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Crystal products of lamotrigine–citric acid for improvement of *in vitro* drug release in simulated gastric fluid

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Abstract: Crystal engineering is an integral part of the drug development research. Crystal forms can modify the physicochemical properties of the parent drug molecule. The present work was aimed at the synthesis and characterization of crystalline product of lamotrigine (LT), an U.S. Food and Drug Administration approved anti-epileptic drug, with citric acid (CA) to improve its release in gastric region and oral absorption. The crystalline products of LT–CA were developed by solvent evaporation method using ethanol-water as the solvent system. Appearance of new characteristic peaks in the FTIR spectra for the crystal products indicated formation of new crystal state. In DSC thermogram, melting point of the experimental crystal products was different than that of the pure drug. Further, formation of new crystalline phase was confirmed from XRD data through the identification of new sharp peaks for the selected crystal products. A higher cumulative percentage of drug release was observed for the crystal products than for the free drug within 60 min of drug release in simulated gastric fluid. However, *in vivo* studies are warranted for the future technology transfer of the product at industrial scale.

Keywords: anti-epileptic; onset of action; BCS class II; solvent evaporation method; lattice strain.

INTRODUCTION

Crystal engineering has been emerged as an important tool in pharmaceutical industry to improve the dissolution and absorption rate of poorly soluble drugs.[1] Poor dissolution rate directly influences the therapeutic efficacy of pharmaceuticals, and significantly lowers the market value of a drug.[2] Especially, in the case of neurological disorders like epilepsy, timely absorption of drugs is very crucial to elicit prompt therapeutic action. However, many pharmaceutical agents have low aqueous solubility, which delays their absorption and consequently leads to

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delayed onset of action.[3] Thus, enhancing the dissolution rate of poorly soluble drugs without compromising their therapeutic potential or stability has been a major challenge in pharmaceutical industry during crystal product development. Crystals may be described as orderly arrangement of molecules in a geometrical pattern in three-dimensional space, where the molecules are connected through intermolecular bonds.[4] Suitable crystalline product of active pharmaceutical ingredients (APIs) can enhance important physicochemical properties like melting point, solubility, dissolution rate, refractive index, stability etc. without affecting their intrinsic therapeutic property and thus improves the industrial feasibility and patient compliance.[5]

Lamotrigine (LT), a widely used anti-epileptic drug, has poor aqueous solubility with low absorption rate.[6] It has been recommended for the treatment of both partial and generalized seizures (primary tonic/clonic seizure).[6] LT has the advantages of fewer side effects associated with a higher therapeutic index unlike other anti-epileptic drugs, thus it does not require regular blood monitoring after administration. However, LT belongs to class II of Biopharmaceutical System of Classification (BSC). Being a BCS class II drug (low solubility and high permeability), it has poor solubility in aqueous medium (0.17 mg/ml at 25 °C) and also is slightly soluble in 0.1 M. HCl (4.1 mg/mL at 25 °C). It is a weak base having dissociation constant (pK_a)[7] 5.7 with *n*-octanol: water partition coefficient of 1.19 (log *P*) at physiological pH.[8] Thus, absorption of LT after oral administration is dependent on the rate of dissolution and release under physiological conditions. Peak plasma concentrations are achieved after 2.5 h after oral administration. Low aqueous solubility linked with poor dissolution rate delays its absorption and onset of action. Hence improvement in dissolution rate is expected to elicit a faster absorption of the drug leading to a quicker onset of action, which is highly essential to control the seizure episodes in epileptic patients. For quicker onset of action lamotrigine orally disintegrating tablet has been formulated by Patil *et al.*[9] Though, several crystal products of LT has been reported over past years for the improvement of solubility and dissolution rate, however an optimized crystalline product of LT with citric acid (CA) is yet to be reported.[10–12] The present work aimed for the development and characterization of a novel crystalline product of LT with CA to improve its dissolution rate and oral absorption for enhanced clinical outcome. The study involved characterization of the LT–CA crystal products by different analytical techniques along with *in vitro* dissolution studies to report the optimized crystal product for future *in vivo* studies.

EXPERIMENTAL

Chemicals

LT was received as gift sample from Unichem Pvt. Ltd., MP, India. CA was purchased from Sisco Research Laboratories, Maharastra, India. Ethanol was received from Merck Specialties Pvt. Ltd., India. All other chemicals used in the experiment were of analytical grade.

