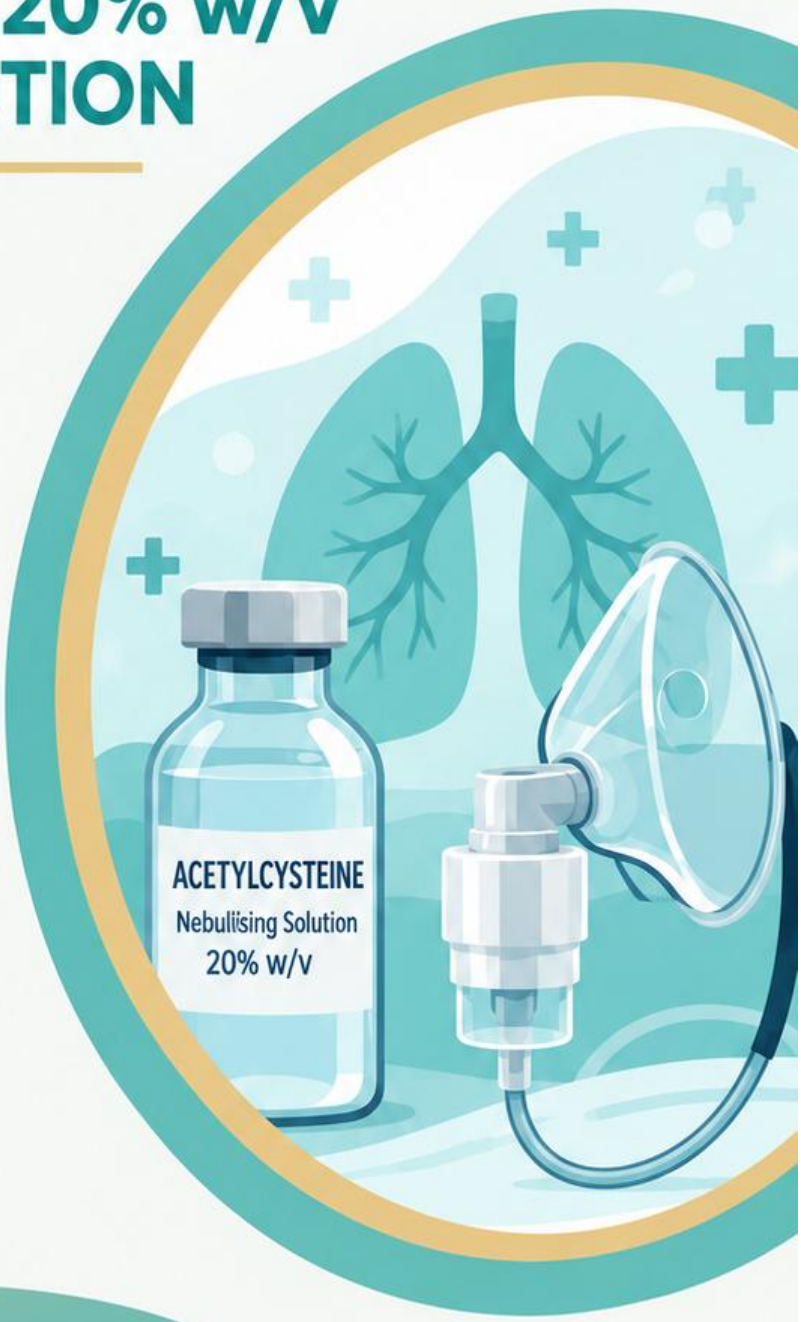
SEPTEMBER 2026

University of the Witwatersrand

INTEGRATED PRODUCT DEVELOPMENT:
ACETYLCYSTEINE 20% w/v NEBULISING SOLUTION



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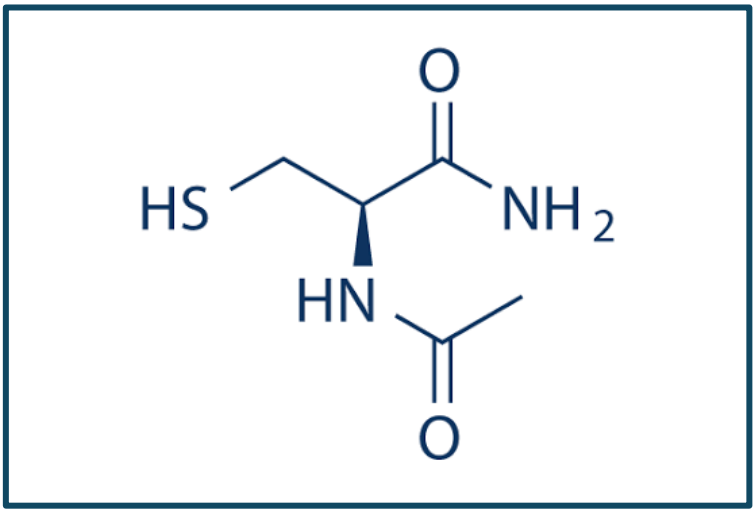
PHARMACEUTICS

INTRODUCTION

Chronic respiratory conditions place a substantial burden on global health, with chronic obstructive pulmonary disease (COPD) alone affecting approximately 4% of the global population and ranking as third leading cause of death worldwide (NCD Alliance, 2024). These conditions, such as cystic fibrosis, emphysema and chronic bronchitis, are characterized by the overproduction of thick, viscous mucus within the airways. Impaired clearance of mucus obstructs airflow, promotes recurrent infections, and significantly reduces patients' quality of life. Effective mucolytic therapy is therefore important to managing these conditions and preventing disease progression.

To address this, our assignment focuses on the development and formulation of an Acetylcysteine 20% w/v Nebulizing Solution, designed for direct delivery to the respiratory tract via inhalation. Acetylcysteine is an N-acetyl derivative of the amino acid L-cysteine, and its free thiol (-SH) group is responsible for its mucolytic action. It cleaves disulfide bonds that cross link mucoprotein stands in the respiratory mucus, reducing its viscosity and making it easier for patients to expectorate (Pfizer, 2023). To ensure the drug remains chemically stable in solution, our formulation incorporates excipients for pH control, tonicity and oxidation.

Solutions are a well-suited dosage form for nebulizing solutions as they form a homogenous, single-phase system that can reliably be aerosolised into a fine mist without the risk of dose non-uniformity that is seen with suspensions and emulsions. Our formulation is designed for direct nebulization, prioritising chemical stability, patient safety, and ease of administration.



DOSAGE FORM

CHOSEN DOSAGE FORM: NEBULISING SOLUTION

The proposed dosage form for this product is a sterile aqueous nebulising solution containing 20% w/v acetylcysteine (200 mg/mL). The formulation is designed for administration via a nebuliser, which converts the liquid solution into a fine aerosol for inhalation into the respiratory tract, where it acts as a mucolytic to decrease mucus viscosity and facilitate expectoration and clearance. (Yan et al., 2025)

WHY WAS THIS DOSAGE FORM CHOSEN?

A nebulising solution was selected as the proposed dosage form because the intended site of action of acetylcysteine is the respiratory tract. Nebulisers are the oldest yet modern method for delivering drugs directly to the lungs (Martin and Finlay, 2015). An aqueous solution was selected because liquid formulations are compatible with nebuliser devices and can be aerosolised to produce droplets suitable for inhalation.

A solution also provides a homogeneous dosage form in which the active pharmaceutical ingredient is dissolved throughout the formulation. Unlike semisolid dosage forms such as creams and ointments, which are designed primarily for application to the skin or mucous membranes, a liquid formulation can be aerosolised for pulmonary delivery. For these reasons, a sterile aqueous nebulising solution was selected as the most appropriate dosage form for the proposed acetylcysteine product

ADVANTAGES TO A NEBULIZING SOLUTION

1. **Homogeneous preparation**
   Acetylcysteine is dissolved in the liquid vehicle, meaning that the API is distributed uniformly throughout the formulation. This helps ensure that each measured volume of solution contains a consistent concentration of acetylcysteine.
2. **No sedimentation or redispersion required**
   Unlike a suspension, the proposed formulation does not contain undissolved solid drug particles that can settle during storage. Therefore, there is no need to shake or redisperse the formulation before administration.
3. **Consistent drug concentration**
   As the API is dissolved uniformly throughout the formulation, each measured volume should contain the same concentration of acetylcysteine. This assists with accurate and consistent dosing when the correct volume is administered.
4. **Suitable for direct aerosolisation**

As the proposed product is already in liquid form, it can be placed into a compatible nebuliser for conversion into an aerosol. No additional dissolution or preparation of a solid dosage form is required before nebulisation.

DISADVANTAGES TO A NEBULIZING SOLUTION

1. **Requires a nebuliser device**
   Unlike some other dosage forms, a nebulising solution cannot be administered without specialised equipment. It requires a compatible nebuliser device to convert the liquid formulation into an inhalable aerosol. This means that the patient must have access to the necessary equipment before the medication can be administered.
2. **Potential physical instability**
   The formulation must remain a true solution throughout its shelf life. Changes in formulation conditions could potentially result in precipitation or the formation of visible particles, which would affect the homogeneity and suitability of the product.
3. **Risk of microbial contamination**
   The presence of water in the formulation may create a favourable environment for microbial growth. This is a major concern because the formulation and aerosol are intended for delivery to the respiratory tract. Therefore, effective microbial control and suitable storage conditions are important for maintaining product quality and safety.
4. **Potential chemical instability**
   A liquid formulation may be susceptible to chemical degradation during storage if appropriate formulation conditions are not maintained. Factors such as pH, temperature, light and exposure to oxygen may therefore need to be controlled to maintain product quality throughout its shelf life.

FORMULATION CONSIDERATIONS

1. **API solubility**

   As the proposed product is a nebulising solution, acetylcysteine must be sufficiently soluble in the chosen vehicle to produce a clear and homogeneous formulation at the required concentration of 200 mg/mL. Acetylcysteine must therefore dissolve completely in the selected vehicle and remain in solution throughout the product's shelf life. The formation of undissolved particles or precipitation should be avoided, as this could affect both the uniformity of the dose and the suitability of the formulation for nebulisation.

2. **Choice of Vehicle**

   The selection of a suitable vehicle is an important consideration in the formulation of the proposed acetylcysteine nebulising solution. As the dosage form is a true solution, the vehicle acts as the medium in which the active pharmaceutical ingredient is dissolved. The vehicle must be suitable for pulmonary administration and must be capable of producing a stable and homogeneous solution at the required concentration of 200 mg/mL. An appropriate vehicle is therefore necessary to maintain the homogeneity, stability and suitability of the final product for nebulisation.

3. **pH**

   The pH of the formulation is an important consideration because it can influence the stability and solubility of the active pharmaceutical ingredient. This could result in degradation or precipitation of the acetylcysteine. pH control is also important because the product is intended for direct administration to the respiratory tract, and an unsuitable pH may contribute to irritation during inhalation.

4. **Tonicity and osmolarity**

   A formulation with an unsuitable osmolality may affect its tolerability and potentially contribute to airway irritation during inhalation. The tonicity of the formulation should be carefully considered during product development to ensure that the final solution is appropriate and well tolerated for pulmonary administration.

5. **Microbial Control**

   Microbial control is an important consideration during the formulation and manufacture of the proposed nebulising solution. As the product is an aqueous formulation intended for administration to the respiratory tract, microbial contamination could affect the quality and safety of the product.

6. **Chemical and Physical Stability**

   Chemical stability is important to ensure that acetylcysteine does not undergo degradation that could reduce the quality of the product. Physical stability is also necessary to ensure that the formulation remains a clear and homogeneous solution without precipitation or other undesirable changes during storage.

USAGE CONSIDERATIONS

1. **Compatibility with the Nebuliser Device**
   The acetylcysteine nebulising solution should be used with a compatible nebuliser device. The patient should follow the manufacturer's instructions to ensure that the formulation is correctly aerosolised and administered.
2. **Correct Dose and Volume**
   The correct prescribed volume of the acetylcysteine solution should be placed into the nebuliser to ensure that the intended dose is administered.
3. **Cleaning of the Nebuliser**
   The nebuliser should be cleaned according to the manufacturer's instructions after use to prevent the accumulation of medication residue and minimize contamination.

4. Prevention of Contamination
Appropriate hygiene and handling should be maintained when preparing and administering the solution to minimize the risk of contaminating the formulation or nebuliser equipment.

TRANSPORT CONSIDERATIONS

1. **Protection from Temperature Extremes**
   The product should be transported under appropriate temperature conditions to prevent excessive heat or cold from affecting the stability of the formulation.
2. **Protection from Physical Damage**
   The packaging should protect the product from breakage, leakage or damage during handling and transportation.
3. **Prevention of Contamination**
   The container should remain securely sealed during transportation to protect the formulation from contamination.
4. **Protection from Light**
   Where required by the product's stability requirements, appropriate packaging should protect the formulation from exposure to light during transportation.

ROUTE OF ADMINISTRATION

The proposed dosage form is a **20% w/v acetylcysteine nebulising solution**, administered by inhalation using a nebuliser. A nebuliser converts the liquid solution into fine aerosol droplets that can be inhaled into the respiratory tract. Acetylcysteine may be administered using conventional plastic or glass nebulisers, with the device required to generate an appropriate range of particle sizes for retention within the respiratory tract. The solution may be administered through a mouthpiece, face mask or tracheostomy, depending on the patient's clinical requirements (DailyMed, 2025).

This route was selected because acetylcysteine is used as a mucolytic acting on respiratory secretions. Inhalation allows the formulation to be delivered directly to the respiratory tract, where acetylcysteine can interact with mucus and reduce its viscosity. A nebulising solution is therefore appropriate for patients requiring acetylcysteine therapy through the respiratory route, while also providing an alternative route for patients who may not be suitable for oral administration.

