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Merged *in silico-in vitro* validations of atenolol and amlodipine besylate with excipient interactions for the design of solid oral dosage forms

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Abstract:

Early understanding and estimation of interactions between the drug and excipients is a crucial step in the preformulation studies. *In silico* studies utilising computational methods were followed by *in vitro* studies and analytical validation to identify stable excipients (SmartEx and Ludipress) for the development of solid oral dosage forms. *In silico* studies were conducted using the FormulationDE machine learning tool, which utilises artificial intelligence to interpret input data. FormulationDE can predict compatibility outcomes based on functional groups using Shapley additive explanation force plots. *In vitro* studies, including isothermal stress testing methods, ATR-FTIR, DSC and drug stability profiles, were conducted to assess the potential for drug-excipient interactions under long-term storage and physiological conditions. These studies were demonstrated, and samples were analysed by high-performance liquid chromatography to quantify the percentage of drug degradation over time. *In silico* studies showed that ATN was compatible with lactose and mannitol, whereas AMB was incompatible with both. *In vitro* IST results showed that ATN was compatible with SmartEx, whereas AMB were incompatible with Ludipress and SmartEx. Drug stability profiles were conducted under gastric pH (1.2) and incubated at 37 °C for four hours to evaluate the resilience of the drugs under physiological conditions and to mimic the gastric environment. The drug stability profile showed that AMB was unstable and ATN was stable under the gastric physiological conditions. This article highlights the importance of integrating *in silico* with *in vitro* experimental techniques in preformulation to minimise risk during later stages of formulation development. The results of various studies have guided the future development of ATN-based solid oral dosage forms using SmartEx QD 100 thermoplastic excipients.

Keywords: *In silico* - *In vitro* studies, Drug-excipient interactions, Compatibility studies, pH instability studies, Isothermal stress testing.

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1. Introduction

Drugs and excipients are integral parts of formulations (such as tablets, capsules, suspensions, emulsions, and many others). The selection of compatible excipients for the selected drug(s) is a crucial part of preformulation studies, as it is desirable for the long-term stability, safety, and efficacy of the pharmaceutical product. In the dosage form, the selected excipients should facilitate the administration, release, and protection of the APIs from external environmental conditions (pH, moisture, and temperature)[1]. However, pharmaceutical products are probably less stable than their active pharmaceutical ingredients (APIs). In an ideal world, excipients should be chemically inert and not interact with APIs. There should be no possible interactions between the selected APIs and excipients that could increase the degradation of susceptible APIs[2]. The selection of compatible excipients and API(s) is a critical step in developing safe dosage forms that may enhance patient compliance and extend their shelf life[3]. The undesirable effects, which can be toxic and may compromise pharmacological activity, include chemical incompatibility between the API(s) and excipients. Compatibility screening of selected excipients for chosen API(s) is a vital aspect of preformulation studies in pharmaceutical product development[4]. A complete understanding of the physicochemical interaction of excipients with API(s) in the dosage form is regarded under Quality by Design. It is encouraged by the United States Food and Drug Administration and other competent regulatory bodies worldwide[5]. The drugs and excipients were formulated (e.g., tablets, capsules, or suspensions), and an imperative step is to assess potential interactions between the drug and excipients. However, there are several instrumental techniques used to confirm the probable drug–excipient interactions (DEIs). Techniques applied to ensure the compatibility of drugs and excipients include Differential Scanning Calorimetry (DSC), thermogravimetry, x-ray diffraction, chromatographic methods, and Isothermal stress testing (IST)[6, 7]. The techniques are widely used at the laboratory scale due to their rapid analysis, reduced material consumption, and, importantly, the ability to detect reactive/nonreactive degradation/impurities in products[8].

The DEI studies, also known as compatibility studies, involved screening one or more suitable excipients for drug(s). Isothermal stress testing (IST) is a widely used method for evaluating potential interactions between a drug and its excipients. Generally, the proportion of the excipient in a BM(s) (Binary mixtures) of the drug and the excipient is kept high (1:1 w/w). The proportion of excipients is slightly high, as it may increase the likelihood of interactions between the drug and excipients. Understanding the role of water and temperature

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in the IST method is essential[9]. The water involved in the IST method increases the chances of interaction between excipients and the drug, altering its properties, while temperature accelerates degradation[10]. These samples were equilibrated at elevated temperature and humidity, conditions that can enhance DEIs. Furthermore, samples were observed for physical changes, including colour, physical state, agglomeration, and more. Additionally, the same samples were further analysed qualitatively and quantitatively using spectroscopic and thermoanalytical techniques[11].

In silico studies have become essential tools for screening DEIs during the preformulation stage of solid oral dosage form development[12]. Conventional compatibility assessments rely on time-intensive "stress testing," in which physical mixtures are subjected to accelerated conditions and examined by thermal (DSC) and spectroscopic (FTIR) techniques. Nonetheless, computational methods now facilitate rapid, cost-effective predictions of potential interactions before the initiation of laboratory experiments. The advancement of solid oral dosage forms will increasingly depend on such *in silico* tools to optimise excipient selection, reduce the number of experimental iterations, and accelerate the time-to-market while maintaining formulation stability and efficacy. Ouyang and his team developed an advanced version of PharmDE[13], an AI tool based on machine learning for assessing interactions between excipients and drugs. They named that advanced system “FormulationDE”, which provided results in the form of a risk assessment and the compatibility probability of the selected drug and excipients. FormulationDE comprises records of 1,105 datasets, consisting of 579 compatible and 526 incompatible study datasets. They achieved performance ratios with accuracies and precisions of 0.75 each, a recall of 0.75, a Matthews correlation coefficient of 0.50, an F1 score of 0.74, and an AUC of 0.82. This DEIs checker can predict interacting functional groups based on SHAP (Shapley Additive Explanations) force plots. They showcase that FormulationDE has the potential to serve as a valuable tool in the pharmaceutical field. It helps reduce the costs associated with preformulation studies and DEI assessments, as well as the time required for the drug development process[14].

