

TITLE

“Co-crystals of non-steroidal anti-inflammatory drugs, Lysine and Gabapentin, pharmaceutical compositions and their medical use”

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DESCRIPTION

The present invention relates to co-crystals of non-steroidal anti-inflammatory drugs (NSAIDs) belonging to the class of phenylpropionic or phenylacetic acids, Lysine and Gabapentin, to a process for their preparation, to a pharmaceutical composition comprising said co-crystals and to the use of said co-crystals or pharmaceutical
10 compositions in the treatment of acute or chronic pain, in particular in the treatment of neuropathic or inflammatory pain.

BACKGROUND ART

Pain is the means by which the body signals tissue damage. According to the definition of the IASP (International Association for the Study of Pain - 2020) and the World Health
15 Organization, pain is an "unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage".

The experience of pain is different for every person, and there are various ways to feel and describe it. Generally, pain conditions can be divided in acute and chronic.

Acute pain is an intense pain that lasts for a short period of time, typically less than 3
20 months, and is commonly associated with tissue injury or localized tissue damage and inflammation. In particular, there are three different types of acute pain: somatic pain (a person feels pain on the skin or the soft tissues just below the skin), visceral pain (pain in the internal organs and the linings of cavities in the body) and referred pain (pain referred at a location other than the source of tissue damage).

Chronic pain lasts far longer than acute pain, usually more than 3 months. Chronic pain
25 lacks the acute warning function of physiological nociception. In general, chronic pain can be mild or severe, continuous (such as in arthritis) or intermittent (such as with migraines). In particular, the peripheral nervous system (PNS) and central nervous system (CNS) are responsible for generating and sustaining the sensitization of nociceptive neurons leading
30 to chronic pain.

Chronic pain may have different etiologies and includes neuropathic pain, chronic inflammatory pain, for example arthritis, or pain of unknown origin, as fibromyalgia and restless leg syndrome. Chronic neuropathic pain is caused by a lesion or disease of the

somatosensory nervous system that provides information about the body including skin, musculoskeletal, and visceral organs.

The pharmacological treatment of chronic inflammatory pain usually includes the use of non-steroidal anti-inflammatory drugs (NSAIDs).

5 Non-steroidal anti-inflammatory drugs are commonly grouped, according to the chemical structure, in different classes, for instance in salicylates such as Aspirin, Sodium Salicylate and Diflunisal, propionic acid derivatives such as Ibuprofen, Ketoprofen, Flurbiprofene and Naproxen, acetic acid derivatives such as Diclofenac, Ketorolac, Indomethacin, Sulindac and Zomepirac, alkanones such as Nabumetone and oxicams
10 such as Piroxicam and Tenoxicam (J Clin Pharmacol 1988 Jun;28(6):512-7).

NSAIDs have antipyretic, analgesic, and anti-inflammatory effects, obtained thanks to the inhibition of prostaglandin synthesis. In particular, NSAIDs inhibit cyclooxygenases (COXs) which are the enzymes that catalyze the synthesis of cyclic endoperoxides from arachidonic acid to form prostaglandins. Non-selective NSAIDs inhibit the activity of both
15 COX-1 and COX-2, whereas selective COX-2 inhibit only cyclooxygenase COX2.

Although NSAIDs are commonly used, they can cause troublesome undesired effects as increased risk of gastrointestinal ulcers and bleeds, heart attack and kidney disease.

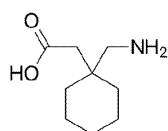
It is therefore felt the need to identify a new therapeutic solution for the treatment of chronic pain not causing the above mentioned severe side effects is required.

20 Chronic inflammatory pain conditions are often associated with neuroinflammation which is a physiological/pathological condition characterized by infiltration of immune cells, namely leukocytes, releasing inflammatory cytokines.

Neuroinflammation also implies activation of glial cells and production of inflammatory mediators which modulate the pain sensitivity. In particular, Schwann cells are activated
25 in the nerve, satellite glial cells in the ganglia and microglia, and astrocytes and oligodendrocytes in the spinal cord and brain.

Today there is no effective therapy for the treatment neuroinflammatory conditions. Non-steroidal anti-inflammatory drugs are largely ineffective in neuropathic pain.

Gabapentin is an anticonvulsant synthetic analogue of the neurotransmitter gamma-aminobutyric acid (GABA) of formula
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Gabapentin, is an anticonvulsant medication primarily used to treat partial seizures and neuropathic pain. Gabapentin has recently been approved for the treatment of chronic pain, in particular neuropathic pain conditions. Neuropathic pain is caused by a lesion or disease of the somatosensory system, including peripheral fibres and central neurons, and affects 7–10% of the general population. In particular, neuropathic pain is caused by imbalances between excitatory and inhibitory somatosensory signaling, alterations in ion channels and in the release of modulators. Neuropathic pain is a chronic pain characterized by complex symptoms, poor outcomes and difficult treatment decisions.

The mechanism of action of Gabapentin includes binding to calcium channels in several areas of the central nervous system and spinal cord in which these channels are expressed. Calcium channels are localized on presynaptic terminals, where they control neurotransmitter release.

Gabapentin is neither inhibited nor metabolized by hepatic enzymes. It is expelled by the renal system and its excretion half-life is roughly 6 hours. Thus, Gabapentin is characterized by short half-life and, as a consequence, need to be administered three times a day (tds).

Rapid titration can be achieved with doses of 300 mg once daily (often at bedtime to minimize sedation) on the first day followed by 300 mg twice daily on the second day and 300 mg tds on the third day. Dosage can be further increased if efficacy is not achieved at this dose.

An unsatisfactory pharmacological and pharmacokinetic profile has been observed when Gabapentin is used alone in pain therapy. In other words, when Gabapentin is used in monotherapy for the treatment of pain is not completely effective. Indeed, a delayed onset of the response was registered. Gabapentin is absorbed slowly after oral administration, and it has an utmost level in plasma within 3–4 hours (Quintero, Journal of Experimental Pharmacology 2017:9 13–21). Furthermore, Gabapentin is little active on inflammatory pain, as also confirmed in the present experimental part in the Carrageenan inflammatory rat model.

In addition, Gabapentin is not recommended for the treatment of lower back pain (*Low back pain and sciatica in over 16s: assessment and management*, National Institute for Health and Care Excellence NICE Guidelines 2016).

Considering the disadvantages of using Gabapentin in monotherapy for the treatment of pain, the Applicant has undertaken several studies to improve the properties of Gabapentin for use in the treatment of pain conditions.

In particular, the Applicant has carried out investigations on Gabapentin combined with non-steroidal anti-inflammatory drugs, specifically with non-steroidal anti-inflammatory drugs belonging to the class of phenylpropionic and phenylacetic acids.

SUMMARY OF THE INVENTION

5 The Applicant with the aim to improve the therapy of pain conditions has surprisingly found that non-steroidal anti-inflammatory drugs belonging to the class of phenylpropionic or phenylacetic acids form stable co-crystals with Gabapentin and Lysine.

During these investigations, the Applicant has found that the co-crystals of non-steroidal anti-inflammatory drugs belonging to the above mentioned classes, Lysine and
10 Gabapentin, show a surprising synergistic effect on inflammation and pain.

In fact, when these active ingredients are associated in the co-crystals of the invention, they show an anti-inflammatory and analgesic activity greater than that obtained with their co-administration as separate active molecules (NSAID, Gabapentin)

Hence the present co-crystals allow to use significantly lower therapeutic doses of either
15 NSAID or Gabapentin or both, thus minimizing the side effects. Additionally, in comparison to Gabapentin alone, a prolongation of the efficacy over time was observed. Finally, the co-crystal improves dissolution rates of NSAIDs, especially if dissolving in an aqueous physiological surrounding, and enhances the absorption and/or the bioavailability of the two active molecules Gabapentin and NSAID.

20 It is thus an object of the present invention a co-crystal of a non-steroidal anti-inflammatory drug (NSAID) belonging to the class of phenylpropionic or phenylacetic acids, Lysine and Gabapentin, providing that said non-steroidal anti-inflammatory drug is not Ketoprofen.

According to an embodiment of the invention, said non-steroidal anti-inflammatory drug
25 is a phenylpropionic acid derivative selected from Ibuprofen, Flurbiprofen, Fenoprofen, Indoprofen, Loxoprofen, Pelubiprofen, and Naproxen.

According to an embodiment of the invention, said non-steroidal anti-inflammatory drug is a phenylacetic acid derivative selected from Diclofenac, Felbinac, Ibufenac, Fenclofenac, Tifurac, and Ketorolac.

30 According to a particularly preferred embodiment of the invention, said non-steroidal anti-inflammatory drug is selected from Ibuprofen and Flurbiprofen.

The co-crystals according to the invention of Flurbiprofen-Lysine-Gabapentin (FLG) and Ibuprofen-Lysine-Gabapentin (ILG) are respectively characterized by the XRPD

diffraction patterns reported in Figure 1 and by XRPD positions and intensities depicted in Tables 2-3.

The Applicant has surprisingly observed that the co-crystals of Flurbiprofen-Lysine-Gabapentin and of Ibuprofen-Lysine-Gabapentin were of striking resemblance to each other, with the most intense XRPD diffraction peaks in the region between 9 - 10 degrees 2-theta, 15 - 25 degrees 2-theta and 27 - 28 degree 2-theta. The same pattern was observed with a previous co-crystal of Ketoprofen – Lysine – Gabapentin, described in international patent application PCT/EP2021/060421, which is not part of the present invention.

This similarity was taken as clear evidence of isostructurality of the co-crystals. Isostructurality refers to different components with similar crystal packing. When closely related molecules are introduced in the crystal lattice of a single component, the corresponding binary system may exhibit close packing [G. Portalone, Crystals, 2020, 10, 999–1012].

A further object of the present invention is a process for the preparation of the co-crystals of the invention, which comprises:

- a) suspending a non-steroidal anti-inflammatory drug (NSAID) belonging to the class of phenylpropionic or phenylacetic acids, Lysine, and Gabapentin in a suitable solvent,
- b) dissolving said NSAID, Lysine, and Gabapentin, optionally by heating the suspension, possibly under stirring, until a clear solution is obtained,
- c) optionally cooling the solution obtained, and/or
- d) optionally adding an anti-solvent, thus providing the NSAID, Lysine, and Gabapentin co-crystal.

A further object of the present invention is a pharmaceutical composition comprising the co-crystals of the invention and at least a pharmaceutically acceptable excipient.

A further object of the present invention is a pharmaceutical composition comprising a co-crystal according to the invention and at least another pharmaceutically active ingredient.

A further object of the present invention is a co-crystals according to the invention for use in the treatment of pain and/or inflammation.

A further object of the present invention is a method for the treatment of pain and/or inflammation comprising administering to the patient an effective amount of a co-crystal according to of the invention.

DEFINITIONS

For the purpose of the present invention, the term “co-crystal” means a multi-component system, in which all components are solid under ambient conditions when in their pure form. The components coexist at a molecular level within a single crystal. At least some
5 the components are connected by non-covalent, non-ionic interactions.

