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Application of DSC, IST, and FTIR study in the compatibility testing of nateglinide with different pharmaceutical excipients

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Abstract Experiments were done to assess the compatibility of nateglinide (NTG) with selected excipients in the development of immediate release tablets of NTG by thermal and isothermal stress testing (IST) techniques. To evaluate the drug excipient compatibility, different techniques such as differential scanning calorimetric (DSC) study, infrared (IR) spectrophotometric study, and IST were adopted. The results of DSC study showed that magnesium stearate exhibited some interaction with NTG. However, the results of IR and IST studies showed that all the excipients used in the formula were compatible with NTG. The optimized formulation developed using the compatible excipients were found to be stable after 3 months of accelerated stability studies (40 ± 2 °C and 75 ± 5% RH). Overall, compatibility of excipients with NTG was successfully evaluated using the combination of thermal and IST methods and the formulations developed using the compatible excipients was found to be stable.

Keywords Immediate release · Stability · Incompatibility · Drug–excipient interaction · Nateglinide

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Introduction

Nateglinide (NTG), a meglitinide derivative, is mainly indicated for the treatment of type-2 diabetics. It lowers the blood glucose levels by stimulating insulin secretion from the pancreas. This action is dependent upon functioning beta-cells in the pancreatic islets. The chemical structure of NTG that correspond to (2R)-2-[trans-4-isopropyl-cyclohexanecarbonyl)-amino]-3-phenyl-propionic acid is given in Fig. 1 [1].

The excipients are generally used in dosage form to ease in administration of drug, to facilitate the formulation of the drug product, to increase the stability of the formulation, for aesthetic reasons or for identification [2]. Excipients may interact with drug that gives rise to changes in the chemical nature, solubility, absorption and therapeutic response of drugs. Therefore, the stable and effective solid dosage form depends on the selection of the excipients which can be achieved through the study of the interaction between drug and excipients in the solid state [3]. The universally accepted protocol is not available for evaluating the compatibility of drug with different excipients yet [4]. Recent works highlighted the application of differential scanning calorimetry (DSC) for the rapid evaluation of the drug–excipient compatibility [5–8]. However, caution need to be exercised in the interpretation of DSC results. This is because of the requirement of high temperature conditions and the lack of moisture in conducting the DSC experiments. Hence, conclusions based on the DSC results alone may be misleading [7].

Another method that is commonly employed for evaluating the drug–excipient compatibility is isothermal stress testing (IST). IST involves storage of drug–excipient blends with or without moisture at a temperature (>50 °C) for a specific period of time (normally 3–4 weeks) to accelerate the ageing of drug and interaction with

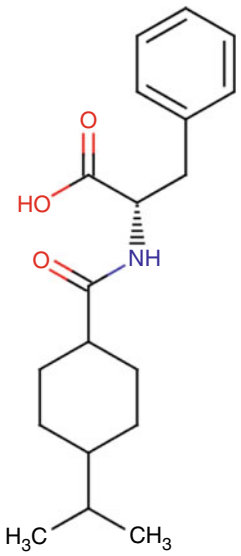

Fig. 1 Chemical structure of nateglinide

excipients. The IST has specific application in pharmaceutical industry where the interaction between drug and excipients is visually observed and the drug content determined quantitatively [9–12]. However, the disadvantage of this method is time consuming and laborious. Ideally, both the techniques, DSC and IST should be used in combination during the compatibility studies for the selection of the excipients.

In recent years, despite of increasing interest in controlled release drug delivery systems scientists are paying more attention to develop the effective formulations for the poorly soluble drugs which easily disintegrate and dissolve their medicaments rapidly in the GIT for better bioavailability. Bioavailability of the poorly soluble drugs from the solid oral dosage forms depends on the disintegration and dissolution of the drug substance from the dosage forms [13].

In this study, DSC was used for evaluating the compatibility of NTG with selected excipients for the formulation of its immediate release tablet. In suspected cases of incompatibility, infrared (IR) spectrum of pure drug was compared with that of drug–excipient mixture and pure excipient. Excipients found to be compatible with each other and with the drug were used for different trial formulations. Excipients which were included in the prototype formula were tested using the technique of IST. Finally, the developed formulations were evaluated after storage at accelerated stability conditions (40 ± 2 °C and 75 ± 5% RH) for 3 months.

