# Oxidation and hydrolysis of acetylcysteine

Acetylcysteine is an antidote used for paracetamol overdose. Different tests had been chosen and conducted to ensure the quality of the product in the drug preparation is within the acceptable range as stated in the British Pharmacopoeia (hereafter referred to as ‘ the BP’). The level of purity and changes in the structure/condition of acetylcysteine can be known based on the value of specific optical rotation; a negative value suggests that the compound has an anticlockwise rotation, and a positive value suggests otherwise. The amount of acetylcysteine in a sample can be determined by iodine and sodium hydroxide titration respectively. The results obtained were then being analysed via HPLC analysis, where the peaks on the chromatogram reflect the level of impurities present in the sample. On top of that, a standard addition plot was constructed to determine the level of zinc in acetylcysteine. On the other hand, the weight of ‘ pure’ acetylcysteine can also be known based on the calculation of weight loss upon drying, a low percentage allows us to assume almost little to no impurities were present in the sample before drying.

# Introduction

Acetylcysteine is a derivative of the natural amino acid L-cysteine. It is a mucolytic agent and is used in combination with hypromellose to treat tear deficiency and impared or abnormal mucus production. (1) It is also used to treat paracetamol overdose. It acts as a dietary supplement as well as it has been shown to increase the levels of glutathione within the body which is a powerful antioxidant. (2) Acetylcysteine is a white powder and freely soluble in water.

Acetylcysteine can used to treat paracetamol overdose by helping protecting the liver and restore the levels of glutathione. It helps to bind toxic metabolites produced from the large amount of paracetamol being metabolised. (2) The exact mechanism however is not known. It is used a mucolytic agent too where it helps to reduce the viscosity of the mucus produced by breaking the disulfide bonds present. (2)

Below is the structure of acetylcysteine.

Acetylcysteine can undergo oxidation and hydrolysis. When oxidation occurs, a sulphur bond is formed between two sulphur atoms from two different acetylcysteine molecules. Under hydrolysis, the COCH3 is substituted with a hydrogen atom resulting in a secondary amine.

## Oxidation and hydrolysis of Acetylcysteine

In this experiment, different tests had been conducted to ensure the quality of acetylcysteine lies within the acceptable range as required in the BP. Quality control is a part of Good Manufacturing Practice (GMP) to ensure that a product will be yielded complying to its specifications and its level of safeness, well-being and protection had been increased to maximum for the patients.

In this experiment, the optical rotations for acetylcysteine in old and freshly prepared solutions were obtained using a polarimeter. The readings are then converted to specific optical rotations using the equation below.

The specific optical rotation values directly reflect the direction of rotation of the molecule, where a positive value would mean the molecule rotates light

in a clockwise direction when light is passed through it, and a negative value suggests otherwise. If zero value is obtained, it would mean the sample was a racemic mixture.

Other than that, assay for acetylcysteine was conducted as well to determine the percentage impurity of the sample. Two types of titration were used to test the percentage of acetylcysteine in the sample which are iodine titration and sodium hydroxide titration. Starch is used as the indicator for iodine titration while phenol red and phenolphthalein are used in sodium hydroxide titration. Different indicators will give different value of percentage of acetylcysteine containing in the sample due to their sensitivity at different pH range. Therefore, factors causing the difference in value are going to be discussed. Based on the chromatograms obtained through the HPLC analysis, the level of impurities in acetylcysteine can be identified and calculated based on the peaks present on the chromatograms.

The content of zinc in the acetylcysteine sample can be determined as well by using the standard addition method where a graph of absorbance against concentration of zinc added into solution is plotted. From the standard addition plot, the amount of zinc contained in the sample can be known and compared to the limit stated in the BP.

Weight of ‘ pure’ acetylcysteine in sample, assuming no impurities were present in it initially, can also be calculated based on the amount of weight loss upon drying where water is being removed from the sample via evaporation from the surface.