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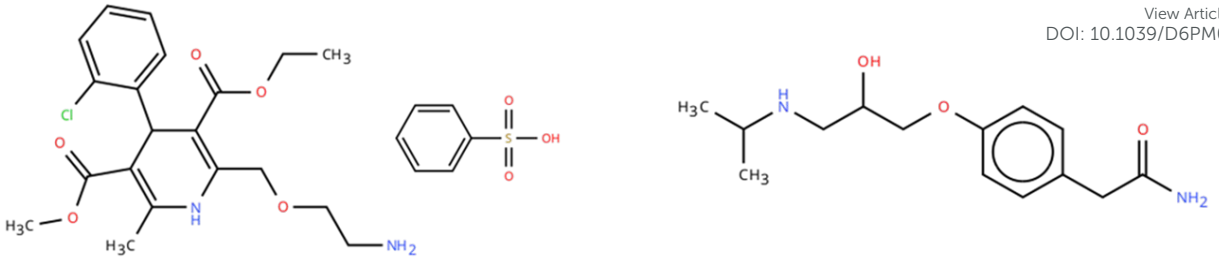

(a) (b)

Figure 1: Chemical structure of (a) amlodipine besylate (AMB), and (b) atenolol (ATN).

Over 1.28 billion people worldwide suffer from hypertension, commonly known as high blood pressure. This condition is often called "the silent killer" because it can cause serious health problems without obvious symptoms. Hypertension is a major risk factor for strokes, heart attacks, heart failure, and kidney disease. Managing is not only of medical importance, but it is also a crucial step in saving lives. Antihypertensive drugs are powerful medicines that help fight this silent threat from within the body. Amlodipine besylate (AMB) is a pharmacologically active 1,4-dipyridamole derivative and belongs to the subclass of L-type calcium channel blockers. Chemical structure shown in **(Figure 1)**, and chemically known as benzenesulfonic acid;3-*O*-ethyl 5-*O*-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate. It appears as a white crystalline powder, weighs 567.1 g/mol, and has a pKa of about 8.8. Although it doesn't dissolve easily in water, it dissolves well in alcohol. Its moderate lipid affinity helps it cross cell membranes, providing a long-lasting effect in the blood for up to 50 hours and allowing once-daily doses. It's well absorbed when orally administered, around 65%, ensuring it works effectively despite the liver's first-pass effect. The photostability of AMB and nifedipine was studied, and the results showed that AMB is more stable than nifedipine. AMB was found to be safe and showed no signs of degradation under refrigerated (4 °C) and room temperature (25 °C) conditions[15]. Atenolol (ATN) is a pharmacologically active drug that belongs to the class of selective beta-1 adrenergic receptor blockers. Chemical structure shown in **(Figure 1)**, and chemically known as 2-[4-[2-hydroxy-3-(propan-2-ylamino)propoxy]phenyl]acetamide. By blocking beta-1 receptors, ATN reduces heart rate and the strength of heart contractions without significantly affecting the lungs, making it a safer choice than non-selective beta-blockers. ATN is water-loving, with a logP of 0.23 and a molecular weight of 266.3 g/mol. Its pKa of about 9.6 means it is ionised at body pH, limiting its entry into the brain and reducing nervous system side effects. Because it doesn't dissolve well in fat, it doesn't easily cross the blood-brain barrier, making it better tolerated than other antihypertensives. ATN's limited gut absorption (~50%)

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underscores the importance of precise dosing. Its hydrophilic nature can also complicate delivery methods. Both medications need strict quality control during manufacturing to ensure consistent strength, proper dissolution, and reliable performance, which can increase production costs[16].

This article focuses on validating *in silico* results and performing *in vitro* methods to assess interactions between selected drugs and excipients in continuation of our earlier *in-silico* exercise[17]. This study aims to evaluate the use of the *in silico* computational model FormulationDE by formulation scientists for the future acceptance as drug-excipient interaction software. This manuscript integrates *in silico* with *in vitro* experimental techniques in preformulation studies to minimise risk during later stages of formulation development. The results of various studies have guided the future development of anti-hypertensive-loaded solid oral dosage forms using a compatible thermoplastic polymeric excipient.

2. Materials and Methods

2.1 Materials

ATN and AMB were purchased from the Tokyo Chemical Industry (India), Pvt. Ltd., Japan. The SmartEx QD 100 (SmartEx), a co-processed thermoplastic polymer (constituents include: D-mannitol (93.10% w/w), L-hydroxy propyl cellulose (5.90% w/w), and polyvinyl alcohol (0.2% w/w)), was purchased from Shin-Etsu Pharma, Pvt. Ltd., Japan. Ludipress® (Ludipress), a co-processed thermoplastic polymer (constituents include: Lactose (93% w/w), povidone (3% w/w), and crosspovidone (3% w/w)), was obtained from BASF, India. All the solvents used for HPLC were of analytical grade, and the chemicals used were of pharmaceutical grade. The triple-distilled water used was obtained from a Milli-Q assembly equipped in our laboratory. Buffer solutions with a pH of 1.2 were prepared using pharmaceutical-grade hydrochloric acid (37 % w/w) obtained from Merck, India.

2.2 *In silico* studies

FormulationDE has drug-excipient incompatibility data from more than 300 peer-reviewed publications. To establish FormulationDE for machine learning modelling, 1,105 drug-excipient incompatibility entries, including 579 compatible and 526 incompatible pairs. The SMILES strings of all drugs and excipients were subsequently retrieved from the PubChem database for model training. It showed that a drug-excipient pair was classified as "incompatible" if DSC thermograms exhibited significant peak shifts, peak disappearances, or the emergence of new peaks; if FTIR spectra showed shifts or alterations in characteristic

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functional group bands; or if HPLC analysis detected substantial chemical degradation and impurity formation. Pairs without such changes were labelled as "compatible"[14]. The selected drug-excipient pairs were entered, and the compatibility probability prediction results were obtained. All collected data were rigorously cleaned and deduplicated to ensure the quality of results and uniqueness.

