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# Development of Method for Determination of Acetylcysteine in Hard Gelatin Capsules by Hplc

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***SUMMARY:*** *An HPLC-DAD method was developed and validated for determination of acetylcysteine in hard gelatin capsules. The chromatographic conditions are as follows: Column: L1-C18 (4.6 mm x 250 mm; 5 µm). Detector UV, λ = 214 nm. Mobile phase: Dissolve 6.8 g of monobasic potassium phosphate in 1000 mL of water, filter through a membrane filter (0.45 µm porosity), and degas. Adjust with phosphoric acid to a pH of 3.0. Flow rate: 1.5 mL/min. Injection volume: 5 µL. Temperature: room temperature.*

***Keywords:*** *Acetylcysteine, hard gelatin capsules, HPLC, method validation, quantititation*

## I. Introduction

In some countries like Vietnam or other ASEAN countries, acetylcysteine is mainly indicated as a mucolytic agent, supportive treatment in respiratory diseases [1, 3]. In the world, depending on different countries, this substance has been approved with a variety of indications such as antidote for acetaminophen and certain toxics, or in contrast-induced nephropathy, as well as diagnostic bronchial studies [5]. This study is conducted to provide a reference method to help domestic drug manufacturers to check the quality of their product with available conditions at the facility, and serve as a tool in the field of drug research and development.

## II. Materials and analytical method

**Instruments**

Instruments have been calibrated according to ISO/IEC 17025 and GLP includes:
- HPLC model Agilent 1200, Shimadzu LC-20A
- Analytic scale Sartorius BSA 224S
- Glassware.

## III. Reference standard and Reagents

- Reference standard supplied by Cassel Research Laboratories PVT. LTD (India), Assay 99.79%, Lot No.: CA/WS/197.
- Solvents for HPLC: Phosphoric acid, monobasic potassium phosphate, DL-phenylalanine.

## IV. Methodology

*Chromatographic condition*
- Column Luna RP-C18 (4.6 mm x 250 mm; 5 µm)
- Detector: UV, λ = 214 nm
- Flow rate: 1.5 mL/min
- Injection volume: 5 µL
- Column temperature: room temperature
- Mobile phase: Dissolve 6.8 g of monobasic potassium phosphate in 1000 mL of water, filter through a membrane filter (0.45 µm porosity), and degas. Adjust with phosphoric acid to a pH of 3.0.
- *Diluting medium*: Dissolve 1.0 g of sodium metabisulfite in 2000 mL of water.
- *Internal standard solution*: Dissolve about 1 g of DL-phenylalanine in 200 mL of freshly prepared *Diluting medium*.

***Analytical Method***

- *Standard preparation*: Dissolve an accurately weighed quantity of Acetylcysteine RS in *Diluting medium* to obtain a solution having a known concentration of about 10 mg per mL. Pipet 10.0 mL of this solution and 10.0 mL of *Internal standard solution* into a 200 mL-volumetric flask, dilute with *Diluting medium* to volume, and mix to obtain a *Standard preparation* having a known concentration of about 0.5 mg/mL.

- *Assay preparation*: Take not less than 20 capsules and weigh accurately the contents. Take accurately weighed portion of capsule contents, equivalent to about 1 g of acetylcysteine, to a 100 mL-volumetric flask, dissolve with *Diluting medium* and mix. Add *Diluting medium* to volume and filter. Pipet 10 mL of the filtrate and 10 mL of *Internal standard solution* into a 200 mL-volumetric flask, dilute with *Diluting medium* to volume, and mix.
- *Procedure*: Separately inject equal volumes (about 5 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for major peaks.
+ The relative retention times are about 0.5 for Acetylcysteine and 1.0 for DL-phenylalanine.
+ Chromatograph the *Standard preparation*: the relative standard deviation for replicate injections is not more than 2.0%, and the resolution, R, between Acetylcysteine and DL-phenylalanine is not less than 6.
- *Cauculation*: Calculate the content, in %, of $C_5H_9NO_3S$ in capsule taken by the formula:

$$(\%) = C \times \frac{R_U}{R_S} \times \frac{2000}{m} \times \frac{W_{Caps}}{200} \times 100$$

in which:
C: the concentration, in mg per mL, of Acetylcysteine RS in the *Standard preparation*; $R_U$ and $R_S$: the ratios of the peak response of Acetylcysteine to that of DL-phenylalanine obtained from the *Assay preparation* and the *Standard preparation*, respectively; m: the weight of sample (mg); $W_{Caps}$: the average weight of capsule contents (mg); 200: the labeled amount of Acetylcysteine per capsule (mg).