For the purpose of the present invention, the term “pain” means pain caused by disturbances of different nature and origin, such as, for example: headache or cephalalgia: both primary and therefore not related to other factors or diseases, and secondary and therefore dependent on trauma, injury and distinct diseases; toothache:
10 in case of abscesses or caries that create pain in the dental pulp, with numerous blood vessels and nerves; menstrual pains: abdominal and lower abdominal pain and headaches caused by hormonal changes typical of the period of menstruation; neuralgia, or intense nerve pain due to strains, trauma and infections; pain in the muscles, or myalgia: pains located at the level of muscles when using or touching them, due to
15 sudden contractions or traumas; osteoarticular pains, such as joint inflammations (to the bones, cartilages, ligaments and tendons) following traumas, old age, strains and injuries. For the purpose of the present invention, the term “inflammation” means the local response of an organism to cellular injury that is marked by capillary dilatation, leukocytic infiltration, redness, heat, and pain and that serves as a mechanism initiating the
20 elimination of noxious agents and of damaged tissue.

For the purpose of the present invention, the term “pharmaceutically acceptable excipient” refers to a substance devoid of any pharmacological effect of its own and which does not produce adverse reactions when administered to a mammal, preferably a human.

25 For the purpose of the present invention, the term “room temperature” means a temperature range of 18 to 25 °C.

For the purpose of the present invention, the term “anti-solvent” means a solvent in which a compound is insoluble or little soluble.

In the present description and in the figures attached to the description, the abbreviation
30 “Gaba” indicates Gabapentin.

The terms “approximately” and “about” herein refers to the range of the experimental error, which may occur in a measurement.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Powder X-Ray diffraction pattern of Flurbiprofen-Lysine-Gabapentin and Ibuprofen-Lysine-Gabapentin co-crystals.

5 Figure 2: DSC thermogram of Flurbiprofen-Lysine-Gabapentin 1:1:1 co-crystal.

Figure 3: DSC thermogram of Ibuprofen-Lysine-Gabapentin 1:1:1 co-crystal.

Figure 4: TG thermograms of Flurbiprofen-Lysine-Gabapentin 1:1:1 co-crystal.

Figure 5: TG thermograms of Ibuprofen-Lysine-Gabapentin 1:1:1 co-crystal.

Figure 6: Raman spectrum of Flurbiprofen-Lysine-Gabapentin 1:1:1 co-crystal.

10 Figure 7: Raman spectrum of Ibuprofen-Lysine-Gabapentin 1:1:1 co-crystal.

Figure 8: FT-IR spectrum of Flurbiprofen-Lysine-Gabapentin 1:1:1 co-crystal.

Figure 9: FT-IR spectrum of Ibuprofen-Lysine-Gabapentin 1:1:1 co-crystal.

Figure 10: ^1H -NMR spectrum (400 MHz, D_2O) of Flurbiprofen-Lysine-Gabapentin 1:1:1 co-crystal.

15 Figure 11: ^1H -NMR spectrum (400 MHz, D_2O) of Ibuprofen-Lysine-Gabapentin 1:1:1 co-crystal.

Figure 12: ^{13}C CPMAS spectra of Flurbiprofen-Lysine-Gabapentin 1:1:1 co-crystal.

Figure 13: Detail of the carboxylic region of the ^{13}C CPMAS spectra of FLU-LYS-GAB, FLU-SALA, FLU-PICA, NaFLU, pure FLU, NaGAB, pure GAB, DL-LYS $\cdot$ 2HCl, L-LYS acetate and pure L-LYS.

20 Figure 14. ^{15}N CPMAS spectra of Flurbiprofen-Lysine-Gabapentin 1:1:1 co-crystal.

Figure 15. Comparison among the ^{15}N CPMAS spectra of FLU-LYS-GAB, NaGAB, GAB, DL-LYS $\cdot$ 2HCl, L-LYS acetate and pure L-LYS.

Figure 16: ^{13}C CPMAS spectra of Ibuprofen-Lysine-Gabapentin 1:1:1 co-crystal.

25 Figure 17: Detail of the carboxylic region of the ^{13}C CPMAS spectra of IBU-LYS-GAB, IBU-L-proline, NaIBU, pure IBU, NaGAB, pure GAB, DL-LYS $\cdot$ 2HCl, L-LYS acetate and pure L-LYS.

Figure 18. ^{15}N CPMAS spectra of Ibuprofen-Lysine-Gabapentin 1:1:1 co-crystal.

Figure 19. Comparison among the ^{15}N CPMAS spectra of IBU-LYS-GAB, NaGAB, GAB, DL-LYS $\cdot$ 2HCl, L-LYS acetate and pure L-LYS.

30 Figure 20. ^{13}C (150.91 MHz) CPMAS spectra of Flurbiprofen-Lysine-Gabapentin and Ibuprofen-Lysine-Gabapentin, acquired at room temperature at a spinning speed of 20 kHz.

Figure 21. ¹⁵N (60.83 MHz) CPMAS spectra of Flurbiprofen-Lysine-Gabapentin and Ibuprofen-Lysine-Gabapentin, acquired at room temperature at a spinning speed of 12 kHz.

Figure 22: Effect of Flur/Lys/Gaba co-crystal in comparison with the admixture of Flur + Lys + Gaba and with single actives, respectively Flurbiprofen, Gabapentin and Indomethacin, on carrageenan-induced rat paw edema. In the present Figure “Gaba” indicates Gabapentin.

Figure 23: Effect of Flur/Lys/Gaba co-crystal in comparison with the admixture of Flur + Lys + Gaba and with single actives, respectively Flurbiprofen, Gabapentin and Indomethacin, on mechanical allodynia in a rat carrageenan-induced paw edema model. In the present Figure “Gaba” indicates Gabapentin.

DETAILED DESCRIPTION OF THE INVENTION

An object of the present invention is a co-crystal of a non-steroidal anti-inflammatory drug (NSAID) belonging to the class of phenylpropionic or phenylacetic acids, Lysine and Gabapentin, providing that said non-steroidal anti-inflammatory drug is not Ketoprofen.

According to a preferred embodiment of the invention, the molar ratio of the components in the co-crystal is 1:1:1.

In line with the analysis reported in the experimental part, in the co-crystal according to the invention the carboxylic group of the non-steroidal anti-inflammatory drug (NSAID) belonging to the class of phenylpropionic or phenylacetic acids is deprotonated and interacts with protonated Lysine ϵ -NH₃⁺ group through ionic bonds forming a neutral salt. The salt of Lysine with the non-steroidal anti-inflammatory drug (NSAID) belonging to the class of phenylpropionic or phenylacetic acids, in particular Flurbiprofen or Ibuprofen salt with Lysine, interacts with Gabapentin through non-ionic bonds, forming stable co-crystals.

According to an embodiment of the invention, said non-steroidal anti-inflammatory drug is a phenylpropionic acid derivative selected from Ibuprofen, Flurbiprofen, Fenoprofen, Indoprofen, Loxoprofen, Pelubiprofen and Naproxen.

According to an embodiment of the invention, said non-steroidal anti-inflammatory drug is a phenylacetic acid derivative selected from Diclofenac, Felbinac, Ibufenac, Fenclofenac, Tifurac and Ketorolac.

In the co-crystal according to the invention, the chiral carbon of the NSAID, when present, can be optically pure (S) or (R) or racemic (S,R) or in form of any admixture of the stereoisomers.

In the co-crystal of the invention, Lysine can be racemic (S,R) Lysine, (S) Lysine or (R) Lysine, or any admixture thereof, preferably is the natural aminoacid (S)-Lysine also named L-Lysine.

5 According to a particularly preferred embodiment, said non-steroidal anti-inflammatory drug is selected from Flurbiprofen and Ibuprofen. According to another preferred embodiment, said non-steroidal anti-inflammatory drug is Flurbiprofen.

In the co-crystal according to this embodiment, Flurbiprofen can be racemic (S,R) Flurbiprofen, (S)-Flurbiprofen or (R)- Flurbiprofen or any admixture thereof.

10 In the co-crystal according to this embodiment, Lysine can be racemic (S,R) Lysine, (S) Lysine or (R) Lysine, or any admixture thereof, preferably is the natural aminoacid (S)-Lysine also named L-Lysine.

In one embodiment, the co-crystal of the invention comprises (S,R)- Flurbiprofen.

In one embodiment, the co-crystal of the invention comprises (S,R)-Lysine.

15 In one embodiment, the co-crystal of the invention comprises (S,R)- Flurbiprofen and (S,R)-Lysine.

According to another preferred embodiment, said non-steroidal anti-inflammatory drug is Ibuprofen.

In the co-crystal according to this embodiment, Ibuprofen can be racemic (S,R) Ibuprofen, (S)- Ibuprofen or (R)- Ibuprofen or any admixture thereof.

20 Also, in the co-crystal according to this embodiment, Lysine can be racemic (S,R) Lysine, (S) Lysine or (R) Lysine, or any admixture thereof, preferably is the natural aminoacid (S)-Lysine also named L-Lysine.

In one embodiment, the co-crystal of the invention comprises (S,R)- Ibuprofen.

In one embodiment, the co-crystal of the invention comprises (S,R)- Lysine.

25 In one embodiment, the co-crystal of the invention comprises (S,R)- Ibuprofen and (S,R)-Lysine.

The co-crystal of the present invention can exist in non-solvated forms as well as solvated forms, including hydrated forms.

The co-crystals of the present invention are easily obtainable and stable.

30 The co-crystals of the present invention show improved pharmaceutical properties, pharmacokinetics and efficacy in pain conditions, as described in the Experimental section that follows.

The co-crystals of the present invention are characterized by a common XRPD pattern, in particular by the following common XRPD diffraction peaks: 9.3, 17.1, 18.5, 19.8, 22.1, 24.1, 24.9, 27.9 degrees 2-theta $\pm$ 0.4 degrees 2-theta.

Other polymorphs of the present co-crystals are also within the scope of the invention.

5 According to an embodiment, the co-crystal of Flurbiprofen, Lysine and Gabapentin of the present invention is characterized by the following XRPD diffraction peaks: 9.3, 10.4, 15.2, 16.0, 17.2, 18.3, 18.8, 19.7, 20.7, 21.9, 24.0, 24.8, 27.9, and 29.0 degrees 2-theta $\pm$ 0.2 degrees 2-theta, preferably further characterized by the following XRPD diffraction peaks: 6.9, 10.9, 12.2, 25.5, 26.3, 29.8, 31.5, 33.0, 34.0, 35.9, 37.5, 39.2 and 40.8
10 degrees 2-theta $\pm$ 0.2 degrees 2-theta.

According to this embodiment, the co-crystal of Flurbiprofen, Lysine and Gabapentin of the present invention is further characterized by the DSC thermogram of Figure 2, with the endothermic sharp peak of the co-crystal corresponding to the melting point at 163.48°C $\pm$ 0.5°C with an onset at 161.59°C $\pm$ 0.5°C, the TGA thermogram of Figure 4,
15 FT Raman and FT-IR spectra with typical absorption bands reported in Figures 6 and 8, solution ¹H-NMR spectrum of Figure 10 and relative assignments in Table 10, solid state ¹³C CPMAS of Figure 12, 13 and relative assignments in Table 12 and/or ¹⁵N CPMAS spectra of Figures 14 and 15.