2.3 *In vitro* studies

2.3.1 High-performance liquid chromatography

A reversed-phase Agilent 1260 Infinity II high-performance liquid chromatography (HPLC) system was used for method development and further sample analysis. The chromatograph was equipped with a DAD detector and set to the isobestic point at 230 nm. This method has been used previously, and we have made some slight modifications to the validated method. The non-polar column (Phenomenex, C18, dimensions of 250 × 4.6 mm, and particle size of 5 μm) was used, with a flow rate of 1mL/min. The mobile phase consists of a buffer solution containing ammonium acetate (10 mM) and octane-1-sulfonic acid sodium salt (5 mM), with acetonitrile as the organic solvent. The gradient method was employed, with the following concentration/time conditions: 75/4, 75/4, 6/40, 7/40, 75/9, and 75/12. The runtime was set to 12 minutes, with a 1-minute post-run time. The injected sample volume was set to 5 μL. This same method was used to analyse DEI samples and to conduct pH stability studies[18].

2.3.2 Isothermal stress testing

BMs of AMB and ANT with each excipient (SmartEx and Ludipress) were prepared at a 1:1 (w/w) ratio by mixing well in a pestle and mortar for 5-10 minutes. Then, BMs were transferred to glass vials, and 10% (w/w) water was added to each vial. The mixtures were then mixed using a glass capillary and stored in an oven at 50 °C for three weeks. The samples were analysed to quantify the percentage degradation of the drug using spectral techniques, and the results were validated using spectral (FTIR) and thermal techniques (DSC)[6].

2.3.3 pH stability studies

Methanolic solutions of AMB and ATN (100 mg/mL) were diluted with a pH 1.2 buffer to obtain samples at 20 mg/100 mL for each. The same procedure will be followed for the ATN and AMB in a BM sample. Then, the samples were transferred into 10 mL amber glass reaction vials and incubated at 37 ℃ for 4 hours to simulate the gastric physiological conditions of the

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human body. Samples were taken at specific time intervals and analysed for ATN and AMB degradation using spectral analytical techniques and HPLC. [19].

2.4 Analytical studies

2.4.1 Attenuated Total Reflection Fourier Transform Infrared Spectroscopy

Attenuated Total Reflection Fourier Transform Infrared (ATR–FTIR) spectra were recorded on dry DEI samples using a Bruker Alpha II spectrometer with a diamond ATR attachment. Scans were performed from 4000 to 600 cm^{-1} with a resolution of 4 cm^{-1} and a scanning interval of 2 cm^{-1}. For this study, a sample of DEIs or compatibility was analysed in transmission mode with a complementary surface functional group. It may be helpful to quantify the interaction between the selected excipients and the drugs by observing shifts in band positions. This study helps monitor the chemical stability of the drug and excipients within the formulations.

2.4.2 Differential Scanning Calorimetry

The thermal analysis of the samples was performed using a Differential Scanning Calorimeter (TA-25, TA Instruments-waters LLC, New Castle, USA), equipped with a dedicated cooling system (RCS90). Approximately 5 mg of samples were weighed and sealed within an aluminium pan and lid. The heating rate was set to 10 ℃/min, and the nitrogen purging rate was 50 mL/min. The samples were scanned from 15 ℃ to 400 ℃ in a thermostatically sealed aluminium pan. Changes in the curves analysed for assessing possible interactions in the samples.

DSC analysis was performed to evaluate the physical state and compatibility of the drug and excipients. The changes in the drug's thermal events help to better understand the DEIs and compatibility for selected drugs and excipients. Changes in thermal peaks may indicate the physical changes observed and, subsequently, the formulation's stability.

2.5 Statistical analysis

Statistical analysis of the data was performed using one-way analysis of variance (ANOVA) for the IST method and pH stability studies. Calculations were performed in Excel and GraphPad Prism.

3. Results and Discussions

3.1 *In silico* studies

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The FormulationDE is an AI-based machine learning tool for data analysis, integrated with an expert system (PharmDE). FormulationDE is a highly interpretable platform for assessing DEIs or compatibility. This tool not only predicts interactions between selected drug-excipient pairs but also provides risk assessments based on essential functional groups. The FormulationDE is based on eight algorithms to obtain a better, more optimal model for predicting DEIs. It utilises the SHAP (Shape Additive Explanations) method for model interpretability analysis, assigning each feature a high importance value. It supports one-time prediction of up to ten excipients corresponding to a single drug. The prediction results can be downloaded as a CSV file that includes information on the selected drug and excipients, listed by name or SMILES. However, the results also highlight potential interactions with risk assessment and the probability of compatibility. The probability score reflects the likelihood of the result outcomes, alongside the SHAP-based force plot **(Table 1).** SmartEx and Ludipress are not available as excipient options in the FormulationDE datasets. Hence, we selected mannitol and lactose for screening of drug and excipient studies. Mannitol (93% w/w) is a major constituent of SmartEx, and lactose (93.10% w/w) is the principal constituent of Ludipress.

Table 1. *In silico* compatibility results of drugs (AMB and ATN) with excipients (Ludipress and SmartEx) from the FormulationDE AI-based machine learning tool.