***Method validation***
Performed according to the ASEAN guideline for method validation.

## V. Results

***System suitability***
- Requirement: There is no interfering Acetylcysteine (ACC) peak and Internal standard (IS) peak (DL-phenylalanine) in the placebo sample.
- The placebo sample: only contain excipients, not Acetylcysteine or DL-phenylalanine.
- The placebo sample were prepared and analysed as per the analytical method for Assay to determine whether excipients interfere with Acetylcysteine peak and Internal standard peak.
- *Internal standard solution*: Use *Internal standard solution* of Precision test.
- *Placebo solution:* Weigh 0.2681 g of placebo sample to a 50 mL-volumetric flask, dissolve with *Diluting medium* and mix. Add *Diluting medium* to volume and filter. Pipet 5 mL of the filtrate and 5 mL of *Internal standard solution* into a 100 mL volumetric flask, dilute with *Diluting medium* to volume, and mix.
- *Acetylcysteine standard solution*: Weigh 25.3 mg of Acetylcysteine RS to a 50 mL-volumetric flask, dissolve and make to volume with *Diluting medium* and mix.
- *Standard solution:* Use *Standard solution* of Precision test.
- *Test solution:* Use *Test solution* of Precision test.
- *Procedure:* Separately inject equal volumes of the *blank solution* (*Diluting medium*), the *placebo solution*, the *Acetylcysteine standard solution*, the *standard solution* and the *test solution*. Record the chromatogram for all injections.

Discussion:
- The retention time of Acetylcysteine (ACC) peak from the *Test solution* chromatogram is about 6.8 minutes.
- The retention time of Acetylcysteine (ACC) peak from the *Standard solution* chromatogram is about 6.8 minutes.
- The retention time of Internal standard (IS) peak (DL-phenylalanine) from the *Test solution* chromatogram is about 13.7 minutes.
The retention time of Internal standard (IS) peak (DL-phenylalanine) from the *Standard solution* chromatogram is about 13.7 minutes.
- No significant deviation from the baseline was observed (in both the *blank solution* and the *placebo solution*). So, the excipients have not contributed to any interfering peak.
Specificity Conclusion**:**
The method is specific for Acetylcysteine in Acetylcysteine hard capsule.
***Lineartiy***
- Requirement: Correlation coefficient (r) ≥ 0.998
Preparing mixed standard solutions at concentrations of Acetylcysteine (ACC) of 0.254 mg/mL – 0.752 mg/mL. The concentrations of Internal standard (DL-phenylalanine) in the above standard solutions about 0.25 mg/ml. The concentration of Acetylcysteine was plotted against the ratio of the area of the Acetylcysteine peak to that

of the Internal standard (IS) peak in the linearity graph. Correlation coefficient was calculated between concentration (mg/mL) and the ratio of the area of the Acetylcysteine peak to that of the Internal standard (IS). Results are shown in Table 1 and Figure 1.

***Table 1.*** *The results of Linearity*

| % Level | Weight of ACC (mg) | Concentration of ACC (mg/mL) | Ratio of peak response of ACC to IS |
|---|---|---|---|
| 50% | 25.4 | 0.254 | 0.897 |
| 80% | 40.1 | 0.401 | 1.392 |
| 100% | 50.3 | 0.503 | 1.768 |
| 120% | 60.7 | 0.607 | 2.165 |
| 150% | 75.2 | 0.752 | 2.713 |
| Slope | 3.6619 | | |
| Intercept | -0.0565 | | |
| Correlation coefficient (r) | 0.9996 | | |

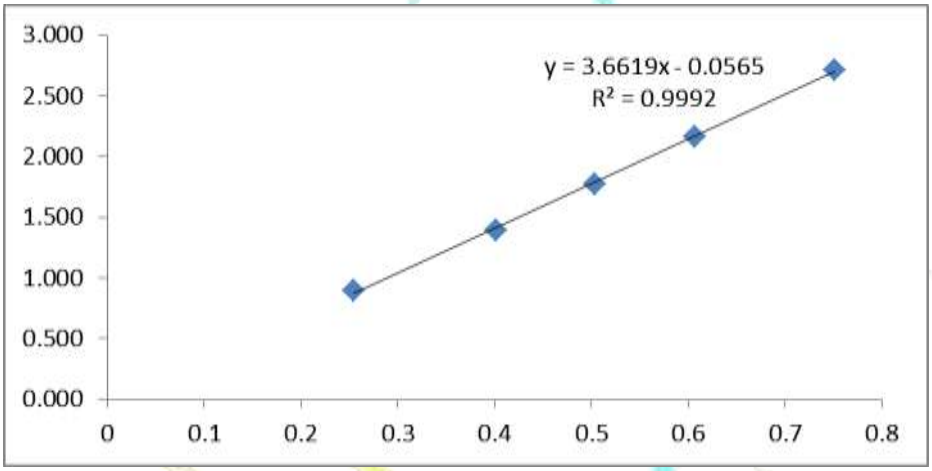


***Figure 1.*** *Linear correlation between Acetylcysteine concentration and the ratio of peak response of ACC to IS*

**Conclusion:** The analytical method for the Assay test of Acetylcysteine hard capsule shows linearity of Acetylcysteine concentrations versus ratio of peak response of ACC to IS at the concentration range of 0.254 mg/mL – 0.752 mg/mL (r = 0.9996).