Experimental

Materials

Nateglinide (purity 99.6%), a gift sample from Glenmark Pharmaceutical Limited, India was characterized against the working standard of NTG (purity 99.7%), which was obtained as gift sample from Novartis Pharmaceutical, USA. The following chemicals and excipients were purchased from commercial sources and used as such. Lactose (Libraw Pharma, New Delhi, India), microcrystalline cellulose (Avicel PH-101, FMC, USA), croscarmellose sodium (Ac-Di-Sol, Maple Biotech, Maharashtra, India), L-hydroxypropyl cellulose (Hyprolose, Zheijiang Joinway Pharm, China), polacrilin K (Kyron T314, Corel Pharm, Ahmedabad, India), polyvinylpyrrolidone (Plasdone K-30, ISP, USA), and magnesium stearate (Mallinckrodt, USA). Methanol and acetonitrile used for the preparation of mobile phase was of HPLC grade (Ranbaxy, India) and water used throughout the HPLC analysis was prepared by water purifier (Arium, 611UF, Sartorius, Germany).

Methods

Differential scanning calorimetry

A differential scanning calorimeter (JADE DSC, Perkin Elmer, USA) was used for thermal analysis of drug and mixtures of drug and excipients. Excipients that were expected to be used in the development of immediate release tablets of NTG at the appropriate ratio were selected for the study. Individual samples of drug and excipients as well as the physical mixtures of drug and selected excipients were weighed directly in the DSC aluminum crucible (Table 1) and scanned in the temperature range of 50–300 °C under an atmosphere of dry nitrogen. The heating rate was 20 °C/min and the curves obtained were observed for any interaction.

IR spectroscopy

IR spectra of drug, and the blend of drug and selected excipient were recorded on a FTIR spectrophotometer (FTIR-8400S, Shimadzu, Japan) in the range of 4000–500 cm^{-1} using potassium bromide discs.

Table 1 Peak temperature and enthalpy values of nateglinide in various drug–excipient mixtures

Sample	Ratio (drug–excipient)	T_{onset}/°C	T_{peak}/°C	ΔH_{fcorr}/J g^{-1}
NTG	–	137.21	144.76	91.0346
NTG + lactose	1:2	139.8	143.48	89.4321
NTG + polacrilin K	1:1	138.51	141.78	51.5828
NTG + PVP	1:1	137.58	140.38	31.8860
NTG + talc	3:1	139.63	142.81	40.9006
NTG + Mg stearate	3:1	–	–	–

$\Delta H_{fcorr} = \Delta H_{f\ obs}$/%drug in sample × 100

Isothermal stress testing

In the IST studies [12], drug and different excipients (Table 2) were weighed directly in 4-mL glass vials ($n = 2$) and mixed on a vortex mixer for 2 min. In each of the vials, water (10% v/w) was added and the drug–excipient blend was further mixed with a glass capillary (both the ends of which were heat sealed). The capillary was broken and left inside the vial to prevent any loss material. Each vial was sealed using a Teflon-lined screw cap and stored at 50 °C in a hot air oven. These samples were periodically examined for any unusual color change, and quantitatively analyzed after 3 weeks of storage at the above conditions. Drug–excipients blends without added water stored in a refrigerator served as controls.

Analysis of IST sample

IST samples were quantitatively analyzed using in house-developed HPLC method. For sample preparation, 2 mL of methanol was added into each vial subjected for IST study. The mixture was vortexed and transferred to 100-mL volumetric flask. Vials were rinsed twice with methanol and the volume made up. The samples were centrifuged and the supernatant filtered through 0.45-μm nylon membrane filters. After appropriate dilutions, samples were analyzed by using a HPLC system and drug content was determined from the calibration curve prepared within the expected range.

For the analysis of drug–excipient mixtures, Waters 600 Pump based HPLC system equipped with Waters quaternary pump, Waters manual injector, Waters on-line degasser AF, CTO-10 AS VP column oven, and Waters 2489 UV/Visible Detector was used. Water empowered software (Version: Empower 2) was used for data acquisition and mathematical calculations. Chromatographic separation of NTG was performed on a C18 hypersil column (4.6 mm × 250 mm; 5 μm particle size; Waters, USA). Mobile phase used was acetonitrile–phosphate buffer (pH 2.5; 25 mM) (35:65 v/v), at a flow rate of 1 mL/min. Temperature of the column oven was maintained at 30 °C. Standard solutions and drug–excipients samples (20 μL) were injected and analyzed at 210 nm using UV–Visible detector.