Synthesis of experimental crystal products

The LT–CA crystal products were prepared by the conventional solvent evaporation method with required modifications. Briefly, accurately weighed amount of LT was mixed with accurately weighed amount of CA in a beaker. The mixtures were then dissolved in 50 vol. % ethanol followed by slow drying at 40–50 °C for 72 h.[13] Slow evaporation of the solvent under controlled conditions induced crystallization. The crystal products were formulated at three different molar ratio of LT with CA, *i.e.*, 1:1, 2:1 and 3:1. After complete removal of organic solvent, the dried crystal products were collected, weighed and stored until further use.

In vitro *characterization*

Fourier transform infrared (FTIR) spectroscopy. FTIR study was carried out with the help of a FTIR spectrophotometer (JASCO, FT/IR-4100). For the study, LT, CA along with selected crystal products, were mixed separately with IR grade potassium bromide (at 100:1 ratio) to prepare thin pellets.[14] A pressure of 5 t was applied in a hydraulic press for 5 min to prepare the pellets. Those were then scanned in the FTIR spectrophotometer over a wave number range of 400 to 4000 cm^{-1}. The spectra manager software (version 2.0) was employed to analyze the peaks.

Differential scanning calorimetry (DSC). For the DSC experiment, required quantity of samples of pure drug (LT), CA and all the selected crystal products were taken in crimped aluminum pans with pin-hole.[15] Calibration of the instrument about heat flow and temperature was done by Indium (m.p. 156.6 °C). Heating rate of 10 °C per min was used within a temperature range of 30–300 °C along with an inert (N_2) atmosphere. The study was carried out by using a DSC-1 (Mettler Toledo DSC) with STAR^e software.

X-ray diffractometry. X-ray diffractometry of free drug along with the synthesized crystal powder products was carried out to obtain idea on the crystallinity, crystal orientations along with other structural parameters. Briefly, dry powdered sample was placed on the glass slide and analyzed with the help of a powder X-ray diffractometer (Ultima, IV, Japan). For the experiment, an X-ray of 40 kV/40 mA was applied on the tested samples at a detection angle (2θ) for 120 s.[16,17]

In vitro drug release study

The release profile of the drug from different crystal products was estimated in a USP XXIII dissolution testing apparatus (Dissolution tester (USP) TDT06L, Electrolab) using rotating paddle method.[10,18] For the experiment, a weighed amount of powder sample of LT (100 mg) and selected crystal products (100 mg equivalent LT) were placed in a dissolution vessel rotated at 50 rpm at 37±0.5 °C. Simulated gastric fluid (900 ml of 0.1 M HCl at pH 1.2) was taken as the release medium. The study was conducted for 60 min duration. During the study, at time intervals of 5, 10, 20, 30 and 60 min, 10 ml of samples were withdrawn from the dissolution chamber through a syringe with simultaneous replenishment of fresh release medium to maintain the sink condition. After collection, the samples were filtered using a membrane filter (0.25 μm). The filtered aliquots following required dilutions were analyzed at 267 nm using a UV–Vis spectrophotometer (JASCO V-630, Japan) against 0.1 N HCl as blank.

Statistical analysis

The experiments were carried out in triplicate for accuracy and reproducibility. Data was expressed as the mean ± standard deviation (*SD*). Model independent statistical methods such as the difference factor f_1 and the similarity factor f_2 were applied for comparison of two dissolution profiles.

RESULTS AND DISCUSSION

Crystal product development

The experimental crystals were synthesized in three different mole ratios of LT and CA such as 1:1, 2:1 and 3:1. In all the crystal products, amount of CA was kept constant, whereas amount of the drug was varied under identical experimental conditions. Out of several crystal product batches, here we have reported three sets of crystal products, *i.e.*, L1S1, L2S1 and L3S1. The composition of various crystal products was depicted in Table I. Simultaneously we have provided a schematic representation of LT–CA crystals (Fig. 1**)** to depict the possible mechanism of development of crystal structure by the formation of strong covalent bond (peptide) between the functional group of LT and CA.

TABLE I. Crystal products of lamotrigine–citric acid by solvent evaporation method in ethanol–water as solvent system

Crystal product code	Mole ratio (LT:CA)	m_{LT} / g	m_{CA} / g
L1C1	1:1	1.87	1
L2C1	2:1	3.75	1
L3C1	3:1	5.62	1

In the schematic diagram, three possible mechanism of crystal formation has been depicted *via* three line diagrams (A, B and C). Considering three numbers of acidic (–COOH) groups present in one molecule of citric acid, there arise three probable types of stoichiometric ratio between the LT and CA molecules, *viz*. 1:1, 2:2 and 3:1 during the formation of crystal structure. Thus, all the three types of possible molecular arrangement in the crystal lattice at different ratio have been schematically represented. The resultant peptide bond formed between the components would make the crystal product more stable and also it would help to improve solubility property. This form of peptide bond will remain stable in hydrolysis, however under acidic conditions (pH 2–4); it can be readily broken into individual components.

