RESEARCH ARTICLE

Comparative Characterization of Reference Market Product and Generic Product Developed using Reverse Engineering Methodology

Jailani S[1*], C K Dhanapal[1], Noohu A Khan[2]

[1]Department of Pharmacy, Faculty of Engineering and Technology, Annamalai University, Chidambaram, Tamil Nadu, India.
[2]Department of Clinical Pharmacy, College of Pharmacy, King Khalid University, Abha, Kingdom of Saudi Arabia.

Received: 27th August, 2023; Revised: 29th September, 2023; Accepted: 09th October, 2023; Available Online: 25th December, 2023

ABSTRACT

The present study is a comparative analysis between the reference market product, Xarelto® 20 mg film-coated tablets, and a generic product developed using reverse engineering methodology, employing low-shear ethanolic granulation. The primary objective in formulating and optimizing this generic product was to attain a formulation that exhibits both physical and chemical stability, closely resembling the innovator or market reference drug product. Both reference and generic products were evaluated using FTIR, TGA, DSC, XRD, SEM, EDX/EDS, Raman mapping, Raman imaging and contact angle measurement studies and were compared. Similar characteristic peaks were observed in the IR spectra of the generic product (RX-LS [AH]) to that of the reference product. Similar crystallinity was detected between reference and generic products. The TG-DSC analysis confirmed the presence of a similar endothermic peak of the generic product to that of the reference product. SEM images showed the presence of deformed granules in both, generic and reference products due to the compression force during tablet production. The specific and major elements present in both samples were C, N, O, Na, Mg, S and Cl. The API and excipient distribution within the reference product and the generic product is closely similar confirmed through the Raman study. Test ($\theta = 17.3$) and reference products ($\theta = 17.9$) showed contact angles around 17 degrees, it indicates that both products have similar wettability capacity, in other words, similar solubility and dissolution behavior. The current study demonstrated the excellent similarity of generic Rivaroxaban 20 mg film-coated tablets with reference products. The exactly similar characteristics of generic products will definitely boost confidence of successful bioequivalence studies and reduce developmental cost and time as there is no a chance of product failure which will lead to redevelopment.

Keywords: Reverse engineering, Rivaroxaban, Raman imaging, Contact angle, Pharmaceutical equivalence, Bioequivalence.

International Journal of Pharmaceutical Quality Assurance (2023); DOI: 10.25258/ijpqa.14.4.26

How to cite this article: Jailani S, Dhanapal CK, Khan NA. Comparative Characterization of Reference Market Product and Generic Product Developed using Reverse Engineering Methodology. International Journal of Pharmaceutical Quality Assurance. 2023;14(4):987-992.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Reverse engineering plays a pivotal role in the development of generic products, particularly in industries like pharmaceuticals and drug manufacturing. It involves the in-depth analysis of existing reference-listed drug (RLD) products to replicate their formulations.[1] In pharmaceuticals, this includes deciphering the chemical composition, structure, and concentration of active ingredients to produce therapeutically equivalent generic drugs.[2] Beyond the final product, reverse engineering encompasses understanding the manufacturing processes, proprietary technologies, and quality control measures involved. This approach aids in navigating patent challenges, allowing generic manufacturers to design alternative processes or formulations that do not infringe on existing patents. Importantly, it contributes to cost reduction by leveraging existing knowledge, accelerating market entry, and facilitating regulatory approval by demonstrating bioequivalence to reference products. Reverse engineering is instrumental in establishing and improving quality control measures, ensuring that generic products consistently meet required standards.[3]

Advanced characterization techniques play a pivotal role in reverse engineering and the development of generic products across industries, offering profound insights into the composition, structure, and performance of existing products.[4] These methods, including spectroscopy (e.g., FTIR, NMR, Raman) for chemical analysis, X-ray crystallography and electron microscopy for structural analysis, and mass spectrometry and chromatography for quantitative analysis, provide a comprehensive understanding of materials.[5] Surface analysis techniques like XPS and AFM delve into surface chemistry and topography, while thermal analysis methods such as differential scanning calorimetry (DSC) and TGA

