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Research

Identification of Teriparatide by Sodium dodecyl sulfate polyacrylamide (SDS-PAGE)

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Running title: Identification of Teriparatide by SDS-PAGE

Abstract

Background: SDS-PAGE is a widely used technique for identifying and characterizing proteins based on their size and charge. Teriparatide, a synthetic form of the N-terminal fragment of human parathyroid hormone (PTH), can be identified through SDS-PAGE by its distinct molecular weight (~4.2 kDa) and migration pattern. This method enables the verification of its purity and confirmation of its protein structure during quality control processes. **Aim:** The aim of this study is to identify teriparatide through SDS-PAGE by evaluating its molecular weight and migration characteristics in comparison to reference standard. This technique allows for the accurate determination of its purity and helps confirm the integrity of the recombinant protein. Additionally, it provides a reliable method for distinguishing teriparatide from other protein contaminants. Ultimately, this approach supports quality control and ensures the consistency of the therapeutic agent. **Material & Method:** In this study, we evaluated teriparatide via SDS-PAGE thereby checking its molecular weight for similarity and their qualitative analysis in comparison to reference standard. **Results:** Teriparatide's molecular weight and purity were evaluated using SDS-PAGE. Teriparatide (~4.2 kDa) is represented by a single band on polyacrylamide gel that has been stained with silver nitrate. No other additional bands were observed, suggesting minimal degradation or presence of impurities. These results confirm the identity and purity of the teriparatide sample. **Conclusion:** In conclusion, SDS-PAGE successfully identified teriparatide by revealing its distinct molecular weight and migration pattern. The technique confirmed the protein's purity, ensuring that it met the required standards for therapeutic use. The results also validated the structural integrity of the recombinant protein, distinguishing it from potential contaminants. SDS-PAGE proved to be an effective and reliable method for quality control in the production of teriparatide. Overall, this approach guarantees the consistency and safety of the therapeutic agent for clinical application.

Keywords: Teriparatide, SDS-PAGE, human parathyroid hormone, electrophoresis

Introduction

Osteoporosis is a progressive skeletal disorder characterized by decreased bone mass, deterioration of bone micro-architecture, and an increased risk of fractures [1]. It is particularly prevalent among postmenopausal women and the elderly, contributing to significant morbidity and reduced quality of life [2]. The primary therapeutic goal in osteoporosis management is to reduce the risk of fractures by improving bone strength [3]. While antiresorptive agents such as bis-phosphonates and selective oestrogen receptor modulators (SERMs) are commonly used to slow bone loss, they do not actively promote new bone formation [4]. This limitation has led to the development of anabolic agents, with teriparatide emerging as a major milestone in osteoporosis treatment [5].

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Teriparatide is a recombinant form of human parathyroid hormone consisting of the first 34 amino acids of the native 84-amino acid peptide, known as PTH (1–34) [6]. These amino acids represent the biologically active region of the hormone, which is responsible for its interaction with the parathyroid hormone receptor (PTH1R) found on osteoblasts and renal tubular cells [7]. Unlike continuous exposure to PTH, which favours bone resorption, intermittent administration of teriparatide has an anabolic effect, stimulating new bone formation [8]. This paradoxical mechanism forms the basis for teriparatide's therapeutic application. It is currently approved by regulatory authorities including the U.S. Food and Drug Administration (FDA) for the treatment of osteoporosis in postmenopausal women, men with primary or hypogonadal osteoporosis, and patients with glucocorticoid-induced osteoporosis who are at high risk for fractures [9].

Despite its advantages, teriparatide therapy comes with certain limitations. It is administered via daily subcutaneous injections, which may affect patient compliance [10]. Additionally, the treatment duration is limited to a maximum of 24 months of a patient's lifetime. This restriction is based on animal studies that showed an increased incidence of osteosarcoma in rats exposed to long-term, high doses of teriparatide [11]. However, similar findings have not been reported in humans. Furthermore, teriparatide is relatively expensive, which can limit its accessibility for some patients [12]. After discontinuation, patients are usually transitioned to antiresorptive therapy to maintain the gains in bone density achieved during teriparatide treatment [13].