Table 2 Results of HPLC analysis of the samples under IST conditions after 3 weeks of storage

Sample	Ratio (drug–excipient)	% Drug remaining[a]	
		Control samples[b]	Stressed samples[c]
NTG	–	100.81 ± 0.72	99.74 ± 2.53
NTG + lactose	1:2	101.42 ± 1.34	100.27 ± 1.65
NTG + polacrilin K	1:1	101.21 ± 2.74	99.78 ± 0.63
NTG + PVP	2:1	101.8 ± 1.48	99.34 ± 1.86
NTG + talc	3:1	100.62 ± 2.68	99.27 ± 2.04
NTG + Mg stearate	3:1	100.84 ± 1.92	99.1 ± 1.62
NTG + CSD	3:1	100.53 ± 1.81	99.62 ± 1.18

[a] Values expressed as average ± standard deviation

[b] Drug excipient blends without added water and stored in refrigerator

[c] Drug excipient blends with 10% added water and stored at 50 °C for 3 weeks

Formulation and stability study of tablets

The detail of the formulation development of the immediate release tablets of NTG is presented in Table 3. In brief, tablets of NTG were prepared by wet granulation using single stroke tablet punching machine (Hardik Engineering Work, Ahmedabad, India) fitted with 8 mm standard concave punches. Lactose, drug, polacrilin K (half of the quantity), and polyvinylpyrrolidone (PVP) were passed through sieve No. 80 and they were mixed for 20 min in a polyethylene bag. Granules were prepared by kneading with ethanol and kept for air drying in tray dryer at 45 °C. The dried granules were passed through sieve No. 20 to get uniform granules. Another half of the polacrilin K and talc were passed through sieve No. 80 and mixed with the granules for 15 min in a polyethylene bag. Magnesium stearate was passed through sieve No. 80 and mixed with the granules for 3 min. The lubricated granules were compressed into tablets.

The assay of the tablets was carried out as follows. Accurately weighed tablets ($n = 6$) was dissolved in 100 mL of methanol. The samples were sonicated (Ultra sonic water bath, Loba Chem, India) for 30 min, after which they were filtered through nylon membrane filter (0.45 μm pore size). The filtered solutions, after appropriate dilution with methanol, were analyzed by a validated UV spectroscopic method [14] at 216 nm (UV-1601, Simadzu, Japan).

Table 3 Composition of immediate release tablets of NTG

Ingredients	%
Nateglinide	40
Lactose	48.6
Polacrilin K	4
PVP	3.4
IPA	Qs
Talc	2.3
Mg stearate	1.7

PVP polyvinylpyrrolidone, *IPA* iso-propyl alcohol

Drug release study of the tablets ($n = 6$) was carried out by using USP-II dissolution apparatus (TDT-06T, Electrolab, India) at 50 rpm. Simulated gastric fluid, pH 1.2 + 0.5% sodium lauryl sulfate (SLS) (900 mL) maintained at 37 ± 0.5 °C was used as dissolution medium. The samples (10 mL) were withdrawn at predetermined time (5, 10, 20, 30, 45, and 60 min) [15] and replaced with an equivalent amount of fresh medium. The samples were filtered through nylon membrane filter (0.45 µm pore size) and analyzed by UV–Visible spectrophotometer at 216 nm λ_{max}. The cumulative percent drug release was plotted against time to determine the release profile.

The optimized tablets of NTG were kept in open petridish and stored in stability chamber maintained at 40 ± 2 °C and 75 ± 5% RH. The samples were withdrawn periodically and subjected to assay and dissolution study.

Results and discussion

Compatibility testing

The selected DSC curves of drug and drug–excipient mixtures are shown in Figs. 2, 3, 4, 5, and 6. The thermal behavior of pure drug, respective excipient, and the combination of drug and excipients are compared in the DSC curves. Peak transition temperature (T_{peak}) and heat of fusion or enthalpy (ΔH_f) of NTG in various excipient mixtures is summarized in Table 1.

The DSC curve of NTG showed a sharp endothermic peak at 144.76 °C. In majority of the cases, melting endotherm of drug was well preserved with slight broadening or shifting toward the lower temperature range. It has been reported that the shape of the peaks of DSC curve and enthalpy may change due to the presence of impurity in the materials used for analysis [7, 12, 16]. Thus, these minor changes in the melting endotherm of drug could be due to the mixing of drug and excipients, which lower the purity of each component in the mixture and may not necessarily, indicate potential incompatibility [8, 17–19].