ADVANTAGES TO INHALATION USING A NEBULISER

1. **Direct delivery to site of action**
   The drug is delivered directly to the respiratory tract, allowing acetylcysteine to act on the mucus where its mucolytic effect is required rather than relying on systemic distribution to reach the airways.
2. **Avoids first-pass metabolism**
   Unlike other acetylcysteine formulations, this one does not go through the GI tract, hence it is not absorbed. This improves local bioavailability and preserves more of the active drug.
3. **Rapid local effect**
   Direct delivery of acetylcysteine to the airways allows the drug to come into contact with respiratory mucus at the site of administration. This makes nebulisation useful when a local mucolytic effect is required, particularly in patients with significant airway secretions.
4. **Lower systemic dose requires**
   Smaller total doses are needed compared to oral or IV, this is because the drug is delivered locally. The decreases dose of nebulizing solution achieves the same therapeutic effect at the target sites, as a larger dose of oral/IV, with reduced risks of systemic side effects.
5. **Alternative to oral mucolytics**
   The nebulizing solution is useful for patients who have difficulty swallowing, is unconscious, intubated or is unable to take oral medication since the solution is administered via a nebulizer

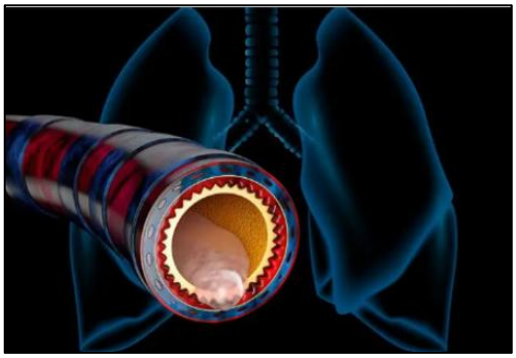
DISADVANTAGES TO INHALATION USING A NEBULISER

- **Variable lung deposition**
  The fraction of drug reaching the lower respiratory tract can vary widely depending on the type of nebulizer, breathing pattern and patient technique. This makes the dose less predictable than other controlled routes.
- **Requires equipment and patient cooperation**
  Effective dosage depends on the functionality of the equipment (compressor, mouthpiece, etc.), the patient's ability to remain appropriately positioned and breathe through the device during treatment, which may be challenging in emergency or resource-limited settings.
- **Risk of bronchospasm**
  Acetylcysteine has a sulphurous odour and can act as a respiratory irritant in some patients. This could trigger reflex bronchospasm or coughing, particularly in those with asthma or hyperreactive airways.
- **Device cleaning and hygiene burden**
  Nebulizing components must be cleaned after every use to prevent microbial growth and biofilm formation. This adds a maintenance burden and risk of infection if not properly cleaned.
- **Not ideal for impaired patients**
  Patients with cognitive impairment, severe respiratory distress or reduced ability to cooperate may have difficulty using a mouthpiece or mask correctly. This can reduce effective aerosol delivery to the respiratory tract.

CHOSEN AILMENT

Viscous mucus secretions in chronic respiratory disorders

THE PHYSIOLOGICAL BACKGROUND



Chronic respiratory disorders are a group of diseases that disrupt the normal functions of the lungs and airways (World Health Organization, 2026). While under normal physiological conditions, mucus secretions play an essential role in the immunity of the respiratory airway by trapping and expelling unwanted and dangerous inhaled particles (Cleveland Clinic, 2024), a range of chronic respiratory disorders can increase mucus viscosity, often resulting in symptoms such as wheezing, shortness of breath, and a wet cough, all of which are commonly seen in practice. The increasing viscosity of mucus relies heavily on the concentration of mucoprotein and deoxyribonucleic acid (DNA). When cells break down, their contents, including DNA, are released into the airway, causing the secretions to become increasingly opaque, discoloured, and thick. This is frequently seen in conditions such as:

- **Cystic fibrosis** — a hereditary condition affecting the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which controls chloride movement in and out of type 2 alveolar cells, responsible for producing mucus. The malfunctioning of this gene results in the production of sticky and thick mucus that clogs the lungs. Unfortunately, cystic fibrosis does shorten life expectancy; with current treatment, the average lifespan for a patient with this condition is 50 to 60 years. Cystic fibrosis presents with symptoms such as wheezing, recurrent respiratory infections, a persistent cough, and exercise intolerance. However, screenings are performed on newborns, so diagnosis often takes place before the baby is 12 months old, prior to the onset of symptoms. Treatment includes loosening tight mucus and initiatives to prevent infections (Mayo Clinic, 2024).
- **Emphysema** — smoking is found to be the main cause of emphysema, a condition in which the walls of the alveoli lose their elasticity over time. When people smoke, substances in the smoke irritate the walls of the lungs, causing them to become chronically inflamed. This inflammation reduces the elasticity of the lung walls, and as a result, the lungs have a reduced clearance ability due to their decreased ability to recoil and force substances out, causing both air and

mucus to become trapped and build up in the lungs. Common symptoms include breathlessness, severe tiredness, and a persistent wet cough. Treatment includes measures to slow the progression of lung damage, such as preventing infections, stopping smoking, and administering corticosteroids to reduce inflammation. Other treatments aim to improve lung function, such as mucolytics and bronchodilators (Cleveland Clinic, 2026).

- **Bronchitis** — a respiratory disorder commonly caused by a virus or smoking, which irritates the airway passage, bringing about swelling and a buildup of mucus in the trachea and bronchi. The main symptom of bronchitis is a cough, caused by the lungs attempting to rid themselves of the irritants causing the inflammation and mucus in the airways. Other symptoms include dyspnoea, fatigue, and fever, as the immune system attempts to kill off the virus responsible. Bronchitis can be contracted via inhalation of irritants such as airborne toxins or smoke; alternatively, virus-induced bronchitis can be spread through the air. Management of bronchitis is usually symptomatic, with medications such as mucolytics and bronchodilators (Cleveland Clinic, 2022).
- **Tuberculosis** — an illness caused by *Mycobacterium tuberculosis*, an airborne bacterium that mainly affects the lungs. When the bacterium destroys cells in the lungs, it leads to an increase in viscous sputum production (Cleveland Clinic, 2026). Tuberculosis affects approximately a quarter of the population. It can exist in two stages: an inactive form and an active form, with only the active form being contagious. Key symptoms include chills, night sweats, coughing up sputum or blood, weight loss, and chest pain. Tuberculosis can be treated with antibiotics, as well as medications to manage symptoms, including mucolytics.
- **Chronic obstructive pulmonary disease** — a group of chronic lung disorders that restrict airflow in the respiratory tract as a consequence of an inflammatory response from the immune system, as well as an increase in mucus secretions, congesting the lungs. COPD results in an increase in lung compliance, a condition in which the elasticity of the lungs decreases. Air flows easily into the lungs, but because the lungs are unable to recoil, air and secretions cannot be forced out, further clogging the lungs. Symptoms include wheezing, breathlessness, and a chronic cough. Treatment depends on the specific nature of the illness, although symptoms are generally managed with mucolytics, bronchodilators, and corticosteroids (Kanchustambham & Brown, 2026).

These conditions are severely worsened by acute viral infections, heavy air pollutants, and tobacco smoke; if left untreated, they can result in life-threatening complications and death (Abrami, 2024).

OUR PRODUCT

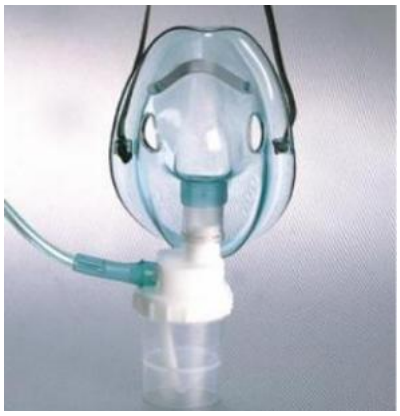
We have developed a 20% w/v acetylcysteine nebulizing solution. This solution falls under a class of drugs called mucolytics — medications that loosen thick mucus in the chest, making it easier to cough up (Cleveland Clinic, 2023). At the molecular level, acetylcysteine specifically uses its sulfhydryl group to split disulphide bonds of mucoproteins — proteins largely responsible for the viscosity of mucus — making mucus less viscous and easier for the cilia in the respiratory tract to expel.

Furthermore, acetylcysteine has been shown to lessen the frequency of flare-ups in chronic obstructive pulmonary disease (Division of Clinical Pharmacology, University of Cape Town, 2022), and it acts as an antioxidant; this is especially useful in protecting the lungs from free radicals produced by cells during an inflammatory response (J.K. Aronson, 2016). Acetylcysteine is unaffected by the presence of DNA, maximizing its efficacy (DailyMed, 2026).

Formulating acetylcysteine as a nebulizing solution allows the aqueous-based solution to be turned into a mist that is inhaled through a mouthpiece or a mask (Cleveland Clinic, 2026). Acetylcysteine is frequently administered through a jet nebulizer, a type of nebulizer that creates a mist by compressing air and effectively administers medication through tidal breathing.

When medication is inhaled, it is delivered directly to the surface of the lungs where it is active, allowing a greater concentration to reach the site of action, as well as fewer side effects due to lower systemic concentration. Inhaling a mist also requires less effort compared to swallowing a tablet or a liquid, making it ideal for compliance in ill patients.

ADMINISTRATION OF THE SOLUTION



Acetylcysteine nebulizing solution is generally packaged in a 30 mL glass vial. When administering via nebulizer, the average dose of the 20% solution for both children and adults is 3–5 mL, 3–4 times a day. The duration of treatment depends on the nature of the condition; in some cases, it may be used as a daily treatment, whereas in others it may only be used during exacerbations.

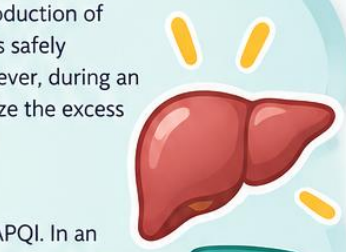
If a mask is used for administration, one must take care to cover and enclose both the nose and mouth within the mask. When a mouthpiece is used, ensure the patient's lips are well sealed around the mouthpiece to optimize effects. When nebulizing, a sticky remnant may be left on the patient's face; this residue can easily be wiped off with water.

Side effects may include fever, nausea and vomiting, stomatitis, headache, and bronchospasm. Caution should be taken in patients with a history of asthma or peptic ulcers, and in those with hypersensitivity to acetylcysteine. Caution is also needed when administering to patients who are unable to cough sufficiently; in this case, sputum should be removed mechanically. Otherwise, it is safe to administer to a range of demographics, including neonates and patients with porphyria (Buckingham, R, 2020).

FUN FACT:

Acetylcysteine solution can be used as a life-saving antidote for acetaminophen poisoning!

Acetaminophen is a common pain-relieving drug, more widely known in South Africa as **paracetamol**. It works by inhibiting cyclooxygenase enzymes, which halts the production of prostaglandins that cause symptoms like pain and fever. Normally, paracetamol is safely metabolized by the liver into non-toxic sulfate and glucuronide conjugates. However, during an overdose, these safe pathways become saturated. The liver is forced to metabolize the excess drug through an alternative pathway, producing a highly toxic metabolite called ***N-acetyl-p-benzoquinoneimine*** **(NAPQI)**.



The liver uses a naturally occurring antioxidant called **glutathione** to detoxify NAPQI. In an overdose situation, the massive flood of NAPQI rapidly depletes the liver's glutathione stores. Once depleted, the unbound NAPQI damages liver cells, which can result in fatal liver failure. Symptoms of this poisoning include nausea, vomiting, and right upper quadrant pain.

Remarkably, the very same vial of **acetylcysteine** used for a nebulizing solution can be administered orally to treat this poisoning! For maximum efficacy, it should be administered **within 16 hours** of the overdose. Acetylcysteine acts as a life-saving antidote by providing the exact chemical **building blocks** the liver needs to restore its glutathione levels, ultimately neutralizing the NAPQI and protecting the liver! (DailyMed,2026)

Did you know?

A solution for injection can be used as a solution for nebulizing but a solution for nebulizing cannot be used as a solution for injection. Although both solutions are sterile, non-pyrogenic, and formulated at a biological pH, the solution for injection is manufactured under stricter conditions and needs to ensure tonicity as well. Due to the macrophages and mucus in the lungs, an additional defence mechanism exists that the bloodstream does not have, hence stricter regulations on substances injected directly into the bloodstream. The solution for injection manages to fill all the requirements for nebulizing substances, resultingly we can use a solution for injection in a nebulizer, however the solution for nebulizing fails to fill criteria for the solution for injections, and consequently cannot be used for injecting.

PRODUCT COMPOSITION

ACETYLCYSTEINE 20% NEBULISING SOLUTION

FORMULATION:	BATCH SIZE
20% w/v Acetylcysteine • Nebulising Solution	**100 mL**

PRODUCT COMPOSITION

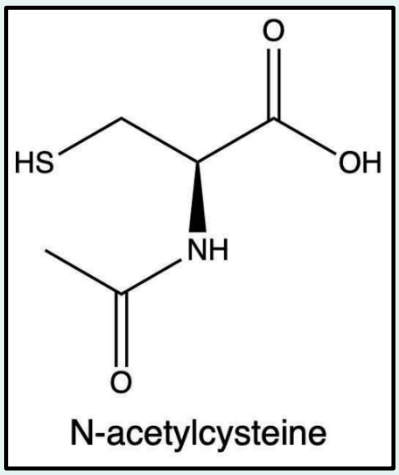
INGREDIENT	AMOUNT
Acetylcysteine	**20.0 g**
Edetate disodium	0.05 g
Sodium hydroxide	q.s. to pH 6.0–7.5
Hydrochloric acid	q.s., if required for pH adjustment
Water for Injection	Up to 100 mL

pH Target: 7

pH Acceptable Range: 6.0–7.5 • Sodium hydroxide for adjustment; hydrochloric acid if required.