*Author for Correspondence: jailanipharmacy@gmail.com

reveal crucial thermal properties. Mechanical analysis tools like rheology and mechanical testing evaluate mechanical properties.[6] Particle size and distribution techniques such as dynamic light scattering and laser diffraction are vital for formulations. Nanotechnology methods like TEM and AFM are essential for characterizing nanomaterials, increasingly used in pharmaceuticals and advanced materials. In vivo imaging techniques like MRI and CT contribute to understanding drug distribution within the body, particularly in pharmaceutical development.[7] Computational modeling and simulation, including molecular dynamics and finite element analysis, complement experimental techniques, providing predictive insights into material behavior. This comprehensive knowledge is instrumental in guiding the reverse engineering process, ensuring the quality and equivalence of generic products, and navigating regulatory requirements across diverse industries, from pharmaceuticals to materials science.[8]

Repeated bioequivalence (BE) failures during the product development process might increase a generic medication manufacturer's development cost. Additional research and testing are required, which will take time and money to complete. Such multiple BE failures may contribute to approval delays and diminished profitability, resulting in elevated overall costs for product development. Consequently, it becomes imperative to minimize the likelihood of BE failures to reduce the financial strain on patients and lower the overall expense of creating generic medicines.[9]

The central aim of this product development program was to conduct a comparative analysis between the reference market product, Xarelto® 20 mg film-coated tablets, and a generic product developed through the low shear granulation method employing ethanol. The primary objective in formulating and optimizing this generic product was to attain a formulation that exhibits both physical and chemical stability, closely resembling the innovator or market reference drug product. This endeavor is geared towards the meticulous replication of the active pharmaceutical ingredient (API) and aims to improve the likelihood of achieving bioequivalences, thereby reducing the potential risk of bioequivalence failure.

MATERIALS AND METHODS

Materials

The reference market product, Xarelto® 20 mg film-coated tablets was purchased from medical shop. Rivaroxaban was gifted by Mylan Laboratories, India. Samples of excipients, Lactose monohydrate was from Kerry, Microcrystalline cellulose from JRS pharma, hypromellose (HPMC) was from Shin-Etsu, sodium lauryl sulfate was from BASF, croscarmellose sodium was from DFE pharma, magnesium stearate was from Merck. All the chemicals used in the analysis were of analytical grade.

Methods

The developed generic product (RX-LS [AH]) and Reference market product, Xarelto® 20 mg film-coated tablets were characterized for FTIR, TGA, DSC, XRD, SEM, EDX, Raman mapping, Raman imaging, and contact angle studied as per standard procedures reported in literature. The obtained results of marketed and generic products were compared to identify the sameness between both products. Following are some advanced characterization techniques utilised in this study.

Fourier-Transform Infrared Study

The comparative FTIR study was performed for the reference listed drug and developed generic product (RX-LS [AH]). The analysis was carried out using analyzed using an FTIR (FT/IR-4600, JASCO, Japan) in Attenuated total reflectance (ATR) assembly mode, across a range of 4000–500 cm^{-1}.

XRD Study

XRD analysis was performed for the Reference drug and developed generic product (RX-LS [AH]) with scanning 2θ range of 5–50, using powder X-ray diffraction (Malvern Panalytical Inc, USA) with high score software.[10]

Thermal Analysis Differential Scanning Calorimetry

TG-DSC analysis was performed for reference innovator product and the developed generic product (RX-LS [AH]) using a simultaneous thermal analyzer (NETZSCH - STA 449 F3 Jupiter, Germany) to check any possible interaction between drug and API as well as to evaluate the sameness between RLD and generic product. The sample was heated at a rate of 10^0C/min in flat-bottomed aluminum pans in the presence of nitrogen with a flow rate of 40 mL/min over a temperature range of 30–300^0C. Empty aluminum pans were used as a reference.[11]

Scanning Electron Microscopy (SEM) With Energy Dispersive X-Ray (EDX) Spectroscopy

SEM with EDS (JEOL-JSM – IT 200, JEOL, Japan) of crushed tablet of RLD and batch RX-LS [AH] was studied using SEM. When using an accelerating voltage of 20.0 kV, the working distance was kept constant at 8.6 to 8.8 mm. The crushed tablets have a gold coating that made them electrically conductive. The double-sided adhesive tape was used to adhere these gold-coated objects on a brass tub. Compositional analysis and elemental mapping were conducted using EDS.[12]

Raman Spectral Mapping and Imaging

API and excipients distribution in reference product and test product tablet samples were analyzed by Raman spectral mapping and imaging at 532 nm laser wavelength and a 20X objective lens, using confocal Raman microscope with AFM (Alpha 300 RA, WiTec GmbH, Germany). The Hyperspectral images generated were processed using Control four software. A few milligrams of sample amounts were employed, and the samples were put on a highly reflective sample carrier in order to capture Raman spectra. The recorded spectra were the average of three 10 second exposure scans.

