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Novel Synthesis of *N*-Acetylcysteine Medicine Using an Effective Method

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Abstract: *N*-acetylcysteine (NAC) is mainly administrated as a mucolytic medication, antioxidant supplement, antidote in paracetamol overdose, and a drug for the prevention of diabetic kidney disease. Its effect has been investigated for the treatment of several diseases such as COVID-19. In this work, an effective method for high-yield synthesis of *N*-acetylcysteine is proposed. This drug can be synthesized in a single-batch step instead of using a multi-stage process. The proposed method has shown the potential to be considered as an alternative method for producing NAC. The purification process was carried out using suitable solvents to reach a high level of purity. The characterization of the synthesized drug was undertaken through Elemental analysis, Proton Nuclear Magnetic Resonance (^{1}H NMR), High Performance Liquid Chromatography (HPLC), Fourier Transform Infrared Spectroscopy (FT-IR), and melting point analyses.

Keywords: *N*-acetylcysteine (NAC); drug synthesis; lung anti-inflammatory; cough medicine; antioxidant; pharmaceutical supplement; characterization

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1. Introduction

Although *N*-acetylcysteine is an antioxidant and is used as an antidote to paracetamol (acetaminophen) overdose, it also has many other applications supported by clinical evidence. *N*-acetylcysteine can support the body's antioxidant level during infections, toxic assaults, inflammations, and stresses. It is often prescribed to patients who are at high risk for hepatotoxicity [1]. In addition, *N*-acetylcysteine is a precursor to make glutathione, which is the major antioxidant in the human body. It was shown that taking *N*-acetylcysteine as a supplement also increases the levels of glutathione. Glutathione can detoxify a number of toxic compounds, including peroxide, xenobiotics substances, and other radical species. It might also meliorate lung inflammation occurring in influenza [2].

Several studies demonstrate that *N*-acetylcysteine can be effective in the prevention of diabetic kidney disease (nephropathy). High levels of glucose in the blood due to diabetes can damage kidneys and this may cause a leakage of proteins into the urine. It has been reported that taking *N*-acetylcysteine through an intravenous route can prevent this kind of kidney damage regarding the scavenging of free radicals with its antioxidant features [3,4].

Mucus is considered a viscoelastic substance that comprises glycoproteins mixed with water, lipids, and other proteins. The lung uses mucus secretion clearance as a mechanism to defend itself from particles and pathogens present in the inhaled air. Impaired mucus can cause lung dysfunction, and in some lung diseases such as idiopathic pulmonary fibrosis (IPF) and chronic obstructive pulmonary disease (COPD), mucus possesses a higher viscoelastic feature that is not easily cleared. *N*-acetylcysteine alleviates the mucus thickness by hydrolysing the disulphide bonds of mucus proteins to reduce its viscosity [5].

COVID-19 is a devastating pandemic that has affected humankind worldwide. Lung infection is one of the main clinical manifestations that might cause acute respiratory distress syndrome and cardiovascular alterations. It is demonstrated that an oxidative stress imbalance occurs in patients with the disease [6]. The depletion of glutathione can

cause glucose 6-phosphate dehydrogenase (G6PD) deficiency and facilitate coronavirus infection [7]. *N*-acetylcysteine can be administrated not only as a mucolytic or lung anti-inflammatory drug, but also as an antioxidant, and the precursor of glutathione to replenish the level of glutathione in the body. Because of this feature, *N*-acetylcysteine may be considered as a COVID-19 preventive-therapeutic medicine, as it has previously demonstrated its remedial effects on influenza and influenza-like sicknesses [8].

Regarding anti-oxidation chemistry of *N*-acetylcysteine, intracellular oxidation happens when a reactive nitrogen species or reactive oxygen species is generated beyond the cells' anti-oxidation ability. Excess oxidative stress can result in the oxidation of proteins, lipids, DNA, and even cell death. This oxidation process can lead to many pathological conditions [9]. Either natural or synthetic antioxidants are effective to alleviate the cumulative effects caused by oxidative stress and *N*-acetylcysteine is of remarkable interest, as it is a particular direct antioxidant that reacts with electrophilic groups of free radicals via its thiol side chain [10]. Since N-acetylcysteine reacts rapidly with carbon trioxide ion ($CO_3 \cdot^-$), nitrogen dioxide ($\cdot NO_2$), and hydroxyl radical ($\cdot OH$), it can detoxify the reactive oxygen species generated by white blood cells [11].

