INTRODUCTION

Commercialization of pharmaceutical products involves a lengthy and time consuming process because (i) the product has to be tested for its suitability toward the target application, (ii) safety, (iii) efficacy, and (iv) need to conduct customer satisfaction survey [1]. However, this can be done through the study and investigation of raw drug materials, drug intermediates, final products, formulations, impurities, degradation products, and biological samples containing drugs and their metabolites to know the physicochemical properties even after their post-marketing [2]. Therefore, it is envisaged that proper corrective action should be needed at any phase of the study process to promote its safety and efficacy. Prominently, such investigation discloses the (i) effect of excipients on the drug formulation, (ii) incompatibility between excipient and drug, (iii) suitability of the excipient over drug administration to release the exact active component in the body, and (iv) capability of excipients in stabilizing the drug against degradation due to environmental factors [3].

Further, it is anticipated that the research on discovery of new drugs are being lowering in recent days and the researchers and pharmaceutical companies are majorly focusing in preparation of drug modifications including active pharmaceutical ingredients (APIs), targeted to furnish the pharmacological activity with optimum therapeutic efficacy [4]. Evidently, it is important to mention here that nearly 40% of approved drugs and 90% of pipeline drugs are medications with poor solubility [5]. As an important property in view of pharmacological activity, drug molecules exhibited nearly 100–1000 times solubility, whereas cocrystals and polymorphs exhibited 4–20 times and 2–3 times, respectively. In addition, the solubility leads to the salt formation; a three component system consisted with acid, base, and solvent. However, the easiest approach to modify the properties of parent drug is salt formation where the unenviable features are conquered with ionizable functional groups. Cocrystals and APIs are non-ionizable neutral drugs, restricted with salt formation but fortunately the engineering of intermolecular interactions resulted in the generation of desired properties [6]. The solid drug substance that can be able to exist in more than one crystalline phase with various packing arrangements or molecular conformations in the same molecular species is considered as polymorphism [7]. It is estimated that, more than 50% of drugs exhibited polymorphism depending on the environmental conditions, and each form can significantly exhibited distinct physicochemical properties such as solubility, heat capacity, melting point, and sublimation point. Consequently, critical investigation on polymorphism of pharmaceutical solids during the pharmaceutical unit operations is worthwhile to alter the stability and bioavailability of the final drug product. Hence, it is recommended that...
proper monitoring of different solid-state forms and understanding the drug interactions with water gains paramount importance to ensure the high-quality product [8].

Different analytical techniques were reported in the literature to measure the structural differences of the solid pharmaceuticals such as X-ray diffraction, kinetic techniques, gravimetric, volumetric, electrochemical, spectroscopic, and various separation techniques [9-14]. Among various spectroscopic techniques, Raman spectroscopy has gained much attention and widely used technique for the identification of polymorphs due to non-invasiveness, small amount of sample requirements, and specificity. Besides, this technique is able to identify various physical forms of medicines such as liquid, powder, cream, tablet, and ointment, but is not useful for some medicines as they do not show Raman scattering. Inspired by this, here in this paper, we are compared the experimental results studied for the detection of polymorphism in Lamivudine and Finasteride drugs using Fourier transform (FT)-Raman spectroscopy, to illustrate the advantages of the technique in the detection of polymorphism over other techniques. The IUPAC name of the Lamivudine (Fig. 1) is [(4S)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one], which is a 1,3-oxathiolane nucleoside analog having antiviral activity and exists in at least two polymorphic forms. This drug is used in the medication to treat the HIV-positive and hepatitis B-positive patients. The two polymorphic forms are needle-shaped crystals (Form I) and bipyramidal crystals (Form II) [15]. Similarly, Finasteride (Fig. 1) supplied under the trade name PROSCAR and the IUPAC name is 17β-[3-tert-butyl-carbamyl]-4-aza-5c-androst-1-en-3-one. It is 5α-reductase inhibitor used in the treatment of acne, female hirsutism, and prostatic hyperplasia. Finasteride also exhibited two polymorphic forms in which the orthorhombic crystal structure is Form I and the monoclinic crystal structure is Form II [16].