Beyond osteoporosis, research is expanding the scope of teriparatide's therapeutic applications. It has been investigated for its potential role in enhancing fracture healing, treating osteogenesis, and managing hypoparathyroidism [14]. In orthopaedic settings, teriparatide has shown promise in accelerating the repair of stress fractures and delayed unions [15]. Moreover, the use of proteomic techniques such as SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) in the identification and characterization of teriparatide is significant in the pharmaceutical and biotechnology industries. Such analytical methods help ensure the identity, purity, and integrity of recombinant proteins like teriparatide, which is critical for both clinical efficacy and regulatory compliance [16]. Establishing SDS–PAGE as a quality control tool for teriparatide therefore extends the utility of this conventional protein separation method into the domain of peptide-based biotherapeutics. This not only enables robust assessment of peptide integrity, impurities, and aggregation but also provides a cost-effective orthogonal approach to complement advanced chromatographic and spectrometric methods in regulatory and manufacturing contexts. Teriparatide ~4 kDa, susceptibility to degradation, and limited electrophoretic mobility present unique challenges that require the use of high-percentage gels and optimized staining protocols for reliable visualization [17-20].

In the present study, we aim to show similarity using SDS-PAGE between Teriparatide test sample and World Health Organization–National Institute for Biological Standards (WHO-NIBSC) reference standard.

Materials & Methods

Materials, reagents and chemicals

Acrylamide, Bis-acrylamide, Tris, Sodium dodecyl sulphate, 10% Ammonium persulphate, 100% glycerol, methanol, Glacial Acetic acid, Glycine, TEMED (N, N,N,N'- Tetramethyl ethylene diamine), Coomassie Brilliant Blue (G-250), Conc. Hydrochloric acid, sodium thiosulphate, silver nitrate, formaldehyde, sodium carbonate anhydrous, water for injection were used to carry out the experiment. Low molecular weight marker i.e. See Blue pre-stained protein marker was used as reference (4-200kDa). Teriparatide (~1mg/vial) as test sample and WHO-NIBSC reference standard was used. All the chemicals and solvents used for performing the analysis were of analytical grade.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

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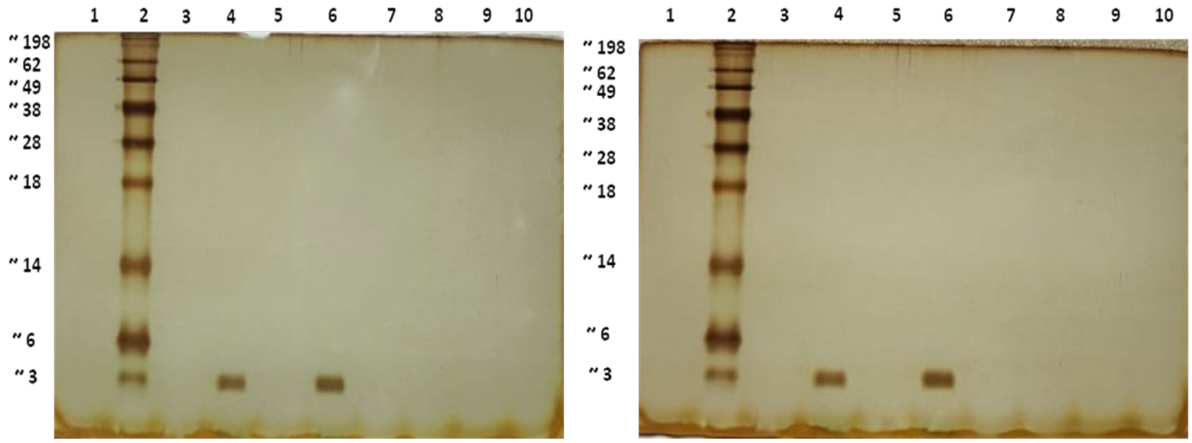


Figure 1: *Two representative Electropherogram of Teriparatide analyzed by SDS-PAGE*

SDS-PAGE was carried out under non-reducing conditions using 20% bis-acrylamide gel. Dilute the preparation under examination with sample buffer to obtain a concentration of 8µg per well. The sample was incubated at room temperature for 10 minutes. The marker, samples and reference standard were loaded in the sequence of Lane 1: empty, Lane 2: molecular weight marker, Lane 3: empty, Lane 4 and 5: Reference standard, Lane 6 and 7: Teriparatide sample. The samples were run at 100V until the sample reached the resolving gel and then increased to 120V until the dye front reached at the bottom of the gel. Silver staining was performed and visualisation was done under white light transiluminator.