Drugs	**Excipient**	**Prediction**	**Compatibility Probability**
ATN	Lactose	Compatible	0.91
	Mannitol	Compatible	0.82
AMB	Lactose	Incompatible	0.10
	Mannitol	Incompatible	0.14

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The results showed that ATN is compatible with both selected excipients (lactose or mannitol), whereas AMB is incompatible with the same. The result showcases the compatibility probability of ATN with mannitol (0.82) and lactose (0.91). On the other hand, AMB showed compatibility probabilities of 0.14 and 0.10 with mannitol and lactose, respectively. AMB was found to be incompatible with the subsequently selected polymers SmartEx (co-processed with mannitol) and Ludipress (co-processed with lactose), indicating that it will also be incompatible with them. A possible reason for AMB showing less compatibility with the same excipients as ATN is the involvement of a chemical reaction with impurities as intermediates. The carbohydrate groups present in reducing sugars (lactose), the aldehyde group, and the ketone group interact with primary or secondary amines present on the AMB. This chemical reaction, commonly known as the Millard reaction, causes incompatibility between the drug and excipients. AMB has both primary and secondary amine functional groups, and lactose contains both aldehydes and ketones, which may increase the tendency for the Millard reaction and reduce compatibility. However, the AMB-mannitol interaction was attributed to physical hydrogen-bond formation between the AMB amine group and mannitol's hydroxyl groups, with no Maillard reaction occurring. ATN lacks a primary amine functional group and does not react with lactose or mannitol. Hence, AMB showed incompatibility with SmartEx and Ludipress, whereas ATN showed compatibility with the same excipients. These results suggest that a solid oral dosage form of ATN, combined with Ludipress or SmartEx, will be more stable and have a longer shelf life than AMB with either excipient.

3.2 *In vitro* studies

3.2.1 High-performance liquid chromatography

The stock solutions were prepared by dissolving 5 mg of ATN in 5 mL of water and 5 mg of AMB in 5 mL of methanol. Then, the stock solutions were diluted with the HPLC mobile phase to obtain samples containing 1 mg of AMB and ATN per 5 mL. Then, the serial dilutions were prepared, and a calibration curve of the simultaneous estimation method was obtained. The retention times of ATN and AMB were 3.37 and 9.24 minutes, respectively. They follow a linear regression over the concentration range of 200 µg/mL to 3.175 µg/mL. The linear equations and other parameters of AMB and ATN **(Table 2).**

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Table 2. Linearity, regression, LOD, LOQ and retention time of the simultaneous method.

Parameters	**ATN**	**AMB**
Linear Regression	y = 4.6879x - 13.499	y = 4.0115x - 12.931
Correlation coefficient	0.999	0.999
Limit of detection	1.353	1.390
Limit of quantification	2.887	3.466
Retention time	3.3 minutes	9.2 minutes

3.2.2 Isothermal stress testing

In the second phase of in *vitro* studies, the compatibility of ATN and AMB with selected excipients (SmartEx and Ludipress) was tested using IST. The HPLC method used in this study was shown to be appropriate for detecting variations in drug concentrations (AMB and ATN) in BM excipients. The degradation of AMB and ATN was analysed in BMs (1:1, w/w) involving each combination of drugs (AMB and ATN) and excipients (SmartEx and Ludipress). The IST method involves high temperatures and humidity, which accelerate the degradation of drugs and excipients, as well as physical interactions and chemical reactions. These interactions or reactions alter the physicochemical properties of the drug and excipients, ultimately leading to the formation of interaction intermediates. The change in the area under the drug concentration-time curve in HPLC of a sample over time was assessed to assess drug degradation. BMs of drug and excipient were set on a control IST method, demonstrating or evaluating for a decrease in percentage of the drug and physical changes with time. The results obtained from the IST method were statistically significant, with a p-value of 0.0004 ($p<0.005$).

Table 3. Compatibility of drugs (AMB and ATN) with excipients (Ludipress and SmartEx) in the presence of 10% (w/w) water after storage at 50 °C for 3 weeks.

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Drugs	Excipients	% Assay	IST Results
ATN	Ludipress	69.74	Incompatible
	SmartEx	81.79	Compatible
AMB	Ludipress	59.27	Incompatible
	SmartEx	74.67	Incompatible

Data presented in **Table 3** indicate that AMB undergo degradation when mixed with SmartEx and Ludipress in BM. More than 75% assay was considered a criterion for compatibility, while less than was classified as incompatible. The results showed that, except for ATN and SmartEx, the other BMs involving drugs (AMB and ATN) and excipients (SmartEx and Ludipress) are incompatible. Excipients with ionizable functional groups, such as carbohydrates containing free aldehyde or ketone groups, like those found in Ludipress (co-processed with lactose), are known as reducing sugars. The Millard reaction is a complex reaction between primary or secondary amines (AMB and ATN) and reducing sugars, producing reaction intermediates, including reducing ketones, aldehydes, and heterocyclic compounds, which may lead to pharmaceutical incompatibility. Therefore, the existence of potential incompatibility due to chemical interactions, specifically the Millard reaction, between AMB or ATN and Ludipress. Wesolowski and Rojek, in their research, demonstrated that ATN was incompatible with the excipients lactose (the primary constituent of Ludipress), using thermogravimetric analysis and Multivariate techniques[20]. Abdoh *et al.* have demonstrated that AMB showed incompatibility with lactose in the solid state. The incompatibility between AMB and lactose results from the Maillard reaction between the reducing sugar (lactose) and the terminal primary or secondary amine group, such as AMB[19]. The AMB and SmartEx demonstrated incompatibility, potentially attributable to a physical interaction, by hydrogen bond formation, between the hydroxyl group of Ludipress and the amine functional group of AMB. Incompatibilities were identified between BMs, including ATN-Ludipress and AMB-SmartEx, likely due to a physical interaction potentially involving hydrogen bonding between the amine group of the drug (ATN or AMB) and the hydroxyl or

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carbonyl group of the excipient (Ludipress or SmartEx). Furthermore, incompatibilities were identified between AMB and Ludipress, caused by a chemical interaction resulting from a Maillard reaction between the carbohydrate group of Ludipress (lactose) and the amine functional group in AMB.