Furthermore, *N*-acetylcysteine can chelate transition metal ions such as Fe^{3+} and Cu^{2+}, as well as heavy metal ions such as Pb^{2+}, Hg^{2+}, and Cd^{2+} via its free thiol group. This chelation assists the removal process of these metal ions from the body [4].

In addition to acting as a direct antioxidant, *N*-acetylcysteine has shown indirect anti-oxidation features. This feature is due to its ability to replenish intracellular glutathione. Regarding the enormous antioxidants feature of glutathione in the intracellular environment and the low concentration of *N*-acetylcysteine inside the cells, it is likely the main anti-oxidation efficacy of *N*-acetylcysteine is related to retaining the glutathione levels inside the cells [12].

Yamamoto et al. [13] have proposed utilizing hyperpolarized [1-^{13}C] *N*-acetyl cysteine as a novel probe to monitor in vivo glutathione redox status in human tumours. Glutathione redox plays an important role in metabolic chemistry and the method they proposed allows us to access oxidative stress in human tumour cells. The real-time monitoring (^{13}C MRS imaging) in vivo illustrates the physiological process and progression of diseases via changes in the metabolic flux.

It is worth mentioning that most of the research studies in the literature are about the synthesis of *N*-acetylcysteine derivatives and their clinical examination. However, published articles that indicate an explicit method for the synthesis of *N*-acetylcysteine are very scarce. This work presents a novel process for chemical synthesis and purification of this drug in a very high yield. The advantage of using the corresponding *N*-acylbenzotriazole as an effective acylation agent is taken into account to form a peptide bond with L-cysteine amino acid [14,15]. It can be considered as an alternative method to be replaced with the complicated multi-stage process, which is used for making this drug.

2. Materials and Methods

2.1. Materials

L-cysteine (99%), thionyl chloride (>99%), acetic acid (>99%), 1*H*-Benzotriazole (99%), acetone (99.5%), methanol (99.8%), ethanol (99.5%), and diethyl ether (99%) were all purchased from Sigma-Aldrich (Sydney, Australia) and used as received. For all experiments, Milli-Q water with a conductivity of 1.2 (μS/cm) was used.

2.2. Methods

2.2.1. Novel Chemical Synthesis of *N*-Acetylcysteine

N-acetyl-1*H*-benzotriazole was prepared by the reaction of acetic acid with the mixture of 1 equiv of 1H-benzotriazole and 1 equiv of $SOCl_2$ at room temperature, 21 °C, for 2 h [16]. $SOCl_2$ is a highly reactive compound that can violently react with water and other reagents. It is recommended to follow the safety regulation to work with this reagent. The product was recrystallized using acetone and diethyl ether (*v*:*v* 50%).

First, 0.02 mol (3.22 g) of *N*-acetyl-1*H*-benzotriazole was dissolved in 30 mL of methanol by stirring at room temperature, 21 °C, then 0.02 mol (2.42 g) of L-cysteine was added to the solution and was stirred for 3 h. The pH of the solution was measured at about 5. Regarding *N*-acetylcysteine is soluble in methanol, the produced solution was left over night to vaporize the solvent. In order to purify the synthesized product, three separation steps were undertaken. In the first step, the dried residual solid was stirred in 50 mL of Milli-Q water for about 5 minutes to isolate the untreated *N*-acetyl-1*H*-benzotriazole from the other compounds as it is insoluble in Milli-Q water. Then, the mixture was filtered out and the residual solution was dried again and prepared for the next step. In the second step, the unreacted L-cysteine was separated by applying 25 mL of dried ethanol as L-cysteine is insoluble in dried ethanol. After 5 minutes of stirring, L-cysteine remained as the precipitation which was filtered out. After this step, the residual solution contained *N*-acetylcysteine as the product and benzotriazole as a by-product. In the third step of purification, 25 mL of diethyl ether was used as a solvent since benzotriazole dissolves in this solvent, however, *N*-acetylcysteine remains insoluble. Therefore, the precipitated solid was filtered and dried out under a vacuum condition and was sent for characterization analyses. Figures 1 and 2 illustrate the schematic synthesis reaction of *N*-acetylcysteine and the isolation process, respectively.

