AN EFFICIENT PROCESS FOR ISOLATION AND PURIFICATION OF VANCOMYCIN HYDROCHLORIDE IN COMPLIANCE WITH INTERNATIONAL PHARMACOPOEIAL STANDARDS

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ABSTRACT

Vancomycin hydrochloride belongs to the glycopeptide antibiotics and has long been used to treat infections caused by methicillin-resistant Staphylococcus aureus (MRSA) in patients with penicillin and cephalosporin allergy. Vancomycin hydrochloride has also long been used to protect patients against infections during surgery. However, Vancomycin hydrochloride has a very complicated structure and is not stable. Thus, isolation of high-purity vancomycin hydrochloride through microbial fermentation broths has long been more difficult when pharmacopeial standards are strictly enforced. This study improved the process of isolating and purifying vancomycin hydrochloride with high purity from Amycolatopsis orientalis fermentation broths. The first step of the process involved cell separation through microfiltration. The second step involved removal of impurities through adsorption resin column chromatography, using 20% methanol as elution solution. Ion exchange resins were used to precisely target the purification step, and then nanofiltration to concentrate the solution. Finally, crystallization or lyophilization steps were carried out to harvest the final material. Results showed that slow pH increase to 8.0 with 10% ammonia solution during base precipitation and subsequent lyophilization produced the highest purity and recovery of vancomycin hydrochloride. Vancomycin content of 95.65% was achieved with purity and recovery exceeding that specified by the United States Pharmacopeia (USP) and European Pharmacopoeia. The content of the impurities was below that specified by the United States Pharmacopeia (USP) and European Pharmacopoeia. This study provides a simple and fast process to isolate vancomycin hydrochloride with high purity that can now be grown commercially. This method meets international pharmaceutical standards.

KEYWORDS

vancomycin, vancomycin hydrochloride, Amycolatopsis orientalis, glycopeptide antibiotics, MRSA treatment, pharmaceutical purification, lyophilization, antibiotic manufacturing

INTRODUCTION

In 1956, *Amycolatopsis orientalis*, also known as *Streptomyces orientalis* and *Nocardia orientalis,* was used to extract vancomycin, which is considered to be the first glycopeptide antibiotic [1, 2]. Gram-positive bacteria are rendered incapable of forming cell walls when vancomycin is present. The treatment of methicillin-resistant Staphylococcus aureus (MRSA) infections and endocarditis in patients who are allergic to penicillin and cephalosporin is a popular application of this medication. As an additional point of interest, vancomycin is the principal therapeutic medication that is utilized for the purpose of preventing MRSA infections during cardiac surgeries that involve artificial implants, orthopaedic procedures, and neurosurgeries for the insertion of a ventriculoperitoneal shunt [3].

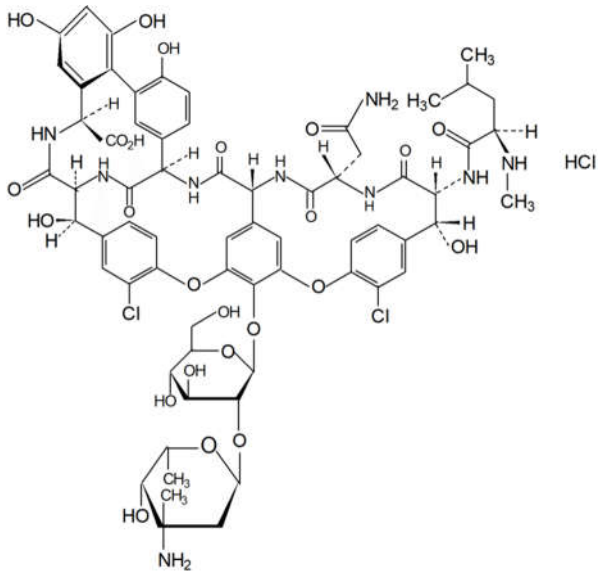

Figure-1: Molecular Structure of Vancomycin Hydrochloride

The vancomycin molecule consists of two basic structures, namely the glycosyl moiety α-o-vancosamine-β-o-glucosyl and the peptidyl moiety central heptapeptide nucleus, whose structure determines its instability, and the vancomycin molecule degradation products are produced by acid or alkali, or high temperature conditions, and the structure also has a plurality of free phenolic hydroxyl groups, which causes them to be easily oxidized into hydrazine. It has been reported in the literature that vancomycin will hydrolyse under acidic conditions and high-temperature conditions, and it will be removed. A glycosyl or a disaccharide group produces a monosaccharide vancomycin or an aglucovancomycin. In a weak acid environment, an amide group may also be degraded to deamino vancomycin, which has two Isomers. Based on these characteristics of vancomycin's chemical structure, it has brought certain difficulties to the manufacture of high-purity vancomycin hydrochloride [4, 5, 9, 10].