Acetylcysteine

20.0 g per 100 mL of the total formulation (20% w/v)



This is the Active Pharmaceutical Ingredient (API) of our formulation. Acetylcysteine is a white or almost white crystalline powder and is the N-acetyl derivative of the naturally occurring amino acid L-cysteine. It is used as a **mucolytic agent** in inhalation therapy. (BP, 2022; DailyMed, 2026).

Acetylcysteine exerts its mucolytic action through its **sulfhydryl group**, which disrupts disulfide linkages in mucus, thereby reducing the viscosity of pulmonary secretions. (DailyMed, 2026).

ALTERNATIVE INGREDIENTS

An alternative to Acetylcysteine would be Ambroxol hydrochloride. Ambroxol hydrochloride is also a mucolytic active pharmaceutical ingredient (API) and is available in formulations for inhalation by nebuliser. Unlike acetylcysteine, which reduces mucus viscosity through its sulfhydryl group and disruption of disulfide linkages in mucus, ambroxol promotes mucus clearance through different secretolytic and mucokinetic actions. It is therefore a suitable alternative API for a nebulised mucolytic formulation (AEMPS, 2020).

SUSTAINABILITY & UNFORESEEN CHALLENGES

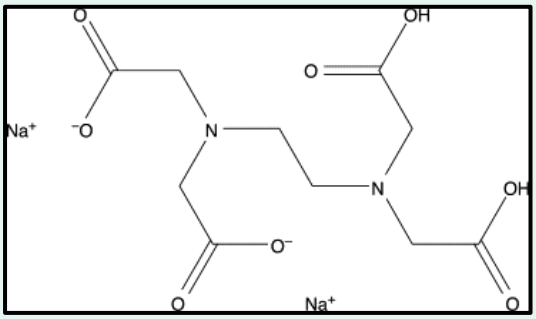
Sustainability: Acetylcysteine has a low potential for bioaccumulation in aquatic organisms, although environmental-fate data indicate that it has high mobility in soil and that biodegradation may not be an important environmental fate process **(PubChem)**. However, the South African Health Products Regulatory Authority (SAHPRA) reports that environmental exposure to acetylcysteine and its metabolites is expected to be very limited under anticipated use and disposal, with no environmental concerns or special environmental labelling considered necessary **(SAHPRA, 2024)**.

Unforeseen challenges: Acetylcysteine presents several formulation and administration challenges. A 1% aqueous solution has an acidic pH of **2.0–2.8**, requiring pH adjustment during formulation. Acetylcysteine is also incompatible with certain metals, including **iron and copper**, as well as **rubber**, and is susceptible to interaction with oxygen and oxidising substances **(Martindale)**.

Acetylcysteine solutions may undergo a **light-purple colour change** during storage; although this does not necessarily indicate significant impairment of safety or efficacy, the change in appearance may affect perception of the product **(Martindale)**. During inhalation, acetylcysteine may additionally cause **bronchoconstriction or bronchospasm** and can increase the volume of liquefied bronchial secretions, which may be problematic in patients with an inadequate cough **(DailyMed, 2024)**.

Disodium Edetate

0.05 g of the total formulation (0.05% w/v)



This is an excipient in our formulation. Disodium edetate is a chelating agent that forms stable, water-soluble complexes with alkaline-earth and heavy-metal ions, thereby sequestering these ions in solution (HEP). Disodium edetate is included in acetylcysteine formulations as a chelating agent, and experimental studies have investigated its role in improving the stability of acetylcysteine-containing solutions.

Acetylcysteine is susceptible to interactions with certain metals, including iron and copper, which are associated with its degradation and oxidation (Anaizi et al., 1997; Sandoz, 2011).

ALTERNATIVE INGREDIENTS

An alternative to **Disodium Edetate** would be **Zinc Gluconate**. Zinc gluconate has been investigated as a stabilising additive in acetylcysteine formulations due to its ability to reduce acetylcysteine dimerisation. In a stability study, zinc gluconate reduced acetylcysteine dimerisation, with **62.5 µg/mL** stabilising a **25 mg/mL acetylcysteine solution** for at least 8 days when stored at **5 ± 3°C** (Primas et al., 2023).

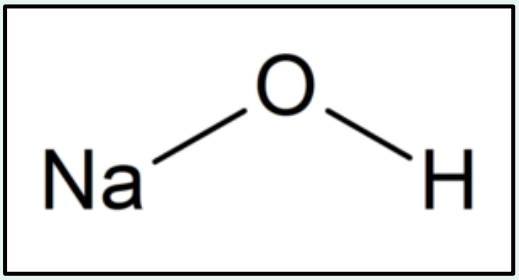
SUSTAINABILITY & UNFORESEEN CHALLENGES

Sustainability: Disodium edetate contains EDTA, which is poorly biodegradable and can therefore remain in wastewater and aquatic environments for extended periods. Once released into the environment, its strong ability to bind metal ions can alter the availability and mobility of these metals, potentially affecting their natural distribution in aquatic systems. This means that although disodium edetate is useful in our formulation for controlling trace metal ions, its environmental persistence should be considered and the release of EDTA-containing waste should be minimised where possible (Sillanpää, 1997; Pinto et al., 2014).

Unforeseen challenges: Disodium edetate requires careful concentration control because its role in the formulation is closely linked to the stability of acetylcysteine. Acetylcysteine is sensitive to oxidation and interactions with trace metal ions, meaning that inadequate sequestration of these ions could contribute to degradation. The stability of acetylcysteine has also been shown to vary with the concentration of disodium edetate present, demonstrating the importance of selecting an appropriate concentration for the formulation (Anaizi et al., 1997). Therefore, the concentration of disodium edetate must be carefully controlled to support acetylcysteine stability while maintaining the required product characteristics.

Sodium Hydroxide

q.s. to adjust pH to 7.0 (range 6.0–7.5)



This is an excipient in our formulation. Sodium hydroxide is a strong alkalising agent that is used in pharmaceutical formulations to adjust the pH of solutions (HPE, 6th ed.). In our formulation, sodium hydroxide is used to increase the pH of the acidic acetylcysteine solution to a target pH of 7.0, within the acceptable range of 6.0–7.5.

Maintaining the appropriate pH is important because the mucolytic activity of acetylcysteine increases as the pH increases, with significant mucolytic activity occurring between pH 7 and 9 (DailyMed, 2024).

ALTERNATIVE INGREDIENTS

An alternative to **Sodium Hydroxide** would be **Potassium Hydroxide**. Potassium hydroxide is also a strong alkalising agent that is used in pharmaceutical formulations to adjust the pH of solutions. It can therefore perform a similar function to sodium hydroxide by increasing the pH of the acidic acetylcysteine solution to the required range (**HPE, 6th ed.**).

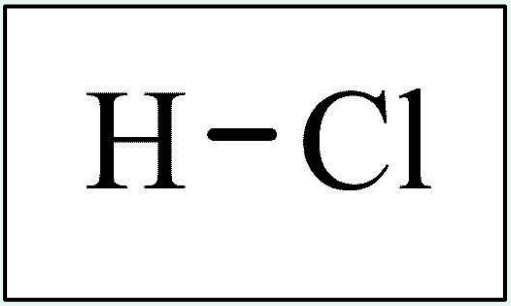
SUSTAINABILITY & UNFORESEEN CHALLENGES

Sustainability: Sodium hydroxide is manufactured through the chlor-alkali process, which uses electrolysis of brine and requires a significant amount of energy. The high energy demand of this production process contributes to its environmental impact. Therefore, improving the energy efficiency of sodium hydroxide production can help reduce its environmental burden (García-Herrero et al., 2017).

Unforeseen challenges: Sodium hydroxide is highly deliquescent, meaning that it readily absorbs moisture from the surrounding environment. It also absorbs carbon dioxide from the air, which can result in the formation of sodium carbonate and affect the composition of the sodium hydroxide. Therefore, sodium hydroxide must be stored in an airtight container to minimise exposure to moisture and carbon dioxide. In addition, sodium hydroxide is a strong base and is corrosive at higher concentrations, requiring careful handling to prevent irritation or burns to the skin, eyes and mucous membranes (HPE, 6th ed.).

Hydrochloric Acid

q.s., if required for pH adjustment



This is an excipient in our formulation. Hydrochloric acid is an acidifying agent used in pharmaceutical formulations to adjust the pH of solutions (HPE, 6th ed.).

In our formulation, hydrochloric acid is used, if required, to decrease the pH after adjustment with sodium hydroxide, allowing the acetylcysteine solution to reach the target pH of 7.0, within the acceptable range of 6.0–7.5. Hydrochloric acid is also used as a pH-adjusting component in marketed acetylcysteine inhalation solutions (DailyMed, 2024).

ALTERNATIVE INGREDIENTS

An alternative to **Hydrochloric Acid** would be **Dilute Sulfuric Acid**. Sulfuric acid is a strong mineral acid that can be used to lower the pH of pharmaceutical solutions. It is also used as a pH-adjusting agent in sterile nebulised inhalation products, demonstrating its use for pH adjustment in formulations intended for pulmonary administration. Therefore, dilute sulfuric acid could potentially perform a similar function to hydrochloric acid by lowering the pH of the acetylcysteine solution to the required range. However, hydrochloric acid remains the preferred choice for our formulation because it has direct precedent in marketed acetylcysteine inhalation solutions. (DailyMed, 2024).

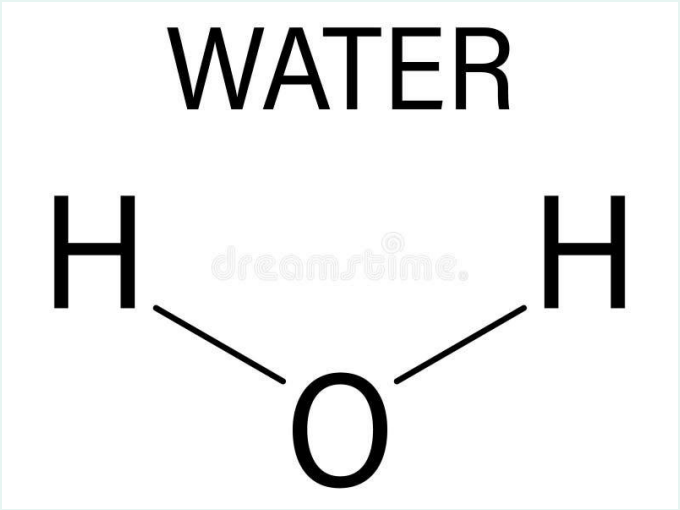
SUSTAINABILITY & UNFORESEEN CHALLENGES

Sustainability: Hydrochloric acid used as a pharmaceutical aid may enter the environment through waste streams. If released into water, hydrochloric acid dissociates readily into chloride and hydronium ions, which can lower the pH of the surrounding water and potentially harm aquatic organisms. However, hydrochloric acid does not bioaccumulate in aquatic organisms. Therefore, unnecessary release of hydrochloric acid-containing waste should be minimised and appropriately managed to reduce its environmental impact (ATSDR, 2002; PubChem/HSDB).

Unforeseen challenges: Hydrochloric acid requires careful handling and concentration control because it is a corrosive acid and reacts strongly with alkalis and certain metals. It should therefore be stored in a well-closed glass or inert container and protected from incompatible materials. During formulation, hydrochloric acid must be added carefully because excessive addition could lower the pH outside the required range, while inappropriate pH in nebulised formulations may contribute to cough or bronchoconstriction. Appropriate control of the quantity added is therefore important to achieve the required final pH without compromising the tolerability or quality of the inhalation formulation (HPE, 6th ed).

Water for Injection

Up to 100mL



This is an excipient in our formulation. Water for Injection (WFI) is highly purified water produced by distillation or a purification process equivalent or superior to distillation for the removal of chemicals and microorganisms and contains no added substances (USP).

In our formulation, WFI serves as the aqueous vehicle in which the acetylcysteine and other excipients are dissolved and is used to bring the formulation to its final volume. The use of WFI is supported by marketed 20% acetylcysteine inhalation solutions, which use WFI as the vehicle (DailyMed, 2025).

ALTERNATIVE INGREDIENTS

An alternative to **Water for Injection** would be **Sterile Water for Inhalation**. Sterile Water for Inhalation is prepared from Water for Injection, subsequently sterilised and suitably packaged, contains no added antimicrobial agents, and is specifically intended for use in inhalators and the preparation of inhalation solutions. It could therefore serve a similar function as the aqueous vehicle in our nebulising formulation (USP).

SUSTAINABILITY & UNFORESEEN CHALLENGES

Sustainability: The production of Water for Injection is resource- and energy-intensive, and the preparation of pharmaceutical-grade water is considered one of the more energy-intensive activities in the pharmaceutical industry. The purification process can therefore contribute to the environmental footprint of pharmaceutical manufacturing through energy consumption and associated water use. Improving the energy and resource efficiency of pharmaceutical water production can help reduce this environmental burden (Rögener, 2024).

Unforeseen challenges: Water for Injection requires strict control of microbiological and endotoxin quality because contamination can compromise the quality and safety of pharmaceutical products. Pharmaceutical water systems can develop microbial contamination and biofilms, which may act as ongoing sources of contamination and endotoxins. Therefore, WFI must be appropriately produced, stored and controlled to maintain its required quality (USP; FDA).

