Identifying amino acids by chromatography

Apparatus

- Empty glass coffee jar
- Lid for glass jar
- Strip of chromatography paper
- Capillary tubes
- Staples
- Oven
- Fume cupboard
- Ninhydrin solution
- Lysine
- Leucine
- Valine
- Glycine
- Threomine
- X acid
- Y acid
- Solvent- 4 parts butan-1-01, 1 part ethanoic acid and 1 part distilled water.

Method

- Fetch and set up equipment.
- Pour a small quantity of the solvent into the glass jar.
- Cover tank with lid.
- On the chromatography paper, make a light pencil mark across the paper 2 cm's from one end.
- Using pencil, place the names of the acids on the paper so you can identify them afterwards.
- Practice using the capillary tubes on normal filter paper
- Using the capillary tubes, place a small amount of each acid on its position along the line on the filter paper.
- Make the spots no bigger than 2 mm's if possible.
- Let the paper dry for a few minutes in air.
- Roll the chromatography paper into a cylindrical form.
- Staple the ends together about a third of the way in from each edge.
- Make sure the edges do not touch, however, otherwise the solvent will flow more rapidly at that point and form an uneven front.
- Quickly place the chromatography paper into the glass jar so it stays saturated in the solvent.
- Cover the glass jar back up.
- Leave to stand for about half an hour, or until the 'solvent front' has rised sufficiently.
- Remove the chromatography paper and mark the solvent front with a pencil.
- Place it on bench to dry.
- When most of the solvent has evaporated rip off the staples and place it in a fume cupboard upside-down to dry.

- When all the solvent has evaporated completely spray the chromatography paper lightly but completely with the ninhydrin solution.
- Place the chromatography paper in the oven at 100- 110 degrees for about 10 minutes- or until all the spots have developed.
- Take the chromatography paper out the oven.
- Circle the spots with pencil.

Safety points

- Safety goggles must be worn at all times.
- Work surface must be clean and tidy before and during the experiment.
- Extra care must be taken when using the capillary tubes with the acids.
- Any spillage must be cleaned up quickly and safely.
- Any breakage must be cleaned up properly.
- Do not run in the laboratory, especially when handling equipment.
- Take care not to get any of the acids of ninhydrin solution on clothes or skin.

Results

Conclusion

From the doing the chromatography test on five known amino acids and 2 unknown amino acids:

Lysine
Leucine
Valine
Glycine
Threonine
X
Y

I have found out that amino acid X is the only acid used in the experiment to contain more than one substance.
All the other amino acids only contain one substance.
I also found out that the solvent front is around 8 cm's.
The Rf values for the seven amino acids are:

Lysine- 0.14
Leucine- 0.73
Valine- 0.6
Glycine- 0.26
Threonine- 0.35
X contains lysine- 0.14 and phenylalanine- 0.68

Evaluation

If I were to do the experiment again I would have done a few things differently.
One thing I would do differently is that I would work more quickly and quieter because I found out I had to rush the experiment as I was running out of time. This was because I was being distracted easily and talking to people.
I would also take more care in the area I was working in because it was very messy during my experiment and I kept on temporarily losing the capillary tubes etc.
I would also take more care in the handling of my chromatography paper because u would have noticed that there is a thumbprint in my results where I have bin handling the paper. Next time I will either use rubber gloves or use tweezers to handle the chromatography paper so my results are not spoilt in any way.
I would also take more care when using the capillary tubes because sometimes I made the dots bigger than they should have been.
I would also be more aware of what is going around me. This is because the person working next to me spilt one of the acids and I didn't notice and some of the acid got on my chromatography paper. This slightly tarnished the results, as u would have noticed that there are random streaks of purple on my chromatography paper.