WATER TOC AMINO

ARACUS

Sample Preparation

Amino Acids Analysis Sample Preparation

Structure of this Talk

- Sample Preparation Method:
  - Acids Hydrolysis (18 Protein Amino Acids)
  - Alkaline Hydrolysis (Tryptophan)
  - Oxidation with Performic Acids (Cystic Acid and Methionine Sulphone)
  - Precipitation (42 AA for Physiological Free Amino Acids)

Standard Acid Hydrolysis with 6N HCL

Hydrolysis with 6 N Hydrochloric Acid

1. Weigh 100 mg (0.1 g) of homogenized sample into reaction tube
2. Add 10ml of 6 N hydrochloric acid [1:1 of 38% HCL and H_2O (Type I water)] and 2 drop of Phenol (AR grade ≥99.0%)
3. Aerate the reaction tube with nitrogen for about 15 min
4. Close tube by melting/ by screw cap
5. Incubate the seal reaction tube in an oven at 110°C for 24 hours
6. Cool the reaction tube to room temperature after hydrolysis
7. The hydrolyzed sample is filtered through a cellulose filter paper in to 50ml volumetric flask. Wash the reaction tube and the filter paper in the funnel few time with pure water.
8. Mark up the filtrate into a 50ml volumetric flask.
9. 1ml of the filtrate from 50ml volumetric flask was taken and dry completely in a N evaporator, water bath or an oven with temperature not > 70 °C
10. Add 1ml of sample dilution buffer (SDB). (It is important that do not add pure water to the dry sample because the AA is more stable in acid solution at pH2.2)
11. The solution is than filtered through a 0.22um or 0.45um syringe filter into an injection vial.
12. The sample is ready for analysis

Alkaline Hydrolysis with Lithium Hydroxide (for TRP)

Hydrolysis with 4N Lithium Hydroxy

1. Weigh 50 – 200 mg (accuracy up to 0.1 mg) of homogenized sample into reaction tube. The sample amount depend on the concentration of the amino acids content in the sample.
2. Add 1.50ml of 4M LiOH
3. Aerate the reaction tube with nitrogen for about 15 min
4. Close tube by melting/ by screw cap
5. Incubate the seal reaction tube in an oven at 110°C for 20 hours
6. Cool the reaction tube to room temperature after hydrolysis
7. The hydrolyzed sample is filtered through a cellulose filter paper in to 50ml volumetric flask. Wash the reaction tube and the filter paper in the funnel few time with pure water.
8. Adjust the pH of the filtrate by adding 1ml of 6N HCL
9. Mark up the 50ml volumetric flask with pure water.
10. 1ml of the filtrate from 50ml volumetric flask was taken and filtered through a 0.45µm syringe filter into a 1.5ml vials.
11. The sample is ready for analysis

Performic Oxidation Prior Acid Hydrolysis

Performic Oxidation

1. Preparing Performic acids by mixing 30% of Hydrogen Peroxide with 88% Formic Acids in ratio of 1:9. For every 1 ml of Performic acids, add 5mg of Phenol (AR grade ≥99.0%)
2. Let the reaction to take place for about an hour. After that cold the solution in ice bath and put into the refrigerator at 0-4 °C for 30min.
3. Weight in between 7.5 – 25 mg of sample (accuracy up to 0.0001g, total sample should not more than 75mg) into reaction tube.
4. After that add 2.0 ml of cold Performic Acids into the sample without shaking and seal it with paraffin.
5. Put the reaction tube in the ice bath and place it into the refrigerator at 0 - 4°C for 16 hour.
6. After the oxidation, add 0.5 ml (drop by drop) sodium metabisulfide (33.6 g sodium metabisulfide + 100ml Ultrapure water in volumetric flask) to stop the oxidation.

Performic Oxidation Prior Acid Hydrolysis

Acid Hydrolysis

1. Add 17.5 ml 6 N HCl into the reaction tube
2. Incubate the reaction (without seal) in an oven at 110 °C for 1 hour
3. After that, seal the reaction tube and put back into the oven at 110°C for 19 hours
4. Cool the reaction tube to room temperature after hydrolysis
5. The hydrolyzed sample is filtered through a cellulose filter paper in to 50ml volumetric flask. Wash the reaction tube and the filter paper in the funnel few time with pure water.
6. Mark up the filtrate into a 50ml volumetric flask.
7. To adjust the pH, take out 1ml of filtrate from the 50ml volumetric flask, dry to 0.5ml with N-evaporator, water bath and oven at 50 °C
8. Mark up the remaining 0.5ml filtrate into a 10ml with sample dilution buffer (SDB)
9. 1ml of the filtrate from 10ml of the solution was taken and filtered through a 0.22µm or 0.45µm syringe filter into a 1.5ml vials.
10. The sample is ready for analysis

Precipitation with Sulfosalicylic Acid (SSA) for Free Amino Acids

Precipitation

1. Into a centrifugal vial, add 400 uL sample and 100uL Sulfosalicyclic Acid in 10%
2. Incubate the vial in a refrigerator at 4°C for 60 min.
3. After, centrifuge at 14,500 rpm for about 15 min
4. Carefully pipette out the supernatant into centrifugal filter of 0.22 um in the vial.
5. Centrifuge at 14,500 rpm at 5min.
6. The supernatant than can be transfer to injection vial and ready for injection.
7. The dilution can be done in between 1:4 – 1:999 depending on the concentration of the amino acids in the sample.