FTIR study

FTIR is an important analytical technique, which is often used as a pre-crystal product study to assess any incompatibility between the drug and excipient. Any significant shifting in the characteristic peaks of the drug or excipient or appearance of new peaks in the crystal product justifies intermolecular interaction and formation of new products. In the FTIR spectra, major functional groups of LT that is at 3448 (N–H aromatic stretching), 3210 (C–H aromatic stretching) 1645 (C=N),[9] 1292 and 1319 cm^{-1} (two weak intensity sharp peaks for C–N bending vibration), 1405–1458 cm^{-1} (four peaks in pairs for aromatic C=C stretch benzene ring), 1051 (C–Cl), 716 (ortho-substituted benzene), 790 cm^{-1} (meta substituted benzene), etc. were all present in the spectrum of pure LT.

However, few changes in the characteristic peaks of experimental crystal products as compared to free drug (LT) were observed, which indicate formation of new crystalline phases (Fig. 2). The sharp peak observed for LT at 3448.21 cm^{-1} (due to N–H aromatic stretching) was found to be shifted at 3360.35 (L1C1), 3329.5 (L2C1) and 3332.39 cm^{-1} (L3C1), respectively. Similarly, the strong peaks observed in the FTIR spectra of the crystal products due to C–N stretching vibration (for aromatic amine), *i.e.*, 1284.36 (L1C1), 1291.11 (L2C1), and 1297.86 cm^{-1} (L3C1) were absent in the FTIR spectrum of the pure LT. Further, the strong peaks at 1754.9 and 1684.55 cm^{-1} (C=O stretching of the COOH group) along with 3364 and 3011 cm^{-1} (O–H stretching) for CA were absent in the crystal products. Particularly, in the crystal product L3C1 (3:1 mole ratio of LT and CA), much changes of the peaks for COOH group was observed, signifying stoichiometric formation of C–N bonds between NH_2 groups (three molecules of LT) with three COOH groups (one molecule of CA). In a nutshell, such type of changes in the peaks justified successful formation of intermolecular bonds in between the components to develop the new crystal state.

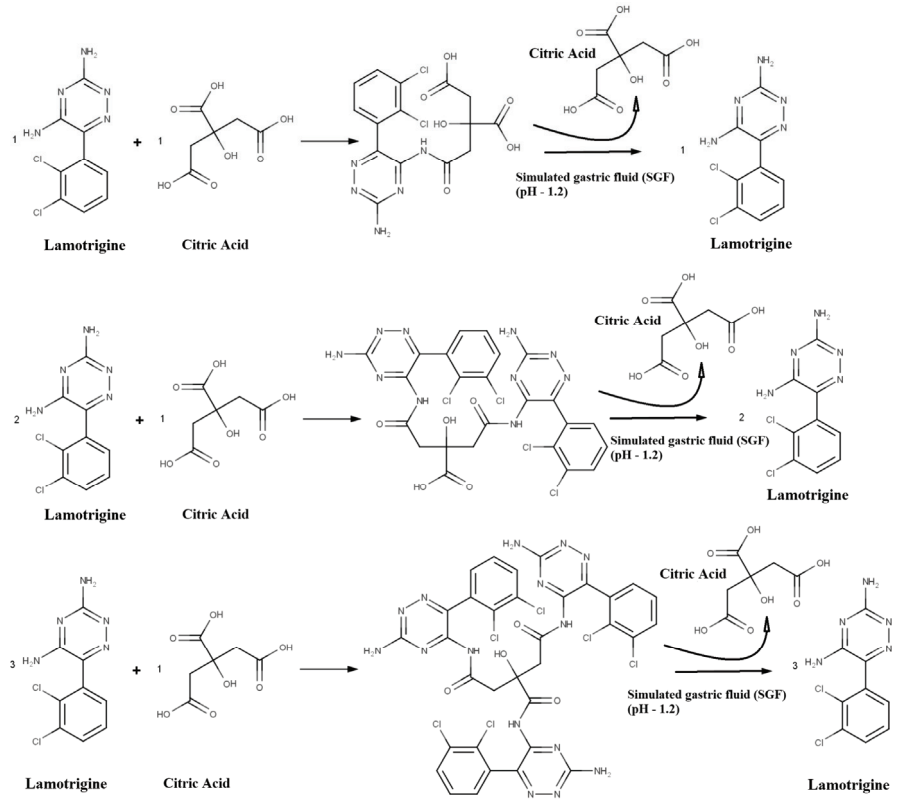

Fig. 1. Schematic representation of LT–CA crystal formation.