Results

Identification by SDS-PAGE

Teriparatide's molecular weight and purity were evaluated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). For the best resolution of low-molecular-weight peptides, a 20% polyacrylamide gel was employed. Teriparatide (~4.2 kDa) is represented by a single band on a 20% polyacrylamide gel that has been stained with silver nitrate. Molecular weight marker (Lane 2); Reference standard (Lane 4, 5); Teriparatide sample (Lane 6, 7). The successful identification and high purity of the peptide are indicated by the observed band matching the expected molecular weight i.e ~ 4 kDa. The identity of Teriparatide is confirmed with the presence of principle band matching to that of the reference standard. No other additional bands were observed, suggesting minimal degradation or presence of impurities. These results confirm the identity and purity of the teriparatide sample.

Discussion

During development and quality control, SDS-PAGE identification of recombinant proteins like Teriparatide provides a reliable and repeatable analytical method for verifying molecular identity and evaluating purity [21]. Teriparatide is a synthetic peptide with a theoretical molecular weight of about 4.2 kDa made up of first 34 amino acids of human parathyroid hormone (PTH 1–34). To ensure sufficient resolution and detection sensitivity, its small size necessitates the use of high-percentage polyacrylamide gels (usually 15–20%) and optimized buffer systems, like Tris-glycine [22].

SDS-PAGE in this investigation showed a clear, sharp band at the anticipated molecular weight (~4.2 kDa), which is in line with teriparatide's monomeric form and WHO-NIBSC reference standard. This finding validates that the recombinant peptide was successfully expressed and folded correctly. High sample purity and structural integrity are suggested by the lack of extra bands, such as high molecular weight aggregates or low molecular weight degradation products [23]. These results support the use of SDS-PAGE as a qualitative identity test for this therapeutic peptide.

SDS-PAGE is a useful orthogonal technique that works well with mass spectrometry and high-performance liquid chromatography (HPLC), despite the fact that it cannot provide sequence-specific information. It is especially useful for batch release testing and in-process control because of its affordability, ease of use, and visual interpretability [24]. Furthermore, SDS-

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PAGE can identify even trace-level contaminants when paired with sensitive staining techniques like silver staining, which is essential for guaranteeing product safety in the production of biopharmaceuticals.

The application of SDS-PAGE to teriparatide analysis is novel because of the unique challenges posed by the molecular size of this therapeutic peptide. Teriparatide, a recombinant fragment of human parathyroid hormone (1–34), is only 34 amino acids long with a molecular weight of approximately 4 kDa. Conventional SDS-PAGE methods are typically optimized for the resolution of larger proteins, often ranging from 10–200 kDa. As a result, adapting the technique for teriparatide requires the use of specialized gel systems such as high-percentage acrylamide or Tris–Tricine gels to achieve proper resolution. This adaptation itself highlights the innovative use of an established protein separation method for a much smaller biomolecule.

Another aspect of novelty lies in positioning SDS-PAGE as a complementary tool to the advanced analytical methods normally employed for peptide therapeutics. High-performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC–MS), and peptide mapping are traditionally considered gold standards for characterizing teriparatide in terms of identity, purity, and degradation products. In contrast, SDS-PAGE offers a simple, low-cost, and visually direct means of assessing these attributes. The ability to demonstrate purity, detect fragments, and identify aggregates using a basic electrophoretic approach provides a fresh dimension to teriparatide quality assessment, particularly in routine laboratory or resource-limited settings.

Furthermore, the use of SDS-PAGE in teriparatide characterization has practical implications for pharmaceutical development and biosimilar validation. Since peptide drugs are prone to degradation and aggregation, stability testing is a critical component of their quality control. By adapting SDS-PAGE to monitor structural integrity, researchers and manufacturers gain a stability-indicating tool that complements more sophisticated assays. In the context of biosimilar development, where regulatory guidelines require comprehensive comparative characterization, incorporating SDS-PAGE into the analytical pipeline represents an innovative application of a classical technique. Use of orthogonal analytical approaches is emphasized in regulatory guidelines (ICH Q6B, EMA, FDA) to ensure comprehensive quality assessment while HPLC and mass spectrometry remain the primary methods for determination of purity, identity, and molecular mass.