All these tests will give an overall conclusion to provide a guarantee for the stability of acetylcysteine in the preparation.

## Reference

[1] British National Formulary 57th Edition, 2009 (pg 29-30, 595)

[2] Ben Venue Laboratories. (2007). Acetylcysteine. Available: (http://www. drugs. com/pro/acetylcysteine-solution. html)

Last accessed 10th Jan 2011

[3] MPH114 Pharmaceutics I, Good Manufacturing Practice, Uni. Of Sunderland, 2009

[4] MPH215 Experiment 7 Lab Handouts, Uni. Of Sunderland, 2010

## Experimental 1: Specific Optical Rotation

## Method

## Specific optical rotation: +21° to +27°

The optical rotations of the freshly prepared and old solutions of acetylcysteine were recorded. These were prepared by dissolving 1. 25g acetylcysteine in a mixture of 1ml of a 10g l-1 solution of disodium edentate, 7. 5ml of 1M sodium hydroxide and sufficient mixed phosphate buffer pH 7. 0 to 25ml.

## Results

## Solution

## Reading (°)

## [Î±]

## Old

-3. 40

-34. 00

## Fresh

+2. 42

+24. 20

The equation below is used to find [Î±],

Where Î± = reading obtained

â„“ = path length = 2dm

c = concentration of sample, expressed in % w/v

Concentration of Sample, c

1. 25 g of acetylcysteine is used to make up the mixture solution of 25mL. Therefore, in 100mL of solution, the amount of acetylcysteine in it is 1. 25 x 4 = 5. 0 g.

Hence, c = 5% w/v

## Calculation of [Î±]

[Î±]old =

= -34. 00

[Î±]fresh =

= +24. 20

## Discussion

## 3

## Assign Chiral Centre as R or S

## 2

## 4

## 1

Assign priority numbers

Place the hydrogen so you are looking down at it

Draw arrow from 1 through 2 to 3.

Clockwise is R and Anticlockwise is S.

Anticlockwise suggests S stereoisomer.

Chiral centre is at the C attached to NH(1), COOH(2), CH2SH(3), and H(4).

There is only one chiral centre present in the molecule, marked with asterisk (*). It is a non-symmetrical R-enantiomer. It may also be called a 2R-

enantiomer because the chiral centre is located at the second carbon in the carbon chain.

The specific rotations for the fresh and old samples are greatly different. This could be due to aggregation of the excess enantiomers in the solution, presence of impurities such as the growth of highly rotating microbes or their production of rotating products, or changes of chemical structure of acetylcysteine under extreme storage conditions, e. g. direct or long term exposure to sunlight.

On the other hand, specific rotation for the fresh acetylcysteine sample, +24. 20° complies with the BP specification, i. e. +21° to +27°. This shows that there is about [(100%)(24. 40/27. 00)] = 89. 63% to [(100%)(24. 20/21. 00)] = 115. 24% of R-acetylcysteine present in the sample. This may suggest high purity, but the accuracy of this method is strongly doubted as any highly rotating impurities present in the solution may affect the outcome greatly. Therefore, other methods were enforced to increase the accuracy in determination of the presence of impurities.

# References

[1] Adapted from:

Lecture handouts of MPH 115, Prof. Roz Anderson, University of Sunderland.

Lecture handouts of MPH 115, Dr. Jonathan Harburn, University of Sunderland.

