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RESEARCH ARTICLE

Investigation of Maillard-Type Drug–Excipient Interaction Between Rivaroxaban and Lactose: Implications for Stability and *In Vitro* Anticoagulant Activity

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Investigation of Maillard-Type Drug–Excipient Interaction Between Rivaroxaban and Lactose: Implications for Stability and In Vitro Anticoagulant Activity

SUMMARY

The study of drug–excipient interactions (DEI) is a critical aspect of the preformulation stage in pharmaceutical formulation development. The Maillard reaction is particularly reported in amine-containing excipients interacting with drug molecules possessing carbonyl groups, or vice versa, forming intermediate products that may be toxic in nature. The present study highlights the interaction between Rivaroxaban (RVI) and lactose monohydrate (LT) through the Maillard reaction. RVI is an oral anticoagulant with potent activity. The RVI–LT adduct was prepared by incubating RVI with LT at 60°C in borate buffer (pH 9.2) for 12 hours. The formation of the adduct was assessed using spectroscopic and thermal techniques such as UV–visible spectroscopy, Fourier-transform infrared spectroscopy, differential scanning calorimetry, high-performance liquid chromatography, and liquid chromatography–mass spectrometry. In silico molecular docking experiments were performed to evaluate the effect of adduct formation on the binding of RVI to its pharmacological target, Factor Xa. A significant loss of binding activity of the adduct, compared with native RVI, was revealed by docking experiments, indicating a potential reduction in anticoagulant potency. These were supported by in vitro chromogenic substrate assays for Factor Xa inhibition, with the adduct demonstrating reduced inhibitory activity. The present study identifies the susceptibility of RVI to Maillard-type interactions when formulated with LT, highlighting the imperative of prudent excipient selection in solid dosage forms to ensure therapeutic activity.

Keywords: *Rivaroxaban, Maillard reaction, drug–excipient interaction, in silico and in vitro study, Factor Xa inhibition.*

Rivaroksaban ve Laktoz Arasındaki Maillard Tipi İlaç-Yardımcı Madde Etkileşiminin Araştırılması: Stabilite ve İn Vitro Antikoagülan Aktivite Açısından Etkileri

ÖZ

İlaç–yardımcı madde etkileşimlerinin (DEI) incelenmesi, farmasötik formülasyon geliştirme sürecinin ön formülasyon aşamasında kritik bir unsurdur. Maillard reaksiyonu, özellikle karbonil grupları içeren ilaç molekülleri ile etkileşime giren amin içeren yardımcı maddelerde veya tersine durumda rapor edilmekte olup, toksik nitelikte olabilen ara ürünlerin oluşumuna yol açabilmektedir. Bu çalışma, Rivaroksaban (RVI) ile laktoz monohidrat (LT) arasındaki etkileşimi Maillard reaksiyonu aracılığıyla vurgulamaktadır. RVI, güçlü etkiye sahip oral bir antikoagülanttır. RVI–LT adüktü, RVI'nin LT ile borat tamponu (pH 9,2) içerisinde 60°C'de 12 saat inkübe edilmesiyle hazırlanmıştır. Adükt oluşumu; UV–görünür spektroskopisi, Fourier dönüşümlü kızılötesi spektroskopi, diferansiyel taramalı kalorimetri, yüksek performanslı sıvı kromatografisi ve sıvı kromatografi–kütle spektrometrisi gibi spektroskopik ve termal teknikler kullanılarak değerlendirilmiştir. In silico moleküler docking çalışmaları, adükt oluşumunun RVI'nin farmakolojik hedefi olan Faktör Xa'ya bağlanması üzerindeki etkisini değerlendirmek amacıyla gerçekleştirilmiştir. Docking deneyleri, adüktün bağlanma aktivitesinde doğal RVI'ye kıyasla belirgin bir azalma olduğunu ortaya koymuş ve bu durum antikoagülan etkinlikte potansiyel bir düşüşe işaret etmiştir. Bu bulgular, Faktör Xa inhibisyonuna yönelik in vitro kromojenik substrat testleriyle desteklenmiş olup, adüktün daha düşük inhibitör aktivite gösterdiği belirlenmiştir. Mevcut çalışma, LT ile formüle edildiğinde RVI'nin Maillard tipi etkileşimlere duyarlılığını ortaya koymakta ve terapötik etkinliğin korunması amacıyla katı dozaj formlarında dikkatli yardımcı madde seçiminin gerekliliğini vurgulamaktadır.

Anahtar Kelimeler: *Rivaroksaban, Maillard reaksiyonu, ilaç-yardımcı madde etkileşimi, in silico ve in vitro çalışma, Faktör Xa inhibisyonu.*

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INTRODUCTION

The four cornerstones of safety, efficacy, quality, and formulation stability are the pillars of any new drug development process. If the physicochemical properties of the drug product are to be consistently preserved in a final dosage form, it is necessary to understand the physicochemical properties of the active pharmaceutical ingredient (API) and all other elements, such as excipients, manufacturing aids, and packaging materials (Patel, Ahir, Patel, Manani, & Patel, 2015). API and other formulation ingredients are typically completely investigated at the preformulation stage of the development of a new drug (Chaurasia, 2013; Mishra et al., 2023b). Drug solubility, dissolution rate, polymorph and salt screening, stability, particle size distribution, ionisation characteristics, and API–excipient compatibility are evaluated during the preformulation stage. Excipients are found in every drug product and contribute to the formation of the dosage form, stability, administration, distribution, and/or other aspects. Choice of excipients relies on their price, compatibility, and functionality. However, such interactions between API and excipient might lead to unstipulated stability problems and compromise the therapeutic performance of the formulation. Thus, conducting preformulation studies is essential to determine drug–excipient compatibility (Bachchhao, Patil, Patil, & Patil, 2017). The most common drug–excipient interactions involve physicochemical incompatibilities, such as acid–base interactions and the Maillard reaction (Monajjemzadeh et al., 2009).