MANUFACTURING PROCESS

ACETYLCYSTEINE 20% NEBULISING SOLUTION

BATCH SIZE	PRODUCT	pH
100 mL	20% w/v Acetylcysteine	TARGET pH: 7.0 Acceptable range: 6.0–7.5

01) PROCESS OVERVIEW

The 20% w/v acetylcysteine nebulising solution is prepared as a **sterile aqueous solution using Water for Injection (WFI)**. Disodium edetate is first dissolved in a portion of WFI, followed by the gradual addition and dissolution of acetylcysteine. The pH is then adjusted to the target of **7.0**, within the acceptable range of **6.0–7.5**, using sodium hydroxide and, if required, hydrochloric acid to correct an overshoot. The solution is subsequently made up to the final volume of **100 mL** with WFI and mixed until uniform. Because acetylcysteine is susceptible to oxidation, exposure to oxygen is minimised during processing and filling, including the use of deaerated WFI and nitrogen protection where applicable. The bulk solution is then passed through a **sterilising-grade membrane filter** into sterile containers and filled aseptically, with oxygen exposure minimised during closure (USP, 2025; US8952065B2, 2015).

02) MATERIALS

- **Acetylcysteine** — 20.0 g
- **Edetate disodium** — 0.05 g
- **Sodium hydroxide** — q.s. to adjust pH to 7.0 (acceptable range 6.0–7.5)
- **Hydrochloric acid** — q.s., if required for pH adjustment
- **Water for Injection** — q.s. to 100 mL

03) EQUIPMENT

- Sterile compounding vessel
- Stirrer / mixer
- Calibrated analytical balance
- Calibrated pH meter
- Measuring equipment
- 0.22 µm sterilising-grade membrane filter
- Sterile receiving vessel
- Nitrogen supply (for oxygen protection)
- Aseptic filling equipment
- Sterile compatible containers and closures\

NO.	STEP	DESCRIPTION
01	**Preparation of WFI**	Transfer approximately 60 mL of deaerated Water for Injection (WFI) into a suitable sterile compounding vessel. Begin controlled mixing and minimise exposure of the WFI to atmospheric oxygen throughout the preparation.
02	**Dissolution of Edetate Disodium**	Accurately weigh 0.05 g disodium edetate and add it to the initial WFI. Mix until the disodium edetate is completely dissolved. Slight heating may be used if necessary to facilitate dissolution, followed by cooling before proceeding.
03	**Dissolution of Acetylcysteine**	Accurately weigh 20.0 g acetylcysteine and gradually add it to the disodium edetate solution while mixing continuously. Continue mixing until the acetylcysteine is completely dissolved and a homogeneous solution is obtained.
04	**pH Adjustment**	Measure the pH using a calibrated pH meter. Add sodium hydroxide solution gradually while mixing until the solution reaches the target pH of 7.0, within the acceptable range of 6.0–7.5. Allow sufficient mixing before confirming the pH. If the pH exceeds the required range, hydrochloric acid may be added carefully, if required, to lower the pH back into the specified range.
05	**Make-Up to Volume**	Add additional deaerated WFI to the solution until the total volume reaches 100 mL. Mix thoroughly to ensure a uniform solution. Minimise exposure to atmospheric oxygen during volume make-up.
06	**Processing / Sterility**	Pass the prepared bulk solution through a sterilising-grade 0.22µm membrane filter into a sterile receiving vessel under aseptic conditions. Minimize oxygen exposure during filtration and subsequent handling.
07	**Filling & Packaging**	Aseptically fill the sterile filtered solution into sterile, compatible single-unit containers. Minimise oxygen exposure during filling and, where nitrogen protection is used, displace the container headspace with nitrogen before closure. Seal the containers promptly and protect the finished product from light during packaging and storage.

Key Formulation and Manufacturing Considerations

1. Appearance

The final product should be a **clear, colourless liquid**.

2. Oxidation Control

Acetylcysteine is highly susceptible to oxidation; therefore, a chelating agent is incorporated to help control trace metal ions, while exposure to oxygen is minimised throughout production.

3. Sterility and Processing Method

Sterility is essential for a product administered directly to the lungs; therefore, a sterile filtration method is selected to minimise exposure of acetylcysteine to potentially damaging high temperatures during sterilisation.

DISCUSSION AND CONCLUSION

DISCUSSION

The development of our 20% w/v acetylcysteine nebulising solution required careful consideration of therapeutic effectiveness, dosage-form suitability, formulation stability and manufacturing requirements. Acetylcysteine was selected for its mucolytic action, which occurs through its sulfhydryl group disrupting disulfide linkages within mucoproteins and reducing the viscosity of respiratory secretions. This makes acetylcysteine suitable for the management of respiratory conditions associated with excessive and viscous mucus secretions.

The choice of a nebulising solution was guided by the intended site of action in the respiratory tract. Nebulisation allows the aqueous solution to be converted into an aerosol for inhalation, while maintaining the API in a homogeneous solution. This provides a consistent concentration throughout the formulation and allows the acetylcysteine to be delivered directly to the lungs.

The selection of excipients was based on their individual functions within the formulation. Water for Injection provides the aqueous vehicle, while disodium edetate was incorporated to control trace metal ions associated with acetylcysteine instability. Sodium hydroxide and hydrochloric acid allow the pH to be adjusted to the required range, with a target of pH 7.0. Together, these excipients support the chemical stability and quality of the final product.

From a manufacturing perspective, acetylcysteine presents additional challenges because it is susceptible to oxidation and the product is intended for direct administration to the lungs. Oxygen exposure therefore needs to be minimised during manufacture and filling, while sterile processing is essential. The selected process incorporates controlled pH adjustment, volume make-up with WFI, sterilising-grade membrane filtration and aseptic filling to produce a sterile product while limiting unnecessary exposure to conditions that may compromise acetylcysteine stability.

Although the formulation provides the advantages of direct pulmonary delivery and a homogeneous dosage form, several limitations remain. Acetylcysteine requires careful control of oxygen exposure, trace metals and pH, while the aqueous nature of the formulation makes microbiological control important. In addition, acetylcysteine solutions may undergo changes in appearance and can present administration-related challenges such as bronchospasm and increased liquefied secretions in patients with an inadequate cough.

Sustainability was also considered during formulation development. While acetylcysteine itself has limited environmental concern under anticipated use and

disposal, disodium edetate presents concerns relating to environmental persistence, and the production of pharmaceutical-grade water is resource- and energy-intensive. These considerations demonstrate the importance of considering environmental impact alongside therapeutic effectiveness and product quality during pharmaceutical development.

Overall, the proposed formulation represents a balance between therapeutic efficacy, formulation stability, sterility, manufacturing feasibility and patient safety. The choice of acetylcysteine, nebulising dosage form, functional excipients and controlled manufacturing process are interconnected and collectively support the development of a suitable product for respiratory administration.

CONCLUSION

The development of the **20% w/v acetylcysteine nebulising solution** demonstrates the importance of integrating therapeutic effectiveness with appropriate pharmaceutic considerations. Acetylcysteine provides effective mucolytic activity, while the nebulising solution allows direct delivery to the respiratory tract. The selected excipients fulfil specific roles in maintaining the formulation's pH, chemical stability and suitable vehicle, while sterile filtration and controlled manufacturing help ensure the safety and quality of the final product. Despite challenges related to oxidation, microbial control, pH and environmental considerations, the proposed formulation provides a rational approach to developing a **stable, sterile and effective acetylcysteine product for pulmonary administration**.

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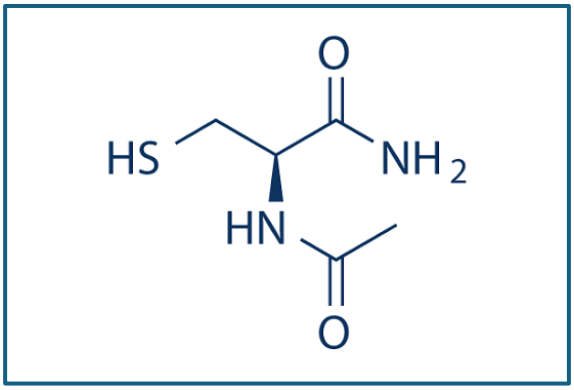
PHARMACEUTICAL CHEMISTRY

INTRODUCTION

The Acetylcysteine 20% w/v Nebulizing Solution discussed in the Pharmaceutics component of this assignment requires a validated analytical method to confirm that the active pharmaceutical ingredient is present in the labelled concentration and has not degraded during the manufacturing process or storage. Quantification of acetylcysteine content is a critical quality control measure because acetylcysteine's free thiol (-SH) group is highly reactive and susceptible to oxidation, forming inactive degradation products such as the disulfide dimer, N,N'-diacetylcystine (Pfizer, 2023). Therefore, an assay must be both accurate and sufficiently selective to distinguish intact drug from these degradation products.

Since no British Pharmacopeia Monograph exits specifically for an acetylcysteine nebulizing solution, the methodology described in the BP Monograph for Acetylcysteine Injection was adapted for this essay (British Pharmacopeia Commission, 2022). This is the closest official recognised sterile aqueous acetylcysteine preparation.

This section outlines the analytical procedure developed to quantify acetylcysteine in the nebulizing solution using High-Performance Liquid Chromatography (HPLC) with UV detection. HPLC was selected for its high sensitivity and specificity, allowing clear resolution of the intact drug from potential degradation products and excipient interference (British Pharmacopoeia Commission, 2022). The following sections define the sample, describe the sampling procedure, and detail sample preparation, dilution and the full analytical method. To strengthen the confidence in this assay's accuracy and results, the HPLC result is further cross validated in the assay validation section against iodometric titration, which is the official British Pharmacopeia assay method for acetylcysteine content (British Pharmacopoeia Commission, 2022).



1. SAMPLE

1.1 DEFINITION AND ORIGIN OF THE SAMPLE

The sample selected for analysis is the finished Acetylcysteine 20% w/v Nebulising Solution produced as the final dosage form of the proposed pharmaceutical product. The sample therefore originates from the completed manufacturing batch, after formulation, filling, and packaging, rather than from the raw active pharmaceutical ingredient (API) or an intermediate manufacturing stage.

The proposed product is an aqueous solution intended for administration by nebulisation. A 20% w/v acetylcysteine solution contains 200 mg of acetylcysteine per mL of solution. Commercial acetylcysteine inhalation solutions are available as sterile, unpreserved solutions and contain acetylcysteine together with edetate disodium in Water for Injection, with sodium hydroxide and/or hydrochloric acid used for pH adjustment (DailyMed, 2026).

For this project, the proposed formulation for a 100 mL batch is:

COMPONENT	PROPOSED QUANTITY	FUNCTION
Acetylcysteine	20.0 g	Active pharmaceutical ingredient
Edetate disodium	0.05 g	Chelating agent
Sodium hydroxide	Quantity sufficient to adjust pH to 6.0–7.5	pH-adjusting agent
Hydrochloric acid	Quantity sufficient, if required, to adjust pH to 6.0–7.5	pH-adjusting agent
Water for Injection	Sufficient to make 100 mL	Vehicle/solvent

The analytical sample is therefore the finished formulation as a whole, containing the target drug together with its formulation matrix. This is important because the analytical method must quantify acetylcysteine specifically in the presence of other substances present in the finished product.

1.2 COMPOSITION OF THE SAMPLE

The finished sample consists of the API, excipients, and aqueous vehicle.

ACETYLCYSTEINE:

Acetylcysteine, also known as N-acetyl-L-cysteine, is the API being quantified. It is the N-acetyl derivative of the naturally occurring amino acid L-cysteine. It has the molecular formula $C_5H_9NO_3S$ and a molecular weight of approximately 163.2 g/mol. It is described as a white crystalline powder in official product information (DailyMed, 2026).

Acetylcysteine contains a free sulfhydryl (-SH) group. This functional group is responsible for an important part of its mucolytic action, because acetylcysteine can disrupt disulfide bonds within mucus, reducing the viscosity of respiratory secretions.

The sulfhydryl group is also important from a pharmaceutical chemistry perspective because acetylcysteine is oxygen-sensitive and can undergo oxidation. Consequently, the conditions under which the sample is collected, stored, and handled must minimise unnecessary changes to the analyte before analysis (DailyMed, 2026).

EDETATE DISODIUM:

Edetate disodium is included as an excipient in the formulation. It functions as a chelating agent, binding trace metal ions that could otherwise participate in undesirable chemical reactions.

It is therefore part of the sample matrix and must be considered when assessing the specificity of the HPLC assay.

The concentration is 0.05 g per 100 mL, equivalent to 0.5 mg/mL or 0.05% w/v.

SODIUM HYDROXIDE AND HYDROCHLORIC ACID:

Sodium hydroxide is used to raise the pH when the formulation is too acidic, while hydrochloric acid may be used to lower the pH when the formulation is too alkaline. These substances are formulation components rather than the target analyte; they nevertheless contribute to the overall sample matrix.

Commercial acetylcysteine solution information describes adjustment to approximately pH 7, within a range of 6.0–7.5 (DailyMed, 2026).

WATER FOR INJECTION:

Water for Injection forms the aqueous vehicle in which the acetylcysteine and soluble excipients are dissolved.

The resulting formulation is therefore a single-phase aqueous solution.

1.3 PHYSICAL CHARACTERISTICS OF THE SAMPLE

The selected dosage form is a homogenous aqueous solution rather than a suspension, emulsion, cream, or ointment.

This means that acetylcysteine is dissolved throughout the aqueous vehicle rather than being intentionally present as dispersed solid particles or droplets.