Contact Angle

Sessile drop measurement was used for contact angle (θ) measurement.[13] A droplet of water was placed on the solid surface of powdered samples (reference or test product) and an image of the drop is recorded. The contact angle measurements

were conducted using a contact angle Meter equipped with a color CCD camera. About 10 µL of water was dropped on the crushed powder film of reference and test product samples. The contact angles were then obtained. This measurement entails using an optical tensiometer to place a drop of liquid and capture images of the droplet sitting stationary on the surface. The images are then fit with an appropriate algorithm, typically based on Young's equation, to determine the contact angle between the droplet and solid by the software.

$$\gamma SV = \gamma Sl + \gamma lv \cos\theta Y$$

The interfacial tensions, γ_{sv} (surface tension of the liquid), γ_{sl} (the interfacial tension between solid and liquid), and γ_{lv} (the surface tension of the solid i.e. surface free energy), form the equilibrium contact angle of wetting, many times referred to as Young's contact angle.

RESULTS AND DISCUSSION

In generic product development, pharmaceutical equivalence plays major role in order to comply regulatory requirements as well as to establish successful bioequivalence. Advanced characterization techniques are very important in reverse engineering to decode the reference product.[14] The data obtained in reverse engineering can be utilized during generic product development. Pharmaceutical equivalence stands as a pivotal concept in the development and assessment of generic products, embodying the similarity in API, dosage form, strength, route, and intended use when compared to their corresponding reference (innovator or brand-name) products. In summary, pharmaceutical equivalence is fundamental to the development, approval, and acceptance of generic products. It not only ensures regulatory compliance but also plays a vital role in guaranteeing the safety, efficacy, and consistency of generic drugs, establishing them as valuable alternatives in healthcare systems globally.[15]

FTIR Analysis

The FTIR spectrum of reference product showed characteristic peaks at 581.51, 757.22, 1026.68, 1416.31, 1643.79 and 1733.51 cm^{-1} (Figure 1A). All these peaks were attributed to the various functional groups present in rivaroxaban drug. Similar characteristic peaks were also observed in the IR spectra of generic product (RX-LS [AH]) (Figure 1B). These observations clearly indicated that the generic product is equivalent to the reference product in terms of IR pattern.

XRD Analysis

The XRD spectrum of reference product presented in (Figure 2A) shows distinct and sharp peaks of Rivaroxaban at diffraction angles of 12.39, 16.34, 18.96, 19.42, 19.81, 21,07, 22.39, and 25.52 degrees indicating crystalline nature of Rivaroxaban. However, in the (RX-LS [AH]) formulation shown in (Figure 2B), sharp and distinct peaks were also observed at 12.41, 16.25, 18.97, 19.43, 19.81, 21.08, 22.28, and 25.47 degree with same crystalline nature. These observations provide clear evidence that the developed generic product showed peaks at the same diffraction angles to that of the reference product.