3.2.3 pH stability studies

This study was conducted because the literature lacked information on the gastric pH stability of AMB and ATN. The above-mentioned HPLC method was used to investigate the decrease in concentration of AMB and ATN due to degradation at gastric pH (1.2). ATN and AMB were incubated for 4 hours at 37 ºC in gastric pH (1.2) to assess their stability and mimic the physiological gastric environment. The pH degradation profile of ATN and AMB till four hours **(Fig. 3).**

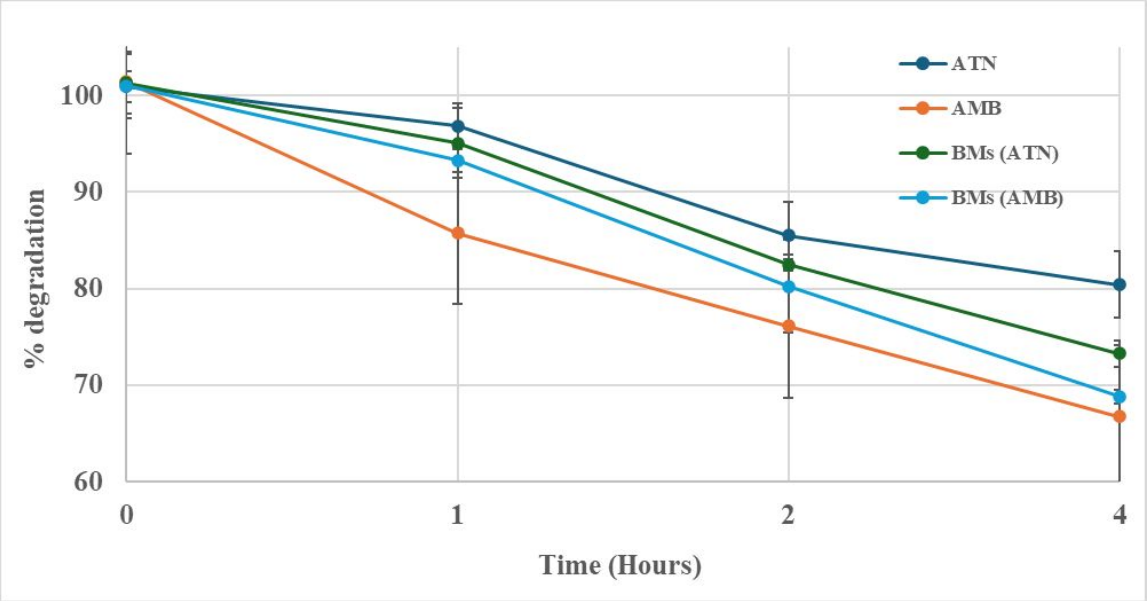

Figure 2: Impact of pH (1.2) on native as well as BMs (1:1) of ATN and AMB degradation after 4 hours of heating at 37 °C. (n=3)

AMB showed greater degradation in a 0.1 M HCl gastric pH (1.2) buffer solution within 4 hours. It degraded to 66.78% individually in a gastric pH (1.2) buffer solution and 68.81% in a BM. However, ATN only degraded to 80.39% in its native state, whereas it degraded to 73.3 % in BM. This clearly demonstrated that ATN was more stable at a gastric pH (1.2) than AMB. It also showed that there might be possible chances of drug–drug interactions or ATN increases the degradation of AMB at gastric pH. AMB is a basic drug and found to be unstable or exhibits higher degradation in gastric pH, both in native and in the BM of drugs. Hence, the development of a solid oral dosage form of AMB for gastric delivery may be less stable and

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more prone to instability than ATN. The degradation products that occurred in a 0.1 M gastric pH (1.2) buffer solution were not detected in the HPLC chromatogram. This means that HPLC does not detect different degradation products. The obtained results of the pH stability studies were found to be statistically significant with a p-value (< 0.0001) less than ($p < 0.005$). Abdoh *et al.* in their research work demonstrated the same and found that AMB is more stable at pH 5[19]. This study showed that the ATN is more stable than AMB under gastric physiological conditions. This study suggested that ATN-based solid oral dosage forms for gastric delivery will be more stable than oral AMB formulation. In the future, bilayer solid oral dosage forms are more suitable for delivering ATN and AMB than fixed-dose combinations of the same drugs.

3.3 Analytical techniques

3.3.1 Attenuated Total Reflection Fourier Transform Infrared Spectroscopy

The ATR-FTIR spectra of native drugs, selected excipients, and BMs were analysed to assess possible interactions or compatibility among them. The broad and intense IR band at 1633 cm^{-1} in ATN is assigned to the C=O stretching due to the acetamide group (Fig. **4)**. Different H-bonding arrangements of the ATN molecules in the solid state explain the doubling of this amide band. [21]. The distinct peaks at 3348 cm^{-1} and 3160 cm^{-1} may be due to functional groups, such as secondary amines and hydroxyl groups. The characteristic bands of AMB at 3257 cm^{-1}, 1671 cm^{-1}, and 1091 cm^{-1} are attributed to primary amine (broad peak), carbonyl group, and a sulphonate group (SO^{3-}), respectively. [22] However, SmartEx and Ludipress represent the IR bands of their primary constituents, mannitol [23] and lactose [24], respectively. The broad peaks at 3279 cm^{-1} and 3241 cm^{-1} in SmartEX and Ludipress, respectively, are due to OH stretching. This suggests that SmartEx and Ludipress contain significant or higher amounts of mannitol and lactose, respectively. Hence, the ATR-FTIR spectra also verified the chemical integrity of excipients, as co-processed polymers.