SDS–PAGE provides an essential complementary technique because it is based on a distinct separation principle—electrophoretic mobility in a denaturing environment (17). This orthogonality enhances confidence in results by reducing method-specific bias and enabling detection of quality attributes not easily resolved by chromatographic or mass-based methods, such as visible peptide degradation fragments, aggregation, or unexpected high-molecular-weight impurities. Optimized SDS–PAGE (e.g., tricine or high-percentage gels) has demonstrated suitability for small peptides like teriparatide, and its adoption contributes to a multi-layered control strategy consistent with regulatory expectations for peptide and protein therapeutics.

To sum up, SDS-PAGE is a useful and reliable technique for teriparatide identity verification and purity evaluation. Particularly in light of regulatory requirements for biosimilars and recombinant peptide therapies, its application in the analytical workflow helps guarantee the final drug product's quality, consistency, and compliance.

Conclusion

The identification of teriparatide by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) represents a critical analytical step in the quality control and characterization of this recombinant peptide therapeutic. These findings are consistent with pharmacopoeial guidelines, which emphasize the importance of orthogonal methods in the verification of biopharmaceutical identity. While SDS-PAGE is inherently limited in providing detailed structural or sequence-specific data, its value lies in its simplicity, speed, and visual clarity. When integrated with complementary techniques such as mass spectrometry and chromatographic profiling, SDS-PAGE serves as a

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foundational tool for comprehensive peptide characterization.

In summary, SDS-PAGE provides an effective and reliable platform for the identity verification and purity assessment of teriparatide. Its application supports regulatory compliance, manufacturing consistency, and product quality assurance in both research and industrial settings. The method's continued relevance underscores its importance in the analytical toolbox for protein and peptide therapeutics.

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Conflicts of interest

NiL.

References

1. Falaschi, P., Marques, A., & Giordano, S. (2021). Osteoporosis and fragility in elderly patients. Orthogeriatrics: The management of older patients with fragility fractures, 35-52.
2. Kothiyal, P., & Sharma, M. (2017). Post menopausal quality of life and associated factors—a review. American Journal of Pharm Research, 3(9), 7175-7183.
3. Qaseem, A., Hicks, L. A., Etxeandia-Ikobaltzeta, I., Shamliyan, T., Cooney, T. G., Clinical Guidelines Committee of the American College of Physicians, & Tufte, J. E. (2023). Pharmacologic treatment of primary osteoporosis or low bone mass to prevent fractures in adults: a living clinical guideline from the American College of Physicians. Annals of internal medicine, 176(2), 224-238.
4. Russell, G. (2024). Introduction: bone metabolism and its regulation. In Bone Markers (pp. 1-26). CRC Press.
5. Jha, S. S. (2023). Biologics: Teriparatide and Newer Anabolics. Indian Journal of Orthopaedics, 57(Suppl 1), 135-146.
6. Chilakapati, R., Mannully, C. T., & Pulicherla, K. K. (2019). Prospects of parathyroid hormone in therapeutic intervention. International Journal of Peptide Research and Therapeutics, 25, 961-977.
7. Guerreiro, P. M., Renfro, J. L., Power, D. M., & Canario, A. V. (2007). The parathyroid hormone family of peptides: structure, tissue distribution, regulation, and potential functional roles in calcium and phosphate balance in fish. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 292(2), R679-R696.
8. Osagie-Clouard, L., Sanghani, A., Coathup, M., Briggs, T., Bostrom, M., & Blunn, G. (2017). Parathyroid hormone 1-34 and skeletal anabolic action: The use of parathyroid hormone in bone formation. Bone & joint research, 6(1), 14-21.
9. Raterman, H. G., Bultink, I. E., & Lems, W. F. (2019). Current treatments and new developments in the management of glucocorticoid-induced osteoporosis. Drugs, 79(10), 1065-1087.
10. Dore, R. K. (2013). Long-term safety, efficacy, and patient acceptability of teriparatide in the management of glucocorticoid-induced osteoporosis. Patient preference and adherence, 435-446.
11. Watanabe, Atsushi, Shigeki Yoneyama, Mikio Nakajima, Norihiro Sato, Ryoko Takao-Kawabata, Yukihiro Isogai, Aki Sakurai-Tanikawa et al. "Osteosarcoma in Sprague-Dawley rats after long-term treatment with teriparatide (human parathyroid hormone (1-34))." The Journal of toxicological sciences 37, no. 3 (2012): 617-629.
12. Tashjian Jr, A. H., & Gagel, R. F. (2006). Teriparatide [human PTH (1-34)]: 2.5 years of experience on the use and safety of the drug for the treatment of osteoporosis. Journal of bone and mineral research, 21(3), 354-365.
13. Cosman, F., Nieves, J. W., & Dempster, D. W. (2017). Treatment sequence matters: anabolic and antiresorptive therapy for osteoporosis. Journal of bone and mineral research, 32(2), 198-202.
14. Chandran, M., Akesson, K. E., Javaid, M. K., Harvey, N., Blank, R. D., Brandi, M. L., & Silverman, S. (2024). Impact of osteoporosis and osteoporosis medications on fracture healing: a narrative review. Osteoporosis International, 35(8), 1337-1358.
15. Yoon, B. H., & Kim, K. C. (2020). Does teriparatide improve fracture union?:a systematic review. Journal of bone metabolism, 27(3), 167.
16. Kovács, P., Schäfer, T., Háda, V., Hevér, H., Klingelhöfer, S., Nebel, M., & Urbányi, Z. (2020). Comparative physicochemical and biological characterisation of the similar biological medicinal product teriparatide and its reference medicinal product. BioDrugs, 34, 65-75.
17. Chunxiao, W., Jingjing, L., Yire, X., Min, D., Zhaohui, W., Gaofu, Q., Xiangchun, S., Xuejun, W., Jie, W., Taiming, L. Study on preparation and activity of a novel recombinant human parathyroid hormone(1-34) analog with N-terminal Pro-Pro extension. Regul Pept. 2007;141(1-3):35-43.
18. Fernández-Carneado, J., Vallès-Miret, M., Arrastia-Casado, S., Almazán-Moga, A., Macias, M.J., Martin-Malpartida, P., Vilaseca, M., Díaz-Lobo, M., Vazquez, M., Sanahuja, R.M., et al (2024). First Generic Teriparatide: Structural and Biological Sameness to Its Reference Medicinal Product. Pharmaceutics. 16(4):537.
19. Ughade, S., Rana, S., Nadeem, M., Kumthekar, R., Mahajani, S., &Bhambure, R (2024). Mechanistic Modeling of Size Exclusion Chromatography-Assisted In Vitro Refolding of the Recombinant Biosimilar Teriparatide (PTH-34). ACS Omega. 9(3):3204-3216.
20. Couchman, L., Taylor, D. R., Krastins, B., Lopez, M. F., & Moniz, C. F. (2014). LC-MS candidate reference methods for the harmonisation of parathyroid

