COLOURFUL EVIDENCE
Identifying colouring agents in food

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Languages available
English

Summary
"No artificial colours or flavours." Wording like this is appearing on many food products, especially sweets, cakes and soft drinks. But how do we know? What are the concerns that have led to this trend? You'll find out answers to both questions in this activity. You will use thin layer chromatography to identify colourings extracted from food products. After checking this against what the packaging says, you will write a statement to be read out in a court of law summarising your evidence that some products tested contained different ingredients to those listed on the packaging.

Activity type **B**
Use of scientific knowledge and understanding to solve problems
Working in teams to solve problems
Communication

Techniques
Thin layer chromatography

Field
Food science

Time
Practical lessons: 120 minutes
Theory lessons: 60 minutes
Out of class time: 90 minutes

StandardBase procedures
None

StandardBase techniques
Chromatographic analysis (Thin layer chromatography)

Other resources
LGC (www.lgc.co.uk)
VAM (www.vam.org.uk)
See also in the activity.

Student's document

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The labels on food products must list any additives. Food colourings are one group of additives. Only certain ones are allowed and only in certain amounts. The European Food Safety Authority (EFSA) collaborates with national authorities to provide independent scientific advice about food safety. In December 2006, it called for data on food colours as part of a re-evaluation of all authorised food additives in the EU.
In the UK the Food Safety Act 1990 protects consumers and fair traders. It is enforced by Local Authorities. Typically, Environmental Health Officers and Trading Standards Officers collect samples for analysis by Public Analysts. The work of these analysts may end up as evidence in a court of law.

Your brief

You will work in a group. The group will be given:

- solutions of pure food colourings and two reference mixtures of food colourings
- commercial food colourings
- samples of colourings extracted from food products.

Your brief is to:

- use thin layer chromatography (TLC) to identify colourings extracted from food products, checking them against what the packaging says
- write a statement to be read out in a court of law saying what evidence you have that some of the food products tested contained the wrong ingredients compared to those listed on the packaging.

Your investigation

The group's tasks are to run chromatograms of:

- the reference mixtures to identify which spots match which food colourings
- commercial food colouring mixtures to confirm the colourings listed on the labels are present
- food colourings extracted from confectionery products to find out if:
  - the food colourings stated on the packaging are present
  - any food colourings not given on the packaging are present.

Decide which analysis or analyses each member in the group will do. Use **Student sheet***: Planning grid* to show who does what.

A risk assessment must be carried out before starting practical work.

Task 1

Using **Student sheet***: Standard procedure for TLC of food colourings*, carry out TLC on the two reference mixtures:

- Reference mixture A containing Tartrazine, Sunset Yellow and Carmoisine
- Reference mixture B containing Ponceau 4R, Brilliant Blue FCF and Green S.

Run each reference mixture alongside solutions of the pure food colourings in it. Identify which spot corresponds to which food colouring. You will use these two reference mixtures to identify the food colourings in food products.

Task 2
Using **Student sheet***: Standard procedure for TLC of food colourings*, carry out TLC on:

- each of the commercial food colouring solutions you are given, running them alongside the two reference mixtures
- the food colourings you are given that were extracted from the confectionery samples. Again, run them alongside the two reference mixtures.

Student sheet*: Products composition* gives the ingredients listed on the packaging of the food products you have been asked to analyse.

For tasks 1 ands 2 you could take photographs of the chromatograms to include as an appendix to your court statement (as an item of evidence).

Task 3
Each member should summarise the group's results (use **Student sheet***: Reporting form*) and write a statement to be read out in a court of law explaining which of the extracted samples gave you cause for concern.

Your teacher will tell you when the analyses are to be carried out. You need to collect the apparatus and chemicals needed and set out your work area. Remember that a risk assessment must have been carried out before you start work.

To find watchdog organisations try these websites:

- European Food Safety Authority: http://www.efsa.europa.eu/en/about_efsa.html
- Food Standards Agency: http://www.food.gov.uk/ and http://www.eatwell.gov.uk/foodlabels/understandingenumbers/
- The Association of Public Analysts: http://www.the-apa.co.uk/
- List of UK laboratories: http://www.the-apa.co.uk/Laboratories/index.html

Use your favourite search engine to find out about food colouring and other food additives. Much information is factual, for example, types of additives and assigned E-numbers. Be wary though. Some sites are biased about the use of food additives – for and against. Make sure you check enough sites to get a balanced view.