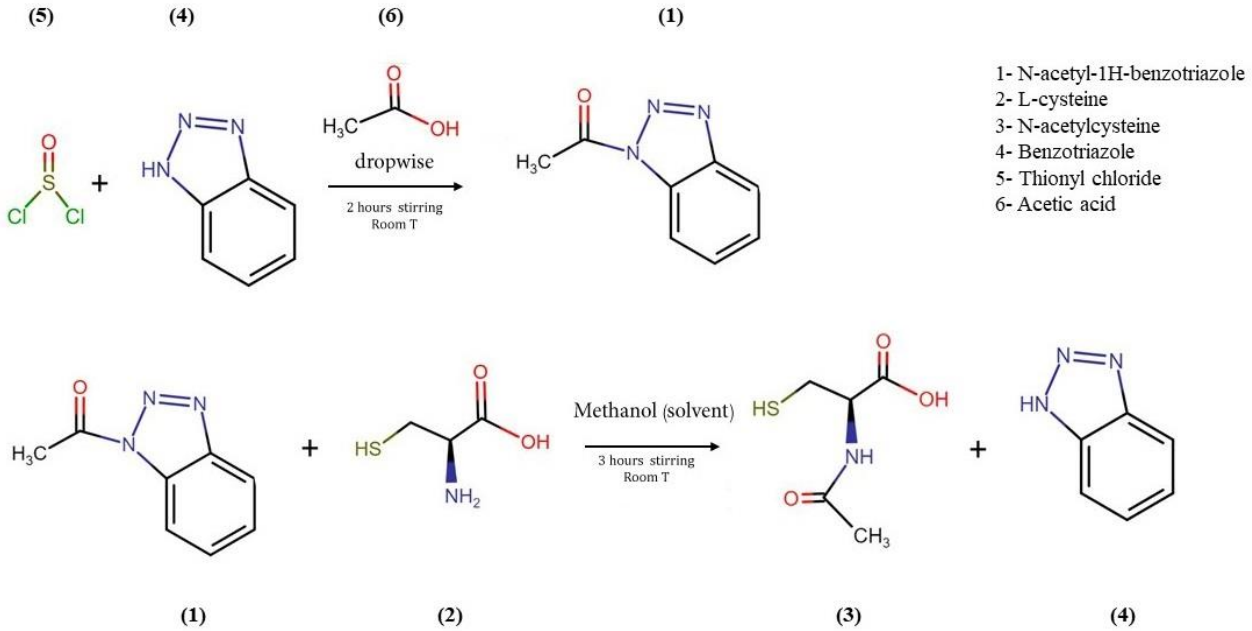

Figure 1. The schematic chemical synthesis of *N*-acetylcysteine.

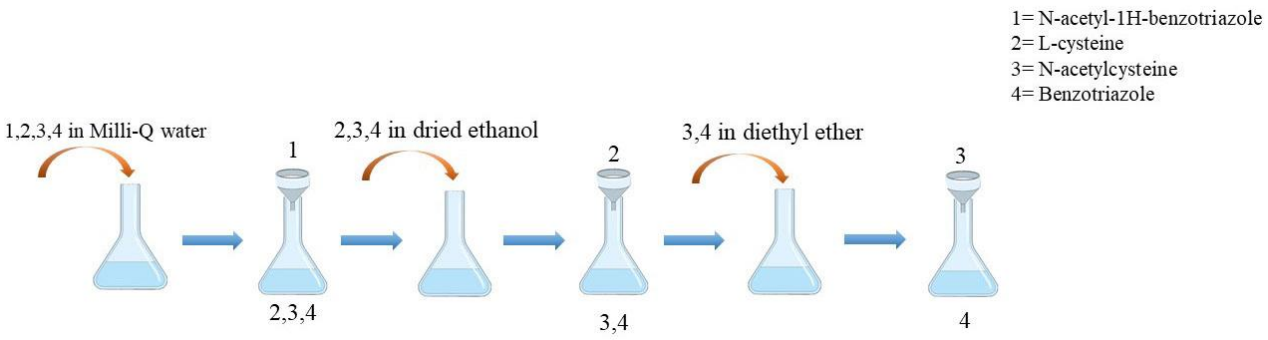

Figure 2. The schematic of three-step isolation process of *N*-acetylcysteine.