The relevant physical characteristics of the finished sample are therefore:

- **Dosage form:** aqueous solution
- **Physical state:** liquid
- **System:** single-phase solution
- **Target strength:** 20% w/v
- **Target acetylcysteine concentration:** 200 mg/mL
- **Expected appearance:** a uniform solution without unexpected visible particulate contamination
- **pH:** approximately 6.0–7.5 for the proposed/reference formulation
- **Chemical stability consideration:** oxygen sensitivity

The commercial product information identifies the 20% acetylcysteine solution as containing 200 mg/mL acetylcysteine and specifies a pH range of 6.0–7.5.

Because the formulation is a solution, there is no expected sedimentation of suspended drug particles or phase separation comparable to that encountered in suspensions and emulsions.

This physical characteristic directly influences the sampling procedure discussed later.

1.4 CHEMICAL CHARACTERISTICS RELEVANT TO ANALYSIS

The chemical characteristics of acetylcysteine are important because the objective of the assay is to determine the concentration of intact acetylcysteine in the finished product.

CONCENTRATION:

The proposed formulation contains:

20% w/v = 20 g/100 mL = 200 mg/mL

Therefore, the analytical procedure must be capable of accurately measuring acetylcysteine around the concentration expected in the finished product.

SULFHYDRYL GROUP:

Acetylcysteine contains a reactive sulfhydryl (-SH) group. This group is responsible for important chemical properties of the molecule and contributes to its mucolytic activity.

OXYGEN SENSITIVITY:

Acetylcysteine is described as oxygen-sensitive. Exposure to oxygen can promote oxidation, meaning that poor sample handling could potentially alter the chemical composition of the sample before analysis (DailyMed, 2026).

This makes appropriate sample handling particularly important.

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pH:

The formulation has a specified pH environment. The commercial 20% solution has a pH range of 6.0–7.5 (DailyMed, 2026).

pH is relevant because the chemical environment of a solution can affect the stability and behaviour of the analyte.

1.5 SAMPLE MATRIX

The sample matrix is the collection of substances present in the sample other than the analyte being quantified.

For the proposed acetylcysteine nebulising solution, the matrix includes:

- Water for Injection;
- Edetate disodium;
- Sodium hydroxide and/or hydrochloric acid used for pH adjustment; and
- Any other substances that may arise from degradation during manufacture or storage.
- The target analyte is acetylcysteine.

The distinction between the analyte and matrix is important because the HPLC method must measure acetylcysteine without the other components producing a falsely high or falsely low result.

1.6 IMPACT OF SAMPLE CHARACTERISTICS ON SPECIFICITY

Specificity/selectivity refers to the ability of an analytical procedure to measure the target analyte in the presence of other substances that may also be present in the sample.

ICH Q2(R2) states that specificity/selectivity can be demonstrated by showing the absence of interference or by comparison with an orthogonal analytical procedure. Potential sources of interference include impurities, degradation products, related substances, matrix components, and other substances that may be present in the sample (ICH, 2023).

This is directly relevant to the proposed acetylcysteine solution.

The HPLC method must distinguish the acetylcysteine peak from:

- Edetate disodium and other formulation excipients;
- Potential impurities;
- Potential degradation products, particularly products formed when acetylcysteine undergoes oxidation; and
- Other matrix components.

This is important because the objective is not simply to detect a chemical response. The objective is to demonstrate that the response being quantified corresponds specifically to acetylcysteine.

For example, if an acetylcysteine degradation product co-eluted with the acetylcysteine peak and was detected together with it, the calculated assay could falsely suggest that more intact acetylcysteine was present than actually exists.

Therefore, the HPLC procedure should provide adequate chromatographic separation between acetylcysteine and potentially interfering substances.

ICH Q2(R2) further recognises that specificity can be demonstrated by showing that quantitation is not affected by other substances, such as impurities, degradation products, and matrix components (ICH, 2023).

1.7 ANALYTICAL METHODS USED FOR EVALUATION

For this project, HPLC will be the primary analytical method used to determine the acetylcysteine content of the finished nebulising solution. HPLC is particularly suitable because it can separate acetylcysteine from formulation components, impurities, and potential degradation products, allowing the acetylcysteine response to be evaluated specifically.

In addition, iodometric titration will be used as a complementary assay approach for evaluating acetylcysteine content. Using an independent analytical principle provides an additional means of evaluating the acetylcysteine content and allows comparison with the primary HPLC assay result. Therefore, iodometric titration will serve as a supporting/orthogonal evaluation, while HPLC remains the main analytical focus of this project.

1.7 ANALYTICAL SIGNIFICANCE OF THE SAMPLE

The sample is therefore analytically defined as:

A finished, homogeneous, aqueous Acetylcysteine 20% w/v nebulising solution containing acetylcysteine as the target analyte together with edetate disodium, pH-adjusting agents, and Water for Injection as the formulation matrix.

Its homogeneous liquid nature simplifies sampling compared with a cream or suspension, because there is no dispersed solid or separate phase that needs to be extracted.

However, the chemical sensitivity of acetylcysteine means that sample integrity must still be protected, while the presence of excipients and possible degradation products means that HPLC specificity must be demonstrated.

Therefore:

Finished solution → acetylcysteine + formulation matrix → potential degradation → chromatographic separation → specific acetylcysteine quantification

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2. SAMPLING PROCEDURE

2.1 OBJECTIVE

The objective of the sampling procedure is to obtain a representative selection of finished Acetylcysteine 20% w/v Nebulising Solution units from the manufactured batch for subsequent chemical analysis.

The procedure must ensure that:

- The selected samples adequately represent the batch;
- Sample identity is maintained;
- Contamination is minimised;
- Degradation is minimised;
- The samples remain physically and chemically intact; and
- Complete traceability is maintained from the manufactured batch to the analytical result.

WHO defines sampling as an operation intended to obtain a representative portion of a pharmaceutical product for a defined purpose using an appropriate sampling procedure (WHO, 2005).

2.2 WHY THE DOSAGE FORM AFFECTS THE SAMPLING PROCEDURE

The physical nature of the dosage form must be considered when designing the sampling strategy.

Our product is a homogeneous aqueous solution.

It is therefore different from:

- A suspension, where particles may settle;
- An emulsion, where phases may separate;
- A cream, where components may become non-uniform; or
- An ointment, where the API may be distributed within a semi-solid base.

Consequently, top/middle/bottom sampling of the liquid within one container should not automatically be used for our product.

The main sampling concern is instead obtaining representative finished dosage units from the batch.

This is an important distinction:

Top/middle/bottom sampling refers to different physical regions of a bulk material. Beginning/middle/end sampling refers to different stages or locations in the manufacturing/filling process.

For our homogeneous solution, if the filling process is being considered, units may be selected from different stages of filling to provide representation across the batch.

2.3 SAMPLING PLAN

Sampling must be based on a predefined and justified sampling plan rather than convenience.

WHO guidance states that sampling plans for finished products should be based on defined sampling standards, such as ISO 2859-1, and that when physical and chemical testing is required, the sampling units should consist of whole packs. Individual packs should not be opened merely for the purpose of sampling (WHO, 2005).

Therefore, for this project, the sampling unit will be the intact finished-product container.

The exact number of containers selected should be determined from the applicable sampling plan, batch size and analytical purpose.

An arbitrary statement such as “three vials must always be collected” would not be sufficiently justified because the appropriate sample number depends on the sampling design and intended testing.

2.4 REPRESENTATIVE SAMPLING STRATEGY

For the proposed manufacturing process, finished units can be selected according to a predetermined plan that provides representation across the batch.

Where appropriate, sampling can be stratified across the filling sequence, for example:

Beginning of filling → Middle of filling → End of filling

The purpose is to detect possible variability between units produced at different stages of the filling operation.

For example, if an issue developed during part of the filling process, analysing only one conveniently selected vial could fail to identify the problem.

Selecting units from different stages provides a more representative assessment of the batch.

However, the beginning/middle/end approach is a proposed project sampling strategy, not a universal requirement that applies to every acetylcysteine product. The final number and exact selection pattern should follow the predetermined sampling plan.

2.5 SAMPLING UNIT

The sampling unit is the intact finished-product container.

The selected container should remain closed during collection and transport.

This is particularly appropriate for chemical testing because WHO specifies that finished-product sampling units should consist of whole packs and that individual packs should not be opened merely for the purpose of obtaining a sample (WHO, 2005).

Opening the container belongs to the subsequent sample preparation procedure, where a measured aliquot can be taken under controlled laboratory conditions.

This distinction prevents the sampling procedure from overlapping unnecessarily with sample preparation.

2.6 EQUIPMENT AND MATERIALS

The following materials should be available:

- Appropriate personal protective equipment (PPE)
- Clean sampling area
- Clean and suitable sampling equipment, where required
- Sample labels
- Permanent marker or validated identification system
- Sampling record/form
- Suitable transport container
- Original finished-product containers

The equipment and environment should be suitable for preventing contamination and maintaining sample integrity.

2.7 DETAILED SAMPLE COLLECTION PROCEDURE

Step 1: Verify product identity

Before sampling, verify:

- Product name;
- Dosage form;
- Strength;
- Batch number;
- Manufacturing/filling information;
- Packaging configuration; and
- Approved sampling plan.

This prevents samples from the wrong product, strength or batch from entering the analytical process.

Step 2: Review the sampling plan

The sampler should review the predetermined sampling plan before selecting any units.

The plan should identify:

- Which batch is being sampled;
- The number of units required;
- How the units will be selected;
- Where applicable, the stages/locations from which units will be selected;
- The analytical tests required; and
- The requirements for storage and transport.

WHO guidance emphasises that sampling plans should be appropriately designed rather than based on arbitrary selection (WHO, 2005).

Step 3: Prepare the sampling area

The sampling area should be clean and organised.

Unnecessary materials should be removed to minimise contamination, cross-contamination, sample mix-ups, and accidental substitution.

The sampler should also ensure that all required equipment and documentation are available before beginning.

Step 4: Wear appropriate PPE

The sampler should wear the PPE required by the laboratory or manufacturing site's approved procedure.

PPE helps protect both the product and the person performing the sampling.

Step 5: Select representative finished units

Select the required number of intact finished-product units according to the predetermined sampling plan.

Where the filling process is being evaluated, the units may be distributed across different stages of the filling sequence: Beginning → middle → end

The selection must be planned rather than based on convenience.

Step 6: Inspect the selected units

Each selected unit should be examined for obvious defects before being accepted as a sample.

The following should be checked:

- Container damage;
- Leakage;
- Compromised closure;
- Damaged packaging;
- Unusual appearance;
- Visible particulate matter; and
- Any other evidence of compromised product integrity.

Any abnormality must be documented.

Step 7: Maintain container integrity

Selected units should remain sealed in their original containers during collection and transport.

The product should not be unnecessarily transferred into another container during the sampling process.

This minimises exposure to environmental contamination and unnecessary exposure to air.

Step 8: Assign unique sample identification

Each selected sample should receive an identification that allows it to be traced to the original batch.

The sample identification should link the unit to product name, strength, batch number, individual sample number, sampling date, sampling location/stage (where applicable), and sampler.

Traceability is essential because the final analytical result must be linked back to a clearly identified sample.

WHO good practices for pharmaceutical quality control laboratories emphasise documented sample identification, receipt and traceability throughout the testing process (WHO, 2024).

2.8 SAMPLE LABELLING

Each selected unit should be clearly labelled or otherwise uniquely identified.

The record should include, where applicable:

Information	Purpose
Product name	Identifies the product
Strength	Confirms the correct formulation
Batch number	Links sample to manufacturing batch
Sample/unit number	Identifies the individual unit
Date and time	Establishes sampling history
Sampling stage/location	Establishes where the unit was selected
Sampler identification	Establishes responsibility
Storage requirements	Maintains sample integrity
Observations	Records abnormalities

Clear identification prevents sample mix-ups and supports traceability.

2.9 SAMPLE STORAGE

The selected samples should be stored according to the established storage conditions for the final formulation.

A storage condition should not be invented solely for the assignment if the final product specification has not yet established it.

The product should be protected from conditions that could alter its chemical or physical characteristics.

This includes avoiding:

- Unnecessary heat exposure;
- Unnecessary exposure to air;
- Inappropriate light exposure where relevant;
- Excessive handling; and
- Prolonged storage outside the established conditions.

This is particularly important because acetylcysteine is oxygen-sensitive (DailyMed, 2026).

For a commercial acetylcysteine 20% solution, one current DailyMed presentation specifies storage at 20–25 °C before opening and refrigeration after opening, demonstrating why the storage condition should be tied to the actual product specification rather than assumed universally (DailyMed, 2026).

For our proposed product, the final storage condition should therefore be established as part of the formulation and stability specification.

2.10 SAMPLE HANDLING

The samples should be handled as little as reasonably possible between collection and analysis.

The following principles should be followed:

1. Keep the original container closed
2. Avoid unnecessary exposure to air
3. Avoid unnecessary transfer between containers
4. Avoid excessive heat or other inappropriate environmental conditions
5. Maintain clear sample identification
6. Follow the established storage requirements
7. Record any abnormal event or storage excursion

The purpose is to ensure that the sample reaching the analytical laboratory remains representative of the product that was originally manufactured.

2.11 SAMPLE TRANSPORT

During transport from the sampling site to the analytical laboratory:

- Samples should remain securely closed;
- Containers should be protected from physical damage;
- Required storage conditions should be maintained;
- Samples should remain clearly identified; and
- Unnecessary handling should be avoided.

The transport conditions should preserve sample integrity rather than introduce changes that could affect the assay.

WHO good laboratory practice guidance emphasises appropriate sample receipt, identification and control of conditions affecting sample integrity (WHO, 2024).

2.12 PREVENTION OF CONTAMINATION

Contamination can introduce substances into the sample that were not originally part of the formulation.

This could produce additional chromatographic responses or interfere with acetylcysteine quantification.