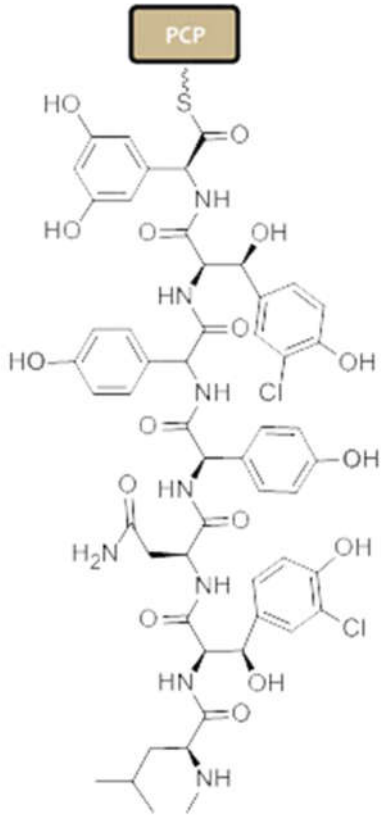

Figure-2: Linear heptapeptide, which consists of modified aromatic rings

The vancomycin hydrochloride product has been developed for decades. The early preparation process is generally carried out by using solvents such as methanol, ethanol, isopropanol, acetone, etc., and precipitation by salting out by adding ammonium chloride or sodium chloride.[6, 7] The preparation of the finished product, but due to the presence of more impurities in the vancomycin fermentation broth, especially vancomycin structural analogs, the purity is generally not high, and to achieve a chromatographic purity greater than 93% of the European Pharmacopoeia standards have a certain Difficulty. [8]

It is necessary to go through a number of different processes in order to purify vancomycin that is produced through microbial fermentation. In the pharmacopoeias of both the United States and Europe, the levels of total and individual pollutants, as well as the vancomycin content, are subjected to stringent monitoring. Within the parameters of the HPLC analysis method that is recommended by the United States Pharmacopeia (USP), the vancomycin content must be greater than 88%, and the content of any other drugs that are present must not be greater than 4%. Vancomycin content must be greater than 93%, as stipulated by the European Pharmacopeia, [11] and any additional ingredient with a content greater than 4% is regulated in a manner that is comparable to that of the United States Pharmacopeia [12]. It is necessary to go through numerous phases of separation and purification in order to comply with these severe regulations.

The isolation of vancomycin from fermentation broth involves several key steps:

- Separation of cells: Cells are separated from the fermentation broth using methods of microfiltration. Solid-liquid separation is the first step of downstream processing after microbial fermentation of vancomycin, to separate whole cells (cell biomass) and insoluble components from the fermentation broth.
- Initial purification: The vancomycin is purified to remove impurities by means of an adsorption resin column chromatography.
- Metabolite-specific purification: The vancomycin was further purified by using an ion exchange resin to remove metabolite-specific purification.
- Final Crystallization: The product is further polished to achieve a high purity level, crystallization is typically used as the final purification stage in the manufacturing process of a pharmaceutical, such as antibiotics, that is extremely pure.

A key technology for the isolation and purification of materials, as well as for controlling their physical properties and shape, crystallization is the process of precipitating a solid from a liquid by evaporation or cooling. Crystallization is a technique that leads to the production of high-value-added products while simultaneously improving the overall quality of the finished goods.

The main challenges regarding the isolation of vancomycin from fermentation broth are-

- Removal of impurities from vancomycin fermentation broth, especially vancomycin structural analogs
- Selection of elution solution for adsorption resin
- Removal of ionic impurities
- Complies with the quality of Vancomycin Hydrochloride with the United States Pharmacopeia and European Pharmacopeia

MATERIAL AND METHODS

Microorganism and cultivation conditions: The vancomycin that was made came from a mutant strain of Amycolatopsis orientalis (ATCC 43491) that made a lot of it. The strain was kept at 37 degrees Celsius on a modified King's medium with the following composition (g/L): maltodextrin, 40; potato protein, 100; K2HPO4.3H2O, 100. The fermentation process took place in a liquid medium for 180 ± 24 hours at 37 degrees Celsius with the following composition (g/L): soya flour, 1000; CaCl2, 30; hydroquinone, 1.0; pyridoxine HCl, 1.0; (NH4)2SO4, 2.4.