The DSC curve of lactose showed endothermic peaks at 145.29 (indicating the dehydration of bound water) and 224.59 °C (melting point). The DSC curve of NTG in the presence of lactose is shown in Fig. 2. Melting endothermic peak of NTG was 143.48 °C in NTG–lactose mixture, which suggested that there was no interaction between lactose and NTG.

The DSC curve of polacrilin K showed a broad endotherm at 117.57 °C (starting from 50.80 °C and ending at 162.63 °C), which may be attributed to the loss of adsorbed water [7, 18]. The curve of NTG–polacrilin K mixture (Fig. 3) showed an endothermic peak of NTG at 141.78 °C, indicating that there NTG is compatible with polacrilin K.

In case of PVP, a broad endotherm was observed at 98.43 °C due to loss of adsorbed moisture (Fig. 4). The curve of NTG and PVP mixture showed broadening and shifting of the peak of NTG to a lower temperature (140.38 °C). This kind of phenomenon may be due to simple mixing of drug with excipient, which lowers the purity of each component [12, 16]. This result signifies that NTG and PVP are compatible with each other.

In the DSC curves of talc, no peaks were observed in the range of 50–300 °C (Fig. 5). However, the endothermic peak of NTG was well preserved at 142.81 °C in the DSC curve of NTG–talc mixture. It was concluded that NTG is compatible with talc.

In the DSC curve of magnesium stearate, an endothermic peak was observed at 98.3 °C with irregular curve. The DSC curve of NTG–magnesium stearate mixture showed disappearance of the peak of NTG (Fig. 6) which suggested that there might have some physical incompatibility between NTG and magnesium stearate. Therefore, NTG–magnesium stearate mixture was subjected to the IR studies and its spectrum was compared with the IR spectra of NTG. The characteristic