Contamination is minimised by:

- Maintaining a clean sampling area;
- Using clean and appropriate equipment;
- Using appropriate PPE;
- Avoiding unnecessary opening of containers;
- Avoiding contact between the product and inappropriate surfaces;
- Keeping samples properly sealed;
- Maintaining correct sample identification; and
- Following the predetermined sampling procedure.

WHO sampling guidance specifically emphasises precautions to prevent contamination and deterioration during pharmaceutical sampling (WHO, 2005).

2.13 PREVENTION OF DEGRADATION

The sampling procedure must also prevent chemical degradation of acetylcysteine before analysis.

This is important because the assay is intended to determine the amount of intact acetylcysteine in the finished product.

If the sample were allowed to degrade between collection and analysis, the analytical result could reflect the condition of the sample after poor handling rather than the condition of the manufactured batch.

Acetylcysteine is described as oxygen-sensitive, making protection against unnecessary oxidative exposure particularly relevant (DailyMed, 2026).

Therefore:

> ***Representative sampling + appropriate storage + controlled handling = preservation of sample integrity before analysis.***

2.14 SAMPLE RECEIPT IN THE LABORATORY

When the samples arrive at the analytical laboratory, the receiving personnel should verify:

1. Product identity
2. Strength
3. Batch number
4. Sample identification
5. Number of units received
6. Container integrity
7. Condition of the samples
8. Relevant storage/transport information
9. Any discrepancies between the received samples and the sampling record

The condition of the sample should be documented.

If a container is damaged, incorrectly labelled, leaking, or otherwise compromised, the laboratory should document the issue and determine whether the sample remains suitable for testing according to the applicable laboratory procedure.

WHO good practices require procedures for delivery and receipt of samples and appropriate sample identification and records (WHO, 2024).

2.15 WHY SAMPLES SHOULD NOT AUTOMATICALLY BE POOLED

The selected finished-product units should not automatically be combined into one composite sample.

If the purpose is to assess variability between individual units, analysing units separately provides more information.

For example:

- Unit A could have a slightly lower assay value.
- Unit B could have a slightly higher assay value.
- Pooling A and B could produce an average value that appears acceptable.

The average result could therefore hide variation between individual units.

Consequently, where the analytical objective includes assessment of unit-to-unit consistency, individual units should be retained for individual analysis.

The final decision regarding individual versus composite analysis should be determined by the analytical objective and approved sampling plan.

2.16 SAMPLING AND SAMPLE PREPARATION ARE DIFFERENT STAGES

This distinction is important for the assignment.

Sampling answers: Which product units do we select from the manufactured batch?

Sample preparation answers: What do we do to the selected unit before injecting it into the HPLC column?

Therefore, during the sampling procedure, the selected unit remains sealed until it is required for controlled laboratory preparation.

This creates a clear analytical workflow:

Manufactured batch → representative finished product sampling → storage and controlled handling → sample preparation → dilution → HPLC analysis → Assay result

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2.17 SUMMARY OF THE SAMPLING PROCEDURE

Parameter	Proposed approach	Rationale
Product sampled	Finished acetylcysteine 20% w/v nebulising solution	Represents the final dosage form
Dosage form	Homogeneous aqueous solution	No routine top/middle/bottom sampling of the liquid is required
Sampling unit	Intact finished-product container	Maintains product integrity
Sampling plan	Predetermined and justified	Prevents convenience sampling
Batch representation	Units selected across the batch/filling sequence where appropriate	Detects possible between-unit variation
Number of units	Determined by the sampling plan	Avoids an arbitrary sample number
Container status	Sealed during collection and transport	Minimises contamination and degradation
Identification	Unique sample identification	Ensures traceability
Storage	According to established product specification	Maintains sample integrity
Transport	Controlled and documented	Prevents deterioration and mix-up
Inspection	Visual examination of container/product integrity	Identifies compromised samples
Documentation	Complete sampling record	Supports traceability and reproducibility
Subsequent procedure	Sample preparation	Separate from sampling

Overall analytical rationale

The selected sample is the finished Acetylcysteine 20% w/v nebulising solution, not the raw API. Its homogeneous aqueous nature means that the major sampling concern is obtaining representative finished-product units rather than correcting for physical separation within a container.

The chemical characteristics of acetylcysteine, particularly its oxygen sensitivity, mean that the selected samples must be handled in a manner that preserves their chemical integrity. The presence of excipients and potential degradation products means that the subsequent HPLC method must demonstrate appropriate specificity.

The sampling procedure therefore provides the foundation for the subsequent analytical stages:

Representative batch selection;

- ➔ Preservation of sample integrity
- ➔ Controlled sample preparation
- ➔ Appropriate dilution
- ➔ Specific HPLC analysis
- ➔ Reliable acetylcysteine assay

3. SAMPLING PREPARATION

3.1 OVERVIEW AND RATIONALE:

Because the sample is a homogenous, single-phase liquid solution rather than a semi-solid or solid dosage form, sample preparation for this assay is considerably less complex than for a cream or tablet matrix. There is no drug imbedded in a solid excipient base requiring sample extraction, levigation or centrifugation to extract the active pharmaceutical ingredient. Acetylcysteine is already fully dissolved in the aqueous vehicle at the point of sampling.

Sample preparation for this HPLC assay therefore focuses on three main objectives:

1. Obtaining an accurately measured, representative volume of the collected sample.
2. Removing any particles from the sample prior to injection.
3. Preserving the chemical integrity of the oxidation-sensitive analyte between collection and analysis.

3.2 WHY NO EXTRACTION STEP IS REQUIRED

In a cream or ointment dosage form, the API is dispersed throughout a multi-phase excipient base (oils, emulsifiers, waxes), so a solvent extraction step is essential to separate the drug from its matrix before it can be chromatographed cleanly. Here, the nebulizing solution is a single-phase aqueous system in which acetylcysteine is already fully dissolved alongside its excipients. Consequently:

1. There is no solid or oily matrix to dissolve.
2. No centrifugation or phase separation is needed, since the sample is already a single homogenous phase.
3. The main specificity concern is not the matrix interference from excipients, but the potential co-elution of acetylcysteine's own oxidative degradation (cystine, cysteine, N,N'-diacetylcystine).

3.3 PRECISE QUANTIFICATION OF SAMPLE VOLUME:

Objective:

To obtain an accurately measured volume of the homogenised nebulizing solution, since the formulation strength is expressed on a % w/v (weight-per-volume) basis. Therefore, precise volumetric measurement, rather than weighing, is the appropriate method of quantification.

Procedure:

1. Ensure the bulk sample container has been thoroughly mixed by gentle inversion prior to sub-sampling, to confirm homogeneity across the collected volume.
2. Using a calibrated Class A pipette, accurately measure the required amount of nebulizing solution (see Question 4 for the exact volume used in the primary dilution).
3. Record the exact volume measure, the pipette used, and its calibration status for traceability.

3.4 FILTRATION AND CLARIFICATION:

Objective:

To ensure that all particles are removed from the sample prior to HPLC injection, protecting the column and detector. This satisfies the general BP requirement (BP Appendix III D) that the “solutions must be free from solid particles”.

Procedure:

1. Draw the measured aliquot into a clean, dry syringe.
2. Fit a 0.45µm HPLC-grade syringe filter to the syringe.
3. Filter the solution directly into a clean, dry sample vial, applying gentle even pressure to avoid forcing air bubbles through the filter membrane.
4. Discard the first few drops of the filtrate to avoid any filter-related particles being present in the filtrate.

Since the sample is already a clear solution rather than a suspension or emulsion, filtration here serves purely as a precautionary clarification step rather than a true separation technique. It remains necessary, since a visibly clear solution can carry sub-visible particles capable of blocking the narrow HPLC column or interfering with detection.

3.5 PRESERVING SAMPLE INTEGRITY DURING PREPARATION:

Because acetylcysteine free thiol (-SH) group is highly susceptible to oxidation, and the compound itself is light-sensitive, the following precautions are built into the preparation procedure itself, rather than addressing it only in the storage step:

1. **Minimize air exposure:** collection, transfer and filtration steps are performed promptly, and containers are kept capped between steps, since prolonged exposure to atmospheric oxygen accelerates thiol oxidation to the disulfide dimer (N,N′-diacetylcystine).
2. **Protect from light:** sample handling is carried out away from direct light where possible and practical, and amber glassware is used from any solution not processed immediately.
3. **Work at controlled temperature:** sample preparation is carried out at room temperature without unnecessary heating, since elevated temperature further accelerates oxidative degradation.
4. **Prepare immediately for analysis:** consistent with the BP's own instruction (for Acetylcysteine Injection related substances test), solutions must "be prepared immediately before use". Therefore, the filtered sample is not held for an extended period before injection.

3.6 STORAGE AND STABILITY PRIOR TO ANALYSIS:

If the prepared sample cannot be injected for analysis immediately:

- Transfer to a tightly sealed amber HPLC vial to exclude both light and air exposure.
- Store at controlled temperature, away for direct light, and analyse within a validated hold time to ensure no significant degradation occurs between preparation and injection.
- Do not store prepared samples for reuse across multiple analytical sessions; fresh dilutions must be prepared for each analytical run. This is consistent with the precautions listed in Section 3.5.

3.7 MATERIALS AND EQUIPMENT SUMMARY FOR SAMPLE PREPARATION:

Item	Purpose
Class A pipette	Accurate measurement of sample aliquot
0.45µm HPLC-grade syringe filter	Removal of particles from sample aliquot
Syringe (clean and dry)	Sample transfer and filtration
Amber HPLC glass vials	Light protection during handling and storage
Notebook	Traceability of volumes, timings and handling

4. SAMPLE DILUTION PROCEDURE

4.1 OVERVIEW AND RATIONALE:

The dilution procedure for this assay serves two purposes:

1. Reducing the nebulizing solution from its labelled 20% w/v strength down to a concentration suitable for HPLC injection.
2. Preparing a series of calibration standards from Acetylcysteine Certified Reference Material (CRM) that bracket the sample concentration, allowing accurate quantification against a calibration curve.

Both the sample and standards are diluted using the same diluent to prevent solvent-mismatch effects on peak shape and retention time.

4.2 DILUENT SELECTION:

The mobile phase (10 volumes methanol: 90 volumes 0.5% ammonium sulphate solution containing 0.02M sodium pentanesulphonate, adjusted to pH 2.0 with 2M hydrochloric acid) was selected as the diluent for both sample and standards, consistent with the method prescribed in the BP monograph for Acetylcysteine Injection (British Pharmacopeia Commission, 2022). This was chosen for three reasons:

1. **Chromatographic compatibility:** diluting with the mobile phase eliminates any solvent disturbance or peak distortion that can occur when a sample is injected in a diluent stronger or more mismatched than the mobile phase.
2. **pH stabilisation:** the low pH (2.0) of the mobile phase supressed base-catalysed oxidation of acetylcysteine's free thiol (-SH) group, which is accelerated at neutral or alkaline pH. This directly protects the analyte between dilution and injection.
3. **Consistency with BP standards:** per Appendix III D, any pH adjustment must be made to the aqueous component of the mobile phase rather than the final mixture.

4.3 HANDLING AND TIMING PRECAUTIONS:

Because Acetylcysteine is oxygen-sensitive, the following precautions must be taken during the dilution process to prevent chemical degradation:

- All dilutions are prepared for immediate use rather than in advance, minimizing the window for oxidative degradation between preparation and injection.
- Volumetric flasks are filled with minimal headspace and capped promptly after mixing to limit air exposure.
- Standards and samples are protected from direct light during preparation and amber glassware for storage should be used.

4.4 EQUIPMENT AND PRECISION CONSIDERATIONS

Class A volumetric glassware is specified throughout, rather than Class B or graduated equipment, because the tolerances of the equipment affect the reliability of the final calculated concentration:

GLASSWARE	TOLERANCES
1ML BULB PIPETTE	±0.007ml
5ML BULB PIPETTE	±0.015ml
10ML VOLUMETRIC FLASK	±0.02ml
100ML VOLUMETRIC FLASK	±0.08ml

Using Class A volumetric glassware keeps the compounded error across a series dilution well under 1%, which matters here because the assay's acceptance criteria (accuracy 95-105%, precision RSD ≤ 2%, per BP) leave little room for dilution error.

4.5 PRIMARY SAMPLE DILUTION PROCEDURE:

At 20% w/v, the nebulizing solution contains 200 mg/ml of acetylcysteine. The target working concentration, consistent with BP monograph for Acetylcysteine Injection, is 0.2% w/v (2000 µg/ml) (British Pharmacopeia Commission, 2022).

Using $C_1V_1 = C_2V_2$:

$$200\ mg/ml \times V_1 = 2\ mg/ml \times 100ml$$

$$\therefore V_1 = \frac{2\ mg/ml \times 100ml}{200\ mg/ml}$$

$$\therefore V_1 = 1.00\ ml$$

Procedure:

1. Accurately pipette 1.00ml of the 20% w/v nebulizing solution into a 100ml Class A volumetric flask using a Class A bulb pipette.
2. Dilute to volume with mobile phase and invert at least 10 times to ensure homogeneity (do not shake vigorously to avoid introducing oxygen/air into the solution).
3. Resulting concentration:

$$(200\ mg/ml \times 1.00ml)/100ml = 2\ mg/ml\ (0.2\%w/v, 2000\mu g/ml)$$

This matches the BP-prescribed working concentration of Acetylcysteine Injection, placing the sample at the same concentration as the calibration midpoint (Section 4.7), which minimizes extrapolation error when reading the result off the calibration curve.