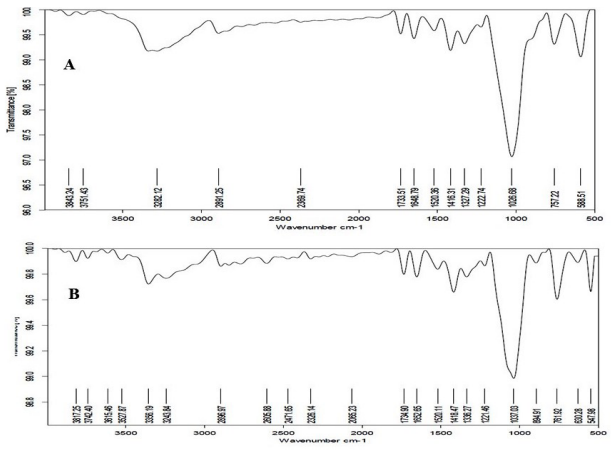

Figure 1: FTIR spectra of A: Reference product and B: generic product

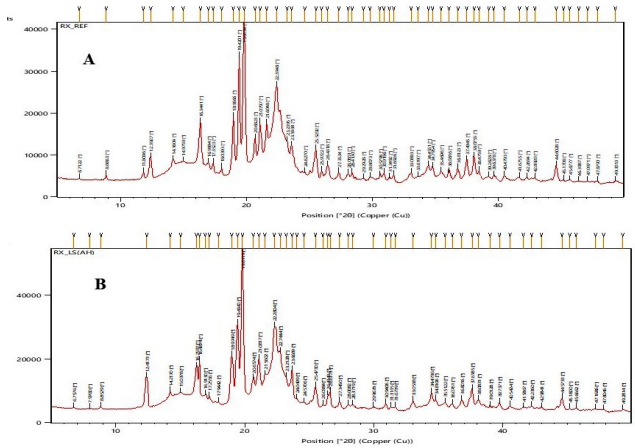

Figure 2: X-ray diffraction pattern of A: Reference product and B: generic product

Thermal Analysis

DSC is a method used in drug development towards identifying various transitions for example melting, glass transition, and crystallization.[16] DSC thermograms of both reference product and generic product are presented in (Figure 3A and 3B), correspondingly. The melting point of pure Rivaroxaban, which is normally between 228 and 234°C, is noticed as a strong endothermic peak at 230°C in the DSC thermogram of the reference product. On the other hand, the DSC thermogram of generic product (RX-LS [AH]) exhibited an endothermic peak within 228–234°C with reduced intensity. Overall, the DSC analysis confirmed the presence of similar endothermic peak of the generic product to that of reference product. Similar Thermogravimetric (TG%) curves and thermal behavior (Figures 3A and 3B), for reference and test products indicate that they are highly identical in composition. Comparable thermal behavior of the reference and test products indicate that they share almost the same composition and exhibit equivalent thermal stability. Furthermore, the consistent mass loss rate observed throughout the temperature range suggests that both products undergo decomposition or weight loss at the same rate, reinforcing the conclusion of their compositional similarity and consistent thermal behavior.

Scanning Electron Microscope and EDS Analysis

Scanning electron microscope (SEM) with EDS analysis is useful in semi qualitatively and semi-quantitatively to compare the elements present in the generic and reference products and also for microstructural and morphological observations.[17] Following the application of conductive layers of gold to their surface, the generic and reference products were photographed. SEM images showed the presence of deformed granules in both, generic and reference products due to the compression force during tablet production (Figures 4A and 4B).

The elemental composition of a material can be ascertained using the highly useful analytical method of energy-dispersive X-ray spectroscopy. These details are crucial for comprehending the chemical structure and properties of the substance. The specific elements present in the both samples are reported to be C, N, O, Na, Mg, S and Cl. EDS provided information showing the almost comparable compositions between generic and reference product with respect to intensity and count (Figure 5A and 5B).

Raman Spectral Mapping and Imaging

A Raman map is a powerful tool that yields valuable insights into various processes and materials involved in sample creation. It can be particularly beneficial in assessing the uniform distribution of an API throughout a tablet. Additionally, it aids in evaluating the chemical consistency between different granules within a granulated drug product.[18] These are just two examples of how this technique can be instrumental in addressing critical aspects of sample analysis. The API and excipient distribution within the reference product and the generic product is closely similar (Figure 6).

Raman microscopy combined with a complementary imaging method was adopted to compare topographical hyperspectral images of generic product with its reference product to correlate Raman spectra with complementary chemical compositional and distributional information. It revealed the showing the API distributional locations within the tablets among the different components (Figure 7).

Contact Angle

The wetting of a solid by a liquid is measured by the contact angle, or. It is described geometrically as the angle created by

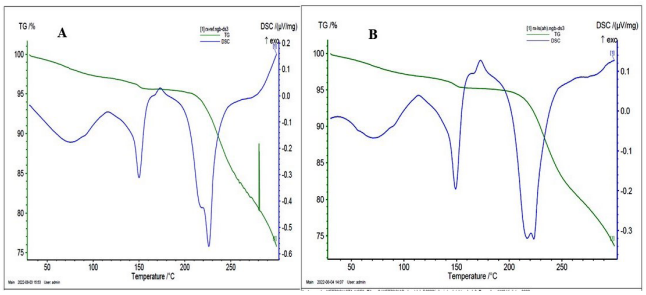

Figure 3: TG-DSC thermograms of A: Reference product and B: generic product

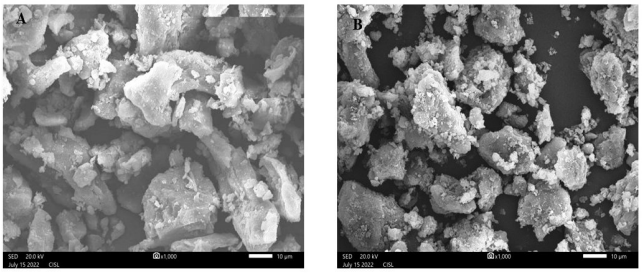

Figure 4: Surface morphology of A: crushed tablet of RLD and B: crushed tablet of batch RX-LS [AH]