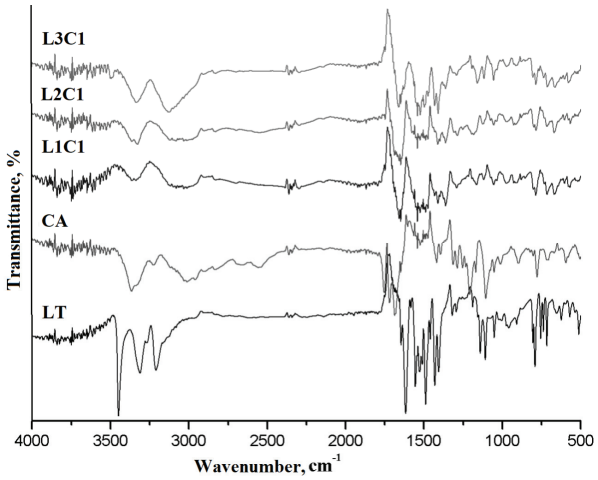

Fig. 2. FTIR spectra of pure drug (LT), excipient (CA) and crystal products (L1C1, L2C and L3C1).

DSC study

Differential scanning calorimetry helps to study the thermal behavior of the crystal form relative to the individual components along with any possible chemical interactions between the drug and excipient.[19] DSC thermogram of LT and experimental crystal products (L1C1, L2C1 and L3C1) were presented in Fig. 3.

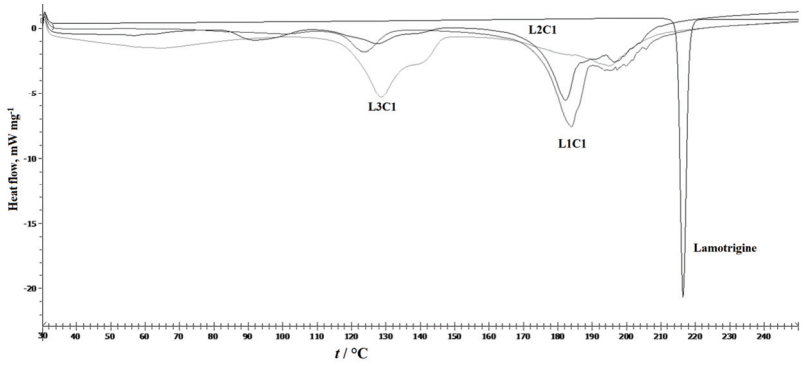

Fig. 3. DSC thermograms of pure drug, excipient (SA) and the crystal products (L1C1, L2C1 and L3C1).

DSC study of the pure drug showed sharp endothermic peak at 215 °C, whereas the experimental crystal products showed sharp peaks at 183 and 125 °C, respectively. From the DSC study, thermal profile of formed crystalline product was found to be different from that of the pure drug, which overall signified formation of new crystalline state. Further, there was no sign of chemical incom-

patibility found in the DSC thermogram, which is another important criterion for successful crystal product development.

PXRD study

To depict the phase identification of a crystalline material and to provide information on unit cell dimensions, XRD study has been used as an important analytical tool.[20] In XRD, a monochromatic beam of X-ray is actually allowed to fall on the powdered sample. Reflected X-rays are then detected by a detector fitted with the machine. Usually, amorphous regions of the samples produce broad peaks in contrast to crystalline regions, which produce relatively sharper peaks. From the experiment, XRD patterns of the experimental crystal products were different from that of the pure drug. For the pure drug, the characteristic crystalline peaks have been identified at 2θ 12.39, 17.35, 25.44, 27.78 and 28.33° (Fig. 4). However, some new peaks were observed in the crystal products. From the XRD data it was clearly found that L3C1 show sharp peak, which was different from that of L1C1 and L2C1, which confirmed the formation of novel crystalline phase. The relatively smaller peaks in case of L1C1 and L2C1 might be due to the phenomenon of amorphization in the product.