Adequate assessment of possible incompatibilities between the drug and excipients, using appropriate analytical strategies, is required to prevent issues during drug development and post commercialization. These analytical techniques also help identify unexpected formulated related while providing valuable information on excipient compatibility with the medicinal compound (Mishra et al., 2023a). In recent years, interest in the interaction between carbonyl-containing excipients and APIs that contain nucleophilic amine groups, particularly due to the potential of Maillard-type reaction, has increased (Ghaderi & Monajjemzadeh, 2020). Since lactose monohydrate (LT) is inert, economical, non-toxic, and provides adequate stability, it is often used as a filler or filler-binder in oral tablets and capsule formulations. Different LT grades are selected based on their physicochemical characteristics, such as flow behavior and particle size distribution (Babazadeh miyandoab, Barghi, Yousefi, & Ghaderi, 2022). LT is, however, capable of participating in Maillard reactions with amine-containing drugs containing primary or secondary amines under specific conditions, i.e., elevated temperature and alkaline pH, to form drug-excipient adducts (Patil & Patil, 2013). Such adducts can alter the physicochemical properties, therapeutic activity, and stability of the original drug molecule. When mixed with reducing sugars such as LT, the functional groups of Rivaroxaban (RVI), a direct oral anticoagulant with specific action on Factor Xa, could be susceptible to such non-enzymatic browning reactions. Although RVI possesses excellent oral bioavailability and steady-state pharmacokinetics, information on its interaction with excipients under stress conditions is lacking (Samama, 2011).

Due to its high mortality and morbidity rate, venous thromboembolism (VTE) is a serious disease that poses a significant public health issue (Fox, Kahn, Langleben, Eisenberg, & Shimony, 2012). VTE refers to blood clots in the veins. Venous thromboembolism, a chronic disease that includes both pulmonary embolism (PE) and deep vein thrombosis (DVT), occurs in nearly 10 million people every year all over the globe. VTE is induced by venous stasis, or reduced venous blood flow, which triggers platelet and fibrin aggregation and thrombosis (Franchini & Mannucci, 2012; Upreti, Gongati, Pandey, Saad, & Vittorio, 2023). DVT is bound to cause PE and be fatal after it occurs. Direct oral anticoagulants are the first choice for treatment of nearly all VTE patients; however, low molecular weight heparins remain widely utilized

(Xu, 2024). Patients with VTE precipitated by a significant transient risk factor may discontinue anticoagulant therapy after the initial three to six months of treatment. Those patients with a greater long-term risk of recurrent VTE compared to major bleeding, including spontaneous VTE or serious malignant disease, should take anticoagulant medication indefinitely. In most cases, pharmacological prophylaxis for VTE is required in patients undergoing major orthopaedic or cancer surgery (Khan, Tritschler, Kahn, & Rodger, 2021).

Without altering existing thrombin activity, several trials with novel oral anticoagulants have shown that factor Xa inhibitors are beneficial in VTE by reducing thrombin generation, thereby decreasing thrombin-mediated platelet activation and coagulation (Ingason et al., 2023; Perzborn, Roehrig, Straub, Kubitza, & Misselwitz, 2011). RVI is the first direct, non-cofactor-dependent inhibitor of factor Xa approved for human use (Kearon et al., 2005). Specific inhibition of Factor Xa prevents fibrin clot formation by blocking both the intrinsic and extrinsic pathways of the coagulation cascade. Unlike traditional vitamin K antagonists, RVI has linear pharmacokinetics and does not require frequent monitoring. Especially at elevated pH or temperature, RVI with aromatic and carbonyl moieties can interact with excipients in covalent or non-covalent manners. An RVI–LT adduct can alter the drug's solubility, binding capacity to Factor Xa, or overall anticoagulant activity. Although LT-containing formulations of RVI are widely available, no extensive studies have investigated the impact of such interactions on drug efficacy.

In this research, the possibility of adduct formation between lactose monohydrate (LT) and RVI of a Maillard-type was explored with a battery of sophisticated analytical tools such as UV–visible spectroscopy, Fourier-transform infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC), high-performance liquid chromatography (HPLC), and liquid chromatography–mass spectrometry (LC–MS). To probe the meaning of this interaction at the molecular level, *in silico* docking experiments were conducted to assess the binding interactions of RVI and its LT adduct to Factor Xa, the drug's major biological target (PDB code: 2W26). In addition, to confirm the functional relevance of adduct formation, an *in vitro* anti-coagulant activity assay was performed, comparing the thrombin generation inhibition effectiveness of RVI with that of the RVI–LT adduct.

MATERIALS AND METHODS

Materials

RVI was obtained as a gift sample from Alembic Pharmaceutical Ltd., Vadodara. The excipients, such as LT, were procured from Merck Specialties Pvt. Ltd., Mumbai, India, Organic solvents were used without purification.

Preparation of adduct

The LT (3 g) was dissolved in 50 mL of alkaline borate buffer, pH 9.2, which provided a buffered alkaline environment for the reaction. This borate buffer had to be used to prevent pH oscillations under heating since the alkaline systems in an unbuffered state can have a pH drift that may result in uncontrolled degradation instead of reproducible adduct formation. Furthermore, it is well known that the borate ion forms reversible complexes with the cis-diol group of LT, thereby offering controlled interaction of the sugar. The quantity of LT was selected in excess with respect to RVI as a model for excipient-rich conditions commonly found in solid oral dosage forms, and also to ensure adequate availability of the reducing sugar for condensation reactions. Then, 0.5 g RVI was added; this amount was selected to maintain adequate solubility of the reactant in the reaction medium and allow reliable physicochemical characterization. The mixture was ultrasonicated in a sonicating bath for 20 min until a clear solution was obtained and then transferred to a round-bottom flask. The solution was heated at 60°C on a water bath under reflux for 12 hours. During heating, the solution gradually discolored indicating adduct formation. After completing

the reaction, the solution was transferred to a Petri dish and dried in a hot-air oven at 50°C. The resulting powder was collected by scraping and stored for further characterization.

Stability under accelerated conditions

The dry physical mixture of RVI and LT (0.5:3 g, w/w) was exposed to 40°C/75% relative humidity for 15 days, simulating the process of Maillard reaction. Afterwards, the color, the content of water, and the thermal behaviour of the new pharmaceutical formulation were evaluated by DSC.