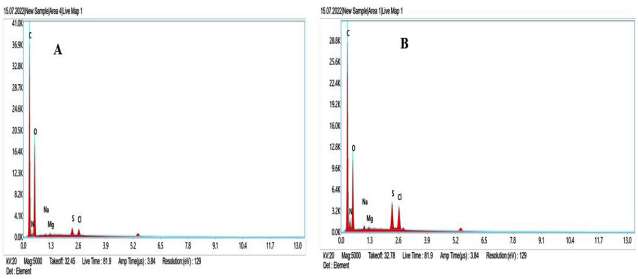

Figure 5: EDS analysis of A: Reference product with intensity and count; B: Test product with intensity count and energy

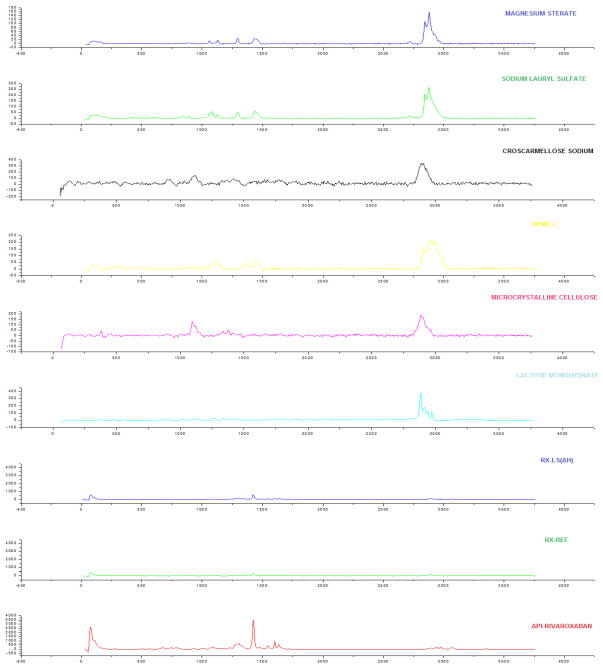

Figure 6: Raman overlay spectrum of excipients, reference product, generic product and pure API

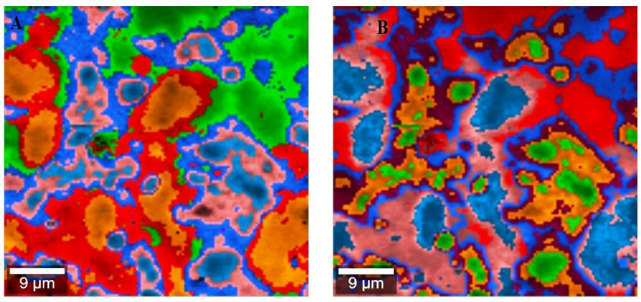

Figure 7: Compositional distribution of components (API and excipients) within A: reference product and B: test product.

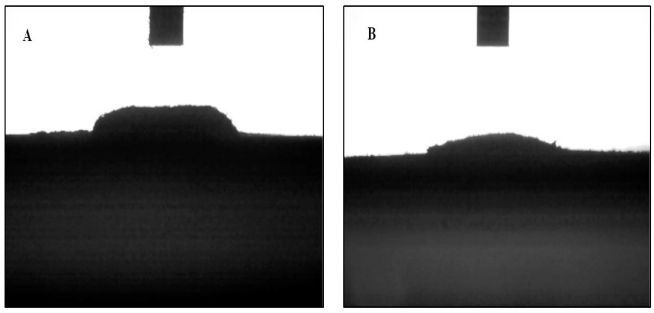

Figure 8: Contact angle of A: Reference product; B: Generic product

a liquid at the point where the three phases of a liquid, gas, and solid converge.[19] A surface must be completely wet to have a contact angle between 0 degrees and 180 degrees.[20] If there is water on a surface, a contact angle of less than 90 degrees designates the surface as hydrophilic, and if the contact angle is greater than 90 degrees, the surface is hydrophobic. Test (θ = 17.3) and reference products (θ = 17.9) showed contact angles around 17 degrees, it indicates that both products have similar wettability capacity, in other words, similar solubility and dissolution behavior, this pre-requisite for in vivo dissolution and absorption.[21] The contact angles of reference and generic product are presented in Figure 8.