***Precision***

- *Internal standard solution*: Weigh 1.0026 g of DL-phenylalanine RS into a 200 mL-volumetric flask. Dissolve and make to volume with *Diluting medium*.
- *Standard solution:* Weigh 50.3 mg of Acetylcysteine RS into a 100 mL-volumetric flask. Take 5.0 mL of *Internal standard solution* in this volumetric flask and make to volume with *Diluting medium*.
- *Test solution:* Take not less than 20 capsules and weigh accurately the contents. Weigh about 0.768 g of capsule contents (equivalent to about 500 mg of Acetylcysteine), into a 50 mL-volumetric flask, dissolve with *Diluting medium* and mix. Add *Diluting medium* to volume and filter. Pipet 5 mL of the filtrate and 5 mL of *Internal standard solution* into a 100 mL-volumetric flask, dilute with *Diluting medium* to volume, and mix.

***System precision***

- Requirement:
+ RSD of replicate injections of *standard solution*: not more than 2.0%.
+ The resolution, R, between Acetylcysteine and DL-phenylalanine: not less than 6.

Six injections of *standard solution* were performed. Results are shown in the table below.

- Results:
+ The relative retention times are about 0.5 for Acetylcysteine and 1.0 for DL-phenylalanine.

+ The relative standard deviation for replicate injections: RSD = 0.65% (not more than 2.0%).
+ The resolution, R, between Acetylcysteine and DL-phenylalanine: R = 6.9 (not less than 6).
Results are shown in Table 2.

***Table 2.*** *The results of System precision*

| No. | Retention time of ACC (min) | Retention time of IS (min) | Ratio of peak response of ACC to IS | Resolution between ACC and IS |
|---|---|---|---|---|
| 1 | 6.798 | 13.649 | 1.772 | 6.86 |
| 2 | 6.826 | 13.622 | 1.746 | 6.84 |
| 3 | 6.781 | 13.691 | 1.774 | 6.82 |
| 4 | 6.789 | 13.725 | 1.775 | 6.84 |
| 5 | 6.785 | 13.716 | 1.776 | 6.89 |
| 6 | 6.811 | 13.748 | 1.764 | 6.86 |
| **Average** | **6.798** | **13.692** | **1.768** | **6.9** |
| **RSD (%)** | **0.25** | **0.35** | **0.65** | **0.35** |

Discussion: Results are within acceptance criteria.

***Method precision***
- Requirement: RSD not more than 2%.
Method precision was determined by preparing 6 samples (*Test solution*) as described in the analytical method. Results are shown in the Table 3.

***Table 3.*** *The results of Method precision*

| Weight of Acetylcysteine RS (mg) = 50.3<br>Ratio of peak response of ACC to IS in *standard solution* = 1.768 | | | |
|---|---|---|---|
| No. | Weight of *sample* (g) | Ratio of peak response of ACC to IS in *test solution* | Result (%) |
| 1 | 0.7772 | 1.789 | 100.1 |
| 2 | 0.7685 | 1.779 | 100.7 |
| 3 | 0.7684 | 1.770 | 100.2 |
| 4 | 0.7712 | 1.749 | 98.6 |
| 5 | 0.7693 | 1.779 | 100.6 |
| 6 | 0.7689 | 1.741 | 98.4 |
| **Mean** | | | **99.8** |
| **RSD (%)** | | | **0.99** |

Conclusion: Results indicate that the method is precise (RSD = 0.99%).

***Intermediate precision***
- Requirement: RSD not more than 2%.
Intermediate precision of the method is determined by analysing 6 samples (*Test solution*) as described in the analytical method by 2 different analysts at different days and/or with different instruments. Results are shown in the Table 4.