[2] Molecular Libraries Roadmap Initiative, National Center for Biotechnology Information (NCBI). (2004). Acetylcysteine – Compound Summary (CID

12035). Available at: http://pubchem. ncbi. nlm. nih. gov/summary/summary. cgi? cid= 12035. [Last accessed on 29 December 2010]

[3] British Pharmacopoeia Commission Secretariat of the Medicines and Healthcare products Regulatory Agency, British Pharmacopoeia 2011 Online. Available at: http://www. pharmacopoeia. co. uk/bp2011/ixbin/bp. cgi? a= displayHYPERLINK " http://www. pharmacopoeia. co. uk/bp2011/ixbin/bp. cgi? a= display&r= YoJDskQKKhY&n= 1&id= 8073&tab= search"&HYPERLINK " http://www. pharmacopoeia. co. uk/bp2011/ixbin/bp. cgi? a= display&r= YoJDskQKKhY&n= 1&id= 8073&tab= search" r= YoJDskQKKhYHYPERLINK " http://www. pharmacopoeia. co. uk/bp2011/ixbin/bp. cgi? a= display&r= YoJDskQKKhY&n= 1&id= 8073&tab= search"&HYPERLINK " http://www. pharmacopoeia. co. uk/bp2011/ixbin/bp. cgi? a= display&r= YoJDskQKKhY&n= 1&id= 8073&tab= search" n= 1HYPERLINK " http://www. pharmacopoeia. co. uk/bp2011/ixbin/bp. cgi? a= display&r= YoJDskQKKhY&n= 1&id= 8073&tab= search"&HYPERLINK " http://www. pharmacopoeia. co. uk/bp2011/ixbin/bp. cgi? a= display&r= YoJDskQKKhY&n= 1&id= 8073&tab= search" id= 8073HYPERLINK " http://www. pharmacopoeia. co. uk/bp2011/ixbin/bp. cgi? a= display&r= YoJDskQKKhY&n= 1&id= 8073&tab= search"&HYPERLINK " http://www. pharmacopoeia. co. uk/bp2011/ixbin/bp. cgi? a= display&r= YoJDskQKKhY&n= 1&id= 8073&tab= search" tab= search. [Last accessed on 29 December 2010]

# Experimental 2: Assay- 98%-101% C5H9NO3S as dried material

## Method

0. 14g of the material was dissolved in approx 60ml of distilled water.

10ml of dilute hydrochloric acid was been added.

The mixture was cooled to room temperature.

10ml of potassium iodide solution was been added into the solution.

It was then been titrated by using 0. 05M iodine with starch as an indicator

## Results

Weight

A

B

Sample +Vial

0. 9918g

1. 0031g

Vial + Residual

0. 8481g

0. 8606g

Amount Used

0. 1437g

0. 1425g

A

B

Initial Volume

0. 01ml

0. 09ml

Final Volume

9. 41ml

9. 32ml

Iodine Used

9. 40ml

9. 41ml

# Calculations

# Sample A

Derivation of equivalent

1ml of 0. 05M I2 = 16. 32mg of C5H9NO3S

8. 8ml of 0. 05M I2 = 143. 7mg of C5H9NO3S

8. 8ml of 0. 0476M I2 = 136. 8mg of C5H9NO3S

8. 8ml of 0. 0476M I2 = 0. 1368g of C5H9NO3S

9. 4ml of 0. 0476M I2 = 0. 1461g of C5H9NO3S

Iodine Used = 9. 40ml

Mass in (g) = 0. 1437g

Percentage yield = (theoretical yield/actual yield) x 100

(0. 1461/0. 1437) x 100 = 101. 67%w/v

## Sample B

Derivation of equivalent

1ml of 0. 05M I2 = 16. 32mg of C5H9NO3S

8. 73ml of 0. 05M I2 = 142. 5mg of C5H9NO3S

8. 73ml of 0. 0476M I2 = 135. 66mg of C5H9NO3S

9. 41ml of 0. 0476M I2= 146. 23mg of C5H9NO3S

Iodine Used= 9. 41mL

Mass in (g) = 0. 1425g

Percentage yield = (theoretical yield/actual yield) x 100

(146. 23/142. 5) x 100 = 102. 62%w/v

# Discussion

Equation of acetylcysteine and iodine:

The values of the percentage purity obtained from the experiment are 101. 67%w/v and 102. 62%w/v for the content of C5H9NO3S. Therefore, it does not comply with the B. P. limits as the B. P range is 98%-101%. The fact that we got higher percentage yields in the assay suggests that there were impurities reacting in it. Acetylcysteine reacts with iodine to produce the desired product in this reaction. Acetylcysteine is prone to oxidation and also to hydrolysis, this is what results in some impurities being formed. When iodine is dissolved in the potassium iodide solution and starch is added as the indicator we achieve a blue/black solution at the end point. When iodine and starch are mixed together in water a starch iodine complex is formed which is blue in colour. This change is due to the polyiodide chains being formed when the starch reacts with the iodine solution. Amylose in starch is what is responsible for the deep blue colour.

Iodine is more specific if compared to that of sodium hydroxide. This is because iodine will only reacts with the thiol group of acetylcysteine (-CH2SH) to change the colour of solution from colourless to blue. Colourless solution indicates that iodine had been broken down to iodide ions. Iodine is more self-indicating and therefore can produce a more reliable endpoint. Compared to sodium hydroxide, sodium hydroxide will react with both functional groups of acetylcysteine and therefore resulting in difficulty in figuring out the correct amount of acetylcysteine in the sample. So, tests have to be repeated by using different indicators to obtain the correct result. Therefore, BP prefers iodine titration rather than sodium hydroxide titration.

# References

[1] British Pharmacopoeia Commission Secretariat of the Medicines and Healthcare products Regulatory Agency, British Pharmacopoeia 2011 Online. Available at:

http://www. pharmacopoeia. co. uk/bp2011/ixbin/bp. cgi? a= displayHYPERLINK " http://www. pharmacopoeia. co. uk/bp2011/ixbin/bp. cgi? a= display&r= YoJDskQKKhY&n= 1&id= 8073&tab= search"&HYPERLINK " http://www. pharmacopoeia. co. uk/bp2011/ixbin/bp. cgi? a= display&r= YoJDskQKKhY&n= 1&id= 8073&tab= search" r= YoJDskQKKhYHYPERLINK " http://www. pharmacopoeia. co. uk/bp2011/ixbin/bp. cgi? a= display&r= YoJDskQKKhY&n= 1&id= 8073&tab= search"&HYPERLINK " http://www. pharmacopoeia. co. uk/bp2011/ixbin/bp. cgi? a= display&r= YoJDskQKKhY&n= 1&id= 8073&tab= search" n= 1HYPERLINK " http://www. pharmacopoeia. co. uk/bp2011/ixbin/bp. cgi? a= display&r= YoJDskQKKhY&n= 1&id= 8073&tab= search"&HYPERLINK " http://www. pharmacopoeia. co. uk/bp2011/ixbin/bp. cgi? a= display&r= YoJDskQKKhY&n= 1&id= 8073&tab= search" id= 8073HYPERLINK " http://www. pharmacopoeia. co. uk/bp2011/ixbin/bp. cgi? a= display&r= YoJDskQKKhY&n= 1&id= 8073&tab= search"&HYPERLINK " http://www. pharmacopoeia. co. uk/bp2011/ixbin/bp. cgi? a= display&r= YoJDskQKKhY&n= 1&id= 8073&tab= search" tab= search

Last accessed: 31 December 2010

[2] http://antoine. frostburg. edu/chem/senese/101/redox/faq/starch-as-redox-indicator. shtml. Last Accessed : 29 December 2010

[3] Paul Hambleton, MPH 215 Lecture Notes, 2010, Uni. Of Sunderland.

## Experimental 3: Assay of titration with Sodium Hydroxide

## Method

a) Approximately 0. 3g acetylcysteine was accurately weighed and dissolved in approximately 50ml distilled water. The solution was then titrated with 0. 1M sodium hydroxide using phenol red indicator.

b) Approximately 0. 3g acetylcysteine was accurately weighed and dissolved in approximately 50ml distilled water. The solution was then titrated with 0. 1M sodium hydroxide using phenolphthalein indicator.