According to another embodiment, the co-crystal of Ibuprofen, Lysine and Gabapentin of
20 the present invention is characterized by the following XRPD diffraction peaks: 9.5, 10.3, 15.9, 17.1, 17.6, 18.7, 20.0, 22.3, 24.1, 25.1, 25.6, 27.9 and 28.6 degrees 2-theta $\pm$ 0.2 degrees 2-theta, preferably further characterized by the following XRPD diffraction peaks: 6.9, 12.0, 14.7, 26.3, 30.6, 31.1, 32.3, 33.1, 34.5, 35.3, 36.6, 38.6, 39.0, 41.1, and 48.9 degrees 2-theta $\pm$ 0.2 degrees 2-theta. According to this embodiment, the co-crystal
25 Ibuprofen, Lysine and Gabapentin of the present invention is further characterized by the DSC thermogram of Figure 3, with the endothermic sharp peak of the co-crystal corresponding to the melting point at 165.60°C $\pm$ 0.5°C with an onset at 161.60°C $\pm$ 0.5°C, the TGA thermogram of Figure 5, FT Raman and FT-IR spectra with typical absorption bands reported in Figures 7 and 9, solution ¹H-NMR spectrum of Figure 11 and relative
30 assignments in Table 11, solid state ¹³C CPMAS of Figures 16, 17 and relative assignments in Table 13 and/or ¹⁵N CPMAS spectra of Figures 18 and 19.

It has been observed that the co-crystals having the XRPDs above indicated, in particular the co-crystals of Flurbiprofen-Lysine-Gabapentin and of Ibuprofen-Lysine-Gabapentin, are isostructural co-crystals.

Isostructurality indicates different components with similar crystal packing. When closely related molecules are introduced in the crystal lattice of a single component, the corresponding binary system may exhibit close packing (G. Portalone, Crystals, 2020, 10, 999–1012).

5 Isostructural co-crystals with similar structural blueprints may exhibit common modified pharmaceutically relevant properties due to the presence of variable coformers and minor changes in their conformations and packing features. Often, isostructural co-crystals have similar lattice parameters including space groups, defined as isomorphs, which generally exhibit identical X-ray diffraction pattern [F. Y. Wang, Q. Zhang, Z. Zhang, X. Gong, J. R. Wang and X. Mei, Cryst. Eng. Comm, 2018, 20, 5945–5948]. The isostructurality of the
10 co-crystals was demonstrated by XRPD pattern comparison and further confirmed by ¹³C and ¹⁵N-CPMAS NMR spectra (see Experimental section).

XRPD diffractograms of the present co-crystals are very similar, with the reflexes of the respective main peaks in the region between 14 and 22 degrees 2-theta having similar
15 intensities (Figure 1 - XRPD). This similarity was taken as clear evidence of isostructurality of the co-crystals.

Furthermore, in the ¹³C and ¹⁵N-CPMAS NMR spectra of two co-crystals (see Figures 20 and 21) many of the resonances match coincides. Careful analysis of the signals points out that all the recurring signals in the aliphatic region (below 80 ppm) correspond
20 to the aliphatic C nuclei of LYS and GAB. The most apparent differences are in the aromatic range (110-170 ppm), which is reasonable, since the aromatic carbons are those of the different NSAIDs. Most notably, the same similarities can be observed in the carboxylic region, with the carboxylic resonances of FLU-LYS-GAB and IBU-LYS-GAB. The same remarkable spectral similarities can be observed in the ¹⁵N CPMAS spectra
25 of the samples.

All these spectral analogies indicate that the crystalline systems of FLU-LYS-GAB and IBU-LYS-GAB are in a homogeneous phase and they are characterized by a very similar local environment, especially regarding LYS and GAB, and comparable vibrational behaviors.

30 A further object of the present invention is the co-crystal of the invention for use as a medicament. The medical use can be curative, prophylactic or palliative.

The Applicant observed that the association of the two active ingredients in the same crystals exhibits several advantages for the present medical use. First, the co-crystals comprising a NSAID, Lysine and Gabapentin behave as a single chemical entity, thus

facilitating the treatments, and causing less side effects with respect to the treatment with Gabapentin and NSAID alone.

The co-crystals comprising a NSAID, Lysine and Gabapentin can be advantageously used in the prevention, reduction or treatment of pain and/or inflammation, said pain being
5 acute or chronic pain.

According to a preferred use, the co-crystals comprising a NSAID, Lysine and Gabapentin are used for neuropathic or inflammatory pain. Indeed, the association of two active ingredients into one unique species may allow for better Pharmacokinetics / Pharmacodynamics (PKPD) including also a better penetration of the blood-brain barrier,
10 which helps in the treatment of neuropathic pain.

Preferably, said pain is selected from headache, toothache, menstrual pain, muscle pain, neuropathic pain, pain associated to neuroinflammation, diabetic neuropathy, cancer pain, osteoarthritis, low back pain, sciatalgia, fibromyalgia, trigeminal neuralgia, post-surgical and post-operative pain, post herpetic neuralgia, rheumatoid arthritis, ankylosing
15 spondylitis, frozen shoulder, phantom limb pain and HIV pain.

According to an embodiment, the daily dosage of the co-crystal for humans preferably provides for Flurbiprofen in an amount between 50 and 250 mg per day, more preferably 100 and 200 mg per day, preferably from 1 to 4 times a day.

According to another embodiment, the daily dosage of the co-crystals for humans preferably provides for Ibuprofen in an amount between 200 and 800 mg per day, more preferably 400 and 600 mg per day, preferably from 1 to 4 times a day.
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The daily dosage of the co-crystal according to the invention for humans advantageously results in a total amount of Gabapentin much lower if compared to the normal dosage of Gabapentin when this is used alone.

25 A further object of the present invention is a process for the preparation of the co-crystals of the invention, which comprises:

- a) suspending a non-steroidal anti-inflammatory drug (NSAID) belonging to the class of phenylpropionic or phenylacetic acids, Lysine, and Gabapentin in a suitable solvent and stirring the resulting mixture at room temperature until a clear solution is obtained,
- 30 b) dissolving said NSAID, Lysine, and Gabapentin optionally by heating the suspension, possibly under stirring, until a clear solution is obtained,
- c) optionally, cooling the solution obtained in b), and/or
- d) optionally, adding an anti-solvent, thus providing the NSAID, Lysine, and Gabapentin co-crystal.

According to an embodiment of the invention, the non-steroidal anti-inflammatory drug according to step a) is a phenylpropionic acid derivative selected from Ibuprofen, Flurbiprofen, Fenoprofen, Indoprofen, Loxoprofen, Pelubiprofen, and Naproxen.

5 According to another embodiment of the invention, the non-steroidal anti-inflammatory drug is a phenylacetic acid derivative selected from Diclofenac, Felbinac, Ibufenac, Fenclofenac, Tifurac, and Ketorolac.

According to a preferred embodiment, said non-steroidal anti-inflammatory drug is selected from Flurbiprofen, Ibuprofen, Diclofenac, and Naproxen, even more preferably from Ibuprofen and Flurbiprofen.

10 In the present process, the starting material for the NSAID can be a NSAID free acid or NSAID salt.

In case of NSAID free acid or a NSAID salt different from the Lysinate, Lysine is added, preferably in its neutral form. Lysine is preferably used in the same molar amount of the NSAID.

15 The NSAID salt may preferably be Flurbiprofen Lysinate or Ibuprofen Lysinate.

In step a) of the present process, the molar ratio of Gabapentin vs NSAID is preferably between 1:1 and 1.5:1, more preferably between 1:1 and 1.2:1, even more preferably is about 1:1.

20 In the preferred embodiment, the molar ratio of NSAID: Lysine: Gabapentin in step a) is about 1:1:1.

Suitable solvents to be used in step a) of the process according to the invention are water, alcohols, preferably methanol and ethanol, esters, preferably ethyl acetate, ethers, preferably tetrahydrofuran and tert-butylmethyl ether or aromatic solvents, preferably toluene.

25 Step b) can be carried out at room temperature or under heating, preferably at the temperature of reflux of the solvent.

Preferably, the clear solution of step b) contains said NSAID at a molar concentration of 1 mol, Lysine at a molar concentration from 1 to 1.5 mol/mol, more preferably 1 mol/mol, as compared to NSAID, and Gabapentin at a molar concentration from 1 to 1.5 mol/mol, 30 more preferably 1 mol/mol, as compared to NSAID.

Preferably the solution from step b) is kept or cooled at room temperature and filtered.

Preferably step b) is carried out under stirring. The stirring of step b) is performed preferably for 10 minutes to 30 minutes.

Optionally in step d) the precipitation of the co-crystal is favored by addition of an anti-solvent, such as ethyl acetate and tetrahydrofuran.

Preferably the anti-solvent can be added in a volumetric ratio from 1:1 to 16:1 with respect to the volume of the solution.

5 According to one embodiment, in step a) of the process according to the invention, NSAID and Lysine can be present as a pre-formed salt or co-crystal, in any polymorphic form.

The starting material for the manufacture of the co-crystals of the present invention can be prepared in accordance with methods of synthesis previously published and well known to the organic chemist of ordinary skill.

10 According to an alternative embodiment, said NSAID is a free acid and/or said Lysine is in neutral form.

In the present preparation process, Gabapentin is preferably used in its neutral form (zwitterionic internal salt) or in any acid or basic salified form, for instance as Gabapentin hydrochloride or Gabapentin Sodium salt.

15 Preferably, Gabapentin is used in its neutral form.

Gabapentin can be in any polymorph form.

The present process provides the co-crystals of the invention with high yields. It is simple and easy scalable at industrial level.

20 The present invention furthermore relates to a pharmaceutical composition comprising a co-crystal of a non-steroidal anti-inflammatory drug (NSAID) belonging to the class of phenylpropionic or phenylacetic acids, Lysine and Gabapentin as above described and a least one pharmaceutically acceptable excipient. According to an embodiment of the invention, said non-steroidal anti-inflammatory drug in the pharmaceutical composition is a phenylpropionic acid derivative selected from Ibuprofen, Flurbiprofen, Fenoprofen, 25 Indoprofen, Loxoprofen, Pelubiprofen and Naproxen.

According to another embodiment of the invention, said non-steroidal anti-inflammatory drug in the pharmaceutical composition is a phenylacetic acid derivative selected from Diclofenac, Felbinac, Ibuprofen, Fenclofenac, Tifurac and Ketorolac.

30 According to a particularly preferred embodiment, the pharmaceutical composition comprises co-crystals of Flurbiprofen-Lysine-Gabapentin.

According to another particularly preferred embodiment, the pharmaceutical composition comprises co-crystals of Ibuprofen-Lysine-Gabapentin.

The non-steroidal anti-inflammatory drug in the co-crystals present in the pharmaceutical composition of the invention is not Ketoprofen.

The composition according to the present invention may contain 6-60% by weight of the co-crystals as defined herein and 40-94 % by weight of one or more pharmaceutically acceptable excipients.

5 The choice of the excipients will to a large extent depend on factors such as the particular mode of administration, the effect on solubility and stability, and the nature of the dosage form.

Pharmaceutical compositions according to the present invention can be in any form suitable for the administration to humans and/or animals, preferably humans including infants, children and adults and can be produced by standard procedures known to those skilled in the art.