2.2.2. Characterization Method

The synthesized *N*-acetylcysteine was characterized by undertaking ^{1}H NMR analysis using a Bruker AVII 600 MHz NMR Spectrometer (Billerica, MA, USA). In addition, FT-IR spectra analysis was accomplished using a Thermo Scientific Nicolet 6700 FT-IR Spectrometer (Waltham, MA, USA). For detecting the purity (%) of the synthesized compound a Reverse Phase Liquid Chromatography was carried out using a Shimadzu Prominence Ultra Performance Liquid Chromatography system (Kyoto, Japan). Furthermore, CHNS-O elemental analysis was performed using an elemental analyzer model PE2400 PerkinElmer (Shelton, CT, USA). Followed by measuring the melting point through an electro-thermal melting point apparatus IA9100, Cole-Parmer (Vernon Hills, IL, USA).

3. Results and Discussion

3.1. Characterization of the Synthesized N-Acetylcysteine

The purified *N*-acetylcysteine was analyzed by ^{1}H NMR spectroscopy. Hydrogen-deuterium oxide was used as a solvent. The result indicates a sharp peak in hydrogen-deuterium oxide (HDO) at 4.70 ppm. Other peaks are δ (ppm) = 4.54 (t, 1H, CHCOOH), 2.90 (t, 2H, CH_2S), 1.98 (t, 3H, CH_3). This analysis confirms the structure of the $C_5H_9NO_3S$. The obtained ^{1}H NMR spectrum is depicted in Figure 3.

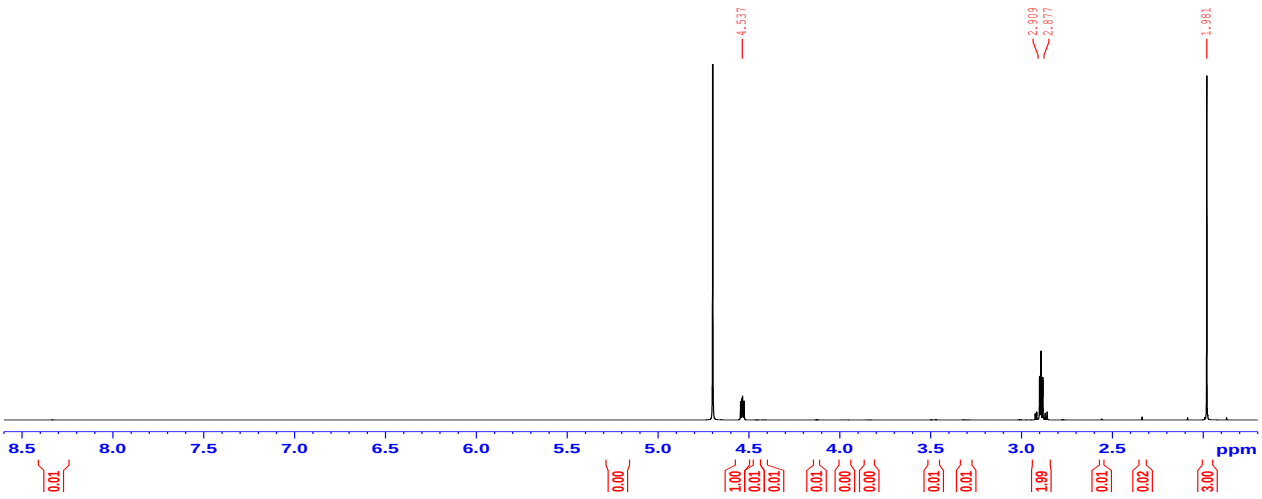

Figure 3. ^{1}H NMR spectrum of purified *N*-acetylcysteine molecule.

Moreover, FT-IR spectroscopy was undertaken to figure out the functional groups of the synthesized molecule. Based on the obtained results illustrated in Figure 4, a strong peak at 3375 (cm^{-1}) represents the N-H bond, and the peaks at 2965, 2900, and 2810 (cm^{-1}) correspond to C-H bonds. The small peaks at 2692 and 2547 (cm^{-1}) are because of O-H functional group of carboxylic acid and S-H bond, respectively. Two relatively sharp peaks at 1916 and 1716 (cm^{-1}) illustrate C=O functional group of acid and amide, respectively. Therefore, the presence of NH, CH, C=O, SH, and COO functional groups is affirmed by FT-IR spectroscopy analysis.