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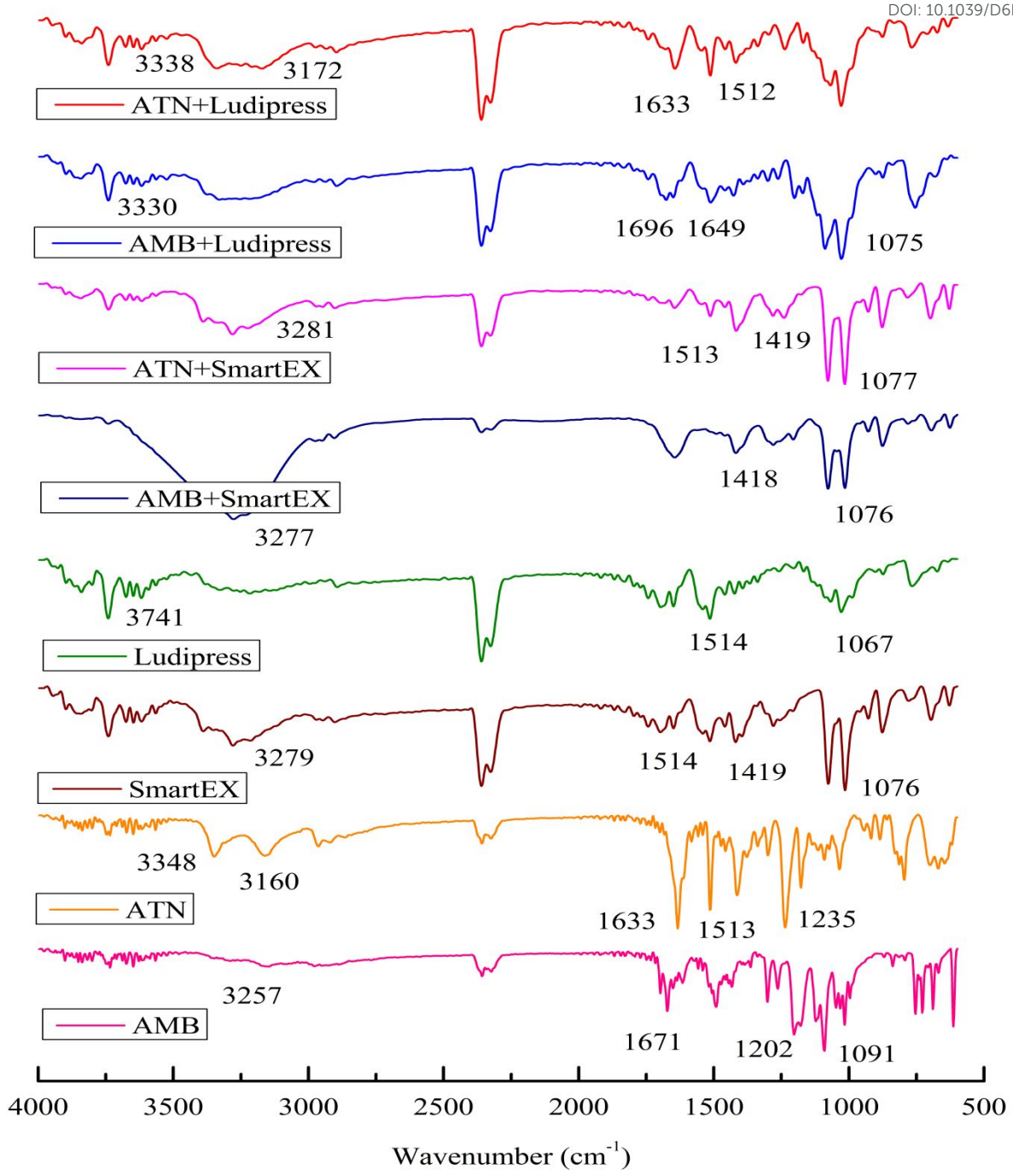

Figure 3: ATR-FTIR spectra of drugs (AMB and ATN), and excipients (Ludipress and SmartEx), displaying different spectral signatures, in the native state as well as in their BMs.

BM(s) of AMB with SmartEx showed a distinct, very broad and intense peak at 3267 cm^{-1}. The subtle shift in the hydroxy stretching might be due to the formation of multiple hydrogen bonds between the mannitol hydroxyl group and the AMB functional group. There is no significant change in the amine bands, indicating that no chemical reaction occurs during this interaction. AMB ester bonds may also undergo transesterification with SmartEx hydroxyl

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groups, leading to incompatibility due to physical interaction between AMB and SmartEx. However, in the BM of AMB and Ludipress, the IR bands of the amine broadened, and the peak at 3330 cm^{-1} might be due to consumption of the amine functional group during the reaction. There is a band shift in the carbonyl region from 1671 cm^{-1} to 1696 cm^{-1}, indicating modification of both the lactose aldehyde and AMB ester groups. Hence, the possibility of the Millard reaction between the amine functional group of AMB and reducing sugar in Ludipress (Lactose) to form aldehyde and ketones as intermediates. This leads to the interactions due to chemical incompatibility between AMB and Ludipress. The characteristic IR bands at 3338 cm^{-1} and 3172 cm^{-1} of BM, including ATN and Ludipress, showed broadening of the hydroxyl and amine stretching region. This shift from native bands may be due to hydrogen-bond formation. There is no significant shift in the amide band, indicating physical interaction (hydrogen bonding) between the ATN and Ludipress. In the BM of ATN and SmartEx, no significant band shift was observed compared to the native ATN and SmartEx. This demonstrated that BM involving ATN and SmartEx is more compatible compared to the other BMs. The shifts in the IR bands of BM(s) from the native IR vibrations of the functional groups are ascribed to interactions between the functional groups of the respective drug and excipients. The ATR-FTIR analysis showed the incompatibilities between ATN and Ludipress (physical interaction in a 1:1 mixture, probably due to a possible hydrogen-bond formation by the amine group of ATN and the hydroxy group of lactose), AMB (decomposition owing to a Maillard reaction taking place between them). The physical interactions between AMB and SmartEx showed incompatibility, possibly due to the hydrogen bond formation by the mannitol hydroxy group and the AMB functional group (amine).