## Results

Using phenol red as indicator,

## Sample

1

2

## Weight of acetylcysteine + Weighing boat /g

3. 7705

3. 8931

## Weight of acetylcysteine + Residual /g

3. 4762

3. 5932

## Weight of acetylcysteine used /g

## 0. 2943

## 0. 2999

## Final burette reading /mL

19. 70

19. 50

## Initial burette reading/mL

3. 00

2. 50

## Volume of NaOH used /mL

## 16. 70

## 17. 00

No. of moles of acetylcysteine = No. of moles of NaOH (1: 1 Reaction)

Molecular mass of acetylcysteine = 163. 2

Concentration of NaOH = 0. 1062M

## Sample 1

Mass of sample used (g) = 0. 2943

No. of moles of acetylcysteine = 1. 7735 X 10-3

Weight of acetylcysteine reacted with NaOH = No. of moles of acetylcysteine X Molecular

mass

= 1. 7735 X 10-3 X 163. 2

= 0. 2894g

Percentage of acetylcysteine in sample = X 100%

= X 100%

## = 98. 34%

## Sample 2

Mass of sample used (g) = 0. 2999

No. of moles of acetylcysteine = 1. 8054 X 10-3

Weight of acetylcysteine reacted with NaOH = No. of moles of acetylcysteine X Molecular mass

= 1. 8054 X 10-3 X 163. 2

= 0. 2946g

Percentage of acetylcysteine in sample = X 100%

= X 100%

## = 98. 23%

Average Percentage of Acetylcysteine in sample using phenol red as indicator

=

=

**= 98. 3%**

Using phenolphthalein as indicator,

## Sample

1

2

## Weight of acetylcysteine + Weighing boat /g

3. 8680

3. 8852

## Weight of acetylcysteine + Residual /g

3. 5674

3. 5849

## Weight of acetylcysteine used /g

## 0. 3006

## 0. 3003

## Final burette reading /mL

20. 40

34. 40

## Initial burette reading/mL

1. 70

15. 80

## Volume of NaOH used /mL

## 18. 70

## 18. 60

No. of moles of acetylcysteine = No. of moles of NaOH (1: 1 Reaction)

Molecular mass of acetylcysteine = 163. 2

Concentration of NaOH = 0. 1062M

## Sample 1

Mass of sample used (g) = 0. 3006

No. of moles of acetylcysteine = 1. 9859 X 10-3

Weight of acetylcysteine reacted with NaOH = No. of moles of acetylcysteine X Molecular mass

= 1. 9859 X 10-3 X 163. 2

= 0. 3241g

Percentage of acetylcysteine in sample = X 100%

= X 100%

**= 107. 82%**

## Sample 2

Mass of sample used (g) = 0. 3003

No. of moles of acetylcysteine = 1. 9753 X 10-3

Weight of acetylcysteine reacted with NaOH = No. of moles of acetylcysteine X Molecular

mass

= 1. 9753 X 10-3 X 163. 2

= 0. 3224g

Percentage of acetylcysteine in sample = X 100%

= X 100%

**= 107. 36%**

Average Percentage of Acetylcysteine in sample using phenolpthalein as indicator

=

=

**= 107. 6%**

## Discussion

Equation of acetylcysteine and sodium hydroxide:

According to BP 2011, the percentage purity content of acetylcysteine is ranging from 98. 0%-101. 0%. Based on the results obtained from the experiment, the average percentage purity of acetylcysteine assay using phenol red as indicator is 98. 3%. This shows that it falls within the range of BP’s requirement. However, compared to that of assay using phenolphthalein as indicator, the percentage purity of the acetycysteine assay is 107. 6% which is higher than the BP requirement for acetylcysteine assay. Both of the assays have the different percentage purities are due to different indicators being used in the titration. Phenol red and phenolphthalein have different pH range where phenol red changes colour from yellow to red over the pH range 6. 8-8. 4 while phenolphthalein changes from colourless to pink in the range of pH 8. 3-10. 0. [1] Both of the indicators have different end points when hydroxide ions are been added to react and remove the hydrogen ions in the assay. Therefore, it causes difference in time for the assay to reach the indicator’s end point and therefore affecting the result of the experiment where the percentage purity of assay using phenol red is lower than that of the assay using phenolphthalein as indicator.

