingly with the ninhydrin solution. It is then heated in an oven at 110°C for 5–10 min when the amino acids should appear as purple spots.

The colour is stable for some weeks if kept in the dark and can be photocopied to give a permanent record.

## Extension

Although aspartame tastes very similar to sucrose, food chemists have to take its chemical properties into account before it is included in a food product in place of sugar. Studies of the stability of aspartame in solution have shown that it is likely to be fully hydrolysed within 9 days at pH 7.4.<sup>2,3</sup> If the aspartame is in a food system when this happens a loss of sweetness will be perceived. This effect could be investigated by dissolving Canderel® in a buffer solution and leaving it for various lengths of time in a warm place.

## References

1. A. D Heaton, *School Sci. Rev.*, 1985, **66**, 728 .
2. *Aspartame: physiology and biochemistry*, D. Lewis, Stegink and L. J. Filer (eds). Marcel Dekker, 1984.
3. *Food additive user's handbook*, J. Smith (ed). London: Blackie, 1991.
4. I. Smith and J. G. Feinberg, *Paper and thin layer chromatography and electrophoresis*. London: Longman, 1972.
5. *Nuffield advanced science chemistry students' book II*. London: Longman, 1984.

## Credits

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