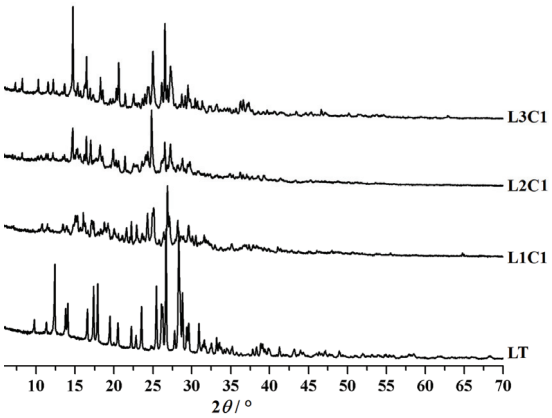

Fig. 4. PXRD data of the pure drug and the crystal products (L1C1, L2C1 and L3C1).

Debye–Scherrer formula was employed to find out other characteristic properties like particle size, strain and dislocation density in the formed crystal products (Table II).

Crystal size was calculated using following equation:

$$D = 0.9\lambda/\beta\cos\theta \quad (1)$$

The particle strain in lattice was determined from the equation:

$$\varepsilon = \beta/\tan\theta \quad (2)$$

where, e = strain, b = full width half maxima (FWHM), D = crystallite size, l = wavelength.

TABLE II. Crystal lattice dislocation and strain arising from crystal imperfections

Crystal product	Particle size, nm	Dislocation density×10^{15}, m^{-2}	Strain
LT	69.54	20.68	0.3528
L1C1	38.90	90.30	0.3455
L2C1	46.68	66.50	0.6338
L3C1	53.55	50.72	0.3518

The dislocation density (δ), which represents the amount of defects in the sample and can be defined as the length of dislocation lines per unit volume of the crystal was calculated:

$$\delta = 1/D^2 \quad (3)$$

The particle size was found lowest in case of L1C1 as compare to the pure drug and other crystal products. The changes in the strain and dislocation value between the pure drug and the crystal products may be due to the bond formation between LT and CA.

In vitro *drug release study*

In vitro drug release study of the experimental crystal products along with the free drug was carried out in simulated gastric fluid (pH 1.2). *In vitro* drug release remains an inevitable piece of study for all solid oral dosage forms, which actually signifies the rate and extent that an API is extracted from the crystal product. Data generated out of *in vitro* release experiments play a crucial role in designing *in vivo* test conditions.[21] In our study, all the selected crystal products showed higher dissolution profile than that of pure LT (Fig. 5). Among the crystal products, L3C1 showed higher percentage of cumulative drug release (97.11 %) within 10 min of experimental release period. The difference factor f_1 and the similarity factor f_2 as the model independent statistical methods were applied for comparison of two dissolution profiles.[3,22] Equivalence or similarity between two dissolution profiles is based on $f_1 \leq 15$ and $f_2 \geq 50$ and so on. f_1 and f_2 factor between LT and L3C1 were found to be 17.807 and 30.964, respectively. Hence, L3C1 may be reported as the optimized crystal product in our study as per *in*

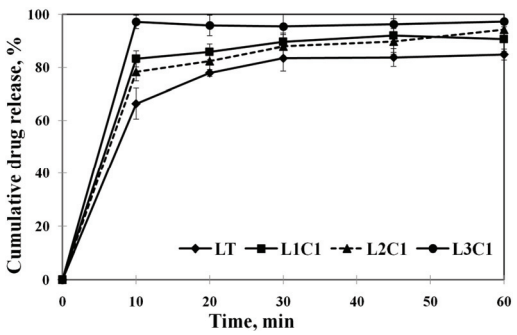

Fig. 5. *In vitro* dissolution data of pure drug (LT), and crystal products (L1C1, L2C1, and L3C1) in simulated gastric fluid (0.1 M HCl, pH 1.2) for 60 min. All set of experiments were performed in triplicate. Data show mean ± *SD* (n = 3). Error bars indicate standard deviation values.

vitro release profile based on the f_1 and f_2 factor. The higher drug release property of the reported crystal product might be due to the well formation of crystal lattice with supramolecular arrangement of molecules in a three-dimensional space.

The uniqueness of the study lies in the formation of strong peptide bond between LT and CA during crystal formation. Such strong covalent bond would help the crystal product to remain stable under normal experimental conditions including hydrolysis, but would readily break under low pH conditions. In the presence of acids or at low pH, peptide bond can be broken easily to free the entrapped drug molecule. As the peptides shorter than five residues are usually soluble in water, thus formation of the peptide bond between the components in our crystal product would increase the solubility of LT, which was the main aim of the work.