10 The pharmaceutical composition of the present invention preferably is an oral solid composition, such as for instance a capsule, pellet, tablet, cachet, chewable dosage forms, powder, lozenge, granules, oral soluble granulate, suspension, emulsion, spray, or as dry powdered form to be reconstituted with a liquid medium.

15 The pharmaceutical composition can additionally contain one or more pharmaceutically acceptable excipients, such as fillers, binders, glidants, disintegrants, flow regulating agents, release agents and the like.

Suitable excipients are for example disclosed in "Handbook of Pharmaceutical Excipients", 3rd Edition, published by A.H. Kibbe, American Pharmaceutical Association, Washington, USA, and Pharmaceutical Press, London.

20 Suitable fillers are for example lactose (monohydrate, spray-dried monohydrate, anhydrous and the like), mannitol, xylitol, dextrose, sucrose, sorbitol, microcrystalline cellulose, starch, dibasic calcium phosphate dihydrate and calcium hydrogen phosphate. Fillers can be present in an amount of 0 - 80% by weight, preferably in an amount of 10 - 25 60% by weight of the total weight of the composition.

Suitable binders are for example polyvinylpyrrolidone, microcrystalline cellulose hydroxypropyl cellulose, hydroxypropyl methylcellulose, methylcellulose, hydroxyethyl cellulose, sugars, dextran, corn starch, gelatin, polyethylene glycol, natural and synthetic gums, pregelatinised starch.

30 Binders can be present in an amount of 0 - 80% by weight, preferably in an amount of 10 - 60% by weight of the total weight of the composition.

Binders are generally used to impart cohesive qualities to a tablet formulation.

Suitable glidants are for example alkaline earth metal salts of fatty acids, like stearic acid such as magnesium stearate, calcium stearate, zinc stearate, sodium stearyl fumarate, and mixtures of magnesium stearate with sodium lauryl sulphate.

5 The glidant can be present for example in an amount of 0 - 2% by weight, preferably in an amount of 0.5 - 1.5% by weight of the total weight of the composition.

Suitable disintegrants are for example crosscarmellose sodium, sodium carboxymethyl starch, crosslinked polyvinylpyrrolidone (crosspovidone), sodium carboxymethylglycolate, sodium starch glycolate, sodium carboxymethyl cellulose, calcium carboxymethyl cellulose, polyvinylpyrrolidone, methyl cellulose, microcrystalline
10 cellulose, lower alkyl-substituted hydroxypropyl cellulose, starch, pregelatinised starch, sodium alginate and sodium bicarbonate.

The disintegrant can be present in an amount of 0 - 20% by weight, preferably in an amount of 1 - 15% by weight of the total weight of the composition.

A suitable flow regulating agent is for example colloidal silica. The flow regulating agent
15 can be present in an amount of 0 - 8% by weight, preferably in an amount of 0.1 - 3% by weight of the total weight of this composition.

A suitable release agent is for example talcum. The release agent can be present in an amount of 0 - 5% by weight, preferably in an amount of 0.5 - 3% by weight of the total weight of the composition.

20 The solid composition can be coated, preferably film coated.

A suitable coating agent are for example cellulose derivatives, poly(meth)acrylate, polyvinyl pyrrolidone, polyvinyl acetate phthalate, and/or shellac or natural rubbers such as carrageenan.

The pharmaceutical composition of the invention can be a solid implant composition
25 wherein the co-crystals are in form of a solid. This composition can be installed into body tissues or cavities.

The implant may comprise a matrix of bio-compatible and bioerodible materials in which particles of the co-crystals of the present invention are dispersed, or in which, possibly, globules or isolated cells of a liquid mixture of the present co-crystals are entrapped.

30 Desirably, the matrix will be broken down and completely absorbed by the body. The composition of the matrix is also preferably selected to provide controlled-, sustained-, and/or delayed release of the co-crystals of the present invention over extended periods.

Alternatively, the co-crystals of the invention can be formulated as a solid, semi-solid, or thixotropic liquid for administration as an implanted depot providing modified release of the active compound.

5 The present composition can be administered topically to the skin or mucosa, that is dermally, epidermally, subepidermally or transdermally.

The present composition can be administered sublingually or via a suppository.

Typical formulations for this purpose include pour-on, spot-on, dip, spray, mousse, shampoo, powder formulation, gels, hydrogels, lotions, creams, ointments, dusting powders, dressings, foams, films, skin patches, wafers, implants, depots, sponges, fibres,
10 bandages, microemulsions, orosoluble granulates. Liposomes may also be used.

The pharmaceutical composition of the present invention can be a solid composition for the extemporaneous preparation of a solution for oral or parenteral administration, for example to be administered by intramuscular, intraperitoneal, or intravenous injection.

15 The pharmaceutical composition of the present invention can be prepared by methods well known to a person skilled in the art.

The composition of the invention can be of immediate-, delayed-, modified-, sustained-, pulsed- or controlled-release type.

According to a further embodiment, the pharmaceutical composition of the invention may comprise the co-crystals of the invention and at least another pharmaceutically active
20 ingredient.

The other pharmaceutically active ingredient will be determined by the circumstances under which the therapeutic agent of the present invention is administered.

According to the invention, the pharmaceutical composition can be used for the prevention, reduction or treatment of pain and/or inflammation, said pain being acute or
25 chronic pain.

According to a preferred use, the composition is used for the prevention, reduction and/or treatment of neuropathic or inflammatory pain.

Said pain is selected from headache, toothache, menstrual pain, muscle pain, neuropathic pain, pain associated to neuroinflammation, diabetic neuropathy, cancer
30 pain, osteoarthritis, low back pain, sciatalgia, fibromyalgia, trigeminal neuralgia; post-surgical and post-operative pain, post herpetic neuralgia, rheumatoid arthritis, ankylosing spondylitis, frozen shoulder, phantom limb pain or HIV pain.

A further object of the present invention is a method for the prevention, reduction or treatment of pain and/or inflammation comprising administering to a subject, preferably a

human, in need thereof an effective amount of the co-crystals of the invention and/or of a pharmaceutical composition comprising said co-crystals.

In particular, said method allows the prevention, reduction or treatment of headache, toothache, menstrual pain, muscle pain, neuropathic pain, pain associated to neuroinflammation, diabetic neuropathy, cancer pain, osteoarthritis, low back pain, sciatalgia, fibromyalgia, trigeminal neuralgia; post-surgical and post-operative pain, post herpetic neuralgia, rheumatoid arthritis, ankylosing spondylitis, frozen shoulder, phantom limb pain or HIV pain. In particular, the patient would benefit from a longer duration of action from the co-crystals treatment than from treatment with Gabapentin or with NSAID or with their combination.

It is within the normal skills of the skilled artisan, such as a physician, to determine the preferred route of administration, the corresponding suitable dosage form and dose regimen.

For example, the daily dosage for humans and animals may vary depending on factors such as age, sex, weight or degree of illness and so forth.

According to an embodiment of the method for the prevention, reduction or treatment of pain and/or inflammation, the co-crystals and/or the pharmaceutical composition can be administered in combination with one or more other drugs useful for the prevention, reduction or treatment of pain and/or inflammation.

EXPERIMENTAL PART

In the following, the manufacture of the co-crystals of NSAIDs, Lysine and Gabapentin, their analytical and biological characterization are described.

1. Synthesis of the co-crystal NSAID-Lysine-Gabapentin

The NSAID (3.3 mmol, 1.0 eq) was dissolved in 6 mL of ethanol. To the resulting solution, 50% w/w D,L-Lysine aqueous solution (1.0 eq.) was added and the solution stirred for 10 minutes. Then, 1.0 eq of Gabapentin was added. The solid precipitation took place in approximately 30 minutes and the suspension was stirred at 25 °C for 5 hours (300 rpm). The solid product was isolated by vacuum filtration on a paper filter and then squeezed under a nitrogen flow for approximately 10 minutes. The solid was gently ground and then dried at 40 °C and 30 mbar overnight affording the desired product as a white solid of NSAID-Lysine-Gabapentin co-crystal (Yield: 75 - 90%).

The co-crystals of Flurbiprofen-Lysine-Gabapentin and of Ibuprofen-Lysine-Gabapentin were synthesized according to the above procedure and subjected to the following analyses.

2. XRPD Analysis

The XRPD analysis was carried out with the following instrument and under the conditions reported in Table 1 below:

Table 1

Instrument type:	Rigaku MiniFlex600
Application SW:	Miniflex Guidance
Measurement Details	
Measurement type:	Single scan
Sample mode:	Reflection
Scan	
Scan range:	3.000 – 40.000 ° (2 θ)
Step size:	0.01 ° (2 θ)
Speed:	10.0 °/min (2 θ)
Scan mode:	Continuous
Used wavelength	
Intended wavelength type:	K α 1
K α 1:	1.540598 Å
K α 2:	1.544426 Å
K α 2/K α 1 intensity ratio:	0.50
K α :	1.541874 Å
K α :	1.392250 Å
Instrument Details	
X-Ray Generator	
Tube output voltage:	40 kV
Tube output:	15 mA
High-voltage generation method:	High-frequency Cockcroft-Walton method
Stability:	Within $\pm 0.05\%$ for both the tube voltage and tube current, with reference to $\pm 10\%$ of input power variation.
X-ray tube	
Name:	Toshiba Analix type A-26L
Anode material:	Cu
Maximus output:	0.60 kW
Focus size:	1 x 10 mm
K β Filter	
Name:	Ni-filter
Thickness (mm):	0.015
Material:	Ni
Goniometer (Angle measuring device)	
Type:	Vertical $\theta/2\theta$
Goniometer radius:	150 mm
Scanning axis:	$\theta/2\theta$ linked
2 θ scanning range:	+2 ° to +140 °
$\theta/2\theta$ axis minimum step angle:	0.005 ° (2 θ)
Position speed:	500 °/min (2 θ)
Scanning speed:	0.01 to 100 °/min

Datum angle:	2 θ = 10 °
X-ray take-off angle:	6 ° (fixed)
Slit	
DS:	1.25 °
IHS:	10.0 mm
SS:	none (open)
RS:	none (open)
Incident side Soller slit:	2.5 °
Receiving side Soller slit:	2.5 °
Detector	
Name:	D/teX Ultra High-speed 1D Detector
Window material:	Be
Effective window size:	13 mm (H) x 20 mm (W)
Dimensions:	80 mm (L)

The Powder X-Ray diffractograms of Flurbiprofen-Lysine-Gabapentin 1:1:1 and Ibuprofen-Lysine-Gabapentin 1:1:1 co-crystals are reported in Figure 1.