hormone (PTH) measurement: a review of recent developments and future considerations. Clinical Chemistry and Laboratory Medicine (CCLM), 52(9), 1251-1263.

21. Sharma, N., Kukreja, D., Giri, T., Kumar, S., & Shah, R. P. (2022). Synthetic pharmaceutical peptides characterization by chromatography principles and method development. Journal of Separation Science, 45(13), 2200-2216.
22. Wagner, W. J., Moyle, A. B., Wagner, N. D., Rempel, D. L., & Gross, M. L. (2024). Evaluating chemical footprinting-induced perturbation of protein higher order structure. Analytical chemistry, 96(23), 9693-9703.
23. Patel, A., & Patel, R. (2023). Analytical method development for biologics: Overcoming stability, purity, and quantification challenges. Journal of Applied Optics, 44(1S), 1-29.
24. Nowakowski, A.B., Wobig, W.J., Petering, D.H. (2014). Native SDS-PAGE: high resolution electrophoretic separation of proteins with retention of native properties including bound metal ions. Metallomics. 6(5):1068-78.
25. Haider, S. R. (2012). Enhanced gel electrophoresis (GE) and inductively coupled plasma-mass spectrometry (ICP-MS) based methods for the identification and separation of proteins and pepides (Doctoral dissertation, Loughborough University).
26. Mitsudome, T., Moribe, M., Obayashi, Y., Uchiyama, A., Aono, M (2020). Influence of low-molecular-weight aggregates on aggregate growth kinetics and physical properties of solid-state proteins during storage. Eur J Pharm Biopharm. 146:10-18.
27. Narayanan, H., Dingfelder, F., Morales, I. C., Patel, B., Heding, K.E, Bjelke, J. R, Egebjerg, T., Butté, A., Sokolov, M., Lorenzen, N., and Arosio, P. (2021). Molecular Pharmaceutics 18: 3843-3853.

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