Analytical methods

UV–visible spectroscopy

The UV–visible spectra of RVI and the RVI–LT adduct were analysed using UV– visible spectrophotometer (UV-1700, Shimadzu, Japan) double-beam spectrophotometer. Solutions of RVI and the RVI–LT adduct at a concentration of 10 µg/mL were analysed in the UV–Vis range of 200–800 nm to determine shifts in absorbance intensity (Monajjemzadeh et al., 2009).

FT-IR

The FT-IR spectra of RVI and the RVI– LT adduct were acquired using an FT-IR spectrophotometer (iD3, Nicolet ST5, ATR, Thermofisher Scientific, Mumbai, India). The prepared samples were analysed in the 4000 – 400 cm^{-1} infrared range to determine the differences in the vibrational intensity.

DSC

DSC analysis was performed to examine thermal behavior and confirm adduct formation. sample of approximately 5–10 mg of the RVI and RVI–LT adduct sample was placed on the pan and heated between 30°C and 300°C at a rate of 10°C/min by employing a DSC apparatus (DSC1, STAR System, Mettler Toledo, USA).

Isocratic HPLC analysis

Chromatographic analysis of RVI and the RVI–LT adduct was performed using an HPLC system (Shimadzu, Japan) equipped with an LC-20AD pump, an SPD-20A UV detector, and a Rheodyne injector. The injection volume was kept constant at 20 µL, and data acquisition and analysis were performed using LC solution software. The Phenomenex Luna C18 (5 µ, 250 mm × 4.6 mm) column was used for the retention and elution of RVI and RVI-LT adduct. The mobile phase consisting of acetonitrile and water (55:45 v/v, pH 6.5) was used for the elution of RVI at a flow rate of 1.0 mL/min (Gouveia et al., 2020; Mestareehi, 2025). For adduct analysis, a water:methanol (95:5 v/v) mobile phase was employed. RVI was detected at 249 nm in both studies.

LC-MS

LC–MS was performed with an electrospray ionization source in positive mode (Varian Inc., USA 410 Prostar binary LC with 500 MS). The adduct was eluted on a Zorbax Eclipse XDB-C18 column (2.1 mm × 150 mm), 5 µm particle size as the stationary phase at a flow rate of 0.2 mL/min. Spectra were acquired between 50 and 2000 amu.

***In silico* evaluation**

Molecular docking studies were conducted using the GLIDE module of Schrödinger (Schrödinger Inc., USA, 2006). The crystal structure of Factor Xa complexed with an inhibitor (PDB ID: 2W26) was obtained from the Protein Data Bank (Kundu & Wu, 2021). The protein was prepared and optimized using the Protein Preparation Wizard in Maestro (version 9.0) through a two-step process involving structure refinement and energy minimization. Ligand structures were generated in the Maestro Build Panel and subsequently processed with LigPrep 2.2 to obtain low-energy conformers based on the OPLS-2005 force field. The most stable ligand conformations were then docked into the receptor's active site using the grid derived from the prepared protein, employing the standard precision (SP) docking protocol.

***In vitro* determination of anti-coagulant activity**

Blood samples of six normal human volunteers were drawn after informed consent. Prothrombin time was determined employing a regular prothrombin time test kit (company: BIOBASE) according to the manufacturer's guidelines. Three treatment

groups were assigned to the samples: untreated control, RVI-treated, and RVI–LT adduct-treated. The treatment was performed at 37°C for 30 min using RVI at a concentration of 10 µg/mL, and an equivalent concentration of the RVI–LT adduct was incubated under the same conditions. All samples were tested in triplicate. Statistical analysis was conducted using one-way ANOVA with Tukey's post hoc test to determine prothrombin times among groups, with $p < 0.001$ as statistically significant.

RESULTS AND DISCUSSION

The present study involved the characterization of a Maillard-type adduct formed between RVI and LT monohydrate. A combination of analytical techniques, including UV–visible spectroscopy, FT-IR, DSC, HPLC, and LC–MS, was employed to confirm adduct formation and evaluate its physicochemical properties. The anticoagulant activity of RVI was compared with that of the synthesized adduct using an *in vitro* Factor Xa inhibition assay. In addition, *in silico* molecular docking was performed to investigate key interactions between RVI and human Factor Xa and to elucidate the impact of adduct formation on target affinity and pharmacological activity.

Analytical methods

UV–visible spectroscopy

UV–visible absorption spectra of the pure RVI and the RVI–LT adduct were obtained with distilled water as a solvent from 200–800 nm, which covers the typical electronic transitions of the aromatic and amide groups in RVI. The adduct exhibited shifts in maximum absorbance and minor changes in intensity relative to the pure drug, suggesting potential alteration in the electronic environment due to Maillard-type interaction. The pure RVI was scanned from 800 – 400 nm in the visible range, showing no increase in the absorbance intensity, as shown in Figure 1. After formation of the adduct, RVI-LT shows an increase in the absorbance spectra post 600 nm, extending towards 400 nm. The increase in the absorbance intensity compared to pure RVI preliminary confirms the formation of an adduct (Han, Yi, Wang, & Huang, 2017). The increase in the absorbance spectra at 420 nm, as shown in Figure 1b, shows the browning effect of adduct formation. In the Maillard reaction, browning is considered an important and initial step after the aldehyde and amine polymerization reaction between amine and carboxylic surface groups. The browning effect was due to the formation of a heterocyclic nitrogen intermediate in the presence of moisture (Wu et al., 2014).