***Table 4.*** *The results of Intermediate precision*

| No. | **Analysist 1**<br>Weight of Acetylcysteine RS (mg) = 50.3<br>Ratio of peak response of ACC to IS in *Standard solution* = 1.768 | | **Analysist 2**<br>Weight of Acetylcysteine RS (mg) = 50.7<br>Ratio of peak response of ACC to IS in *Standard solution* = 1.764 | |
|---|---|---|---|---|
| | Ratio of peak response of ACC to IS in *Test solution* | Result (%) | Ratio of peak response of ACC to IS in *Test solution* | Result (%) |
| 1 | 1.789 | 100.1 | 1.746 | 99.7 |
| 2 | 1.779 | 100.7 | 1.760 | 99.9 |
| 3 | 1.770 | 100.2 | 1.750 | 100.2 |
| 4 | 1.749 | 98.6 | 1.754 | 100.1 |
| 5 | 1.779 | 100.6 | 1.757 | 99.7 |
| 6 | 1.741 | 98.4 | 1.783 | 102.1 |
| **Mean: 99.8%**<br>**RSD: 0.99%** | | | **Mean: 100.3%**<br>**RSD: 0.90%** | |
| **Average: 100.0%**<br>**RSD: 0.94%** | | | | |

***Accuracy***

- Requirement: Recovery rate is in the range of 98.0-102.0%, RSD ≤ 2%.

Accuracy for the Assay test was assessed by spiking nine placebo sample preparations. Three samples were spiked with the equivalence of 80, 100, 120% of the nominal assay concentration: Transfer accurately quantities of Acetylcysteine RS, , equivalent to about 40 mg, 50 mg, 60 mg of Acetylcysteine to 100-mL volumetric flasks which had contained an amount of placebo about 27 mg. Take 5.0 mL of *Internal standard solution* into these above 100-mL volumetric flasks. Dissolve and dilute with *Diluting medium* to volume, and mix. Filter through a 0.45 µm membrane filter. Results are shown in Table 5.

***Table 5.*** *Results for Accuracy*

| **Sample** | **Weight of placebo (mg)** | **ACC added (mg)** | **Ratio of peak response of ACC to IS** | **ACC Recovered (mg)** | **% Recovered** |
|---|---|---|---|---|---|
| 80% | 27.4 | 40.1 | 1.383 | 39.3 | 98.1 |
| 80% | 28.6 | 40.4 | 1.418 | 40.3 | 99.8 |
| 80% | 26.9 | 40.8 | 1.408 | 40.1 | 98.2 |
| | | | | **Mean** | **98.7** |
| | | | | **RSD (%)** | **1.00** |
| 100% | 27.7 | 50.5 | 1.783 | 50.7 | 100.4 |
| 100% | 27.2 | 50.7 | 1.746 | 49.7 | 98.0 |
| 100% | 28.1 | 50.9 | 1.780 | 50.7 | 99.5 |
| | | | | **Mean** | **99.3** |
| | | | | **RSD (%)** | **1.26** |
| 120% | 26.8 | 60.1 | 2.103 | 59.8 | 99.5 |
| 120% | 27.5 | 60.3 | 2.084 | 59.3 | 98.4 |
| 120% | 28.6 | 60.7 | 2.148 | 61.1 | 100.7 |
| | | | | **Mean** | **99.5** |
| | | | | **RSD (%)** | **1.16** |
| ***Mean*** | | | | | ***99.2*** |
| ***RSD (%)*** | | | | | ***1.06*** |

Ratio of peak response of ACC to IS in *standard solution* = 1.768

Discussion: Mean sample recovery is 99.2% of the theoretical content across 80 to 120% of the nominal content, RSD = 1.06%, demonstrating excellent accuracy.

Conclusion: The analytical method for the Assay test of Acetylcysteine in the hard capsule is accurate.

***Range***

As shown in Accuracy of method:

- At the concentration of 80%: Recovery = 98.7%, RSD = 1.00%.
- At the concentration of 100%: Recovery = 99.3%, RSD = 1.26%.
- At the concentration of 120%: Recovery = 99.5%, RSD = 1.16%.

Conclusion: These results show that at concentrations of Acetylcysteine from 80% to 120% of the nominal content, the accuracy and precision are good. The method has a range from 80% to 120% of the nominal content.

## VI. Conclusion

The study has developed and validated a method to determine Acetylcysteine in hard gelatin capsules by HPLC. The experimental results show that the selected chromatographic conditions Column: L1-C18 (4.6 mm x 250 mm; 5 µm); detector UV, λ = 214 nm; mobile phase: dissolve 6.8 g of monobasic potassium phosphate in 1000 mL of water, filter through a membrane filter (0.45 µm porosity), and degas, adjust with phosphoric acid to a pH of 3.0; flow rate: 1.5 mL/min; injection volume: 5 µL; temperature: room temperature, are suitable for determination of Acetylcysteine in hard gelatin capsules.

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