The work is highly expected to have interesting clinical implications in future days. These novel crystal products of LT-CA with higher dissolution rate may be formulated in tablet dosage forms and can be compared with marketed LT tablet crystal products to establish its superiority over other such crystal products. Though, LT tablets are widely available in the market in various doses, the extent of variations in LT serum concentration between the marketed tablets and tablets formed out of LT–CA crystals are yet to be investigated. By dint of its unique developmental feature (*i.e.*, formation of peptide bond, exceptionally stable under normal condition, highly sensitive under acidic condition), the experimental crystal product in suitable dosage forms would certainly provide much faster absorption of the drug with quicker onset of action, which undoubtedly improve its therapeutic efficacy and patient acceptability. A prompt onset of action is highly crucial to control the occurrence of epileptic seizures and is the present need of the hour.

However, our work also has some limitations. As we discussed above, the experimental crystal product has not been compared with any marketed LT tablet crystal products for the *in vitro* drug release study. Further, the crystal geometry or arrangement pattern of molecules/atoms during development of the crystal structure could have been investigated. Moreover, our work is totally restricted to *in vitro* studies; however, to get a clear idea on the therapeutic implications *in vivo* studies, in suitable experimental epileptic animal models, are also required. The data on the *in vitro-in vivo* correlation is also highly needed for its successful clinical transfer. All such experiments are planned to be included in the future course of our work.

CONCLUSIONS

Crystalline products of LT with CA have been developed for the improvement of dissolution rate and oral absorption of the drug. FTIR and DSC studies confirmed absence of any incompatibility between drug and excipient, however,

minor shifting of some characteristic peaks as well as appearance of newer peaks in the crystal product justified the formation of covalent bond (peptide) during the formation of crystal structure. In FTIR spectra, strong peaks observed for the crystal products, *i.e.*, 1284.36 (L1C1), 1291.11 (L2C1) and 1297.86 cm^{-1} (L3C1) due to C–N stretching vibration (for aromatic amine) were clearly absent in the FTIR spectrum of the pure LT. XRD data further confirmed formation of novel crystalline phase. In XRD analysis, L3C1 showed much sharper peaks among all the tested crystal products and pure LT justifying successful formation of crystal phase. *In vitro* drug release study depicted higher dissolution rate for the crystal products than the free LT under the identical experimental conditions. Further, among all the crystal products, L3C1 showed the highest dissolution rate (97.11 % within 10 min) as compared to the pure drug (60.32 % within 10 min) and thus reported as the optimized crystal product in our study. The production steps were kept very simple with optimization of all critical processing parameters, which would help for future technology transfer of the product at industrial scale. Further *in vivo* studies are warranted to establish the crystal product in clinics.

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ИЗВОД

ЕФЕКАТ КРИСТАЛИСАНИХ ПРОИЗВОДА ЛАМОТРИГИНА ИЗ СИСТЕМА ЛАМОТРИГИН–ЛИМУНСКА КИСЕЛИНА НА ПОВЕЋАНО ОСЛОБАЂАЊЕ ЛЕКА У *IN VITRO* СИМУЛИРАНОЈ ЖЕЛУДАЧНОЈ СРЕДИНИ

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Кристал-инжењеринг је саставни део истраживања у области развоја нових лекова. Различите кристалне форме неког лека могу значајно модификовати његова физичко-хемијска својства. Циљ овог рада је синтеза и карактеризација нових кристалних форми ламотригина (LT, од *U.S. Food and Drug Administration* одобреног антилептичког лека) у присуству лимунске киселине (CA) у циљу његовог већег отпуштања у симулираној желудачној средини и боље оралне апсорпције. Нове кристалне форме продуката из система ламотригин–лимунска киселина су добијене применом методе упаравања смеше растварача етанол–вода. Постојање нових форми кристала лека утврђено је на основу карактеристичних сигнала у FTIR спектру. Експериментално одређене тачке топљења добијених кристалних форми ламотригина су биле различите од тачке топљења чистог лека. Такође, формирање кристалних форми лека потврђено на основу постојања нових оштрих сигнала у XRD спектру. Нађен је већи проценат отпуштања лека у симулираној желудачној средини у току 60 min за експериментално добијене кристалне форме лека у односу на чист лек. Међутим, у циљу развоја технолошког процеса за индустријску производњу лека неопходно је урадити одговарајућа *in vivo* испитивања.

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