Isolation and purification of vancomycin: After the fermentation process was over, hydrochloric acid was used to lower the pH of the culture broth to 2.0+0.2. Microfiltration was then used to separate the cell mass from the acidified broth. After that, the product solution is loaded into an adsorption resin column (HP20). The product was eluted by a 20% methanol solution, 20% acetone solution, or 20% IPA solution. A 10% HCl solution was used to change the pH of the eluted product solution to between 2.0 and 3.0. After that, the solution went through anion exchange resin and then cation exchange resin at a rate of 0.5 BV per hour. Nanofiltration was used to make the product solution more concentrated. To separate the base from the concentrated material, a 10% ammonia solution was used to basify it at temperatures between 0 and 5 degrees Celsius. The product was filtered and washed with cold water. The wet base was dried in a vacuum oven at 35 degrees Celsius. The dry base was mixed with water and then acidified with hydrochloric acid up to pH 2.0. The clear solution was then charcolized and passed through a 0.45µ µm filter. The outcome was either crystallized by adding a solution in cooled methanol, filtering the wet cake, washing it with chilled methanol, and drying it under vacuum at 55+5 degrees Celsius or lyophilized at -80 degrees.

Identification: Identification of the components of the obtained substance and their quantification was performed by HPLC. The analysis was performed using an Agilent 1200 chromatographic system (Agilent technologies, USA) with an ODS C-18(250mm x 4.6 x 5µm). The mobile phase was 70:29:1, buffer solution (TEA):acetonitrile:THF, the flow rate was 2.0 mL/min at 25°C. The absorbance was measured at 280nm; the sample volume was 20 µl. A standard preparation of Vancomycin Hydrochloride (CRS) dissolved in water mix up to a concentration of 0.5 mg/mL was used as a reference sample. The retention time for vancomycin hydrochloride was 23 min.

RESULTS AND DISCUSSION

In the course of this study, we evaluated many elution solvents, including 20% methanol, 20% acetone, and 20% isopropyl alcohol, aiming to enhance both the yield and quality of the eluted product. Of the solvents examined, methanol facilitated the best yield and superior product quality. The table displays the comprehensive findings.

Table-1: Comparison of Results in between the different elution solvents in terms of Overall Yield, Output of Resin Stage and Vancomycin Content

Sr. No.	Elution Solution	Output of Resin Stage (%)	Overall Yield of batch	Vancomycin Content
1.	20% Acetone Solution	56.74%	37.85%	91.23%
2.	20% IPA solution	65.85%	40.54%	92.15%
3.	**20% Methanol solution**	**80.92%**	**57.82%**	**95.65%**

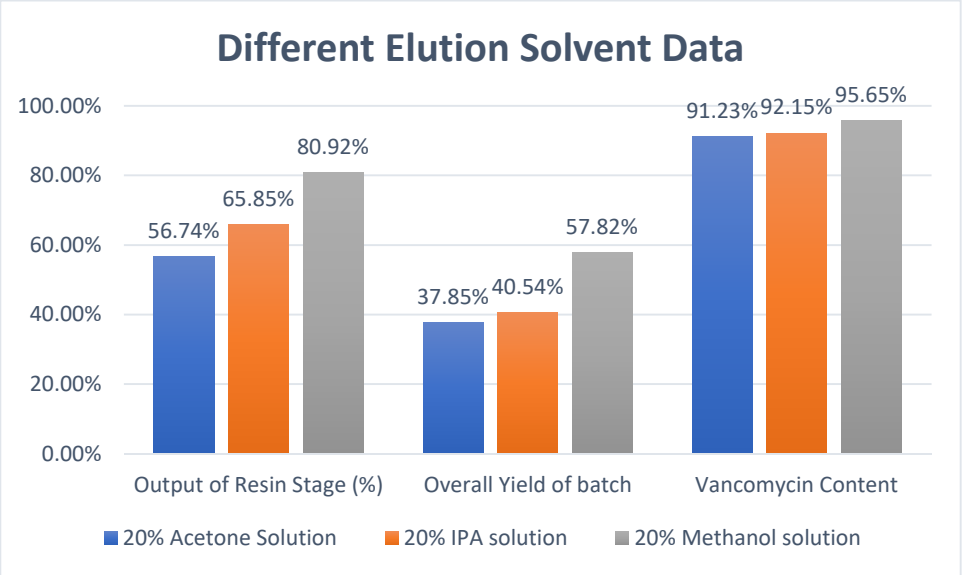

Figure-3: Comparison of Results (Table-1) in between the different elution solvents in terms of Overall Yield, Output of Resin Stage and Vancomycin Content