4.6 CRM STOCK SOLUTION PREPARATION:

Procedure:

1. Accurately weigh 40.0 mg of acetylcysteine CRM.
2. Transfer to a 10.0ml volumetric flask and dissolve in a small amount of mobile phase.
3. Dilute to volume with mobile phase to give a 4.0 mg/ml (0.4% w/v, 4000 µg/ml) stock solution.
4. Prepare fresh. Do not store beyond working session, given the oxidation risk discussed in Section 4.3.

4.7 CALIBRATION STANDARD SERIES:

A bracketing calibration series (80-120% of the target working concentration) was constructed from the CRM stock solution, using dilution equation $C_1V_1 = C_2V_2$, where C_1 and V_1 are the concentration and volume of the stock solution, and C_2 and V_2 are the concentration and volume of the final standard:

% of Target	Target Concentration (µg/ml)	Volume of 4000 µg/ml Stock Required (ml)	Diluted to (ml)
80%	1600	4.00	10.00
90%	1800	4.50	10.00
100%	2000	5.00	10.00
110%	2200	5.50	10.00
120%	2400	6.00	10.00

Each standard is mixed by inversion and transferred to an amber HPLC vial immediately prior to injection.

Worked example (100% of standard):

$C_1 \times V_1 = C_2 \times V_2$

$\therefore 4000\ \mu g/ml \times V_1 = 2000\ \mu g/ml \times 10.00ml$

$\therefore V_1 = 5.00\ ml$

4.8 WHY AN 80-120% BRACKETING RANGE WAS CHOSEN:

This calibration range was chosen deliberately, based on the following considerations:

1. The sample is expected to measure at approximately 100% of its labelled concentration, a range of 80-120% places this expected value at the centre of the calibration curve rather than near either extreme. Measurements towards the edge of a calibration curve/range are inherently less reliable, so centring the expected result within the curve support a more accurate and reliable determination.

2. The range reflects standard practice for an assay of this type. Because the purpose here is to confirm drug content against the label claim, rather than to detect trace impurities, the expected result will always lie close to 100% of the stated strength. A narrow, targeted calibration range is therefore more appropriate than a broad range designed to capture widely varying concentrations, as would be used in an impurity-focused method.

5. ANALYTICAL PROCEDURE

Formulation: 20% w/v Acetylcysteine Nebulizing Solution

Batch size: 100ml

Objective:

The objective of this analytical procedure is to accurately quantify the acetylcysteine content of the 20% w/v Acetylcysteine solution using High-Performance Liquid Chromatography (HPLC) and access the presence of any related substances (oxidative degradation products) against acceptance limits found in the British Pharmacopeia.

5.1 INTRODUCTION TO THE ANALYTICAL PROCEDURE

An analytical procedure can be described as a step-by-step process that is used to qualify (identify), quantify or characterize a certain chemical substance or mixture (American Chemical Society, n.d). In this case, we are qualifying and quantifying Acetylcysteine in the 20%w/v Nebulizing solution.

To qualify and quantify acetylcysteine, High-Performance Liquid Chromatography (HPLC) is used as the primary assay. This is an analytical technique that is used to separate, identify and, therefore, quantify specific individual components in a liquid mixture. The process is carried out by passing a mobile phase through a column that is tightly packed under high pressure. The acetylcysteine nebulising solution is treated as liquid mixture, and the aim is to separate, identify and quantify the acetylcysteine content present.

It is important to understand the main principle behind HPLC. It is a method of chromatographic separation based on the different ways in which species distribute between 2 non-miscible phases — the stationary phase and the mobile phase (British Pharmacopoeia Commission, 2022).

This method uses reversed-phase chromatography, meaning the stationary phase is non-polar and the mobile phase is polar. The stationary phase consists of octadecylsilyl silica gel (a C18 -bonded silica), a non-polar, chemically modified material tightly packed within the column. The mobile phase is a polar aqueous mixture of methanol and buffered ammonium sulphate solution, flowing consistently through the system. Separation occurs based on polarity: more polar species interact less strongly with the non-polar stationary phase and are carried through the column more quickly by the polar mobile phase, while less polar species are retained longer.

As the sample passes through the column, molecules with greater affinity for the stationary phase will move slowly, while those with greater affinity for the mobile phase move more quickly. As a result of these different speeds, the different species separate from one another and reach the detector at different times.

5.2 MATERIALS REQUIRED:

Reference standards and reagents:

- Acetylcysteine Standard Solution
- Methanol (HPLC Grade)
- Ammonium Sulphate
- Sodium pentanesulphonate
- Hydrochloric acid (2M, for mobile phase pH adjustment)
- Purified water

For the iodometric cross-validation:

- Glacial acetic acid
- Iodine volumetric solution (0.05M iodine VS)

Consumables:

- HPLC grade syringe filters (0.45µm)
- Amber HPLC glass vials
- Volumetric flasks (Grade A): 10ml, 100ml
- Class A bulb pipettes (1ml, 5ml as required for standard/sample dilution)
- Clean, dry syringes for sample transfer and filtration

Calibration Standards:

A bracketing series of calibration standards, prepared from the acetylcysteine CRM stock solution (Section 5.2), by diluting with the mobile phase, spanning 80-120% of the target working concentration (0.2% w/v acetylcysteine or 2000µg/ml).

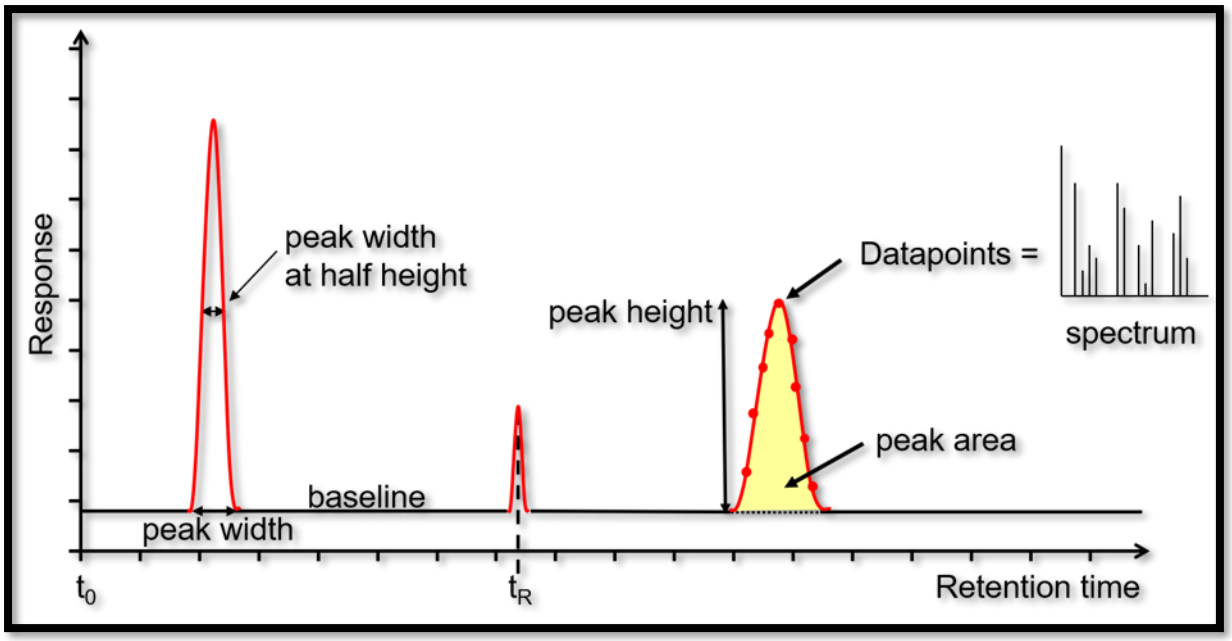
% of Target	Concentration (µg/ml)
80%	1600
90%	1800
100%	2000
110%	2200
120%	2400

HPLC Equipment:

- **A pumping system:** Delivers the mobile phase at a known and controlled flow rate. In HPLC, the mobile phase is pumped under high pressures.
- **An injector:** It is the sample solution that enters into the mobile phase at the column head through the injection. This injection can be used under high pressures, making it perfect for HPLC.
- **A chromatographic column:** It is in this column where the mobile and stationary phases are found and where the separation of the species occurs. These columns must be controlled by temperature regulators since temperature can affect different aspects of HPLC, such as retention time, the viscosity of the mobile phase, the pressure of the column as well as the final sharpness of the peak.
- **A detector:** More than one type may be used; the UV/Vis spectrophotometer is most common, though fluorescence spectrophotometers, electrochemical detectors, and others also exist. The detector registers molecules leaving the column at different times and generates an electrical signal.
- **Data system:** Uses the electrical signals from the detector and turn them into a graph with different peaks that represent the different species in the sample. This graph is known as a chromatogram.

Additionally, there are several terms that are important to consider when it comes to HPLC:

- **Retention Time:** Exact time taken for a species to reach the detector once being injected. Most species will have the same retention time under the same specific conditions. In this case, acetylcysteine will have the same retention time when HPLC is done under the exact same conditions. It is through this method that qualification of Acetylcysteine can also be done.
- **Area of the peak:** The peak area corresponds to the concentration of a species in the sample, enabling quantification.
- **Isocratic vs. Gradient:** Isocratic elution keeps the mobile phase constant for the duration of the run. On the other hand, gradient elution is where the solvent mixture is changed by the machine during the run in order to speed up the slower species.



5.3 HPLC CONDITIONS:

- Stationary phase: A stainless-steel column (25cm x 5mm), packed with octadecylsilyl silica gel for chromatography (5µm particle size).
- An isocratic elution must be used.
- The mobile phase: 10 volumes of methanol and 90 volumes of 0.5% w/v ammonium sulphate containing 0.02M sodium pentanesulfonate. The pH adjustment of the mixture should be to pH 2.0 using 2M of hydrochloric acid.
- Flow rate of 1ml per minute
- Detection wavelength of 205nm (UV/Vis).
- Injection volume of 20µL per solution
- Run time of three times the retention time of acetylcysteine.

5.4 ANALYTICAL PROCEDURE FOR THE STANDARD SOLUTION

It is important to have a standard to which a comparison can be to ensure reliability and consistency. It provides a reference point when performing any procedure. Therefore, creation of a master stock solution is required. This procedure includes the following:

- Accurately weigh 40.0 mg of acetylcysteine CRM.
- Transfer to a 10.0ml volumetric flask and dissolve in a small amount of mobile phase.
- Dilute to volume with mobile phase to give a 4.0 mg/ml (0.4% w/v, 4000 µg/ml) stock solution.
- Prepare fresh. Do not store beyond working session, given the oxidation risk discussed in Section 4.3.

After the creation of this stock solution, the calibration curve is built using the equation $C_1V_1 = C_2V_2$.

The volume of stock solution required, and the intricacies required for each target concentration, the table under 4.7 is used.

5.5 ANALYTICAL PROCEDURE FOR SAMPLE PREPARATION

The Acetylcysteine nebulizing solution contains 200mg/ml of Acetylcysteine (20%w/v). According to the BP monograph for an Acetylcysteine Injection, the target concentration is 0.2%w/v (2000ug/ml.)

Using the $C_1V_1 = C_2V_2$ equation, a volume of 1.00ml of the nebulizing solution is required, as shown in the calculations under Section 4.5.

Procedure:

- Accurately pipette 1.00ml of the 20% w/v nebulizing solution into a 100ml Class A volumetric flask using a Class A bulb pipette.
- Dilute to volume with mobile phase and invert at least 10 times to ensure homogeneity (do not shake vigorously to avoid introducing oxygen/air into the solution).
- Resulting in a concentration of 2mg/ml, which is equal to 2000µg/ml and 0.2%w/v.

Due to this working concentration matching what is prescribed in the BP, the sample is placed at the same concentration as the calibration midpoint. This minimises extrapolation errors when it comes to reading off the final calibration curve.

5.6 PREPARATION OF REFERENCE AND SYSTEM SUITABILITY SOLUTIONS

In addition to the test solution described above, the British Pharmacopoeia requires several reference solutions to be prepared so that the system suitability criteria and related substances limits in Section 5.7 can actually be assessed against a chromatogram. With the exception of solution (3), all solutions below should be prepared immediately before use.

- **Solution (1) Test solution:** The nebulising solution diluted with mobile phase to 0.2% w/v acetylcysteine, as described in Section 5.5.
- **Solution (2) Reference solution:** A freshly prepared 0.2% w/v solution of N-acetyl-L-cysteine in the mobile phase. This represents acetylcysteine before any oxidative degradation has had time to occur.
- **Solution (3) Aged reference solution:** A 0.2% w/v solution of N-acetyl-L-cysteine in the mobile phase, stored at room temperature for at least 2 hours before use. Allowing this solution to stand permits partial oxidation to N,N'-diacetylcystine, giving a chromatogram against which degradation in the test solution can be compared.
- **Solution (4) System suitability solution:** Dissolve 20 mg of L-cysteine and 20 mg of L-cystine in 10 mL of 1M hydrochloric acid, add 40 mg of N-acetyl-L-cysteine, and immediately dilute to 100 mL with the mobile phase. Dilute 10 mL of the resulting solution to 200 mL with the mobile phase. This solution deliberately contains cysteine and cystine alongside acetylcysteine so that the resolution between these peaks can be verified before testing samples.
- **Solution (5) Dilute system suitability solution:** Dilute 1 volume of solution (4) to 10 volumes with the mobile phase. This dilution is used as the reference point for the disregard limit and secondary peak thresholds specified in Section 5.7.

Each solution above is injected under the chromatographic conditions given in Section 5.3, generating the chromatograms referenced in the system suitability criteria and limits (Section 5.7).

5.7 SYSTEM SUITABILITY AND LIMITS

The concept of system suitability is important in HPLC as it ensures that the entire setup of the procedure is working properly and meets the criteria of the BP before testing the samples created above.