According to BP 2011,

## Retention time of impurities in acetylcysteine

Compound

Time (min)

Acetylcysteine

About 6. 4

L-cystine

About 2. 2

L-cysteine

About 2. 4

2-methyl-2-thiazoline-4-carboxylic acid, originating in test solution

About 3. 3

N, N’-diacetyl-L-cystine

About 12. 0

N, S-diacetyl-L-cysteine

About 14. 0

The chromatograms obtained from the HPLC analysis of a fresh solution of acetylcysteine and an old solution of acetycysteine was examined.

**In cysteine 0. 5mg/mL,**

**Peak Retention Time/ min**

**Predicted Compound**

**Area**

**Concentration / %**

2. 018

L-cystine

87001

5. 2956

2. 323

L-cysteine

38097

2. 3189

2. 650

L-cysteine

39167

2. 3840

3. 008

Unknown

409128

24. 9029

3. 207

2-methyl-2-thiazoline-4-carboxylic acid, originating in test solution

1069503

65. 0987

## In Acetylcysteine 2. 5mg/mL [Old Sample]

## Peak Retention Time/ min

## Predicted Compound

## Area

## Concentration / %

2. 110

L-cystine

62935

0. 7214

3. 256

2-methyl-2-thiazoline-4-carboxylic acid, originating in test solution

78046

0. 8946

3. 756

2-methyl-2-thiazoline-4-carboxylic acid, originating in test solution

64342

0. 7375

5. 447

Unknown

1642356

18. 8255

5. 893

Unknown

1902857

21. 8115

6. 340

Acetylcysteine

2942118

33. 7241

13. 415

N, N’-diacetyl-L-cystine

1337263

15. 3284

16. 308

N, S-diacetyl-L-cysteine

694171

7. 9569

## In Acetylcysteine 8. 57mg/mL [Fresh Sample]

## Peak Retention Time/ min

## Predicted Compound

## Area

## Concentration / %

1. 930

L-Cystine

238606

0. 5948

3. 250

2-methyl-2-thiazoline-4-carboxylic acid, originating in test solution

31861

0. 0794

3. 786

2-methyl-2-thiazoline-4-carboxylic acid, originating in test solution

67023

0. 1671

4. 653

Unknown

151529

0. 3778

5. 023

Unknown

1433178

3. 5728

6. 972

Acetylcysteine

38007110

94. 7507

13. 623

N, S-diacetyl-L-cysteine

158211

0. 3944

16. 451

N, S-diacetyl-L-cysteine

25230

0. 0629

**Percentage Impurity =**

**In cysteine 0. 5mg/mL,**

**Peak Retention Time/ min**

**Total Area = 1642895**

**Percentage Impurity / %**

**Predicted Compound**

**Area**

2. 018

L-cystine

87001

5. 2956

2. 323

L-cysteine

38097

2. 3189

2. 650

L-cysteine

39167

2. 3840

3. 008

Unknown

409128

24. 9029

3. 207

2-methyl-2-thiazoline-4-carboxylic acid, originating in test solution

1069503

65. 0987

## Percentage Impurity of L-cystine =

= 5. 3%

## Percentage Impurity of L-cysteine =

= 4. 7%

## Percentage Impurity of Unknown =

= 24. 9%

## Percentage Impurity of 2-methyl-2-thiazoline-4-carboxylic acid =

= 65. 1%

## In Acetylcysteine 2. 5mg/mL [Old Sample]

## Peak Retention Time/ min

## Total Area : 8724087

## Percentage Impurity/ %

## Predicted Compound

## Area

2. 110

L-cystine

62935

0. 7214

3. 256

2-methyl-2-thiazoline-4-carboxylic acid, originating in test solution