METHODS
The pure crystalline polymorphic forms of Lamivudine (Form I and Form II) and Finasteride (Form I and Form II) drugs were synthesized in APL Research Centre, Aurobindo Pharma Limited, Hyderabad, India.

Preparation of polymorphic mixtures
Initially, the pure forms of Lamivudine and Finasteride drugs were ground with the help of porcelain mortar and pestle. Later, polymorphic mixtures of Form I and Form II for both Lamivudine and Finasteride drugs were prepared with different weight ratios for the study [15, 16]. In addition to the test sample, calibration standards were also prepared to compare the results and it was observed from the FT-IR data that the grinding process does not induce the polymorphism.

Instrumentation
Polymorphism in Finasteride drug was investigated using NXR FT-Raman spectrophotometer, Thermo Electron Corporation, USA, with the slit aperture of 50 mm and the laser power of 1.0 W. The CaF2 crystal as beam splitter with InGaAs detector with a 180-degree reflective configuration for sample position adjustment was used. A total of 512 scans at resolution of 4 cm⁻¹ were acquired for each of standards and samples. Similarly for Lamivudine drug, Thermo Nicolet 5700 NXR-FT Raman spectrometer equipped with Nd-YAG laser source at a wavelength of 1064 nm was used. The InGaAs detector with 150 scans at 0.7 w exciting power and 4 cm⁻¹ spectral resolution and the scans were collected between 4000 and 200 cm⁻¹. In addition, polarized light microscopy, FT-IR spectroscopy, differential scanning calorimeter, thermo gravimetric analysis, and X-ray powder diffraction studies were performed to further evidence the FT-Raman spectroscopic results.

Preparation of sample for Raman spectroscopy
A powdered sample was placed in a sample holder and it was a gold coated round shape central cavity and the cavity extended to other side of the holder. The holder was kept in such a way that the top face was placed over the stainless steel base and nearly 30 mg of the sample was poured into the cavity. The sample was gently pressed with the finger press and was placed in the mount holds, which holds the sample at the correct height in the sample compartment. For tablet samples, the tablet was cut with a razor blade and placed in a spring action clamp holder in the sample compartment [15, 16].

RESULTS
To ensure that, the Raman spectroscopy is one of the best experimental techniques in detection and identification of polymorphic forms in drug substances and here, in this paper, we compared the Raman spectroscopic experimental results of Lamivudine and Finasteride drugs [15, 16]. First of all, reference standards for Form I and Form II polymorphs of Lamivudine were thoroughly characterized by FT-IR spectroscopy, differential scanning calorimeter, thermo gravimetric analysis, and X-ray powder diffraction studies including Raman spectroscopy and confirmed that the purity of the polymorphs were 100%. Raman spectrum of Form I showed a characteristic peak at 697 cm⁻¹ and showed no peak at 463 and 798 cm⁻¹ which further confirms the absence of Form II (Fig. 2).

Later, the drug mixture containing 6 % (w/w) was analyzed by grinding at various time intervals, that is, 5, 10, 15, and 30 min to test the effect of grinding on polymorphism and was characterized by FT-Raman spectroscopy. As shown in Fig. 3, all the above ground samples of different time intervals show the same peak intensity at 798 cm⁻¹ Raman shift which confirmed that the grinding process does not induce polymorphic conversion for both polymorphic forms as well as for the mixture containing 6% (w/w) of Form II in Form I. Therefore, there is no effect of grinding for the ground samples of polymorph standard mixtures, which were prepared with the help of porcelain mortar and pestle [15].

Again, FT-Raman spectra of both Form I, Form II, and mixture containing 4% (w/w) of Form II were studied. The spectra showed different pattern, and observed a Raman band particularly at 798 cm⁻¹ characteristic of Form II in polymorphic mixture containing 4% (w/w) of Form II in Form I (Fig. 4).