System suitability identifies issues that could exist with the equipment or the process before actual testing occurs and, in the end, saves time and resources.

According to the British Pharmacopoeia for Acetylcysteine Injections, when it comes to the detection of impurities caused by the degradation products of Acetylcysteine as a result of oxidation, the HPLC test that is being performed is not valid if it does not meet the following criteria:

- In the chromatogram obtained with **solution (4)**, the height of the trough separating the peaks due to cysteine and cystine is less than one quarter of the height of the peak due to cysteine.
- In the chromatogram obtained with **solution (3)**, a peak due to N,N'-diacetylcystine appears with a retention time of about 13 minutes, and the area of this peak is greater than the area of any corresponding peak in the chromatogram obtained with **solution (2)**, demonstrating the system's ability to detect this degradation product as it forms.

As with any procedure, there are limits that exist. Limits are important to consider as they explain the reliability and the accuracy of this particular method. Being aware of these limits ensures there is an understanding of whether a method is actually able to detect any impurities that exist, can qualify and quantify the active pharmaceutical ingredient and meet the required standards or not.

Limitations related to detection of impurities, according to Acetylcysteine Injection Monograph (BP)

In the chromatogram obtained with solution (1):

- The area of any peak corresponding to **N,N'-diacetyl-L-cystine** is **not greater than** the area of the peak corresponding to acetylcysteine in the chromatogram obtained with **solution (4) (1%)**.
- The area of any peak corresponding to **cysteine or cystine** is **not greater than** the area of the corresponding peak in the chromatogram obtained with **solution (4) (0.5%)**.
- The **sum of the areas** of any other **secondary peaks** is **not greater than** the area of the peak corresponding to acetylcysteine in the chromatogram obtained with **solution (4) (1%)**.

- **Disregard** any peak with an area less than the area of the peak due to acetylcysteine in the chromatogram obtained with **solution (5) (0.1%)**.

The retention times of cystine, cysteine and acetylcysteine are about 3.6 minutes, 4 minutes and 6 minutes respectively (British Pharmacopoeia Commission, 2022).

5.8 CALIBRATION CURVE CONSTRUCTION:

Once calibration standards have been prepared, the calibration curve is constructed as follows:

- Inject 20µL of each calibration standard into HPLC system, using the chromatographic conditions specified in Section 5.3.
- Record the peak area corresponding to each calibration standard.
- Plot the recorded peak areas against their known concentrations to construct the calibration curve and determine the regression line.

The calibration curve serves as reference against which the sample's peak area is later compared to determine acetylcysteine content.

5.9 SAMPLE ANALYSIS:

Once the test solution and the reference/system suitability solutions have been prepared (Sections 5.5 and 5.6), the following injection sequence is used:

- Inject 20 µL of **solution (4)** (system suitability solution) and confirm the resolution between the cysteine and cystine peaks meets the criterion in Section 5.6 before proceeding. If this criterion is not met, the system is not suitable and the run must not continue.
- Inject 20 µL of **solution (2)** (freshly prepared N-acetyl-L-cysteine reference) and **solution (3)** (aged 2 hours), recording the N,N'-diacetylcystine peak, if any, in each.
- Inject 20 µL of **solution (5)** (diluted system suitability solution) to establish the reference peak area for the 0.1% disregard limit.
- Inject 20 µL of the **test solution (1)** , the prepared sample dilution, under the same chromatographic conditions used for the calibration standards.

- Record the peak area corresponding to acetylcysteine in the test solution, together with any secondary peaks corresponding to cysteine, cystine, or N,N'-diacetylcystine.
- Confirm the identity of the acetylcysteine peak by comparing its retention time to that of the calibration standards.

5.10 DATA ANALYSIS:

Before the sample's acetylcysteine content is calculated, the chromatograms obtained from solutions (2) to (5) must first be reviewed to confirm the system is suitable for use:

- From the chromatogram of **solution (4)**, measure the height of the trough separating the cysteine and cystine peaks and the height of the cysteine peak. Confirm the trough height is less than one quarter of the cysteine peak height.
- Compare the chromatograms of **solution (2)** (freshly prepared) and **solution (3)** (aged 2 hours). Confirm that a peak corresponding to N,N'-diacetylcystine (retention time ≈ 13 minutes) is present in solution (3) with a greater area than any corresponding peak in solution (2), demonstrating the system can detect this degradation product as it forms.
- Use **solution (5)** as the reference point for the 0.1% disregard limit: any peak in the test solution's chromatogram with an area less than 0.1% of the acetylcysteine peak area is disregarded.

Only once these criteria are satisfied is the test solution's chromatogram used for quantification:

- Read the sample's acetylcysteine concentration directly from the calibration curve.
- Calculate the %w/v content, accounting for the dilution factor applied during sample preparation, and compare this to the declared 20% w/v label claim.
- Access related substances by expressing the area of each secondary peak (cysteine, cystine, or N,N′-diacetylcystine) as a percentage of the acetylcysteine peak and compare each against the corresponding limit specified in Section 5.6.
- Confirm that all system suitability and limit criteria have been satisfied before accepting as valid.

5.11 REPORTING:

Acetylcysteine content is reported as % w/v of the nebulizing solution, alongside the % of label claim. Any related substances detected are reported as a percentage of the acetylcysteine peak area, referenced against the British Pharmacopoeia limits for Acetylcysteine Injection (British Pharmacopoeia Commission, 2022).

5.12 CROSS-VALIDATION USING IODOMETRIC TITRATION

Acetylcysteine is a derivative of the amino acid L-cysteine and possess a sulfhydryl group (-SH). Due to having this additional hydrogen atom, acetylcysteine is in a reduced state and has additional electrons that can be donated. Iodine reacts readily with free thiol groups, oxidizing them in a redox reaction. However, this reaction is not selective for acetylcysteine specifically. Iodine will oxidize any free thiol present in the sample, including thiol-containing degradation products.

This lack of specificity is precisely why iodometric titration is unsuitable as the primary quantification method and is instead used here as an independent cross-validation check against the HPLC result, following the British Pharmacopoeia's official assay method for Acetylcysteine Injection.

Procedure:

- Measure out a volume of the nebulizing solution theoretically containing 0.4 g of acetylcysteine.
- Add 20 mL of glacial acetic acid to create the acidic environment required for the redox reaction.
- Titrate with 0.05M iodine volumetric solution (VS).
- Iodine is continuously consumed by the free thiol groups present until no further reducible thiol remains. The next drop of iodine added is then in excess, producing a permanent pale-yellow colour that marks the endpoint.
- Each mL of 0.05M iodine VS is equivalent to 16.23 mg of acetylcysteine (British Pharmacopoeia Commission, 2022).

6. ASSAY VALIDATION

Assay validation is the process used to prove that an analytical procedure is reliable, repeatable, and suitable for its intended purpose. It is a crucial process because it ensures data reliability, complies with regulatory authorities (such as the ICH), guarantees patient safety, and reduces costly errors.

There are four key validation parameters that are set out (Pharmaffiliates, 2026):

1. **Linearity:** This refers to the ability to get test results that are directly proportional to the concentration of the sample in a particular range.
2. **Accuracy:** How close is the test result to the true reference value set out?
3. **Precision:** How close the measurements and results are to each other when performed under the exact same conditions.
4. **Specificity:** This refers to the ability of a particular assay to measure only the substance intended for analysis without any interference.

6.1 LINEARITY:

This parameter can be evaluated by plotting the detector signals as a function of analyte concentration. To assess linearity, a minimum of five concentrations distributed across a specified range is required. For the procedure to be reliable, the obtained analyte signals must be directly proportional to the true sample concentrations.

Test results should be evaluated using appropriate statistical methods, such as calculating the linear regression line, to provide mathematical estimates of linearity. Additional reporting requirements include a plot of the data, the correlation coefficient, the y-intercept, and the slope of the regression line. Lastly, analysing the deviation of actual data points from the regression line is useful, as all points impact the overall linearity.

6.2 ACCURACY:

Accuracy is shown by comparing results obtained from the procedure with expected values. It is important that accuracy is demonstrated with test conditions that remain consistent in the procedure. In this case, the concentration of Acetylcysteine obtained from the HPLC can be compared to a reference material, such as comparing it to the concentration of the standard. The measured versus the theoretically expected results can then be evaluated. For the above comparison of actual and measured concentrations, calculating the percentage stated is required using the following formula:

$$\%\ stated = \frac{actual\ value}{stated\ value} \times\ 100\%$$

As per the British Pharmacopoeia for Acetylcysteine Injection, the percentage stated is required to be at 95.0 to 105.0%.

6.3 PRECISION:

Validation tests for assays usually include an evaluation of precision. One of the ways in which precision can be evaluated is through repeatability. According to the ICH Q2 (R2) Guideline, there are two ways repeatability can be assessed:

- Obtaining a minimum of 9 determinations along the desired range, such as having 3 concentrations and 3 replicates each time.
- Having a minimum of 6 determinations at 100% of the test concentration.

Proceeding this, using the results obtained from the analytical procedure, a percentage relative standard deviation (%RSD) can be calculated using the following formula:

$$\%RSD = \frac{standard\ deviation}{mean} \times 100\%$$

For the results to be considered precise, the %RSD has to fall within the BP range, which is ≤2%.

6.4 SPECIFICITY:

In this procedure, using HPLC means having an inherently selective procedure on interferences of impurities. In this case, by looking at the chromatogram obtained at the end of the procedure and comparing it to reference material, determining that there is no presence of impurities and no impact of them on the identification or quantification of the analyte can be done. This can be done by comparing the relative peaks of the chromatograms as well as determining whether there are any additional peaks present or not.

6.5 CROSS-VALIDATION:

Finally, a cross-validation comparison can be done by using the Iodometric titration. Due to its ability to bind to the thiol group in Acetylcysteine, the quantification of Acetylcysteine can be made via this back-titration and be compared to that of the results obtained from HPLC.
It is important to note that Iodometric titration is used to validate the results from HPLC- it is a precautionary measure. However, it is non-selective, as discussed under Section 5.11, therefore, it does not exclude any potential impurities that could be present, which can skew the results obtained. Therefore, the Iodometric titration is used purely as a precaution for cross-validation.

DISCUSSION AND CONCLUSION

Discussion

The analytical technique developed for this assignment was primarily shaped by the nature of the sample and the specific chemical degradation risk associated with Acetylcysteine. Because the sample is a homogenous, single-phase aqueous system, sample preparation did not require the API extraction step typically used with semi-solid dosage forms. Instead, the analytical method must focus on distinguishing the chemical degradation products of Acetylcysteine—principally N,N'-diacetylcystine, cysteine, and cystine—formed via the oxidation of the free reactive thiol (-SH) group. Therefore, High-Performance Liquid Chromatography (HPLC) was chosen as the primary method, since chromatographic separation allows the intact drug to be measured independently of any degradation products present in the sample.

Since there is no official British Pharmacopoeia monograph for an Acetylcysteine Nebulising Solution, the official Acetylcysteine Injection monograph was adapted for this assignment. This approach was considered appropriate given that both preparations are sterile, unpreserved aqueous acetylcysteine solutions sharing the same excipients (disodium edetate as a chelating agent, with pH adjustment to limit oxidation). The chromatographic conditions and working concentrations used in the injection monograph were also applied to this assay.

Notably, the official assay method used for acetylcysteine content in aqueous preparations is iodometric titration, with HPLC reserved for the Related Substances (Impurity) test. The rationale for choosing HPLC as the primary method lies with a specific limitation of iodometric titration. Because iodine oxidises any free thiol present, titration measures the total reducible sulfhydryl content; consequently, it cannot measure the acetylcysteine content specifically. HPLC was therefore chosen as the primary method due to its superior specificity, while iodometric titration was incorporated as an independent cross-validation check.

The validation strategy for this assay was designed to address four core parameters — linearity, accuracy, precision, and specificity — in line with recognised pharmaceutical validation principles (Pharmaffiliates, 2026; ICH, n.d.). Linearity was addressed through a five-point calibration range with statistical evaluation via regression analysis, while accuracy and precision were designed to be assessed against the British Pharmacopoeia's acceptance criteria for Acetylcysteine Injection (95.0–105.0% recovery and %RSD ≤2%, respectively). Specificity was addressed inherently through the chromatographic separation itself, since HPLC allows the acetylcysteine peak to be evaluated independently of any co-eluting impurities. To further strengthen the accuracy assessment, cross-validation against iodometric titration was incorporated. However,

because this titration reacts with any free thiol group present rather than acetylcysteine specifically, it was deliberately used only as a supporting precautionary check rather than as a specificity-confirming method in its own right, since its non-selective nature means it cannot itself rule out interference from thiol-containing degradation products.

Certain limitations must be acknowledged. Acetylcysteine's oxidative instability means that even minor delays or inconsistencies in sample handling between preparation and injection could introduce variability. Precautionary measures built into Sections 3 and 4 were intended to mitigate this, but cannot completely eliminate it. Additionally, because this method was adapted from a parenteral monograph rather than one specific to nebulising solutions, its performance has not been independently validated against an official reference.

Conclusion:

The HPLC method developed for this assignment is designed to provide a specific, accurate, and precise means of quantifying acetylcysteine content in a 20% w/v nebulising solution, while remaining capable of distinguishing between the intact drug and its principal oxidative degradation products. Its performance was intended to be confirmed through a structured validation approach assessing linearity, accuracy, and precision against British Pharmacopoeia acceptance criteria, supported by cross-validation against iodometric titration as an additional, though non-selective, precautionary check. It is suitable for routine quality control testing of the formulation, ensuring patients receive a product that contains the labelled concentration of the drug and is free from chemical degradation products. However, this is only a proposed method that is still awaiting practical confirmation.

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