It may be assumed that the presence of equal amount of critical excipient, SLS present in a comparable quantity in both reference and generic products, so that in both products the reduction of the interfacial tension between Rivaroxaban, the poorly soluble BCS class II drug, and aqueous fluid resulting in low values of contact angle leading to improved wetting behavior.

The reported findings indicate that surface hydrophobicity can be discerned by observing contact angles between particles and the surrounding medium, with contact angles exceeding 65 degrees suggesting a hydrophobic surface, while angles below 65 degrees signify a hydrophilic surface. In the context of poorly soluble drugs, such as indomethacin, interacting with a hydrophilic carrier, the resulting surface of drug particles tends to be less hydrophobic. This, in turn, facilitates improved contact with the aqueous medium, ultimately leading to increased solubility.[22]

The enhanced solubility observed in solid self-nano emulsifying drug delivery system-based formulations is attributed to a reduction in contact angle, an increase in wettability, and the micellar solubilization of drug particles facilitated by the chosen carriers in different formulations.[23]

CONCLUSION

The current study demonstrated excellent similarity and equivalence of generic product, Rivaroxaban 20 mg film-coated tablets with the reference product Xarelto® 20 mg film-coated tablets. The compositional sameness of generic and reference products was confirmed using advanced analytical techniques like IR, TGA,DSC, XRD, SEM, EDS, Raman imaging, mapping, and contact angle. A characterization study of packaging materials used between reference and test products not covered in this paper and was described in our previously published work (Jailani et al., 2023).[24] The physical properties of API observed in the reference product were also observed in the developed generic product. The exactly similar characteristics of generic products will definitely reduce the chances of failure in product performance and stability and thus, can reduce developmental cost and time with higher confidence for success of bioequivalence studies.

ACKNOWLEDGMENT

The authors are thankful to the Centralised Instrumentation and Service Laboratory (CISL), Annamalai University, Chidambaram and National College Instrumentation Facility (NCIF), Trichy, and to the Sophisticated Analytical Instrument Facility (SAIF), Mahatma Gandhi University, Kottayam, for the instrumental analytical support rendered.

REFERENCES

1. Deulkar V, Sampathi S.Vitamin-E TPGS Based Microemulsion: An Approach for Solubility Enhancement of Poorly Water-Soluble Drugs. International Journal of Drug Delivery Technology. 2023;13(3):1065-1068.
2. Bansal A, Koradia V. The role of reverse engineering in the development of generic formulations. Pharmaceutical Technology. 2005 Aug 2;29(8).
3. Parashar T, Kalra K, Kalra JM, Singh N, Saha S, Singh A, Morris S, Jakhmola V. Formulation and In-vitro Evaluation of Bilayer Tablet of Olmesartan Medoxomil for Biphasic Drug Release. International Journal of Pharmaceutical Quality Assurance. 2023;14(2):388-392.
4. Pham Minh D, Accart P, Boachon C, Calvet R, Chesnaud A, Del Confetto S, Dirion JL, Dong J, Ephraim A, Haurie L, Lyczko N. Generic and advanced characterization techniques. Handbook on characterization of biomass, biowaste and related by-products. 2020:31-497.
5. Sun D, Rouse R, Patel V, Wu Y, Zheng J, Karmakar A, Patri AK, Chitranshi P, Keire D, Ma J, Jiang W. Comparative evaluation of US brand and generic intravenous sodium ferric gluconate complex in sucrose injection: Physicochemical characterization. Nanomaterials. 2018 Jan 5;8(1):25.
6. Ferreira AP, Gamble JF, Leane MM, Park H, Olusanmi D, Tobyn M. Enhanced understanding of pharmaceutical materials through advanced characterisation and analysis. AAPS PharmSciTech. 2018 Nov;19:3462-80.
7. Moravkar KK, Shah DS, Magar AG, Bhairav BA, Korde SD, Ranch KM, Chalikwar SS. Assessment of pharmaceutical powders flowability and comparative evaluation of lubricants on development of gastro retentive tablets: An application of powder flow tester. Journal of Drug Delivery Science and Technology. 2022 May 1;71:103265.
8. Rajalahti T, Kvalheim OM. Multivariate data analysis in pharmaceutics: a tutorial review. International journal of pharmaceutics. 2011 Sep 30;417(1-2):280-90.
9. Bhoop BS. Quality by Design (QbD) for holistic pharma excellence and regulatory compliance. Pharm times. 2014;46(8):26-33.
10. Butreddy A, Sarabu S, Almutairi M, Ajjarapu S, Kolimi P, Bandari S, Repka MA. Hot-melt extruded hydroxypropyl methylcellulose acetate succinate based amorphous solid dispersions: Impact of polymeric combinations on supersaturation kinetics and dissolution performance. International Journal of Pharmaceutics.

