An experiment to identify substance X using thin layer chromatography.

Introduction

In this experiment the method being used is chromatography. Chromatography is an important method for finding out more about mixtures. Chromatography allows substances to be separated with a solvent and indicates whether a substance is pure, meaning it contains only one single substance, as opposed to a mixture of substances, allowing for concentrations to be made. By using think layer chromatography (TLC), the aim of the experiment is to find out whether 'substance X' is either Anadin which contains aspirin and caffeine, or Anadin extra, which contains aspirin, caffeine and also paracetamol. Thin layer chromatography is a technique involving the distribution of a mixture of two or more substances between a stationary phase (involving a solid or a liquid) and a mobile phase (involving a solid or a gas). The thin layer of absorbent coated on the chromatography paper is normally a silica get, cellulose or alumina. The mobile phase is a developing liquid. The developing liquid (Butyl Ethanolate), travels up the stationary phase, moving the samples with it, in this case over the counter analgesics. The samples will separate on the stationary phase according to how much they absorb on the stationary phase against how much they dissolve in the mobile phase.

Hypothesis

It is to be expected that substance x will be able to be identified using thin layer chromatography.

Safety assessment

Goggles	Prevents from any splashes getting in the eyes.
Lab coats	Prevents staining of clothes.
Stools and bags	Should be put away, preventing trips and falls.
U.V light source	The U.V light is strong, so it's important not to look directly at the light source.
Hazardous liquids	Care must be taken when measuring and the correct procedures must be carried out when cleaning up any spillages. Liquids should not be poured down the sink. If any of the liquids were to get on the skin, hands must be washed immediately. Avoid any inhalation of the hazardous liquids.
Fume cupboard	Must be switched on and remain switched on throughout the experiment. Helping to remove any toxic fumes produced by the hazardous liquids.

Method

1. A strip of chromatography paper was taken and a thin pencil line was made at the bottom of the paper – three equally spaced marks were also made, ensuring that the paper was not touched.
2. Four test tubes were taken and labeled A(aspirin), P(paracetamol), C(caffeine) and X(substance X)

3. 1-2cm³ of the drug solvent (Ethanol) was added to each tube. Then carefully shaken from side to side, to ensure the drug samples were dissolved.
4. A spot of solution from each of the drug samples were carefully placed on the chromatography plate using a fresh capillary tube each time. This was repeated three times to ensure enough of the drug sample was placed on the chromatography plate and was then allowed to dry in fume cupboard.
5. 0.5 – 0.75cm³ of the developing solvent (Butyl Ethanolate) was poured into a beaker – ensuring that the solvent does not touch the pencil line drawn on the bottom of the plate. Foil was then placed on top of the beaker.
6. The chromatogram was allowed to develop until the solvent was 1cm from the top of the plate.
7. The plate was allowed to dry in the fume cupboard for 10 minutes.
8. The plate was then placed under the UV light source, with the spots being marked in pencil.

Set up of the experiment

Results

Aspirin = 6.3cm
Paracetamol = 4.5 cm
Caffeine = 2.6cm
Substance X =(1^{st})2.6cm, (2^{nd})4.3cm, (3^{rd})6cm
Solvent front = 7.7cm

Calculations

R_f = Distance from start to centre of spot
Distance from start to solvent front

Analgesic	Aspirin	Paracetamol	Caffeine	Substance X		
Distance from start to centre spot (cm)	6.2	4.5	2.6	2.4	4.5	6
Distance from start to solvent front (cm)	7.7	7.7	7.7	7.7	7.7	7.7
	0.81	0.58	0.34	0.31	0.58	0.59

Interpretation

From the results of the experiment, it is proven that the hypothesis stated at the beginning is correct. Substance X was discovered to be Andain extra. This was proven as it had the same spotting in the same area as the other tested analgesics. Each of the substances on the chromatography paper are pure, as they only had one spotting on the paper. Substance X (anadin extra) however had a mixture of the tested substances on the chromatography paper, proving that it was not a pure substance. There was extra spotting on the chromatography paper, which were highlighted by the UV light. These results were anomalies, and were invalid results.

Evaluation

If the experiment was to be carried out again I would not use capillary tubes, because they are very fine and break easily. Instead I would use a wider piece of chromatography paper and use a drop of solution from a pipette. This would also ensure that the results would be more accurate removing the risk of any anomalous results, as you would be able to ensure that the substance hit the mark correctly, whereas, with the capillary tubes it was needed to be repeated several times to ensure it was place on the chromatography plate. Another change I would make in the experiment is that I would use a larger test tube, or even a small beaker. By doing this you would be able to stir the analgesic, ensuring that it has fully dissolved in the drug solvent.