The XRPD peak lists of the co-crystals are reported in Table 2 and in Table 3 below:

5

Table 2. XRPD Peak List of Flurbiprofen-Lysine-Gabapentin co-crystal

Position 2 θ (°)	d-spacing [Å]	Intensity
6.88	12.84706	780
9.35	9.44693	22400
10.38	8.51275	2300
10.92	8.09719	511
12.24	7.22378	248
15.20	5.82425	3180
16.01	5.51406	1530
17.21	5.1483	5850
18.30	4.84484	19300
18.83	4.70847	7640
19.74	4.49477	6520
20.66	4.29526	6340
21.93	4.04977	2010
24.03	3.69988	2610
24.77	3.5922	2290
25.53	3.4861	442
26.32	3.383	425
27.86	3.1998	1650
28.95	3.08212	1870
29.78	2.99748	652
31.52	2.83627	248
32.99	2.71279	627
34.06	2.63014	307
35.93	2.49756	410
37.53	2.39471	424
39.18	2.29746	153
40.81	2.20918	330

Table 3. XRPD Peak List of Ibuprofen-Lysine-Gabapentin co-crystal

Position 2 θ (°)	d-spacing [Å]	Intensity
6.86	12.87176	666
9.45	9.35542	34800
10.27	8.61064	1970
11.98	7.37902	249
14.72	6.01471	1010
15.85	5.58853	2280
17.14	5.16973	16100
17.61	5.0328	9120
18.70	4.74233	14500
20.03	4.42983	10600
22.31	3.98153	1960
24.13	3.68462	2200
25.06	3.55052	2510
25.57	3.48078	2430
26.29	3.38739	900
27.87	3.19912	2010
28.56	3.12317	2920
30.62	2.91753	280
31.13	2.87064	284
32.30	2.76943	567
33.09	2.70495	251
34.52	2.59653	570
35.36	2.53672	642
36.57	2.455	791
38.56	2.33313	523
39.03	2.30589	400
41.11	2.19415	530
48.93	1.85987	109

5 XRPD diffractograms of Flurbiprofen-Lysine-Gabapentin and Ibuprofen-Lysine-Gabapentin were very similar, with the reflexes of the respective main peaks in the region between 9 - 10 degrees 2-theta, 15 - 25 degrees 2-theta and 27 - 28 degree 2-theta having similar intensities (see Figure 1 and Tables 2 and 3). This similarity was taken as clear evidence of isostructurality of the co-crystals.

3. Thermal analyses

10 DSC Analysis

The analysis was carried out using the instrument DSC Mettler Toledo DSC1.

The sample was weighed in an aluminum pan hermetically sealed with an aluminum cover. The analysis was performed by heating the sample from 25°C to 320°C at 10°K/min, under the conditions shown in Table 3a below:

Table 3a

Temperature Data	
Temperature range	25 °C to 320 °C
Temperature accuracy	± 0.2° K
Temperature precision	± 0.02° K
Furnace temperature resolution	± 0.00006° K
Heating rate	0.02 to 300° K/min
Cooling rate	0.02 to 50° K/min
Cooling time	5 min (100 °C to 0 °C)
Calorimetric Data	
Sensor type	FRS5
Sensor material	Ceramic
Number of thermocouples	56
Signal time constant	1.8 s
Indium peak (height to width)	17
TAWN resolution	0.12
Sensitivity	11.9
Resolution	0.04 µW
Digital resolution	16.8 million points

The analysis was carried out on samples of Flurbiprofen-Lysine-Gabapentin co-crystal (Figure 2) and Ibuprofen-Lysine-Gabapentin (Figure 3).

- 5 The DSC profile of Flurbiprofen-Lysine-Gabapentin showed an endothermic event at 163.48 °C (onset 161.59 °C, Delta H = 153.58 J/g) that was associated with sample melting and degradation (Figure 2). The DSC profile of Ibuprofen-Lysine-Gabapentin showed endothermic event at 165.60 °C (onset 161.60 °C, Delta H = 157.73 J/g), while above 120 °C, multiple partially overlapped endothermic peaks were detectable due to degradation steps (Figure 3). Surprisingly, Flurbiprofen-Lysine-Gabapentin and Ibuprofen-Lysine-Gabapentin showed similar enthalpy of fusion (153.58 and 157.73 J/g) that further support their isostructural packing. Furthermore, lower melting endotherms of co-crystals are supposed to improve the solubility/dissolution profile of the native drug [(a) M. K. Mishra, P. Sanphui, U. Ramamurty and G. R. Desiraju, Cryst. Growth Des., 2014, 14, 3054–3061; (b) P. Sanphui, N. R. Goud, U. B. R. Khandavilli and A. Nangia, Cryst. Growth Des., 2011, 11, 4135–4145].
- 10
15

Thermogravimetric Analysis TGA

The analysis was carried out using the instrument Mettler Toledo TGA/DSC1.

- The sample was weighed in an aluminum pan hermetically sealed with an aluminum pierced cover. The analysis was performed by heating the sample from 25 °C to 320 °C at 10 °/min, under the conditions shown in Table 4 below:
- 20

Table 4

Temperature Data	
Temperature range	25 °C to 320 °C
Temperature accuracy	±1 ° K
Temperature precision	±0.4 ° K
Heating rate	0.02 to 250 ° K/min
Cooling time	20 min (1100 to 100 °C)
Sample volume	≤100 µL
Special modes	
Automation	34 sample positions
TGA-FTIR	coupled with Thermo Nicolet iS10 spectrometer
Balance data	XP5
Measurement range	≤5 g
Resolution	1.0 µg
Weighing accuracy	0.005%
Weighing precision	0.0025%
Internal ring weights	2
Blank curve reproducibility	better than ±10 µg over the whole temperature range

The TGA analysis of Flurbiprofen-Lysine-Gabapentin co-crystal showed a weight reduction of only 1.686 % before the melting point, confirming that the co-crystal was almost free from solvents (Figures 4).

The TGA analysis of Ibuprofen-Lysine-Gabapentin co-crystal showed a loss of 8.007% by weight confirming the loss of solvation water (Figures 5).

4. FT-Raman and FT-IR

FT-Raman

Raman spectra were recorded with a Nicolet iS50 FT-IR Spectrometer. The excitation source was an Nd-YAG laser (1064 nm) in the backscattering (180°) configuration. The focused laser beam diameter was approx. 50 mm and the spectral resolution 4 cm⁻¹. The spectra were recorded with a laser power at the sample of approx. 100 mW.

FT-IR

The analysis was carried out using an instrument Thermo Nicolet iS50 - ATR module Spectrometer equipped with Smart Performer Diamond, DTGS KBr Detector, IR Source, KBr Beam splitter, under the conditions shown in Table 5 below:

Table 5

Data Collection Information	
Number of sample scans:	32
Number of background scans:	32
Collection length:	47.29 sec
Resolution:	4.000
Levels of zero filling:	2

Number of scan points:	16672
Number of FFT points:	65536
Laser frequency:	15798.3 cm ⁻¹
Interferogram peak position:	8192
Apodization:	N-B strong
Phase correction:	Mertz
Number of background scans:	32
Background gain:	1.0
Sample gain:	8
Aperture	100
Optical velocity	0.6329

The Raman spectra of Flurbiprofen-Lysine-Gabapentin and Ibuprofen-Lysine-Gabapentin co-crystals are reported in Figures 6 and 7 and the peaks lists are reported in Tables 6 and 7.

5 Table 6: Raman peaks list of Flurbiprofen-Lysine-Gabapentin co-crystal

Wavenumber (cm ⁻¹)	Rel. Intensity
3069	0.002
2970	0.001
2919	0.005
2861	0.001
2240	0.001
2147	0.001
1624	0.016
1604	0.007
1513	0.002
1450	0.004
1287	0.007
1161	0.001
1038	0.002
1001	0.005
977	0.001
878	0.001
737	0.003
618	0.002
506	0.001
414	0.001
272	0.001
223	0.002
87	0.002

Table 7: Raman peaks list of Ibuprofen-Lysine-Gabapentin co-crystal

Wavenumber (cm ⁻¹)	Rel. Intensity
3055	0.001
2919	0.006
2868	0.002
1613	0.003
1449	0.005
1396	0.001
1344	0.001
1187	0.003
1119	0.001
1091	0.001
979	0.001
885	0.001
835	0.002
757	0.001
704	0.001
640	0.001
507	0.001
414	0.001
63	0.011

The FT-IR spectra of Flurbiprofen-Lysine-Gabapentin and Ibuprofen-Lysine-Gabapentin co-crystals are shown in Figures 8 and 9 and the peaks lists are reported in Tables 8 and

5

9.

Table 8: FT-IR peaks list of Flurbiprofen-Lysine-Gabapentin co-crystal

Wavenumber (cm ⁻¹)	Rel. Intensity	Wavenumber (cm ⁻¹)	Rel. Intensity
2965	0.011	10612	0.089
2932	0.257	1028	0.030
2849	0.026	1011	0.058
2763	0.017	974	0.055
2596	0.024	944	0.044
2164	0.051	927	0.167
1628	0.189	911	0.036
1581	0.043	873	0.202
1546	0.039	835	0.108
1519	0.013	811	0.076
1504	0.343	793	0.017
1475	0.035	765	0.231
1451	0.063	737	0.086
1413	0.070	715	0.135
1392	0.542	697	0.388
1359	0.094	661	0.043
1340	0.020	644	0.102
1282	0.182	619	0.100

1240	0.022	578	0.073
1232	0.079	557	0.159
1206	0.059	522	0.077
1179	0.022	507	0.109
1156	0.098	478	0.046
1129	0.041	450	0.034
1112	0.025	421	0.349
1089	0.065	379	0.018

Table 9: FT-IR peak list of Ibuprofen-Lysine-Gabapentin co-crystal

Wavenumber (cm ⁻¹)	Rel. Intensity	Wavenumber (cm ⁻¹)	Rel. Intensity
2924	0.146	1090	0.040
2865	0.014	1063	0.023
2596	0.011	1025	0.028
2187	0.004	1002	0.011
2162	0.023	975	0.023
1900	0.006	945	0.036
1854	0.005	929	0.008
1816	0.004	910	0.020
1787	0.006	881	0.080
1765	0.006	848	0.037
1738	0.004	819	0.006
1725	0.010	784	0.040
1710	0.009	743	0.062
1691	0.007	715	0.140
1631	0.109	683	0.032
1581	0.016	633	0.009
1547	0.028	618	0.034
1502	0.194	593	0.011
1475	0.027	579	0.018
1451	0.036	556	0.082
1411	0.016	506	0.031
1392	0.283	495	0.007
1360	0.061	422	0.173
1287	0.062	399	0.029
1242	0.029	376	0.022
1206	0.018		
1183	0.006		
1160	0.042		
1114	0.014		

Even in the Raman and FT-IR spectra, significant similarities among the co-crystals were
5 observed, together with some differences that could be due to the presence of different
NSAIDs. All the spectra seemed to indicate that the two crystalline systems are

characterized by a very similar local environment, especially regarding Lysine and Gabapentin, and show comparable vibrational behaviors.

5. Liquid and solid state NMR

¹H-Nuclear magnetic resonance (NMR) spectra were recorded in the indicated solvent with tetramethylsilane (TMS) as internal standard on a Bruker Avance3 400 MHz instrument. Chemical shifts are reported in parts per million (ppm) relative to the internal standard. Abbreviations are used as follows: s=singlet, d= doublet, t=triplet, q=quartet, m=multiplet, dd=doublet of doublet, br=broad. Coupling constants (J values) are given in hertz (Hz).