Your findings
Write your results and conclusions on the 'Reporting form' you have been given. Use this data to write your statement to be read in court. It needs to explain, with supporting evidence (e.g. photographs of the chromatograms), which confectionery samples gave cause for concern.
Compare the group's results with data given on the 'Products composition' datasheet. For each chromatogram you run, calculate and record the R_f values for each spot.

Student self assessment
This activity was about using scientific knowledge and understanding to solve problems, working in teams to solve problems and communicating scientific results. For each case, write about 50-100 words explaining:

- what scientific skills and knowledge you used and how effectively you used them.

- how you worked as a team, giving examples of how you supported others in your group or were supported by them
- how effective you think your statement might be in a court of law given that it would be heard by people who might not have a science background.

You may decide to use alternative forms of self assessment

Student sheet: Standard procedure for TLC of food colourings

Scope
This method may be used for aqueous solutions of food colourings. The solution should not contain significant quantities of other solutes such as sugars.

Principle
Food colourings present in solution are separated using thin layer chromatography. Cellulose-coated plastic backed TLC sheets may be used. Water is an effective eluting solvent, but others may be tried. Specimens under examination are compared with standard samples. The coloured spots are easily visible and the intensity of colour allows quite low concentrations to be recognised.

Apparatus
Equipment
- glass capillary tubes
- chromatography tank and lid
- TLC plate - cellulose on glass or plastic (10 cm x 5 cm).

Materials
- six solutions of pure food colourings:
  - Tartrazine
  - Sunset Yellow
  - Carmoisine
  - Ponceau 4R
  - Brilliant Blue FCF
  - Green S
- two reference mixtures: Reference mixture A (Tartrazine, Sunset Yellow and Carmoisine) and Reference mixture B (Ponceau 4R, Brilliant Blue FCF and Green S)
- samples of commercial food colouring products containing one or more of the colourings listed above
- samples of food colourings extracted from confectionery products
- distilled water

Procedure
Remember, a risk assessment must be carried out before starting work. Work with care. Keep equipment, including the TLC plates, clean. Hold the TLC plates at their top corners. Only lay them down clean sheets of paper.

1 Pour distilled water into the chromatography tank to a depth of about 5 mm. Cover with a lid and leave while you prepare the TLC plates.
2 Mark the TLC plate using a soft lead pencil. Draw a baseline 1 cm from the bottom of the plate. Mark the positions where the spots will be applied and label them (again using a soft lead pencil. Leave at least 1 cm between spots.
3 Spot the solutions being examine. Dip a clean capillary tube into the solution being tested and apply a small drop to the plate. Use a new, clean capillary tube for each solution. Allow spots to dry.
  Note: if necessary, practice on a piece of filter paper until you can produce spots not more than 0.3 cm in diameter.
4 Place the plate in the TLC tank. Do this carefully to avoid the water splashing around. Leave until the water front reaches about 2 cm from the top of the plate.

5 Remove the TLC plate from the tank. Lay the plate on a clean piece of paper. Mark the position of the water front and leave the plate until it has dried. At this stage you may wish to take a photograph of the chromatogram.

Calculations

Record the identities of the separated spots. For each spot of food colouring, calculate its retention value, R_f., using:

$$R_f = \frac{\text{distance moved from the baseline by the food colouring}}{\text{distance moved from the baseline by the water front}}$$

Take the position of each spot from its centre.

Expression of results

For each chromatogram run, record the identity and R_f. value for any spots observed.

Student sheet: Planning grid

Use the pictures of blank TLC plates to plan your work. In each case write the name of the group member responsible for running the chromatogram and list the samples to be spotted, including their positions on the baseline.

Samples to be used:	Samples to be used:	Samples to be used:	Samples to be used:	Samples to be used:	Samples to be used:
Analyst's name:	Analyst's name:	Analyst's name:	Analyst's name:	Analyst's name:	Analyst's name:
Samples to be used:	Samples to be used:	Samples to be used:	Samples to be used:	Samples to be used:	Samples to be used:
Analyst's name:	Analyst's name:	Analyst's name:	Analyst's name:	Analyst's name:	Analyst's name:

Student sheet: Products composition

	Food colourings found in products					
Food product	Tartrazine	Sunset Yellow	Carmoisine	Ponceau 4R	Brilliant Blue FCF	Green S
Langdale Blue			✓		✓	
Langdale Green	✓				✓	
Langdale Scarlet		✓		✓		
Langdale Yellow	✓	✓				
SuperCook Blue			✓		✓	
SuperCook Green	✓					✓
Extract from confectionery A		✓		✓		
Extract from confectionery B	✓		✓		✓	
Extract from confectionery C	✓		✓		✓	✓
Extract from confectionery D		✓		✓	✓	
Extract from confectionery E	✓				✓	✓
Extract from confectionery F	✓				✓	✓

Student sheet: **Reporting form**

Indicate clearly which analyses you carried out and which ones were done by other members of your group (note: you may want to stick your chromatograms to the back of this reporting form or on a separate sheet of paper

Sample	**Food colourings found to be present**	**Food colourings listed on packaging**	**Comments**

Teacher's document

Overview

Students work as analysts in a laboratory specialising in the analysis of food products. Students are put in the position of working as a Public Analyst. They carry out thin layer chromatography to identify food colourings extracted from food products. They also write a statement to be read out in court explaining which samples gave them cause for concern. To be effective they need to have appropriate scientific skills and knowledge, be able to work together as a team and to communicate effectively.

Students learn to:

- tackle scientific problems by applying their practical skills and their knowledge and understanding of thin layer chromatography
- observe, measure and analyse data collected from thin layer chromatography, and to evaluate these
- work with others and managing relationships with people
- communicate by writing a statement to be read out as evidence in a court of law, bearing in mind the audience, and using visual images to support the statement.

Developing students' skills

Each student will carry out three TLCs. They should receive feed back after each one.
Appropriate questions should be asked as prompts to think about.
This will depend on the required assessment objectives for the course being followed by the students. The completed 'Reporting form', photographs of chromatograms and the statement to be read in a court of law may all provide summative assessment evidence, judged against published grading criteria.

Organising and managing the class

Students should have experience of paper and/or thin layer chromatography, in particular the technique of spotting chromatograms.

Standards

Solutions of the following food colourings should be prepared, each about 1 g of colouring in 100 cm^3 of distilled water:

Tartrazine	E102	Sunset Yellow	E110
Carmoisine	E122	Ponceau 4R	E124
Brilliant Blue FCF	E133	Green S	E142

Reference mixtures

Make the following reference mixtures by mixing equal volumes of the appropriate standards.
Standard mixture A: Tartrazine + Sunset Yellow + Carmoisine
Standard mixture B: Ponceau 4R + Brilliant Blue FCF + Green S

Samples

Samples 1-6 are commercial food colourings products which are available as solutions in UK supermarkets and other food shops. This provides a cheaper alternative to using pure food colours, although these could be used. Other branded food colours can be used (see below for examples of combinations to use)

Ref	Sample	Food colourings stated on packaging
1	Langdale Blue	Brilliant Blue FCF; Carmoisine
2	Langdale Green	Tartrazine; Brilliant Blue FCF
3	Langdale Scarlet	Ponceau 4R; Sunset Yellow
4	Langdale Yellow	Tartrazine; Sunset Yellow
5	SuperCook Blue	Brilliant Blue FCF; Carmoisine
6	SuperCook Green	Tartrazine; Green S

Samples 7-12 are simulated extracts from confectionery products, prepared by mixing roughly equal volumes of the appropriate commercial food colourings. Some samples are 'genuine', i.e. the food colourings identified match those listed on the packaging. Others are not. They either have ingredients not listed or have an ingredient missing (see details below).

Ref	Sample	Food colourings stated on label	Food colourings actually present
7	Extract from confectionery A	Ponceau 4R; Sunset Yellow	Ponceau 4R; Sunset Yellow; Tartrazine (mix Langdale Scarlet and Langdale Yellow) So sample contains tartrazine which it should not
8	Extract from confectionery B	Brilliant Blue FCF; Carmoisine; Tartazine	Brilliant Blue FCF; Carmoisine; Tartazine (mix Langdale Blue and Langdale Green) So sample is genuine
9	Extract from confectionery C	Brilliant Blue FCF; Carmoisine; Tartazine; Green S	Brilliant Blue FCF; Carmoisine; Tartazine; Green S (mix SuperCook Blue and SuperCook Green) So sample is genuine
10	Extract from confectionery D	Brilliant Blue FCF; Ponceau 4R; Sunset Yellow	Brilliant Blue FCF; Carmoisine; Ponceau 4R; Sunset Yellow (mix Langdale Blue and Langdale Scarlet) So sample contains carmoisine which it should not
11	Extract from confectionery E	Tartrazine; Brilliant Blue FCF; Green S	Tartrazine; Brilliant Blue FCF (Langdale Green) So sample does not contain Green S which it should
12	Extract from confectionery F	Tartrazine; Brilliant Blue FCF; Green S	Tartrazine; Brilliant Blue FCF; Greens (mix Langdale Green and SuperCook Green) So sample is genuine

If different brands are used you will need to work out how to combine the food colours to give five unique combinations of ingredients. A sixth could contain the same ingredients as one of the others but in different ratios. When writing the labels list the ingredients in each sample, but for a couple of samples leave out one ingredient, for one add a colour to the list which it doesn't contain.