In continuation, FT-Raman analysis was performed for the two polymorphic forms of Finasteride, as shown in Fig. 5, which divulges that the Raman shifts between the two polymorphic forms were clearly distinguishable. The Raman spectra of Finasteride Form I and Form II tablets prepared at APL and the Raman spectra of the placebo were

![Fig. 1: Structure of lamivudine and Finasteride drugs](image-url)
recorded (Fig. 6). The two polymorph forms showed a clear remarkable difference in the Raman shift region between 1750 and 1550 cm\(^{-1}\). It was observed that no placebo interference was noticed in this region and therefore this region is suitable for identification and detection of
the polymorphic forms of Finasteride in the tablets. The characteristic
peaks of the polymorphic Form I were present at 1670 and 1695 cm\(^{-1}\),
and for polymorphic Form II at nearly 1656 and 1677 cm\(^{-1}\). The Raman
spectral peak at 1598 cm\(^{-1}\) was common for Finasteride polymorphic
forms and was clearly indicated that there was no interference
from placebo in the region between 1750 and 1550 cm\(^{-1}\). The major
characteristic peak of the polymorphic Form I was observed nearly at
1670 cm\(^{-1}\) and the minor peak nearly at 1695 cm\(^{-1}\). The major peak is
used for detection of the polymorph Form I in Form II tablets. In the
same way, the major characteristic peak of polymorphic Form II was
observed at 1656 cm\(^{-1}\) and the minor peak at 1677 cm\(^{-1}\), simultaneously
the major peak of Form II was used for detection of the polymorph
Form II in Form I tablets [16].

After studying above Raman spectral patterns, FT-Raman spectroscopy
was finalized for the quantification of Lamivudine as well as Finasteride
drug substances, and the method was developed by the preparation of
e polymorphic mixtures. The advantages of Raman spectroscopy over
other analytical techniques are (i) very little sample is required for
analysis, (ii) ease of sample preparation, which includes the fact that
both glass and water show very little Raman scattering. (iii) Raman spectra showed invariably sharp, well resolved bands comparable to IR spectra due to the minor contribution of overtone vibrations in Raman spectra, resulting in much less broadening and a better resolution of bands compared to IR spectra [17], and (iv) Raman spectroscopy does not suffer from the sampling problems that are common in X-ray powder diffraction, where preferred orientation and specimen displacements are serious restrictions for the application of quantitative method [18].

**DISCUSSION**

The existence of the drugs in more than one solid form is termed as polymorphism, which could become one of the hot research focus in recent days to investigate the most stable and effective form of drug material for its quality assurance. Among several techniques, FT-Raman spectroscopy method is the best one for the detection of polymorphism in medicines and here we compared the results obtained for Lamivudine and Finasteride drugs [15,16]. Initially, X-ray powder diffraclometry (XRPD) technique was used for the study, as it requires small quantity of drug sample and the sample is non-destructive during the identification and quantification of polymorphic forms. However, the technique has the limitation for unwanted polymorphic forms, as it do not exhibit distinct X-ray powder pattern and more intense characteristic peaks of unwanted polymorphic form of drug substance. In case of Lamivudine polymorphic forms, Form II was not having the distinct X-ray powder pattern and is not having the interference free regions, so it is difficult to quantify the Form II with low limit of detection by XRPD. Since, it is difficult to quantify the unwanted form with low limit of detection by XRPD [15].

Similarly, IR spectra of lamivudine forms I and II are well correlated with the previously published spectra and it was observed that polymorphic Form I exhibits a strong absorption band, but no bands were observed for polymorphic Form II [16]. Differential scanning calorimetry thermograms disclosed that the melting and crystallization temperature values of polymorphic forms of Lamivudine were different. As an advanced analytical method, FT-Raman spectroscopy has the advantage of direct investigation of solid samples without sample preparation. On investigation, Raman spectrum of Lamivudine Form I shows characteristic peak at 697 cm\(^{-1}\), as shown in Fig. 2. However, there were no such peaks observed at 463 and 798 cm\(^{-1}\) indicates the absence of Lamivudine Form II. Later, to reduce the source of error occurred due to the heterogeneity of the sample, pure polymorphic forms were ground in a porcelain mortar with the help of pestle for 5 min and were characterized by FT-Raman spectroscopy to identify the affect of grinding for polymorphic changes of pure polymorphs. Fig. 3 showed the FT-Raman spectra of Lamivudine Form I in Form I mixture containing 6% (w/w) which was studied by grinding it at different time intervals of 5, 10, 15, and 30 min. Similarly, Fig. 4 showed the FT-Raman spectra of Form I, Form II and a mixture containing 4% (w/w) of Form II, and Raman band observed at 798 cm\(^{-1}\) is the characteristic of Form II found in polymorphic mixture containing 4% (w/w) of Form II in Form I. It may be noted that this method used in this quantification work has been optimized in such a way that is found to be more sensitive to quantify Form II content in Lamivudine Form I leading to the lower detection levels [15].