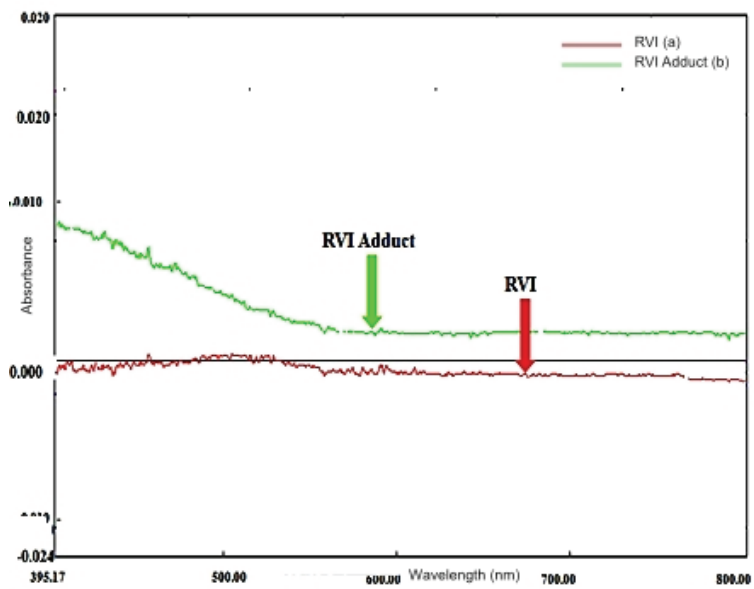

Figure 1. UV spectra of RVI and RVI-LT adduct.

FT-IR

The FT-IR absorption patterns of RVI and the RVI–LT adduct were recorded as shown in Figure 2. The vibration spectrum of pure RVI was recorded and depicted in Figure 2a, showing a distinct and sharp peak at about 3400 cm^{-1}, which represents the N-H stretching vibrations, typical of amide or amine functionalities in the molecule. Nevertheless, within the range of the RVI–LT adduct (Figure 2b.), this peak can be observed distinctly broadened and weaker, indicative of the engagement of hydrogen bonding interactions between RVI's N-H groups and LT's hydroxyl groups. Furthermore, a stretching vibration peak of C=O appears at 1750 cm^{-1} in the RVI spectrum, indicative of carbonyl groups (Shaligram et al., 2023). In the adduct, there is a shift and decrease in intensity of the carboxylic (C=O) peak, implying a modification of the electronic surrounding of the carbonyl functionality, possibly on account of contact with LT. Additionally, the spectral zone between 1600–1500 cm^{-1} similarly displays significant modification in peak position and intensity, further confirming intermolecular contact formation (Ozon et al., 2024). These spectral changes establish the formation of an RVI–LT adduct by non-covalent interactions, i.e., hydrogen bonding, as opposed to simple physical mixing (C-N stretching vibrations typically appear in the range of 1020–1350 cm^{-1}, while 1750 cm^{-1} is characteristically attributed to C=O stretching (particularly from esters or lactams). Thus, the peak at 1750 cm^{-1} in RVI is more accurately assigned to a carbonyl (C=O) group rather than C-N (Xu, Wu, Liu, Zhang, & Lu, 2017).

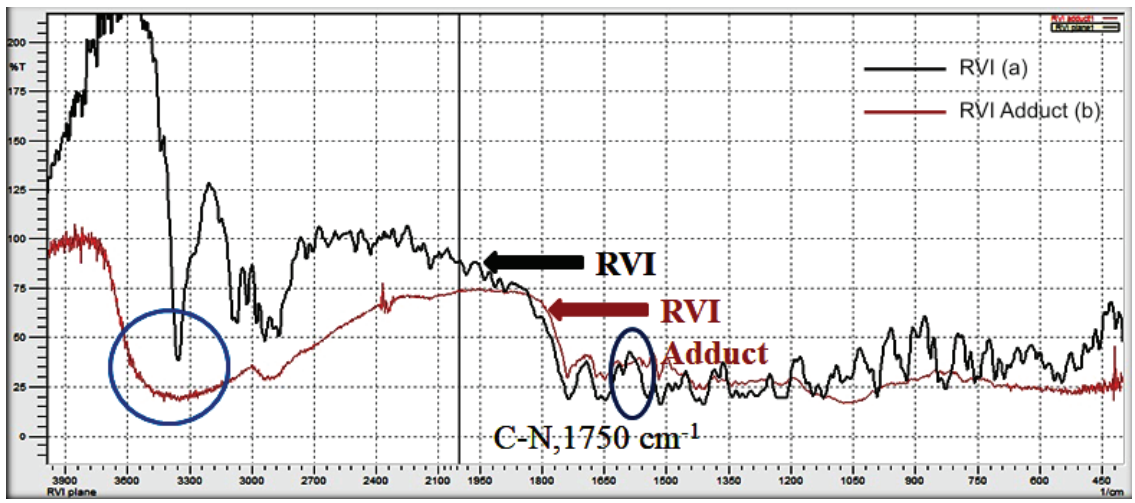

Figure 2. FT-IR spectrum of RVI (a) and RVI-LT adduct (b)

DSC

DSC is a crucial technique for figuring out whether an adduct was successfully produced. Figure 3a and 3b display the DSC curves of RVI and RVI-LT adduct. A sharp endothermic peak of RVI is displayed at 232°C, which corresponds to its melting point (Sherje & Jadhav, 2018). The clear, narrow peak illustrates the drug's crystalline structure and thermal purity. Unlike the pure form, the combination of RVI and LT shows a wide range of temperature changes instead of a clear melting point. The melting peak of RVI after the formation of the adduct decreased sharply, suggesting a loss of crystallinity, followed by the disappearance of the peak at 232°C (Ding, Chen, Zhai, Fu, & Sun, 2017). The adduct curve also has a small, broad endothermic hump in the region of 100 and 150°C. Desorption of adsorbed water or low-energy interactions, such as hydrogen bonding with LT, are likely the cause of this. The finding indicates that after coming in contact with LT, RVI has become an amorphous or largely amorphous state and also gives confirmatory evidence for the formation of the RVI–LT adduct.