The solid-state ¹³C CPMAS spectra of the samples were acquired with a Jeol ECZR 600 instrument, operating at 600.17 and 150.91 MHz, respectively for ¹H and ¹³C nuclei. The powder samples were packed into a cylindrical zirconia rotor with a 3.2 mm o.d. and a 60 µl volume. A sample was collected from each batch and used without further preparations to fill the rotor. The ¹³C CPMAS spectra were acquired at room temperature, at a spinning speed of 20 kHz, using a ramp cross-polarization pulse sequence with a 90° ¹H pulse of 2.1 µs and a contact time of 3.5 ms. An optimized recycle delay from 5.7 for co-crystal to 100 s was used, for a number of scans from 2200 to 20. For every spectrum, a two-pulse phase modulation (TPPM) decoupling scheme was used, with a radiofrequency field of 108.5 kHz. The ¹³C chemical shift scale was calibrated through the methylene signal of external standard glycine (at 43.7 ppm). As for the ¹³C T₁ ¹H analysis, 12 spectra were acquired for 350 scans with different relaxation delays, included in the range 0.1-60 s and calculated by the Delta v5.2.1 software through an exponential algorithm. The spectra were acquired at a spinning speed of 20 kHz at room temperature using a ramp cross-polarization pulse sequence with a 90° ¹H pulse of 2.1 µs and a contact time of 2 ms.

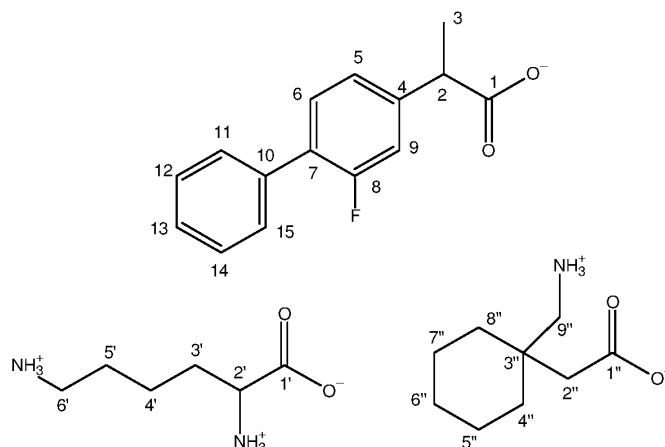
¹H-NMR spectra of Flurbiprofen-Lysine-Gabapentin

¹H-NMR spectrum of the co-crystal confirmed the concomitant presence in the sample of the Flurbiprofen-Lysine-Gabapentin with 1:1:1 stoichiometry.

The multiplicity and the assignment of the signals in line with the atoms numbering shown in the scheme 1 are reported in Table 10:

Scheme 1. Chemical structure and atom numbering of Flurbiprofen-Lysine-Gabapentin

Scheme 1

Table 10: ¹H-NMR

δ ppm	Multiplicity	Assignment
7.62-7.64	m, 2H	Ar FLU
7.44-7.55	m, 4H	Ar FLU
7.19-7.25	m, 2H	Ar FLU
3.69-3.77	m, 2H	CH (2') LYS; CH (2) FLU
3.00-3.03	m, 4H	CH ₂ (6') LYS; CH ₂ (9'') GAB
2.42	s, 2H	CH ₂ (2'') GAB
1.87-1.93	m, 2H	CH ₂ (3') LYS
1.72	quint., J = 7.6 Hz, 2H	CH ₂ (5') LYS
1.37 -1.51	m, 15H	5 CH ₂ (4'', 5'', 6'', 7'', 8'') GAB; CH ₂ (4') LYS; CH ₃ (3) FLU
Flurbiprofen-Lysine-Gabapentin Ratio 1:1:1		

The ¹H-NMR spectrum (400 MHz, D₂O) of Flurbiprofen-Lysine-Gabapentin co-crystal is shown in Figure 10.

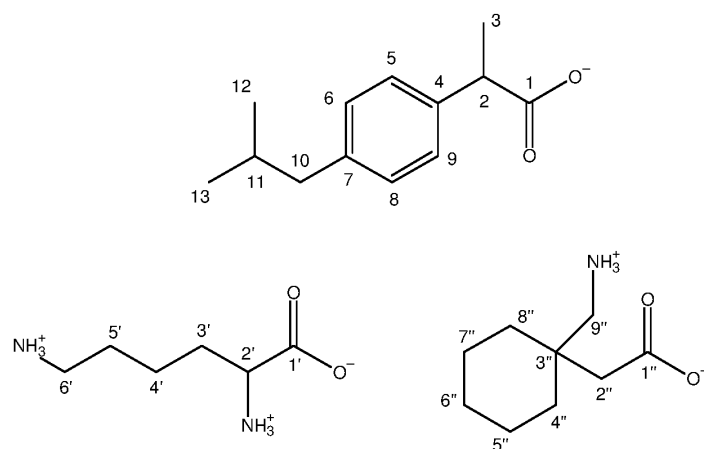
¹H-NMR spectra of Ibuprofen-Lysine-Gabapentin

¹H-NMR spectrum of Ibuprofen-Lysine-Gabapentin co-crystal confirmed the concomitant presence in the sample of Ibuprofen-Lysine-Gabapentin with 1:1:1 stoichiometry.

The multiplicity and the assignment of the signals in line with the atoms numbering shown in the Scheme 2 are reported in Table 11:

Scheme 2. Chemical structure and atom numbering of Ibuprofen-Lysine-Gabapentin

Scheme 2

Table 11: ^1H -NMR

δ ppm	Multiplicity	Assignment
7.21-7.28	m, 4H	Ar IBU
3.75	t, $J = 6.1$ Hz, 1H	CH (2') LYS
3.62	quart., $J = 7.2$ Hz, 1H	CH (2) IBU
3.00-3.03	m, 4H	CH ₂ (6') LYS; CH ₂ (9'') GAB
2.48	d, $J = 7.1$ Hz, 2H	CH ₂ (10) IBU
2.43	s, 2H	CH ₂ (2'') GAB
1.81-1.93	m, 3H	CH (11) IBU; CH ₂ (3') LYS
1.72	quint., $J = 7.6$ Hz, 2H	CH ₂ (5') LYS
1.37 -1.49	m, 15H	5 CH ₂ (4'', 5'', 6'', 7'', 8'') GAB; CH ₂ (4') LYS; CH ₃ (3) IBU
0.88	d, $J = 6.6$ Hz, 6H	2 CH ₃ (12, 13) IBU
Ibuprofen-Lysine-Gabapentin Ratio 1:1:1		

5

The ^1H -NMR spectrum (400 MHz, D_2O) of Ibuprofen-Lysine-Gabapentin co-crystal is shown in Figure 11.

Solid state ^{13}C CPMAS spectra of co-crystal Flurbiprofen-Lysine-Gabapentin

A new homogeneous phase of Flurbiprofen-Lysine-Gabapentin was confirmed by ^{13}C -CPMAS spectra. The stoichiometry was assessed to be 1:1:1, with one independent molecule of Flurbiprofen, Lysine and Gabapentin in the unit cell.

10

In Table 12 below the characteristic solid state ^{13}C NMR signals are summarized:

Table 12: solid state ^{13}C NMR

^{13}C δ (ppm)	Assignment
181.3	1
179.5	1"
178.2	1'
160.1	8
148.2	Aromatic C_q
136.0	Aromatic C_q
132.3	Aromatic C_q
130.0	2 Aromatic CH
128.3	4 Aromatic CH
125.0	Aromatic CH
113.0	9
54.4	2'
49.2	9" + 2
42.6	6'
38.6	2" + 4" or 8"
34.4	3' + 6" + 4" or 8"
30.7	5' + 3"
26.1	4'
23.1	5" or 7"
21.4	3
20.7	5" or 7"

From ^{13}C SSNMR analysis, the carboxylic/carboxylate moieties of the substances are involved in a variety of different interactions (Figure 12 and 13):

- Pure Flurbiprofen (FLU) displays carboxylic homodimers in its crystal structure, leading the associated signal to 183.4 ppm.
- Flurbiprofen sodium salt (NaFLU, Figure 13) displays a carboxylate peak falling at 183.9 ppm;
- In the two Flurbiprofen co-crystals reproduced from literature (salicylamide FLU·SALA and picolinamide, FLU·PICA), the neutral COOH moiety of Flurbiprofen is involved in hydrogen bonds with the aromatic N of the two amides. The corresponding signals resonate at 178.6 (FLU·SALA) and 177.8 (FLU·PICA) ppm respectively.
- Pure Gabapentin (GAB) is a zwitterion, with a COO^- moiety resonating at 179.0 ppm; as sodium salt (NaGAB) a neutral NH_2 moiety is present instead of a NH_3^+ group, but it still has a carboxylate group that resonates at 183.4 ppm.
- Zwitterionic L-Lysine (L-LYS) is characterized by a carboxylate signal at 176.7 ppm, while the double hydrochloride salt of DL-lysine (DL-LYS·2HCl) displays a neutral COOH group, whose peak falls at 171.7 ppm.

The spectrum of Flurbiprofen-Lysine-Gabapentin (FLU-LYS-GAB) features three distinct peaks in the carboxylic region, at 181.3, 179.5 and 178.2 ppm. Given the chemical shifts of the starting materials, it is reasonable to assume that they belong to Flurbiprofen, Gabapentin and Lysine, respectively.

5 ¹⁵N CPMAS analysis was performed on the co-crystal, as well as on NaGAB. A comparison of their spectra, together with those of L-LYS and GAB, is displayed in Figures 14 and 15.

Flurbiprofen-Lysine-Gabapentin displays three distinct ¹⁵N resonances, at 37.6, 30.2 and 21.6 ppm, which confirms the presence of a single independent molecule of Lysine (accounting for 2 signals) and of Gabapentin (accounting for 1) in the unit cell. The chemical shifts suggest that in the co-crystal the α-N and ε-N of Lysine are protonated (i.e. in the form of NH₃⁺) as well as the nitrogen of Gabapentin (Figures 14 and 15).

Solid state ¹³C CPMAS spectra of co-crystal Ibuprofen-Lysine-Gabapentin

A new homogeneous phase of Ibuprofen-Lysine-Gabapentin was confirmed by ¹³C-CPMAS spectra. The stoichiometry was assessed to be 1:1:1, with one independent molecule of Ibuprofen, Lysine and Gabapentin in the unit cell. In Table 13 below the characteristic solid state ¹³C NMR signals are summarized:

Table 13: solid state ¹³C NMR

¹³ C δ (ppm)	Assignment
182.8	1
181.3	1
179.1	1"
178.0	1'
143.4	Aromatic C _q
142.9	Aromatic C _q
141.5	Aromatic C _q
139.9	Aromatic C _q + CH
129.0	Aromatic CH
127.6	Aromatic CH
54.3	2'
49.6	9'' or 2
49.2	9'' or 2
46.1	10
42.4	6'
38.5	2'' + 4'' or 8''
34.3	3' + 6'' + 4'' or 8''
30.4	5' + 3'' + 11
29.8	5'' or 7''
25.9	4'
23.1	5'' or 7'' + 12 + 13
16.4	3

Figure 16 displays the ^{13}C CPMAS NMR spectrum of Ibuprofen-Lysine-Gabapentin co-crystal.

Regarding ^{13}C CPMAS NMR of Ibuprofen-Lysine-Gabapentin, two signals ascribable to
5 Ibuprofen (IBU) appear, at 182.8 and 181.3 ppm (Figure 17). The first chemical shift is very similar to that of pure Ibuprofen (182.9 ppm), in which the COOH moiety is involved in homodimeric interactions, and to that of the carboxylate group of Ibuprofen sodium salt (NaIBU) at 183.4 ppm. This suggests that it corresponds to a carboxylate moiety, as further implied by the very much lower chemical shift of a neutral COOH of Ibuprofen in
10 the co-crystal with L-proline (176.7 ppm). As for the other three carboxylic signals, all the considerations done for Flurbiprofen-Lysine-Gabapentin apply here as well. The same can be said for the ^{15}N CPMAS spectra, displayed in Figures 18 and 19.