78046

0. 8946

3. 756

2-methyl-2-thiazoline-4-carboxylic acid, originating in test solution

64342

0. 7375

5. 447

Unknown

1642356

18. 8255

5. 893

Unknown

1902857

21. 8115

13. 415

N, N’-diacetyl-L-cystine

1337263

15. 3284

16. 308

N, S-diacetyl-L-cysteine

694171

7. 9569

## Percentage Impurity of L-cystine =

= 0. 72%

## Percentage Impurity of 2-methyl-2-thiazoline-4-carboxylic acid

=

= 1. 6%

## Percentage Impurity of Unknown =

= 40. 6%

## Percentage Impurity of N, N’-diacetyl-L-cystine =

= 15. 3%

## Percentage Impurity of N, S-diacetyl-L-cysteine =

= 8. 0%

## In Acetylcysteine 8. 57mg/mL [Fresh Sample]

## Peak Retention Time/ min

## Total Area = 40113072

## Percentage Impurity / %

## Predicted Compound

## Area

1. 930

L-Cystine

238606

0. 5948

3. 250

2-methyl-2-thiazoline-4-carboxylic acid, originating in test solution

31861

0. 0794

3. 786

2-methyl-2-thiazoline-4-carboxylic acid, originating in test solution

67023

0. 1671

4. 653

Unknown

151529

0. 3778

5. 023

Unknown

1433178

3. 5728

13. 623

N, S-diacetyl-L-cysteine

158211

0. 3944

16. 451

N, S-diacetyl-L-cysteine

25230

0. 0629

## Percentage Impurity of L-cystine =

= 0. 59%

## Percentage Impurity of 2-methyl-2-thiazoline-4-carboxylic acid

=

= 0. 25%

## Percentage Impurity of Unknown =

= 4. 0%

## Percentage Impurity of N, N’-diacetyl-L-cystine =

= 0. 39%

## Percentage Impurity of N, S-diacetyl-L-cysteine =

= 0. 063%

There are 3 peaks mixed together in old sample where a new peak was formed before the previous peak had completed its round. This may cause some inaccuracy in the results obtained of finding the level of impurities.

## References

[1] Acid Base Indicators. Available at:

http://www. chemguide. co. uk/physical/acidbaseeqia/indicators. html

Last accessed: 31 December 2010

[2] British Pharmacopoeia Commission Secretariat of the Medicines and Healthcare products Regulatory Agency, British Pharmacopoeia 2011 Online. Available at:

http://www. pharmacopoeia. co. uk/bp2011/ixbin/bp. cgi? a= displayHYPERLINK " http://www. pharmacopoeia. co. uk/bp2011/ixbin/bp. cgi? a= display&r= YoJDskQKKhY&n= 1&id= 8073&tab= search"&HYPERLINK " http://www. pharmacopoeia. co. uk/bp2011/ixbin/bp. cgi? a= display&r= YoJDskQKKhY&n= 1&id= 8073&tab= search" r= YoJDskQKKhYHYPERLINK " http://www. pharmacopoeia. co. uk/bp2011/ixbin/bp. cgi? a= display&r= YoJDskQKKhY&n= 1&id= 8073&tab= search"&HYPERLINK " http://www. pharmacopoeia. co. uk/bp2011/ixbin/bp. cgi? a= display&r= YoJDskQKKhY&n= 1&id= 8073&tab= search" n= 1HYPERLINK " http://www. pharmacopoeia. co. uk/bp2011/ixbin/bp. cgi? a= display&r= YoJDskQKKhY&n= 1&id= 8073&tab= search"&HYPERLINK " http://www. pharmacopoeia. co. uk/bp2011/ixbin/bp. cgi? a= display&r= YoJDskQKKhY&n= 1&id= 8073&tab= search" id= 8073HYPERLINK "