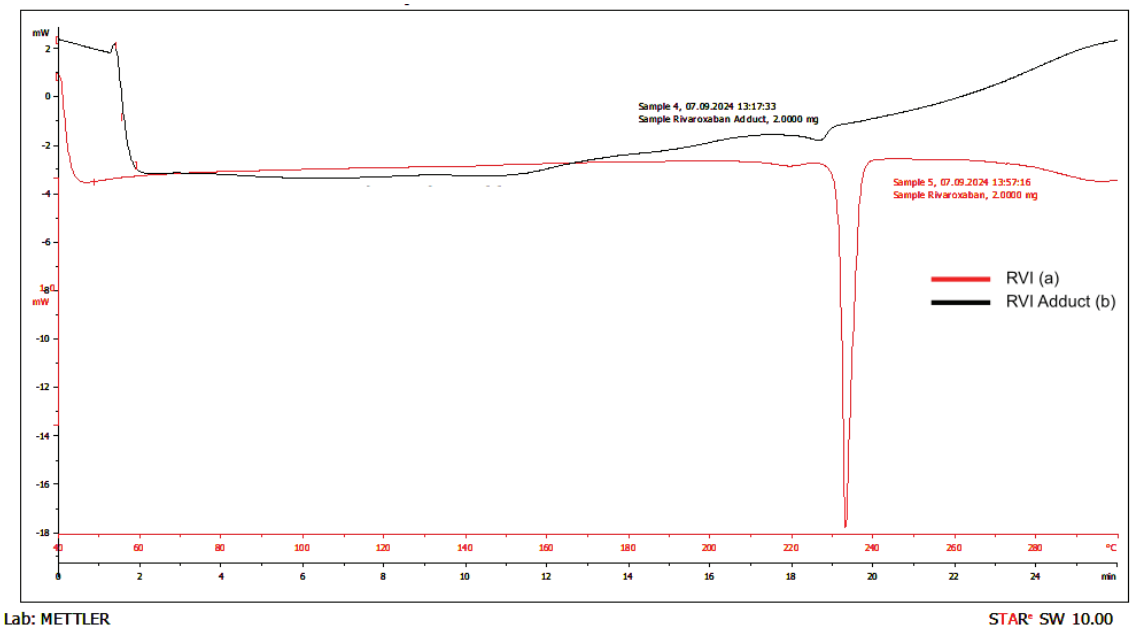

Figure 3. DSC Spectrum of RVI and RVI-LT adduct

HPLC analysis

HPLC was used to determine the chromatographic behavior of RVI and its Maillard adduct with LT, as shown in Figures 4a and 4b. The method was operated in isocratic mode using a Phenomenex Luna C18 column (250 × 4.6 mm, 5 µm, 100 Å) and a mobile phase containing ACN: Water (55:45 v/v) ratio. The same mixture was utilized as the diluent. The column temperature and sample temperatures were set to 25°C, at a 1.0 ml/min flow rate. The 20 µL injection volume was set, and detection occurred at a wavelength of 249 nm during a 10-minute runtime. Pure RVI produced a single, sharp peak on the chromatogram at a retention time of 4.203 minutes, ensuring the analyte was pure (Figure 4a.) (Rao et al., 2023). The RVI–LT adduct produced multiple peaks (Figure 4b.). The main peak of the RVI–LT Maillard adduct was found to occur at 6.988 minutes retention time, which is different from the pure compound. Other low-intensity minor peaks were at 0.142, 0.493, 1.628, 4.169, and 7.280 minutes, showing evidence of intermediate or side products that are generated under the Maillard reaction (Choi et al., 2023).

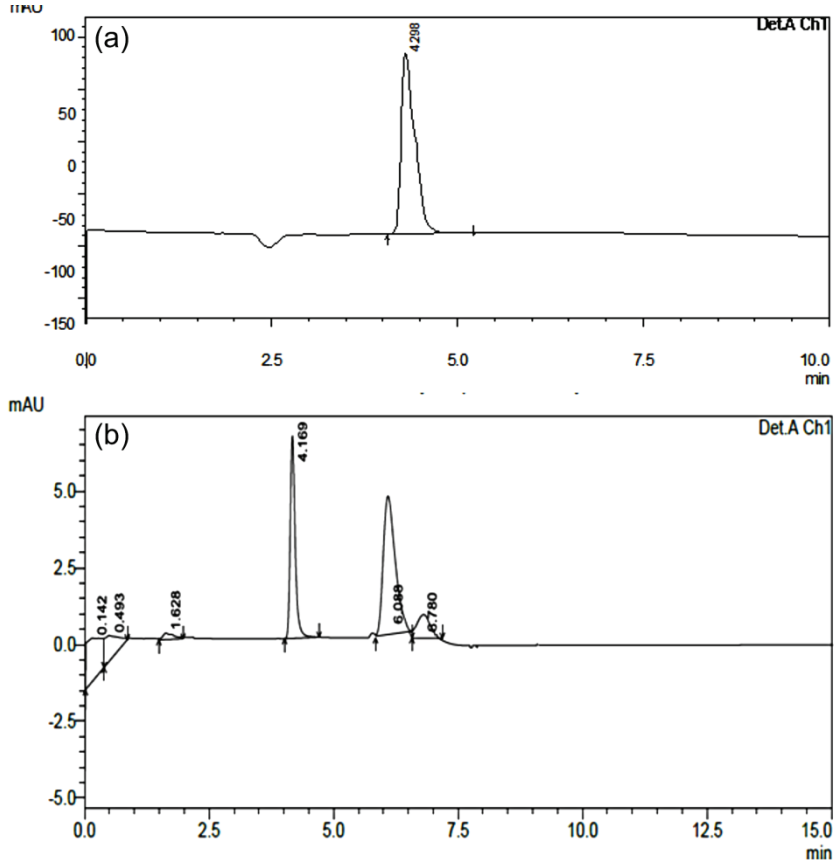

Figure 4. HPLC chromatogram of RVI (a), RVI-LT adduct (b).