The ^{13}C CPMAS spectra of, Flurbiprofen-Lysine-Gabapentin and Ibuprofen-Lysine-Gabapentin co-crystals are very similar as shown in Figure 20. Many of the signals of the
15 two co-crystals (highlighted in Figure 20 by means of dashed lines) coincide. Careful analysis of the signals points out that all the recurring signals in the aliphatic region (below 80 ppm) correspond to the aliphatic C nuclei of Lysine and Gabapentin. The most apparent differences relate to the aromatic range (110-170 ppm), which is reasonable, since the two different NSAIDs are those containing aromatic carbons. Most notably, the
20 the same similarities can be observed in the carboxylic region, with the carboxylic signals of Flurbiprofen-Lysine-Gabapentin appearing also for Ibuprofen-Lysine-Gabapentin.

The same remarkable spectral similarities (highlighted by dashed lines) can be observed in the ^{15}N CPMAS spectra of the samples (Figure 21).

From ^{13}C and ^{15}N data, the local environment of the Lysine and Gabapentin fragments
25 seems to be the same for the two crystal forms. In all cases, Gabapentin is a zwitterion, while Lysine displays the usual COO^- and $\alpha\text{-NH}_3^+$ groups. The $\epsilon\text{-N}$ of Lysine and the COOH group of the NSAID share the carboxylic proton.

The results strongly suggested the occurrence of a protonic transfer from the COOH moiety of Flurbiprofen and Ibuprofen, which turns into a carboxylate moiety, to the only
30 possible acceptor, the $\epsilon\text{-NH}_2$ of Lysine.

In line with the solid state ^{13}C and ^{15}N NMR analysis, in the present co-crystals Flurbiprofen and Ibuprofen carboxylic group is deprotonated and interacts with protonated Lysine $\epsilon\text{-NH}_3^+$ group through ionic bonds forming a neutral salt. The Flurbiprofen and

Ibuprofen Lysine neutral salt interacts with Gabapentin through non-ionic bonds forming a stable co-crystal.

6. In vivo studies

Inflammatory pain induced by carrageenan intraplantar injection in rats

5 Male Wistar rats (270-280 g) (Envigo, Italy), were housed 2 - 3 per cage under controlled illumination (12:12 h light : dark cycle; light on 06.00 h) and standard environmental conditions (room temperature 22 ± 1 °C, humidity $60 \pm 10\%$) for at least 1 week before experimental use. Rat chow and tap water were available ad libitum. The procedures were approved by the Animal Ethics Committee of University of Campania "Luigi Vanvitelli". Animal care was in compliance with Italian Legislative Decree (D.L. 116/92) and European Commission Directive (O.J. of E.C. L358/1, 18/12/86) regulations on the protection of laboratory animals. All efforts were made to minimize animal suffering and the number of animals used.

Carrageenan-induced Rat Paw Edema test method

15 Peripheral inflammatory pain was induced in the left hind paw of each animal by a single intraplantar injection of 1% λ -carrageenan (100 μ l for each rat in 0.9% NaCl) (Sigma-Aldrich, St. Louis, MO), by using a 30G insulin syringe, accordingly with previous studies (Hajhashemi V et al., The role of central mechanisms in the anti-inflammatory effect of amitriptyline on carrageenan-induced paw edema in rats. Clinics (Sao Paulo). 2010).
20 Vehicles or drugs were orally administered 1 h before the carrageenan injection. The paw volume was measured by Plethysmometer (Ugo Basile, Varese, Italy) before (0 h) and after injection of carrageenan at different time intervals (1, 3 and 5 h post-carrageenan). Edema was expressed as the mean increase in paw volume (ml) relative to control animal group. The inhibition percentage of edema was calculated by the following equation:

25
$$\% \text{ inhibition of edema} = [(V_c - V_t) * 100 / V_c]$$

where V_c is the edema volume in the control group and V_t is the edema volume in treated group. After 5 h, the animals were sacrificed with a lethal dose of urethane and paw and stomach were dissected for morphological and biochemical evaluations.

Mechanical allodynia

30 Mechanical allodynia was assessed using the up and down method (Chaplan, S. R., et al., (1994) Quantitative assessment of tactile allodynia in the rat paw. J. Neurosci. Methods 53, 55–63). All animals were allowed to acclimate for ~30-45 minutes on an elevated mesh platform in an enclosure (Ugo Basile, Italy). Calibrated von Frey filaments for rats (Stoelting, Wood Dale, IL, ranging from 4 g to 100 g bending force) were applied

to the midplantar surface of the hind paw for 3–4 s (Nour Elhouda Saidi et al., Unilateral 6-Hydroxydopamine-Lesioned Rat as Relevant Model to Study the Pain Related to Parkinson's Disease, NEUROLOGY AND NEUROBIOLOGY (2019). ISSN 2613-7828). The threshold was captured as the lowest force (g) that evoked scratching or licking of the stimulated hind-paw. Animals were tested at baseline (0 h) before the carrageenan injection and at 1, 3, and 5 h post-carrageenan.

Experimental groups and drugs

Vehicle 1 (torpac capsules filled with Avicel PH101, 1cps) (n=4); Vehicle 2 (ethanol/0.9% saline, 1:19, 100ul) (n=4); Indomethacin (10mg/Kg, 100ul) in Vehicle 2 (n=8); Flurbiprofen (5 mg/Kg, 1 cps) (n=8); Gabapentin (3.51mg/Kg, 1cps) (n=8); Flur+Lys+Gaba mixture (11.50 mg/Kg, 1cps) (n=8); Flur/Lys/Gaba co-crystal (11.50 mg/Kg, 1 cps) (n=8). The weight ratio of Flurbiprofen, Lysine and Gabapentin in the Flur+Lys+Gaba mixture is 1:1:1.

Comparative effect of single oral administration of Flur/Lys/Gaba co-crystal and Flurbiprofen alone on carrageenan-induced paw edema in rats

Single administration of both vehicles (capsules or ethanol/0.9% saline, i.g.) did not affect the increase in the rat left paw thickness induced by carrageenan intra-plantar injection, as compared to the untreated contralateral paw (4.71 ± 0.17 mm, 3 h post-carrageenan). In particular, a time-dependent paw swelling was observed starting from 1 h with a peak of edema at 5 h (9.55 ± 0.47 mm, $p < 0.0001$; $n = 8$) post-carrageenan injection (Figure 22). Single administration of Flurbiprofen (5 mg/Kg), performed 1 h before carrageenan injection, significantly reduced rat paw thickness (6.5 ± 0.28 mm at 5 h post-carr, $p = 0.028$, $n = 8$), as compared to vehicle-treated group (Figure 22, Table 14), with a reduction of paw volume of -32.0 % (Table 15).

In Table 14 below, the statistic of paw thickness in different group of animals treated with Vehicle, Flurbiprofen alone, Gabapentin, Indomethacin, the Flur+Lys+Gaba mixture or the Flur/Lys/Gaba co-crystal after intra-plantar injection of 1% of carrageenan, is reported. Each time point represents the mean $\pm$ SEM of 8 rats per group. $P < 0.05$ was considered as statistically significant and calculated by using Two-way ANOVA followed by Tukey's post-hoc test.

^{****} $p < 0.0001$ vs Contra, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ vs Vehicle and [#] $p < 0.05$ and ^{##} $p < 0.01$ vs Gaba. In table 14 below, "ns" stands for "non significant".

Table 14: Statistic of paw thickness in different group of animals

	Vehicle	Flurbiprofen	Gaba	Indomethacin	Flur+Lys+Gaba	Flur/Lys/Gaba
1h	oooo	*	ns	**	*	**
3h	oooo	**	ns	**	**	*** ##
5h	oooo	**	*	**	**	*** #

Table 15: volume reduction of paw edema in rats (%)

	Flurbiprofen	Gaba	Indomethacin	Flur+Lys+Gaba	Flur/Lys/Gaba
1h	-18.20	-1.93	-17.68	-17.57	-21.80
3h	-31.50	-16.21	-35.27	-29.32	-39.91
5h	-32.00	-17.27	-40.45	-35.47	-42.27

5

Whereas, the administration of Flur/Lys/Gaba (11.5 mg/Kg) co-crystal greatly reduced the paw thickness (5.51 ± 0.42 mm at 5h post-carr; $p=0.0003$, $n=8$), with an inhibition percentage of paw volume of -42.27 %, as compared to the vehicle-treated rats (Figure 22, Table 15). Similarly, Indomethacin (10 mg/Kg, i.g.), used as a reference drug in this study, reduced paw thickness in a significant way at all time points of observation (5.69 ± 0.27 mm at 5h post-carr; $p=0.0002$, $n=8$) with a reduction in percentage of paw volume of -40.45 %, respect to vehicle-treated group (Figure 22, Table 15). Finally, Gabapentin alone at 3.51 mg/Kg was able to reduce paw swelling only at 5 hours after carrageenan injection (7.45 ± 0.26 mm at 5h post-carr; $p=0.0284$, $n=8$) with a volume reduction in percentage of -17.27 % (Figure 22, Table 15). Two-way ANOVA analysis showed a significant effect of treatments ($F_{6, 49} = 57.48$, $P < 0.0001$), of time ($F_{3, 147} = 45.10$, $P < 0.0001$), and a significant interaction for factors time $\times$ treatment ($F_{18, 147} = 7.042$, $P < 0.0001$) was observed.