3.3.2 Differential Scanning Calorimetry

DSC is a crucial thermal technique for gaining a deeper understanding of the interactions between the drug and excipients. DSC provides complete, detailed information on thermal transitions, including amorphisation, phase changes, and melting. DSC thermogram plays a crucial role in assessing DEIs and compatibility **(Figure 5)**. The DSC thermograms of native AMB and ATN showed sharp melting points at 203.85 °C[25] and 153.21 °C[26], respectively. The DSC thermogram of excipients, including SmartEx (co-processed with mannitol), showed a sharp, distinct endotherm at 167.66 °C. The characteristic peak of crystalline mannitol[27] has a similar melting point, confirming mannitol as a major constituent of SmartEx[28]. The DSC thermogram of Ludipress (co-processed with lactose) has two

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endothermic transitions near 160 °C, indicating recrystalization of amorphous lactose and another endothermic peak shows melting at approx. 220 °C[29].

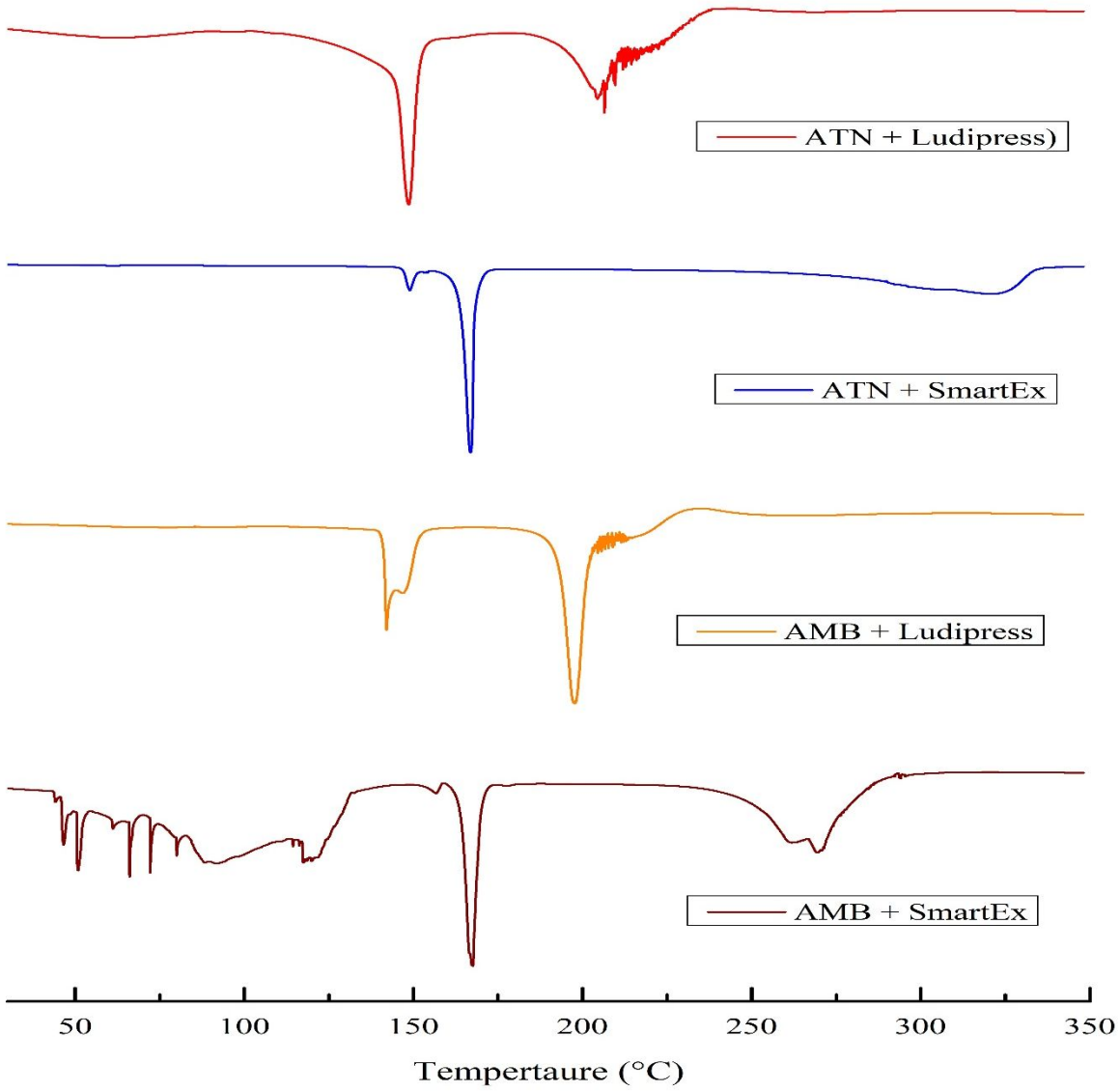

Figure 4: DSC thermograms of the physical mixture of the selected drug and excipients.

The thermograms of BM, including ATN and SmartEx, showed endothermic peaks at 149.78 °C and 166.28 °C, respectively. The sharp melting point at 166.28 °C showed the crystalline nature of SmartEx, and after 300 °C, degradation of BM. However, the sharp, distinct endothermic peaks for ATN at 149.78 °C and SmartEx at 166.28 °C suggest little or no interaction between the two. The thermograms of BM with ATN and Ludipress showed a clear, discrete peak at 149.62 °C and a broad peak at 204.9 °C, respectively. The ATN intensity exceeds that of the BM, including the ATN with SmartEx, which may be attributed to the superimposed endothermic peak of Ludipress with ATN. The small shift in the endothermic