LC-MS

LC-MS is commonly utilized to characterize amine–LT adducts (Babazadeh miyandoab et al., 2022; Gouveia et al., 2020). The adduct was investigated using the electrospray ionization method in positive ion mode. LC MS spectra revealed the precursor ion of the RVI– LT adduct to be a protonated molecule ([M + H] +) at m/ z 751.03 (Figure 5a.). The proposed structure of the RVI– LT adduct is illustrated in Figure 5b. The molecular weight of the RVI–LT adduct mass was in agreement with the Maillard condensation product adduct. The loss of a water molecule from a parent compound results in the production of the Maillard adduct (Wingert et al., 2015; Wirth et al., 1998). The interaction between RVI and LT was investigated to assess potential excipient–drug adduct formation. Mass spectrometric analysis (Figure 5a.) shows an ionization peak at m/z 751.03 [M+H]+, corresponding to a molecular weight of 750.018 Da. This mass aligns with the theoretical molecular weight of a covalent adduct formed between RVI ($C_{19}H_{18}O_5N_3S$-Cl; MW = 435.882 Da) and LT ($C_{12}H_{22}O_{11}$; MW = 342.2996 Da), with the elimination of a single water molecule (H_2O; 18.015 Da) via condensation. The proposed reaction mechanism (Figure 5b.) involves nucleophilic attack by the hydroxyl groups of LT on an electrophilic center of RVI, likely its ester or carbamate functional group, resulting in a covalent bond formation. Such adduct formation could have significant implications on the chemical stability, pharmacokinetics, and therapeutic efficacy of RVI when formulated with LT-containing excipients. This finding highlights the importance of thorough excipient compatibility studies during preformulation to mitigate unexpected drug–excipient interactions.

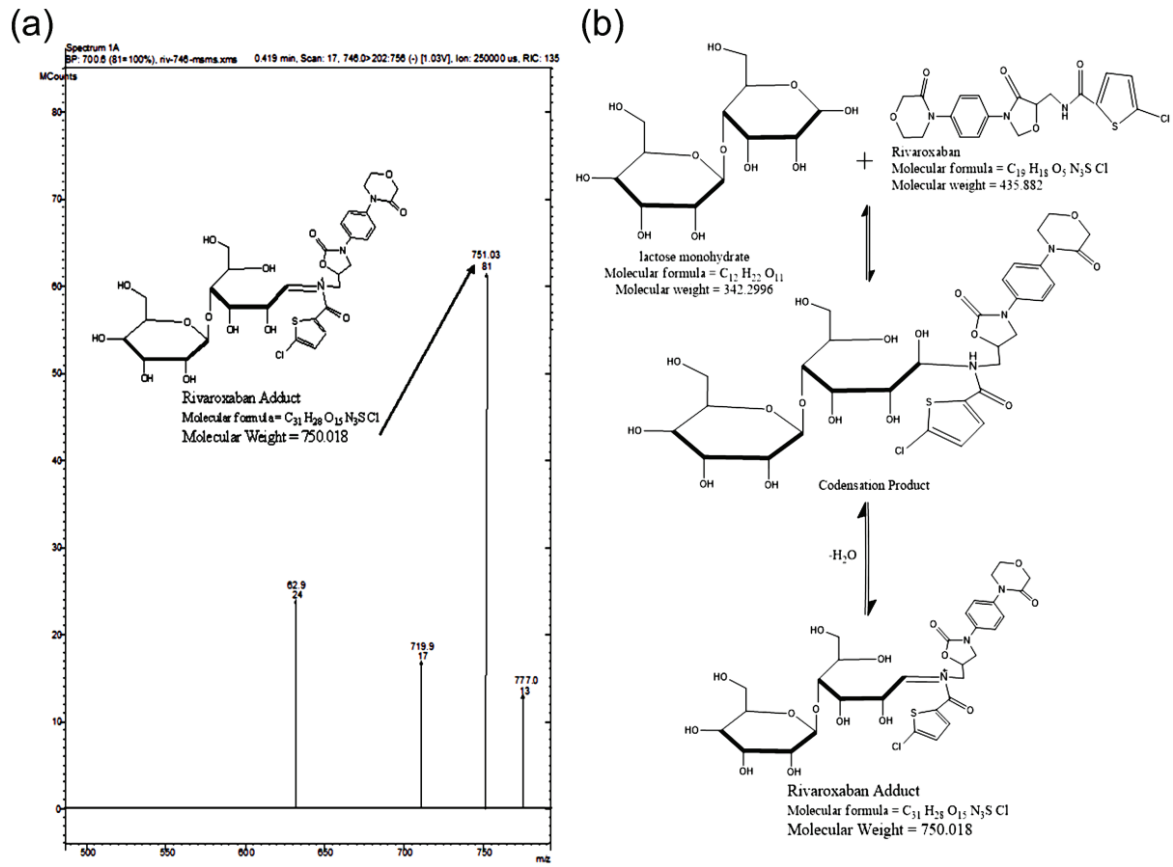

Figure 5. LC-MS spectra of RVI adduct (a), Reaction scheme for adduct formation using Maillard Reaction (b).

Docking study

RVI has an active binding site of Factor Xa in the molecular docking study with the Protein Data Bank (PDB code: 2W26) (Sabbagh, Al-Beik, & Hadid, 2023). It validated its established inhibitory activity against Factor Xa by establishing critical interactions with the ALA-121, GLY-219, and CYS-220 residues of the binding pocket (Schumacher, Luettgen, Quan, & Seiffert, 2010). These amino acid residues assist in keeping RVI stable and properly positioned within the catalytic domain of the enzyme, as depicted in Figure 6. Conversely, a binding orientation change was found when the same protein was docked with the RVI–LT adduct. LYS-148, GLU-147, GLY-216, and LYS-96 each (Figure 6.), which lie outside the traditional active site area, engaged the adduct (Esmon, 2017). These results suggest that RVI's conformation and binding energy are altered by the formation of Maillard-type adducts, possibly compromising its anticoagulant properties. The results *in vitro*, which showed that the adduct was less inhibitory of Factor Xa than the pure drug, were consistent with the *in silico* results. The introduction of the bulky LT moiety appears to interfere with the drug's ability to access and occupy its original binding site. As a result, the adduct failed to form interactions with the key catalytic residues identified for native RVI, indicating a potential reduction in binding affinity and target specificity (Du, Qian, Yao, & Xue, 2020).