2022 Mar 5;615:121471.
11. Hubert S, Briancon S, Hedoux A, Guinet Y, Paccou L, Fessi H, Puel F. Process induced transformations during tablet manufacturing: phase transition analysis of caffeine using DSC and low frequency micro-Raman spectroscopy. International journal of pharmaceutics. 2011 Nov 25;420(1):76-83.
12. Abd Mutalib M, Rahman MA, Othman MH, Ismail AF, Jaafar J. Scanning electron microscopy (SEM) and energy-dispersive X-ray (EDX) spectroscopy. InMembrane characterization 2017 Jan 1 (pp. 161-179). Elsevier.
13. Bachmann J, Ellies A, Hartge KH. Development and application of a new sessile drop contact angle method to assess soil water repellency. Journal of Hydrology. 2000 May 29;231:66-75.
14. Junnuthula V, Kolimi P, Nyavanandi D, Sampathi S, Vora LK, Dyawanapelly S. Polymeric Micelles for Breast Cancer Therapy: Recent Updates, Clinical Translation and Regulatory Considerations. Pharmaceutics. 2022 Sep 3;14(9):1860.
15. Pathak SM, Ruby PK, Aggarwal D. In vitro and in vivo equivalence testing of nanoparticulate intravenous formulations. Drug research. 2013 Nov 7:169-76.
16. Newman A, Zografi G. Commentary: Considerations in the measurement of glass transition temperatures of pharmaceutical amorphous solids. Aaps Pharmscitech. 2020 Jan;21:1-3.
17. Scimeca M, Bischetti S, Lamsira HK, Bonfiglio R, Bonanno E. Energy Dispersive X-ray (EDX) microanalysis: A powerful tool in biomedical research and diagnosis. European journal of histochemistry: EJH. 2018 Jan 1;62(1).
18. Vašková H. A powerful tool for material identification: Raman spectroscopy. Int. J. Math. Model. Methods Appl. Sci. 2011;5:1205-12.
19. Giridhar G, Manepalli RK, Apparao G. Contact angle measurement techniques for nanomaterials. InThermal and Rheological Measurement Techniques for Nanomaterials Characterization 2017 Jan 1 (pp. 173-195). Elsevier.
20. Merlen A, Brunet P. Impact of drops on non-wetting biomimetic surfaces. Journal of Bionic Engineering. 2009 Dec;6(4):330-4.
21. Vafaei S, Borca-Tasciuc T, Podowski MZ, Purkayastha A, Ramanath G, Ajayan PM. Effect of nanoparticles on sessile droplet contact angle. Nanotechnology. 2006 Apr 24;17(10):2523.
22. Kallakunta VR, Eedara BB, Jukanti R, Ajmeera RK, Bandari S. A Gelucire 44/14 and labrasol based solid self emulsifying drug delivery system: formulation and evaluation. Journal of Pharmaceutical Investigation. 2013 Jun;43:185-96.
23. Damian F, Blaton N, Naesens L, Balzarini J, Kinget R, Augustijns P, Van den Mooter G. Physicochemical characterization of solid dispersions of the antiviral agent UC-781 with polyethylene glycol 6000 and Gelucire 44/14. European Journal of Pharmaceutical Sciences. 2000 Jun 1;10(4):311-22.
24. Jailani. S, C. K. Dhanapal, Noohu Abdullah Khan, 2023. Selection Of Packaging In Generic Product Development. European Chemical Bulletin,12, 476 – 484. doi: 10.31838/ecb/2023.12.si5.057.