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peaks of Ludipress from 160°C to 150°C may be due to physical interactions (hydrogen bonding) between them. The second endothermic peak of Ludipress shifts from 220 °C to 205 °C and broadens, suggesting possible chemical incompatibility. This clearly depicts the incompatibility between ATN and Ludipress in their respective BM. Furthermore, the examination of the endothermic peaks of BM (AMB with SmartEx) reveals a SmartEx peak at 167.8 °C and a broad AMB peak at 262.5 °C and 269.7 °C. The shift and branding of AMB from 204 °C to above 260 °C indicate the formation of new chemical entities, such as impurities. The decrease in SmartEx intensity (compared to BM of ATN with SmartEx) supports the degradation of AMB in the presence of SmartEx or vice versa. This thermogram clearly showcases incompatibility in BM due to the interaction between AMB and SmartEx. The resulting thermogram of BM (AMB and Ludipress) shows an endothermic peak of AMB at 197.05 °C, and Ludipress at 146.68 °C. The primary endothermic peak of Ludipress showed broader, distorted peaks at 146.68 °C, with an onset at 142.36 °C and an end at 158.91 °C. indicating an interaction between Ludipress and AMB. This thermogram revealed an interaction between the drug (AMB) and excipients (Ludipress), as indicated by a broadening of the primary Ludipress peak. The broadening and shift of the peak indicated the occurrence of new thermal events in the physical mixture, reduced crystallinity, and a transition in the state of the excipients. The DSC results revealed that the physical mixture of AMB and SmartEx exhibits higher incompatibility and drug-excipient interactions. ATN and SmartEx exhibited sharp, well-defined, clear, and distinct peaks on the thermogram, indicating minimal or no interaction and greater drug-excipient compatibility. These changes, along with the shift, broadening, and decrease in enthalpy, indicate strong molecular interactions between the drug and excipients. The DSC results showed that Ludipress interacts with AMB and ATN, whereas SmartEx interacts only with AMB. This leads to the conclusion that a solid oral dosage form of ATN and SmartEx will be stable and have a higher shelf life.

4. Conclusions

The present study investigated the preformulation compatibility profile of two pharmacologically complementary antihypertensive agents (AMB and ATN), with a panel of pharmaceutical thermoplastic polymeric excipients (Ludipress and SmartEx) to develop a stable solid oral dosage form. The rationale includes validating *in silico* results by performing *in vitro* methods to assess interactions between selected drugs and excipients. *In silico* studies were conducted using FormulationDE, a machine-learning tool for DEIs. It showed that ATN was compatible with lactose and mannitol, whereas AMB was incompatible with these

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excipients. The possible Millard reaction between reducing sugar (Lactose) and drugs (AMB) with terminal functional groups (primary and secondary), while AMB-SmartEx and ATN-Ludipress showed incompatibility. *In silico* results of FormulationDE were validated through *in vitro* studies employing the IST method for DEIs or compatibility studies, as well as analytical methods (FTIR and DSC). IST compatibility results showed that ATN was compatible with SmartEx thermoplastic polymer. However, AMB-SmartEx, AMB-Ludipress, and ATN-Ludipress showed incompatibility due to physicochemical interaction in their respective BMs. In FTIR studies, incompatibilities were found between AMB and ATN with Ludipress. The physical interactions between AMB and SmartEx showed incompatibility. The ATN and SmartEx showed little or no interaction, so they were deemed compatible. The DSC thermogram revealed that incompatibilities due to physicochemical interactions between AMB and ATN with Ludipress. Due to physical interactions in BM, including AMB and SmartEx, incompatibility was observed. The overall *in vitro* results provide a clear and mechanistic approach to quantifying interactions among AMB, ATN, and the excipients (Ludipress and SmartEx) under investigation. By combining all the findings from DSC, ATR-FTIR, and IST, it establishes an incompatibility profile, each grounded in a specific and identifiable physicochemical mechanism.

The final results indicated that the incompatibility observed between AMB and Ludipress was attributed to a chemical interaction, by the Maillard reaction between the amine functional group of AMB and the free carbonyl group of Ludipress. The incompatibility observed between AMB and SmartEx was identified as a physical interaction, possibly driven by hydrogen-bond formation between the hydroxyl groups of SmartEx and the amine functional group of AMB. This finding is consistent with the known hydrogen-bond donor capacity of SmartEx (mannitol) polyol hydroxyl groups and confirms that the BM, including AMB–SmartEx, was incompatible. The interaction was physical rather than chemical (Maillard reaction), and it also established that mannitol, a non-reducing sugar, cannot initiate the Maillard reaction. The ATR-FTIR and DSC clearly represent a covalent, chemically driven incompatibility, distinct from the physical hydrogen-bond interaction observed, and the manuscript now clearly distinguishes between the two BMs. The incompatibility observed between ATN and Ludipress was characterised as a physical interaction, most likely due to hydrogen-bond formation between the ATN amine group and the Ludipress hydroxyl group. All these physicochemical interactions in BMs were sufficient to compromise their stability and were accordingly flagged as an incompatibility during the preformulation screening.

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The pH stability studies showed that ATN was more stable than AMB at gastric physiological pH. Hence, the development of an ATN-based solid oral dosage form for gastric delivery using SmartEx yields a more stable formulation with a higher shelf life. In the future, bilayer solid oral dosage forms will be more suitable for delivering ATN and AMB than fixed-dose combinations of the same drugs. This study suggested that FormulationDE is also used in the pharmaceutical industry at the preformulation stage to screen excipients for the development of final pharmaceutical products. The preformulation studies should not be limited to the excipients selected for this study. Multicomponent DEIs or compatibility studies should be considered to minimise instability in the final developed product. The results of various studies have guided the future development of anti-hypertensive-loaded solid oral dosage forms using a compatible thermoplastic polymeric excipient.

Availability of data and material

Data will be made available on request.

Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that might have influenced the results reported in this work.

Consent for publication

All authors contributed to the development, writing, and editing of the manuscript submitted. All authors read and approved the final manuscript.

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CRediT authorship contribution statement

Gaurav Awasthi: Investigation, Data curation, methodology, writing-original draft, writing-review, and editing. **Subham Banerjee:** Conceptualisation, methodology, supervision, writing–review, and editing.

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Availability of data and material

Data will be made available on request.