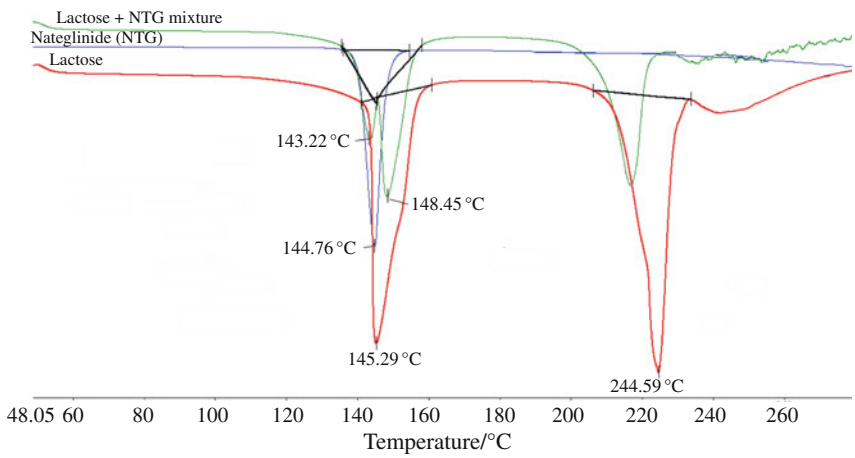

Fig. 2 DSC curve of NTG with lactose

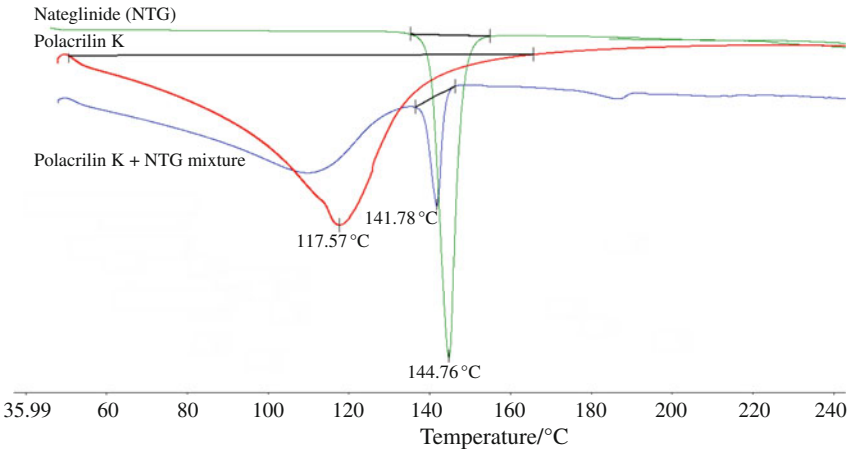

Fig. 3 DSC curve of NTG with polacrilin K

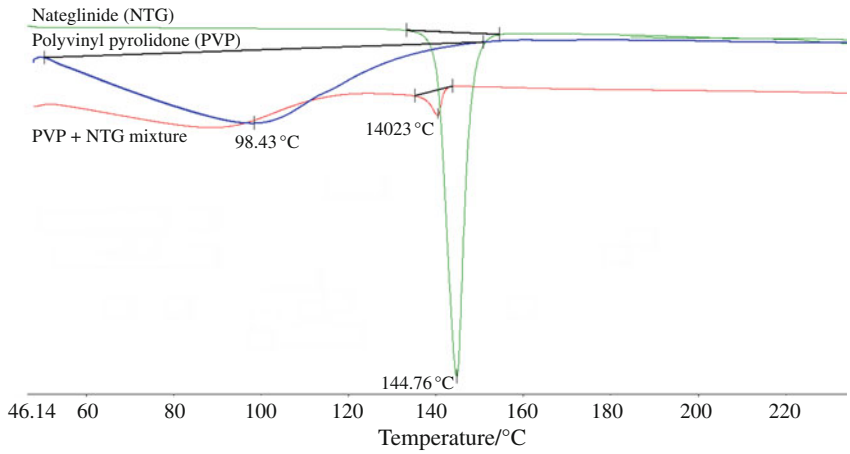

Fig. 4 DSC curve of NTG with PVP

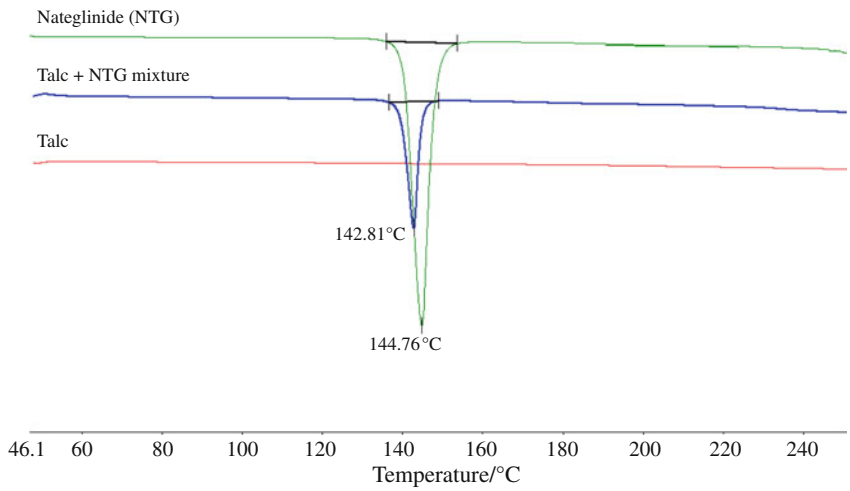

Fig. 5 DSC curve of NTG with talc

bands of NTG (Fig. 7) were observed at 1647 cm^{-1} (–C=O), 1713 cm^{-1} (–COOH), 2862–3096 cm^{-1} (–CH_2–cycloalkane), and 3296 cm^{-1} (–N–H stretching). However, IR spectrum of NTG–magnesium stearate mixture are shown in Fig. 8, and the characteristic bands were observed at 1647 cm^{-1} (–C=O), 1712 cm^{-1} (–COOH), 2850–2953 cm^{-1} (–CH_2–cycloalkane), and 3300 cm^{-1} (–N–H stretching), which corresponds to the structure of NTG. The results confirmed that there is no chemical interaction between NTG and magnesium stearate.