It is suggested that students work in groups of three or six.

If they work in a group of six, each student might run three chromatograms:
- one of the standard mixtures alongside solutions of the pure food colourings in it
- one the commercial food colouring solutions alongside the two standard mixtures
- one of the food colourings mixture extracted from the confectionery samples alongside the two standard mixtures.

Some may prefer that each student investigates two commercial food colouring solutions and two food colourings mixture extracted from the confectionery samples. It still means each student runs three chromatograms.
Make sure students have sufficient time to share and discuss their results before starting to write the statement for use in a court of law.

Students need to recognise the importance of taking care and pride in their work. They need to be gently reminded of this while they are working. They may need three types of help.
- Help with technical details, including manipulative skills and correct handling of chemicals and equipment.
- Help with, and vigilance over, health and safety issues.
- Help with working relationships in their group.

Students from different groups could compare their results to look at differences in measurements. They could comment on how accurate they feel their results are.

Session plan for teachers
A suggested approach is:
- Introduce the activity and check prior knowledge. (about 30 minutes)
- Arrange students into groups to discuss and allocate the work. (about 10 minutes)
- Carry out the practical work. (about 120 minutes)
- Group comes together to share and discuss results. (about 20 minutes)
- Complete reporting forms and draw conclusions. (about 30 minutes)
- Prepare court statement. (about 60 minutes)

Technical notes

Preparation

Solutions of the following food colourings should be prepared, each about 1 g of colouring in 100 cm^3 of distilled water:

Tartrazine	E102	Sunset Yellow	E110
Carmoisine	E122	Ponceau 4R	E124
Brilliant Blue FCF	E133	Green S	E142

The two reference mixtures may be made by mixing equal volumes of the appropriate standards.
Reference mixture A: Tartrazine + Sunset Yellow + Carmoisine
Reference mixture B: Ponceau 4R + Brilliant Blue FCF + Green S

For the simulated extracts from confectionery products various solutions need to be made from the commercial food colourings:
Extract from confectionery A: equal volumes of Langdale Scarlet and Langdale Yellow
Extract from confectionery B: equal volumes of Langdale Blue and Langdale Green
Extract from confectionery C: equal volumes of SuperCook Blue and SuperCook Green
Extract from confectionery D: equal volumes of Langdale Blue and Langdale Scarlet
Extract from confectionery E: Langdale Green
Extract from confectionery F: equal volumes of Langdale Green and SuperCook Green
These need to be provided in sample tubes labelled with the ingredients for authentic samples of A-F (see table in Teachers Guide).

Equipment and materials

Materials and their CAS numbers

Tartrazine	1934-21-0
Sunset Yellow	2783-94-0
Carmoisine	53026-69-9
Ponceau 4R	2611-82-7
Brilliant Blue FCF	3844-45-9
Green S	3087-16-9

These may be obtained from
Sensient Colors UK, Oldmedow Road, Kings Lynn, Norfolk, PE30 4LA
or
Town End (Leeds) plc, Silver Court. Intercity Way, Stanningley, Leeds, LS13 4LY www.dyes.co.uk
Both will deliver within Europe and can provide all dyes necessary for the activity.

It is easier to make up your own mixtures of pure food colours. However it is possible to use commercial food colourings from supermarkets. Two commonly available brands in the UK are SuperCook and Langdale. If others

are used containing different ingredients you will need to change the list of samples and their ingredients appropriately.

Using a mixture of propanone and water as the eluent will give the best results. Students might try different ratios of the two (for example 75:25, 50:50 or 25:75).