Based on the above experimental results for Lamivudine Form II in Form I, FT-Raman spectroscopy was again used for the identification of Finasteride polymorphic mixtures [16]. It is observed from Fig. 5 that the Raman shifts between the two forms were clearly distinguishable. Later, the Raman spectra for Form I, Form II, and placebo were studied and shown in Fig. 6. The two polymorphic forms show a clear remarkable difference in the region between 750 and 1550 cm\(^{-1}\). No placebo interference was observed in this region and therefore this region is suitable for identification and detection of the polymorphic forms of Finasteride. The characteristic peaks of the polymorph Form I were present at 1670 and 1695 cm\(^{-1}\) and for the polymorph Form II at about 1656 and 1677 cm\(^{-1}\). The peak at 1598 cm\(^{-1}\) was common for Finasteride polymorphic forms [16]. This study showed that the FT-Raman spectroscopic method is useful to detect the very low level of Finasteride polymorph Form II in polymorph Form I even though the drug concentration is less. This method might be employed for routine analysis for observing the polymorph changes during the formulation preparation and drug product.

There were several reports published for the identification and detection of polymorphic forms in pharmaceutical products using FT-Raman spectroscopy. To discuss few examples here, Roberts et al. studied the FT-Raman spectroscopic method for the quantification of a binary mixture containing beta and delta mannnitol [19]. Usually, the polymorphic epimict of the mannnitol is its beta form while the other alpha and delta polymorphs are the contaminants. Using this technique, the authors quantified up to the concentration levels down to 2% of the beta form. Another research group demonstrated the quantitative determination of polymorphic mixtures of calcium carbonate, that is, vaterite, aragonite, and calcite [20]. Amado et al. studied the vapor phase induced hydrate-anhydrate pseudopolymorphic transformations of theophylline crystals using a vibrational spectroscopic technique, that is, Raman spectroscopy [8]. Niclosamide is the anthelminthic as well as anti-cancer drug crystallizes into three solvated forms, that is, two monohydrates (NHA and NHB) and one anhydrous (NHN) form [7]. Here, the authors evaluated and compared the application of Raman, near infrared (IR), and mid-IR spectroscopy in identification and quantification of the polymorphic forms of niclosamide. Besides, several reviews highlighted the advantages of Raman spectroscopic method in the analysis of polymorphism in various pharmaceuticals [1,6].

**CONCLUSION**

Earlier days, the analysis of pharmaceuticals using Raman spectroscopy was limited because the technique had limitations with regard to sensitivity, fluorescence, and sub-sampling. Fortunately, these problems have been alleviated after its advancement through the development of FT-Raman spectrometer with charge coupled device, surface-enhanced Raman scattering, and transmission Raman geometry. Afterward, Raman spectroscopy has become notorious technique from the past three decades and has found incredible application in the analysis of environmental and biological samples, including pharmaceuticals. Further, it is important to mention here that the poor Raman scattering property of water makes the technique more superior than IR spectroscopy for the analysis of APIs in formulations especially when the drug concentration is low [1]. More importantly, the detection of polymorphism in the final dosage form using FT-Raman spectroscopic method is highly recommended due to (i) very little sample is required for analysis, (ii) ease of sample preparation, (iii) Raman spectra showed invariably sharp, well resolved bands, and (iv) Raman spectroscopy does not suffer from the sampling problems that are common in X-ray powder diffraction. Evidently, here in this paper, we have investigated and compared the polymorphism in Lamivudine and Finasteride drug substances using FT-Raman spectroscopic method at very low concentration level.

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**AUTHORS’ CONTRIBUTIONS**

All the authors have contributed equally to this research work.

**CONFLICTS OF INTEREST**

The authors declare that they have no conflict of interest.

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