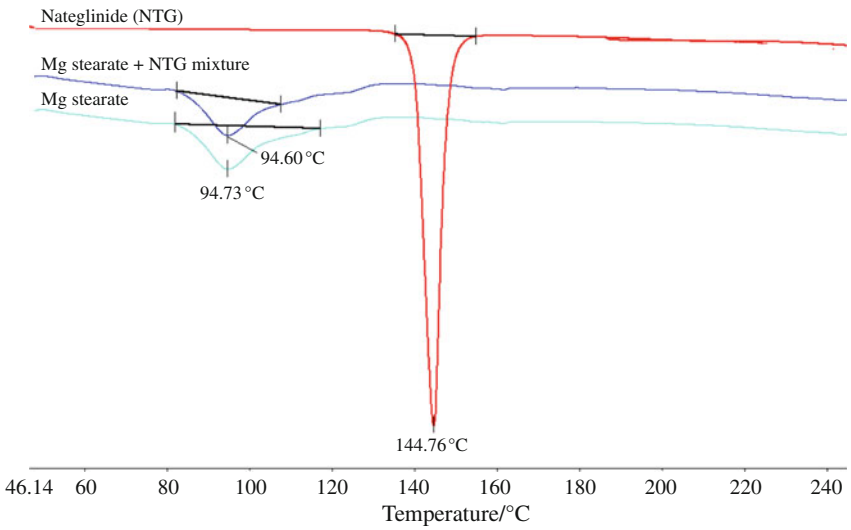

Fig. 6 DSC curve of NTG with magnesium stearate

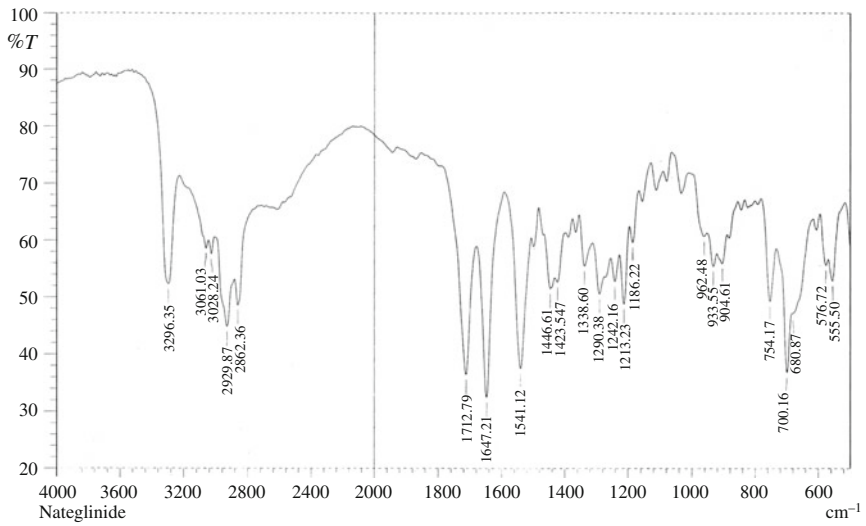

Fig. 7 Fourier transmission-infra red spectrum of NTG

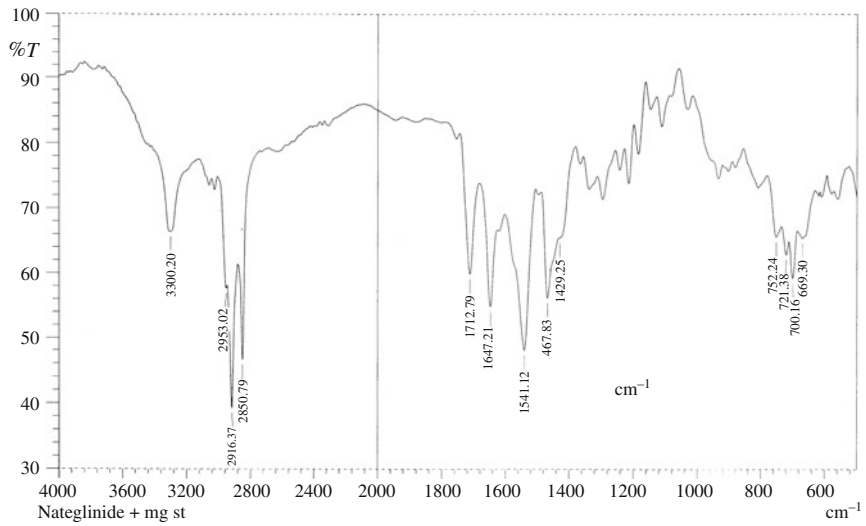

Fig. 8 Fourier transmission-infra red spectrum of NTG with magnesium stearate

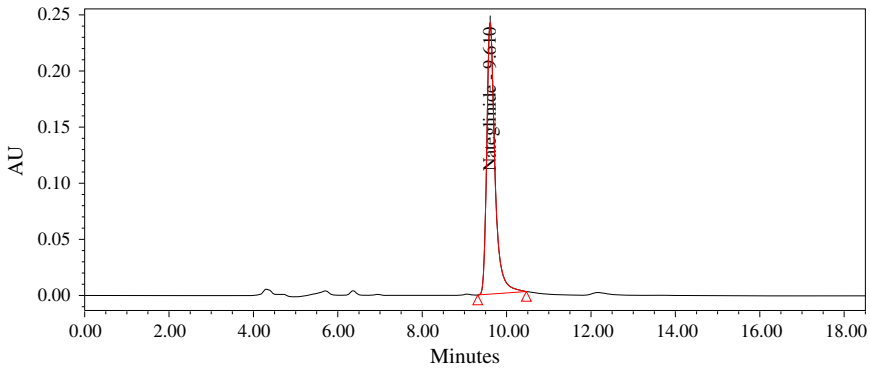

Fig. 9 HPLC chromatogram of NTG

The drug–excipient mixtures were tested using the technique of IST and the quantitative results are shown in Table 2. It has been observed from the Table 2 that there is a little change in the drug content of the samples after storage of drug–excipient blends under stressed conditions of IST studies.

Lactose, polacrilin K, PVP, and talc were used for the formulation of immediate release tablets of NTG as diluent, superdisintegrant, binder, and lubricant, respectively, based on the results of DSC curves and IR spectra. However, as seen from the Table 2, there is little change in the drug content in IST samples after 3 weeks of storage at stressed conditions. Moreover, when the HPLC chromatogram (shown in Fig. 9) of the mixture of NTG and individual excipients was compared with that of pure NTG during the analysis of IST sample, it was found that the retention time (RT) and the shape of the peak of NTG were remained unchanged, and there was not any extra peaks were observed. It indicates that NTG was not degraded in drug–excipients mixture of IST sample. Therefore, it was considered that the NTG and the excipients used are compatible with each other.

In case of NTG–magnesium stearate mixture, a definite conclusion could not be drawn based on the DSC results alone. However, the results of IST studies showed that the residual drug concentration was within the limit and in the IR spectrum of NTG–magnesium stearate mixture, the characteristic bands of NTG were well preserved confirming the compatibility.

Table 4 Evaluation of tablets after 3 months of storage at 40 °C and 75% RH

Parameter	Initial	1 Month	2 Months	3 Months
Drug content[a]	101.58 ± 2.4	100.95 ± 2.8	100.28 ± 3.4	98.46 ± 2.5
Hardness[a]/kg/cm^2	5 ± 3.8	5 ± 5.2	5 ± 6.2	5 ± 5.7
DT[b]/s[a]	32 ± 4.6	36 ± 3.9	44 ± 4.2	46 ± 3.3

[a] Values expressed as average ± standard deviation

Formulation development and stability study