http://www. pharmacopoeia. co. uk/bp2011/ixbin/bp. cgi? a= display&r= YoJDskQKKhY&n= 1&id= 8073&tab= search”&HYPERLINK “ http://www. pharmacopoeia. co. uk/bp2011/ixbin/bp. cgi? a= display&r= YoJDskQKKhY&n= 1&id= 8073&tab= search” tab= search

Last accessed: 31 December 2010

## Experimental 4: Determination of Level of Zinc in Acetylcysteine :

## Not more than than 10ppm Zn

## Method

1) 1. 00g sample was dissolved in 0. 001M HCL and diluted to 50ml with the same solvent (Solution 1).

2) Three solutions were then been prepared for analysis containing

a) 10ml solution 1 diluted to 20ml with 0. 001M HCl

b) 10ml solution 1 and 1ml of 5ppm zinc standard diluted to 20ml with 0. 001 M HCl

c)10ml solution 1 and 2ml of 5 ppm zinc standard diluted to 20ml with 0. 001M HCL.

3) An appropriate AA spectrophotometer was then used to measure the absorbance of each solution at 213. 8 nm giving the results tabulated below.

4) The content of zinc in the sample was calculated using the method of standard addition.

## Zinc AA Results

## Solution

## Absorbance

(a)

0. 056

(b)

0. 115

(c)

0. 173

## Results

## Gradient, m =

=

=

= 0. 234 ppm-1

y-intercept, c = 0. 056

Equation of trend line is therefore y = 0. 234x + 0. 056

| x-intercept| = level of zinc in solution (a)

Since y = 0. 234x + 0. 056

At x-intercept, y = 0

x =

x =

x = – 0. 2393 ppm

Level of zinc in solution (a) = |- 0. 2393|

## = 0. 2393 ppm

Since 10mL of Solution 1 is diluted to 20mL to produce solutions (a), (b) and (c), the level of zinc in solution 1 hence equals to twice of that in the diluted solution, i. e. 0. 2393 ppm x 2 = 0. 4786 ppm.

## Discussion

Standard addition is a method used to eliminate the matrix effect from the experiment, where in this case matrix effect is defined as absorptions caused by components in the solution other than the analyte. For simple matrices, a calibration curve can be used, but when complex or unknown matrices are present, standard addition method is preferred. In this test, zinc acts as the standard addition whereas acetylcysteine remains as the analyte. From the standard addition plot, extrapolation of the plot to the x-axis, where y= 0, gives the level of zinc in solution (a). Since Solution (a) is made from Solution 1 via 1: 2 dilution, the actual level of zinc in the undiluted solution, Solution 1, can be determined by multiplying the level of zinc in solution (a) by a 2. Therefore, the level of zinc in acetylcysteine is 0. 4786 ppm. This complies with the BP specification, i. e. not more than 10ppm.

In the BP monograph, it is also stated that lead, Pb may be used as heavy metals test for acetylcysteine. But a higher concentration of reference

solution of lead is required, i. e. 10 ppm lead is needed. In standard addition, the smaller the amount of standard addition used the better, to minimise disturbance in the matrix. In this case, only a small amount of the reference solution will be needed, but the smaller the amount, the higher the percentage error. Even if a bigger amount of the reference solution is used, a larger dilution factor will be used before the absorbance of the sample solutions can be determined via a spechtrophotometer. Therefore, zinc test is preferred over the lead test in this experiment.

## References

[1] (2005). McGraw-Hill Encyclopedia of Science and Technology. 5th Edition. The

McGraw-Hill Companies, Inc.

[2] British Pharmacopoeia Commission Secretariat of the Medicines and Healthcare products Regulatory Agen