Equipment

Each student will need:
glass capillary tubes
chromatography tank and lid (250 cm^3 beaker with a watch glass will suffice, but it is better to use a tank with a ground glass top that can be sealed on with silicone grease)
three TLC plates - cellulose on glass or plastic (10 cm x 5 cm)
disposable gloves may be used to prevent dye's staining skin

TESTING PRIOR KNOWLEDGE

Thin layer chromatography (TLC) can be used to separate and identify substances in mixtures. Fill in the missing words:

Scientific principle

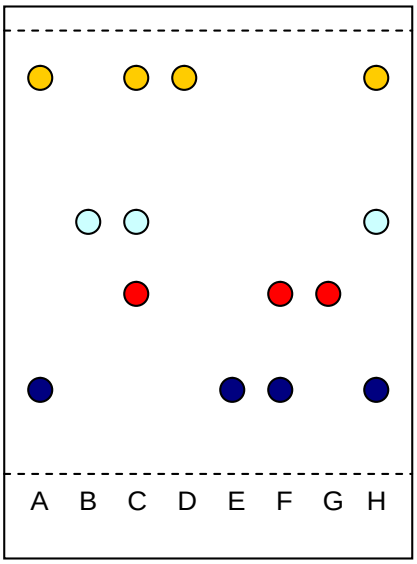
1 A TLC plate consists of a solid on a glass plate or stiff plastic sheet. This solid is called the stationary phase. It can absorb and adsorb substances.
2 A sample is 'spotted' on the TLC plate and left to dry. A solvent, called the mobile phase, moves through the solid.
3 As the solvent passes, it dissolves the sample to give a solution. The dissolved substances are called solutes.
4 The solution passes through the solid and the substances move between the mobile and stationary phases.
5 How quickly substances move through the stationary phase depends on the relative attraction of their molecules to the mobile and stationary phases.
   a A substance attracted relatively strongly to the mobile phase passes through quickly.
   b A substance attracted relatively weakly to the mobile phase passes through slowly.

Interpreting a chromatogram

1 Substances may be identified by comparing spots with those from reference materials.
2 A substance may also be identified by its retention factor (R_f). This does not change provided the same conditions used.

$$R_f = \frac{\text{distance moved by substance}}{\text{distance moved by solvent}}$$

3 Look at this chromatogram and answer the questions alongside it.



Questions

a Which substances are not mixtures? B, D, E, G
b Which two substances have two components? A, F
c What are the R_f values for the components in substance H? 0.20, 0.55, 0.87 (Note: teachers should check these values as the chromatogram may distort when printed.)

Note: answers are given in red. These need to be removed before the test is used.

TESTING PRIOR KNOWLEDGE

Thin layer chromatography (TLC) can be used to separate and identify substances in mixtures. Fill in the missing words:

Scientific principle

1. A TLC plate consists of a solid on a glass plate or stiff plastic sheet. This solid is called the ..... phase. It can absorb and adsorb substances.
2. A sample is 'spotted' on the TLC plate and left to dry. A solvent, called the ..... phase, moves through the solid.
3. As the solvent passes, it dissolves the sample to give a ..... The dissolved substances are called .....
4. The solution passes through the solid and the substances .....the ..... and .....phases.
5. How quickly substances move through the ..... phase depends on the relative attraction of their molecules to the ..... and ..... phases.

   a A substance attracted relatively strongly to the ..... phase passes through quickly.

   b A substance attracted relatively weakly to the ..... phase passes through slowly.

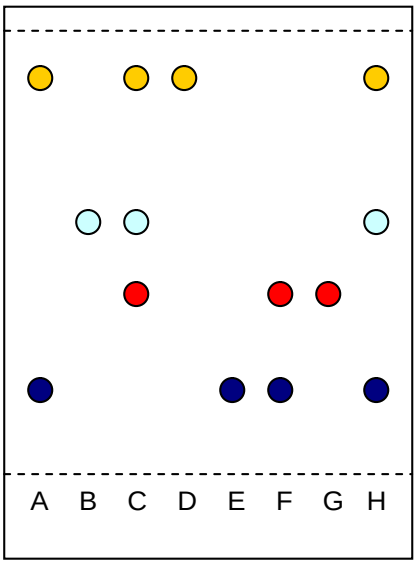
Interpreting a chromatogram

1 Substances may be identified by comparing spots with those from ..... materials.

2 A substance may also be identified by its .....(R_f). This does not change provided the same conditions used.

$$R_f = \frac{\text{distance moved by}\ldots\ldots}{\text{distance moved by}\ldots\ldots}$$

3 Look at this chromatogram and answer the questions alongside it.



Questions

d Which substances are not mixtures?

e Which two substances have two components?

f What are the R_f values for the components in substance H?