Excipients defined in the prototype formula were used for formulation development of immediate release tablets of NTG. Various parameters were varied to optimize the prototype formula. The tablets were evaluated after 3 months of storage at accelerated stability conditions (40 ± 2 °C and 75 ± 5% RH), the results of which are presented in Table 4. It is evident that the formulation is having stability in terms of both drug content and dissolution profile. There was a very little change in the drug content after 3 months of storage at accelerated stability condition (Table 4). Based on the results, it has been concluded that the immediate release tablets of NTG were stable after 3 months of storage at accelerated stability conditions.

Conclusions

The results of the studies confirmed that DSC and IR could be used as the rapid methods to evaluate the compatibility between NTG and excipients. However, the techniques of IST after storage of the mixture of NTG and individual excipients under stressed conditions should also be adopted in conjunction with DSC and IR studies to reach any definite conclusion. In this study, the DSC analysis along with IR spectroscopy and HPLC analysis (for IST studies) were successfully employed to assess the compatibility of NTG with the excipients used in the development of immediate release tablet formulation.

No definite evidence of interaction was observed between NTG and the excipients used in the development of *in house* formulations of immediate release tablets of NTG. From the results of the DSC studies an interaction was suspected between NTG and magnesium stearate. However, based on the results of IR spectroscopy and IST study, the possibility of incompatibility between NTG and magnesium stearate was ruled out. On confirmation of the compatibility of the excipients with NTG by the methods described above, formulation of immediate release tablets

of NTG were developed using the excipients studied. The prototype formulation was found to be stable after 3 months storage at of accelerated stability conditions. Thus, the methods of DSC, IR spectroscopy, and IST have been proved to be successful in the assessment of compatibility of NTG and excipients used in the immediate release tablets of NTG.

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