Furthermore, HPLC analysis was used for further purification and measuring the purity of synthesized *N*-acetylcysteine. Waters X-Bridge BEH C18 column (130 Å, 5 µm, 4.6 × 150 mm) and Waters Atlantis T3 C18 column (3 µm, 4.6 × 50 mm) were used as the stationary phase at a flow rate of 1.0 mL/min. The mobile phase was composed of eluents A (0.1% (*v*/*v*) aqueous formic acid in MilliQ water) and B (0.1% (*v*/*v*) aqueous formic acid in acetonitrile). The obtained result is depicted in Figure 5. The running time for the main peak is 6.1 min and the related surface area (purity %) of the main isolated fraction is 99.2%. Therefore, considering the percentage of the purity, 3.044 g of pure *N*-acetylcysteine is obtained, which gives a synthesis yield of 94 %.

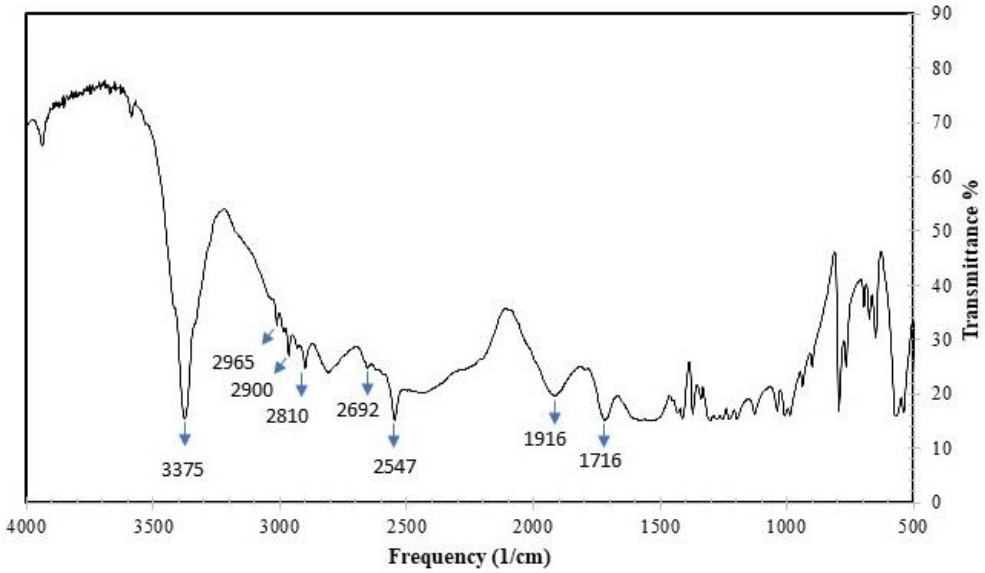

Figure 4. FT-IR spectrum of purified *N*-acetylcysteine molecule.

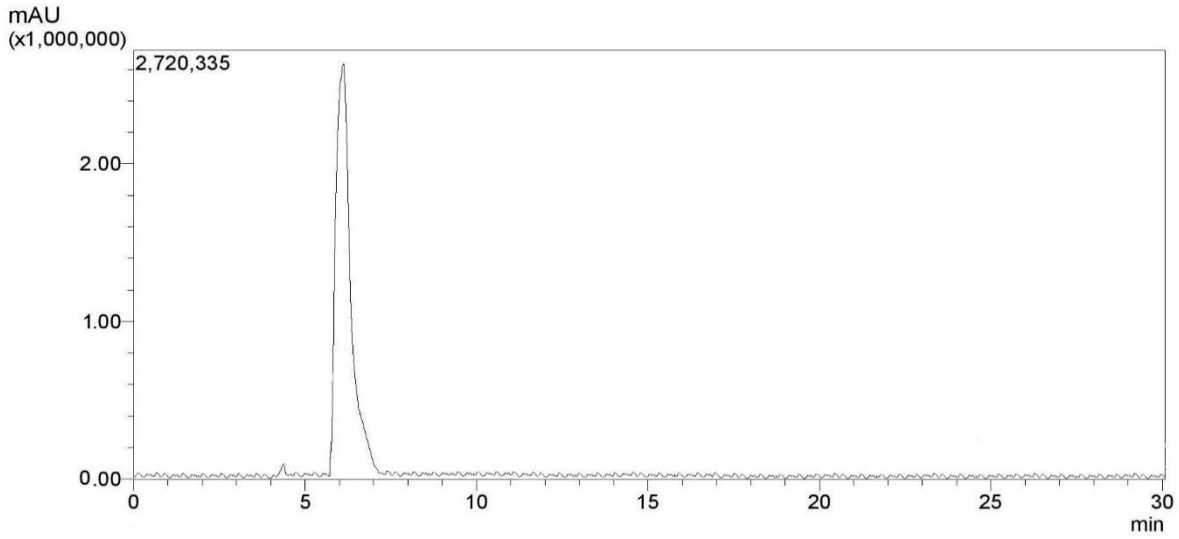

Figure 5. The HPLC analysis of purified *N*-acetylcysteine molecule.