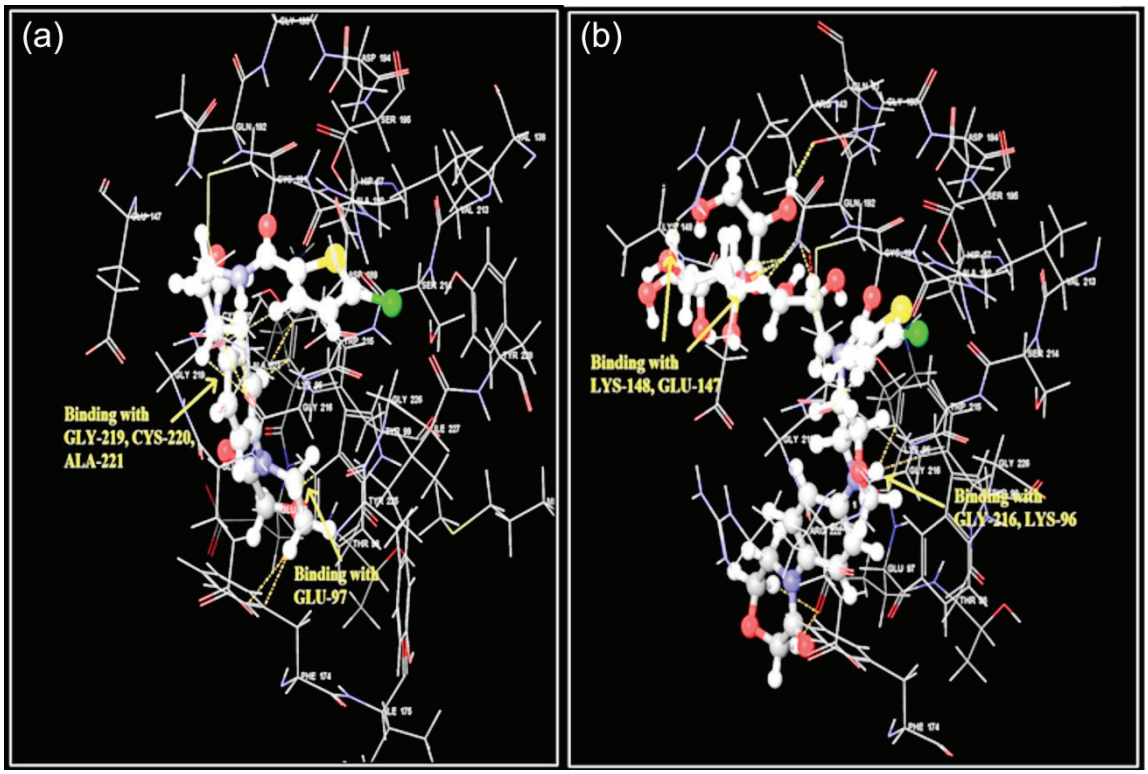

Figure 6. RVI docked with protein 2W26 (a), RVI-LT adduct docked with 2W26 (b)

***In vitro* anticoagulant activity**

By determination of prothrombin time (PT), the anticoagulant effect of RVI and its LT adduct was assessed. Six healthy human volunteers were selected for the research after the clinical practitioners ascertained their initial blood levels and secured their consent. Prothrombin time (PT) is often employed to measure the anticoagulant effect of pharmaceutical drugs and is an important clinical parameter for studying the extrinsic pathway of coagulation. The PT in the control, RVI-treated, and RVI–LT adduct groups was significantly altered in the present study, yielding data regarding the pharmacodynamic consequences of potential drug–excipient interactions (Marlu et al., 2017).

As seen from the graph, the control group had a mean PT of 43.23 ± 1.12 seconds, which was the physiological baseline. When RVI, a direct factor Xa inhibitor, was administered, a significant rise in PT to 60.29 ± 1.45 seconds was evident, an indication of its strong anticoagulant effect by inhibiting the conversion of prothrombin to thrombin (Figure 7.). This extension in PT is consistent with the established pharmacological profile of RVI and confirms its effectiveness in the modification of coagulation parameters. Perhaps most notably, the RVI–LT adduct group showed a decreased PT of 50.22 ± 1.28 seconds, significantly less than that of the pure RVI group. This decrease indicates a potential diminution of anticoagulant activity upon RVI complexation to LT, a frequently used drug excipient. Such modulation may be the result of non-covalent interaction or Maillard-type conjugation between LT and RVI (Marlu et al., 2017; Samuelson, Cuker, Siegal, Crowther, & Garcia, 2017), possibly modifying the bioavailability or molecular structure of the drug and hence its binding capacity to factor Xa. Statistical analysis with one-way ANOVA reveals a notable difference between the groups ($p < 0.001$). These findings indicate that interaction with LT resulted in reduced anticoagulant effect of RVI, as shown by the reduced PT compared to normal RVI (Pailleret et al., 2019).