Comparative effect of single oral administration of Flur/Lys/Gaba co-crystal and Flurbiprofen alone on the mechanical allodynia in a rat carrageenan-induced paw edema model

In another set of experiments, single oral administration (i.g) of Flurbiprofen (5 mg/Kg) and of Flur/Lys/Gaba co-crystal (11.5 mg/Kg, cps) were tested on mechanical allodynia in carrageenan-injected animals as compared to Indomethacin (10 mg/kg), Gabapentin (3.51 mg/Kg) or Vehicles (capsules or ethanol/0.9% saline). In particular, the analysis was performed at baseline (0), before the carrageenan intra-plantar injection, and at 1, 3 and 5 hours after carrageenan by using manual Von Frey filaments. We observed that mechanical withdrawal threshold significantly decreased in the ipsilateral paw starting from 1h post-carrageenan and it was maintained until the end of observation (5h) in

vehicle-treated animals (8.25 ± 1.61 g at 3h post-carr, $p < 0.0001$; $n=8$), as compared to the baseline (80 ± 7.56 g, time 0) (Figure 23). No changes were observed in the contralateral side of carrageenan injection (85 ± 7.31 g at 3h post-carr). Single oral administration of Flurbiprofen showed a strong anti-inflammatory but not significant anti-allodynic effect after paw carrageenan injection. Indeed, Flurbiprofen was not able to increase paw withdrawal threshold in rats 3 h post-carrageenan injection (44.62 ± 14.43 g, $p=0.2802$; $n=8$), as compared to vehicle-injected animals (Figure 23). Moreover, Indomethacin (10 mg/Kg) treated animals showed a non-significant trend in increasing paw thresholds at 3 hours post-carrageenan (36 ± 7.21 g, $p=0.0567$; $n=8$). On the contrary, Flur/Lys/Gaba co-crystal induced a reduction of mechanical allodynia up to the end of the experiment, starting from 1h post carrageenan injection, reaching a peak at 3 h post carrageenan injection (85.75 ± 9.86 g, $p=0.001$; $n=8$) as compared to vehicle-injected animals (Figure 23). Finally, Gabapentin (3.51 mg/Kg) administration showed a trend in decreasing mechanical allodynia, with a peak-time at 3h post-carrageenan injection (66.5 ± 11 g, $p=0.0109$; $n=8$) (Figure 24). Two-way ANOVA analysis showed a significant effect of treatments ($F_6, 49 = 22.13$, $P < 0.0001$), of time ($F_3, 147 = 39.29$, $P < 0.0001$), and significant interaction for factors time $\times$ treatment ($F_{18, 147} = 2.30$, $P = 0.0035$) was observed. Table 16 below indicates the statistic of paw withdrawal threshold (g) in different groups of animals has been represented. $P < 0.05$ was considered as statistically significant and calculated by using Two-way ANOVA followed by Tukey's post-hoc test. $^{\circ\circ\circ}p < 0.0001$ vs Contra, $^*p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ vs Vehicle and $\#p < 0.05$ and $\#\#p < 0.01$ vs Gaba.

In Table 16 below reported, "ns" stands for "not significant".

Table 16: statistic of paw withdrawal threshold (g)

	Vehicle	Flurbiprofen	Gaba	Indomethacin	Flur+Lys+Gaba	Flur/Lys/Gaba
1h	oooo	ns	*	*	* oooo #	* ##
3h	oooo	ns	*	ns	*	***
5h	oooo	ns	*	ns	*	*** ##

7. Determination of the exposure parameters in plasma of NSAIDs and Gabapentin after their oral administration as capsules in the rats

Aim of the study was the determination of pharmacokinetic parameters of NSAIDs, in particular Flurbiprofen and Ibuprofen, and Gabapentin in NSAID-Lysine-Gabapentin co-crystal compared to the physical mixture of NSAID, Lysine and Gabapentin.

Sprague Dawley male rat (body weights 310 gr at the time of the treatment) were used in this study. The animals were originally supplied by Harlan, Italy. Once receipt from the supplier, the animals were subjected to health examinations and acceptance. The animals were housed, in a group of three, in cages suitable for the species and were routinely kept in the following environment except for short periods of time where experimental procedures dictated otherwise. The animals were acclimatized to local housing conditions for approximately 5 days.

The animals were housed in a single, exclusive room, air conditioned to provide a minimum of 15 air changes/hour. The environmental controls were set to maintain temperature within the range 22 °C and relative humidity within the range 50 to 60% with an approximate 12 hour light and 12 hour dark cycle that is controlled automatically. Food (Mucedola Standard GLP diet) and water were available *ad libitum* throughout the study. All animals were weighed on the day of each treatment. Clinical signs were monitored at regular intervals throughout the study in order to assess any reaction to treatment. Each animal was uniquely identified with a coloured spray on the back before the experiment. At the end of the study animals were sacrificed by exsanguination under anaesthesia. The experiment was carried on in agreement with the Italian Law D. L.vo 4 marzo 2014, n. 26.

The experimental protocol consisted in the blood and brain tissue sampling on the animals according to the following Tables 17 and 18 and analysis of samples as described below.

Table 17: Blood Sampling

Animals/Time Point	7 time points
Time points	30 min, 1, 2, 3, 6, 8, and 24h
Fasting Requirements	Not required
Collection Site	Animals were exsanguinated from caudal vein
Collection tube	Li heparin anticoagulant
Target Blood Volume	70 µL
Sample Identification	Label indicating: Study number, animal ID, test item ID, sampling time
Sample Requirements	Stored in ice and centrifuged at +4° C, 3000 g for 10 minutes
Final Sample Storage Conditions	-20 °C until bioanalysis

Table 18: Cmax Flurbiprofen and Gabapentin

	Cmax (ng/mL) Flurbiprofen	Cmax (ng/mL) Gabapentin
Co-crystal	14725±1267	3008±246
Mixture	11495±1088	2560±157

As depicted in Table 18, following oral administration of Flurbiprofen/Lysine/Gabapentin co-crystal and of the physical mixture of Flurbiprofen, Lysine and Gabapentin
5 Flur+Lys+Gaba, given through one capsule/animal, systemic exposure parameters of Gabapentin were higher and reached the statistical significance (t-test=0.017).

For Flurbiprofen, Cmax was higher for the co-crystal compared to the mixture. The difference reached the statistical significance (t-test=0.023).

Thus, the higher Cmax of the co-crystal compared to the mixture means an improved
10 pharmacokinetic profile that can led to a better pharmacological efficacy.

CLAIMS

1. Co-crystal of a non-steroidal anti-inflammatory drug (NSAID) belonging to the class of phenylpropionic or phenylacetic acids, Lysine and Gabapentin, providing that said non-steroidal anti-inflammatory drug is not Ketoprofen.
- 5 2. The co-crystal as claimed in claim 1 wherein the molar ratio of the components in the co-crystal is 1:1:1.
3. The co-crystal as claimed in any one of claims 1 or 2, wherein said non-steroidal anti-inflammatory drug belonging to the class of phenylpropionic acid derivative is selected from Ibuprofen, Flurbiprofen, Fenoprofen, Indoprofen, Loxoprofen, Pelubiprofen, and Naproxen.
- 10 4. The co-crystal as claimed in any one of claims 1 or 2, wherein said non-steroidal anti-inflammatory drug belonging to the class of phenylacetic acid derivative is selected from Diclofenac, Felbinac, Ibufenac, Fenclofenac, Tifurac and Ketorolac.
5. The co-crystal as claimed any one of claims 1 to 3, wherein said non-steroidal anti-inflammatory drug is selected from Flurbiprofen and Ibuprofen.
- 15 6. The co-crystal as claimed in any one of claims 1 to 5, characterized by having the most intense XRPD diffraction peaks in the region between 9 - 10 degrees 2-theta, 15 - 25 degrees 2-theta and 27 - 28 degree 2-theta.
7. The co-crystal as claimed in any one of claims 1 to 6, characterized by the following common XRPD diffraction peaks: 9.3, 17.1, 18.5, 19.8, 22.1, 24.1, 24.9, 27.9 degrees 2-theta $\pm$ 0.4 degrees 2-theta.
- 20 8. The co-crystal as claimed in claim 1 or 2, in which said NSAID is Flurbiprofen, characterized by the following XRPD diffraction peaks: 9.3, 10.4, 15.2, 16.0, 17.2, 18.3, 18.8, 19.7, 20.7, 21.9, 24.0, 24.8, 27.9, and 29.0 degrees 2-theta $\pm$ 0.2 degrees 2-theta, preferably further characterized by the following XRPD diffraction peaks: 6.9, 10.9, 12.2, 25.5, 26.3, 29.8, 31.5, 33.0, 34.0, 35.9, 37.5, 39.2 and 40.8 degrees 2-theta $\pm$ 0.2 degrees 2-theta.
- 25 9. The co-crystal as claimed in claim 8, further characterized by one or more of the following:
- 30 a DSC thermogram as reported in Figure 2, characterized by a melting peak at 163.48°C $\pm$ 0.5°C,
a TGA thermogram as reported in Figure 4,
FT Raman and FT-IR spectra of Figures 6 and 8,
solution ¹H-NMR spectrum of Figure 10 and signals of Table 10,

solid state ^{13}C CPMAS spectrum of Figure 12 and signals of Table 12, and/or ^{15}N CPMAS spectrum of Figure 14.

10. The co-crystal as claimed in claim 1 or 2, in which said NSAID is Ibuprofen, characterized by the following XRPD diffraction peaks: 9.5, 10.3, 15.9, 17.1, 17.6, 18.7, 20.0, 22.3, 24.1, 25.1, 25.6, 27.9 and 28.6 degrees 2-theta $\pm$ 0.2 degrees 2-theta, preferably further characterized by the following XRPD diffraction peaks: 6.9, 12.0, 14.7, 26.3, 30.6, 31.1, 32.3, 33.1, 34.5, 35.3, 36.6, 38.6, 39.0, 41.1, and 48.9 degrees 2-theta $\pm$ 0.2 degrees 2-theta.

11. The co-crystal as claimed in claim 10, further characterized by one or more of the following:

a DSC thermogram as reported in Figure 3, characterized by a melting peak at $165.60^\circ\text{C} \pm 0.5^\circ\text{C}$,

a TGA thermogram as reported in Figure 5,

FT Raman and FT-IR spectra of Figures 7 and 9,

solution ^1H -NMR spectrum of Figure 11 and signals of Table 11,

solid state ^{13}C CPMAS spectrum of Figure 16 and signals of Table 13, and/or ^{15}N CPMAS spectrum of Figure 18.

12. The co-crystal as claimed in any one of claims 1 to 11, wherein said Lysine is (S,R)-Lysine.

13. A pharmaceutical composition comprising a co-crystal as claimed in any one of claims 1 to 12 and a least one pharmaceutically acceptable excipient.

14. The co-crystal as claimed in any one of claims 1 to 12 or the pharmaceutical composition as claimed in claim 13 for use as a medicament.

15. The co-crystal as claimed in any one of claims 1 to 12 or the pharmaceutical composition as claimed in claim 13 for use in the prevention, reduction or treatment of pain and/or inflammation, preferably of acute or chronic pain.

16. The co-crystal or the pharmaceutical composition for use as claimed in claim 15, wherein said pain is neuropathic or inflammatory pain and is selected from headache, toothache, menstrual pain, muscle pain, neuropathic pain, pain associated to neuroinflammation, diabetic neuropathy, cancer pain, osteoarthritis, low back pain, sciatalgia, fibromyalgia, trigeminal neuralgia, post-surgical and post-operative pain, post herpetic neuralgia, rheumatoid arthritis, ankylosing spondylitis, frozen shoulder, phantom limb pain or HIV pain.

17. Process for the preparation of a co-crystal as claimed in any one of claims 1 to 12, which comprises:

- a) suspending a non-steroidal anti-inflammatory drug (NSAID) belonging to the class of phenylpropionic or phenylacetic acids, Lysine, and Gabapentin in a suitable solvent,
- 5 b) dissolving said NSAID, Lysine, and Gabapentin optionally by heating the suspension, possibly under stirring, until a clear solution is obtained,
- c) optionally cooling the solution, and/or
- d) optionally adding an anti-solvent, thus providing the NSAID, Lysine, and Gabapentin co-crystal.

10 18. The process of claim 17 wherein the solvent to be used in step a) is selected from water, alcohols, preferably methanol and ethanol, esters, preferably ethyl acetate, ethers, preferably tetrahydrofuran and tert-butylmethyl ether and aromatic solvents, preferably toluene.

15 19. A method for the treatment of pain and/or inflammation comprising administering to the patient an effective amount of a co-crystal according to any one of claims 1 to 12 or of a pharmaceutical composition according to claim 13.