Post-nanofiltration, the resultant concentrated mass is alkalinized using a 10% ammonia solution (upto three distinct pH levels 8.0, 9.0, and 10.0). The pH level of 8.0 yields optimal output and superior product quality. The ammonia addition pattern during pH adjustment is a crucial factor for achieving optimal yield. Both fast and slow additions significantly affect product degradation, ultimately resulting in decreased yield. The table displays the complete findings. The slow addition yields higher yield means less degradation.

Table 2: Comparison of Results in between the different elution solvents in terms of Overall Yield, Polymyxin B Content, Output of Resin Stage and pH of elution solution

Sr. No.	pH of solution	Addition Pattern	Overall Yield	Vancomycin content
1.	8.0	Fast	46.06%	93.14%
2.	**8.0**	**Slow**	**57.82%**	**95.65%**
3.	9.0	Slow	43.05%	91.12%
4.	10.0	Slow	38.10%	85.05%

Lyophilization and cooled methanol crystallization, both processes are applied to isolate pure vancomycin hydrochloride. While the quantity of vancomycin significantly influences outcomes, the experimental data indicates that the quality of vancomycin remains roughly equivalent in both scenarios. In contrast to crystallization, lyophilization yields a higher quantity of vancomycin hydrochloride during isolation.

Table 3: Comparison of Results in between the different isolation processes

Sr. No.	Isolation Processes	Overall Yield	Vancomycin content
1.	Crystallization	43.02%	94.90%
2.	**Lyophilization**	**57.82%**	**95.65%**

The results obtained prove an adequate degree of isolation and purification of the vancomycin hydrochloride gained by means of the suggested isolation technology to equate the adopted pharmaceutical requirements for vancomycin hydrochloride.

The optimum results with respect to the output of the final product was achieved by using 20% methanol solution as eluent for adsorption resin column, adjusting pH slowly to 8.0 by using 10% ammonia solution during base crystallization and using lyophilization technique to isolate the pure vancomycin hydrochloride. The Vancomycin content observed in the final product is 95.65% well above the limits (not less than 88% as per United States Pharmacopeia (USP) and not more than 93% as per European Pharmacopeia).

CONCLUSION

The research undertaken resulted in the development of an effective and streamlined process of vancomycin hydrochloride separation and isolation appropriate for large-scale production. The process involves the following steps: [1] culture broth pretreatment to give a native vancomycin solution, [2] removal of impurities from the native solution using a adsorption resin column treatment, [3] ionic impurities removal through anion exchange resin and cation exchange resin treatment, [4] solution concentration using nanofiltration, [5] precipitation of the base of vancomycin, [6] isolation of pure vancomycin hydrochloride.

The research indicates an optimized technique for the purification of vancomycin hydrochloride through screening eluents, pH, addition pattern and isolation techniques. Below is the conclusion in a nutshell:

Removal of impurities from vancomycin fermentation broth: The impurities generated during the fermentation broth were removed by using the efficient purification technique. The end product vancomycin hydrochloride with 95.65% content (not less than 88% as per United States Pharmacopeia (USP) and not more than 93% as per European Pharmacopeia) reflect the efficiency of the process.

Elution Optimization: For Adsorption (HP20) Resin column – Methanol as elution solvent gave the maximum output and optimum quality product, the use of a 20% methanol solution improved results significantly, with resin stage output of 80.92%, overall yield of 57.82%, and vancomycin hydrochloride content of 95.65%.

Removal of ionic impurities: The ionic impurities generated during the process were remove smoothly efficiently by using the optimized purification technique. The any other impurities content is 2.14% which is better than the limits (not less than 4.0% as per United States Pharmacopeia (USP) and European Pharmacopeia).

Pharmaceutical Standards: The process efficiently separated toxic impurities in accordance with pharmaceutical standards. The results show the values of vancomycin content and any other impurity content is well better than the pharmacopeial limits.

This purification technique, optimized elution conditions, optimized pH process and efficient isolation technique are recommended for industrial-scale application due to its efficiency and compliance with pharmaceutical standards.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest regarding this manuscript.

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