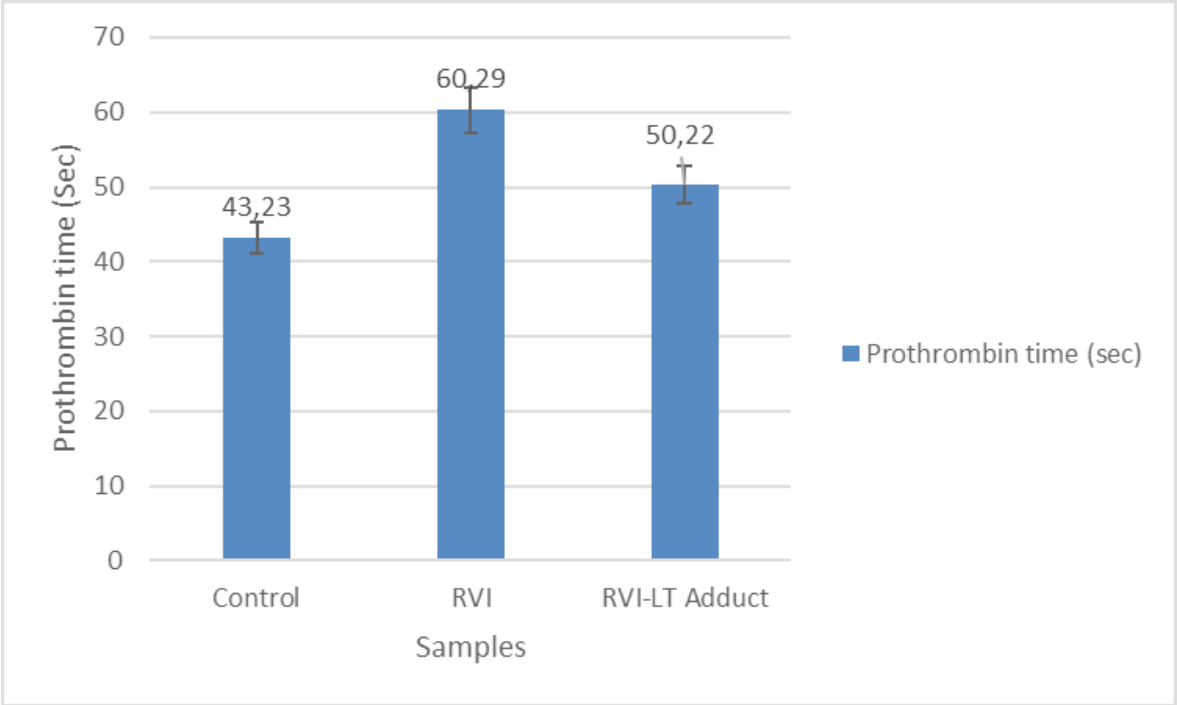

Figure 7. Prothrombin time (seconds) of control, RVI-treated, and RVI–LT adduct-treated blood samples (n = 6). Data are mean ± standard deviation. One-way ANOVA showed a statistically significant difference among the groups ($p < 0.001$)

Assessment of Maillard reaction under accelerated conditions

The physical mixture stored for 15 days underwent slight browning. This indicated that the Maillard reaction started between RVI and LT. The moisture content rose compared to that of the physical mixture. This increased moisture content helped to accelerate the Maillard reaction between the NH group of RVI and the reducing end of LT. The DSC curves of the fresh mixture revealed a sharp endothermic peak around 220°C due to RVI and another small peak due to LT at 148°C (Figure 8.). However, upon accelerated storage conditions, the DSC thermogram revealed widening and mild shift of the endothermic peak of RVI towards 220°C with reduced intensities indicative of its interaction with LT and reduced crystallinity of the remaining mixture due to Maillard reaction product formation. A small new peak emerged around 148°C, corresponding to possibly emerging Maillard reaction products. Thus, based on observations of browning reactions, increased moisture content, and changes in heat diffusivity of the mixture, it is established that the Maillard reaction proceeds in the RVI and LT mixture under accelerated conditions.

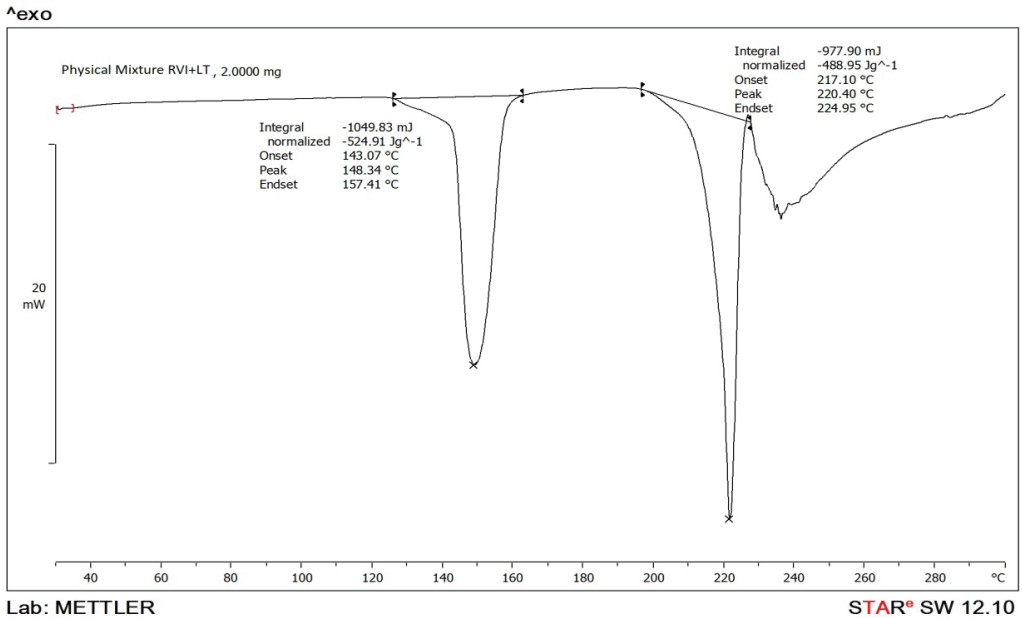

Figure 8. Physical Mixture of RVI-LT under accelerated condition

CONCLUSION

While it has long been recognized that amine-containing drug substances are capable of undergoing Maillard-type reactions with LT, LT continues to be widely used as an excipient because of its economical nature, good compressibility, and cost-effectiveness. The present study highlights the potential formation of a Maillard reaction adduct between LT and RVI in pharmaceutical formulations. Both *in vitro* anticoagulant assays and *in silico* docking studies confirmed that RVI–LT adduct formation reduced the anticoagulant activity of RVI, as evidenced by a reduced prothrombin time relative to the native drug. Given the critical therapeutic role of RVI as an anticoagulant, such excipient interactions are of significant concern. The findings suggest that the use of LT as an excipient in RVI formulations should be reconsidered. Further studies are warranted to investigate RVI–LT adduct formation under storage and environmental conditions and to assess its stability and clinical relevance over time.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

KB – Methodology, investigation, visualization, writing original draft, AG – Formal Analysis, Resources, Supervision, Writing – review and editing, CP – Investigation, statistical investigation, Analysis, DP – Supervision, Writing – review and editing.

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