Elemental analysis was also undertaken and the achieved data is presented in Table 1. This table demonstrates an excellent match of the expected theoretical values and the measured values.

Table 1. The obtained elemental analysis data of the synthesized *N*-acetylcysteine.

Sample	Weight (mg)	C%	N%	H%	S%
Test 1	1.17	37.0	8.5	5.5	20.8
Test 2	1.38	37.1	8.6	5.6	20.9
Expected values		36.8	8.6	5.5	19.6

The melting point *N*-acetylcysteine was detected at 108–109.3 °C confirming the melting point reported in the literature [17].

3.2. Alternative Synthesis Methods

Riera et al. suggested a two-step route to make *N*-acetylcysteine by acylation of L-cystine and then taking the advantage of reduction methods to reach *N*-acetylcysteine.

In their proposed method, the aim of reducing bis-acetyl-L-cystine is to breakdown the L-cystine's S-S bond and convert it into two S-H bonds resulting two *N*-acetylcysteine molecules [18]. For the reduction purpose, a reducing agent such as metallic zinc or an electrochemical reduction process were considered.

The main problem of this method lies on the separation process where metallic zinc converts into Zn^{2+} ions which contaminate the medium with a high concentration of zinc ions. Therefore, isolating *N*-acetylcysteine to reach a high grade can be a challenge. On the other hand, managing the residual aqueous solution containing high contents of Zn^{2+} ions is an environmental issue. In this method, the production of hydrogen gas should also be managed due to its potential explosion issue. Moreover, the presence of unconverted zinc in the final product should be addressed by forming the corresponding lead mercaptan, followed by treatment with H_2S gas, removal of formed lead sulphide, and then recrystallization with suitable solvents to obtain purified *N*-acetylcysteine.

N-acetyl-L-cysteine can be made through direct acylation in the presence of sodium acetate [17,19]. For this purpose, a suspension of L-cysteine hydrochloride monohydrate is stirred in a reaction vessel containing aqueous tetrahydrofuran under a nitrogen atmosphere, and sodium acetate trihydrate is added. The mixture is stirred at room temperature to ensure the neutralization of the hydrochloride salt, resulting in the formation of a suspension of equimolar amounts of cysteine and sodium acetate is obtained. The mixture is then chilled by external cooling and acetic anhydride is added dropwise. The resulting suspension is stirred at room temperature and heated at reflux. The resulting suspension of sodium *N*-acetyl-L-cysteinate is then neutralized by aqueous hydrogen chloride. The resulting sodium chloride is removed by filtration and the product is isolated by distilling the solvent from the filtrate in vacuo. This is followed by crystallization to reach *N*-acetylcysteine as a white solid.

Besides, following the method proposed by Katritzky et al. [20] failed to synthesize *N*-acetylcysteine. Apparently, this is due to the presence of water in their method, as water hydrolyses *N*-acetylbenzotriazole. Additionally, the presence of acetonitrile in their method is another issue, since acetonitrile may compete with L-cysteine to form a peptide bond.

4. Conclusions

This research presents an efficient and uncomplicated method for the chemical synthesis of *N*-acetylcysteine, which is mainly administrated as a mucolytic medication, antioxidant supplement, and an antidote in paracetamol overdose. This work has improved the synthesis process of this drug, using a peptide-making route to achieve a high yield of the product, as the obtained characterization data shows a reaction yield of 94%. The purification process was undertaken in three steps using suitable solvents to reach an excellent purity. Since the proposed method does not use any reduction agents, it can address the concerns that may arise due to the presence of metals such as zinc in the final product.

Author Contributions: F.Z. contributed to carrying out experiments, characterization analyses, interpretation obtained analysis data, writing the manuscript. M.Z. contributed to undertaking experiments, characterization analyses, interpretation obtained analysis data, writing the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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