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Sparfloxacin Crystals And Pharmaceutical Salts Containing Dicarboxylic Acid: Increasing Sparfloxacin's Solubility And Permeability To Enhance Its In-Vitro Antimicrobial Activity

Pappu Lal Meena
Assistant Professor department of Chemistry,
MSJ GOVT. PG College Bharatpur Rajasthan

Abstract

Two new pharmaceutical salts/cocrystals of sparfloxacin (SPX) with oxalic acid and malonic acid have been designed, synthesized, and characterized to enhance its antimicrobial activity in vitro. The current research showed the co crystalalization of sparfloxacin with oxalic acid and malonic acid (SPX-OXA and SPX-MLO). The FTIR and XRD confirm the stable formation of target compound. Thermal analysis showed the water loss during the production of these co crystals. The crystal exhibit considerable antimicrobial activity against *Escherichia.coli*, *Staphylococcus. aureus* and *Candida albicans* as compared to sparfloxacin alone. This suggests that oxalic acid and malonic acid-prepared pharmaceutical salts or cocrystals of Spx can boost antibacterial action and improve solubility and permeability.

Key words: Sparfloxacin, Oxalic acid, Malonic acid, Co-crystallization, FTIR, Antimicrobial acrivity, XRD

1. Introduction

Despite the advancements in antibiotic discovery and production techniques, bacterial diseases causing untreatable infections pose a significant threat to public health in the 21st century (Sohail et al., 2021). Drug research aims to create solid pharmaceutical ingredients with optimal properties, safety, and reliability through rational design, while addressing solubility and permeability issues for maximum therapeutic efficacy (Zhang et al., 2022).

"Quinolones" refers to a class of synthetic antibacterial drugs called 4-quinolones, or 4-quinolonecarboxylic acids, which have a 4-oxo-1, 4-dihydroquinoline structure and a piperazine ring at position 7 and a fluorine atom at position 6 were shown to significantly broaden the range of action (Turel, 2002). Many fluoroquinolones, including ciprofloxacin, ofloxacin, levofloxacin, Sparfloxacin, and Gatifloxacin, were synthesized and tested following the significant discovery of norfloxacin. These fluoroquinolones exhibit broad-spectrum activity against a variety of pathogenic microorganisms in humans and animals that are resistant to aminoglycosides,

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Penicillins, cephalosporins, tetracyclines, and other antibiotics (Vieira et al., 2009). A third generation fluoroquinolone antibiotic drug Sparfloxacin is frequently used to treat mild to severe infections brought on by Gram-positive and Gram-negative bacteria (Beberok et al., 2015). It is mainly used to treat acute bacterial exacerbations of chronic bronchitis and community-acquired pneumonia (Joshi et al., 2018). Their action against DNA gyrase (topoisomerase II) and topoisomerase IV leads to the inhibition of prokaryotic DNA replication.

A potential result of co-crystallization is a supramolecular complex known as cocrystals, which are created by a variety of non-covalent interactions between the coformers, such as hydrogen bonds, π-π interactions, Van der Waals interactions, etc. (Abidi et al., 2017).It has been hypothesized that the pharmaceutical cocrystal technique can be used to manufacture sparfloxacin salts or cocrystals, which will increase the drug's permeability and solubility and boost its therapeutic efficacy (Liu et al., 2020).

2. Materials and methods

2.1. Materials Sparfloxacin (Spx), oxalic acid, malonic acid Ethanol (AR), DMSO (AR) n-octanol (AR) and DMSO (AR) were purchased from Himedia Scientific. All other chemicals and reagents were of pure and analytical grade.

2.2. Preparation of SPX-OXA and SPX-MLO

The SPX-OXA complex and SPX MLO complex was prepared according to the method of Liu et al., 2020 (Liu et al., 2020) with slight modifications A mixture of SPX (0.10 mmol) and oxalic acid (0.1 mmol) was dissolved into 10 mL mixture solution of ethanol and water (v/v = 4:1). The resulting mixture was sufficient stirred for 4 h under 40 °C thermostatic water bath. After filtration, the filtrate was evaporated slowly at room temperature. After three days, colorless transparent block crystals of SPX-OXA were harvested. A mixture of SPX (0.10 mmol) and malonic acid (0.1 mmol) was dissolved into 10 mL mixture solution of ethanol and water (v/v = 5:1). The resulting mixture was sufficient stirred for 3 h under 55 °C thermostatic water baths, and remaining steps are similar to the preparation of SPX-OXA. After three days, off white colored block crystals of SPX-MLO were harvested. The structure of active pharmaceutical ingredients used in study is shown in figure.1.

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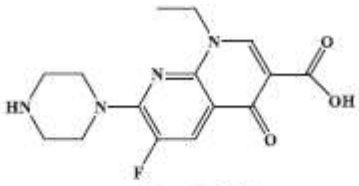
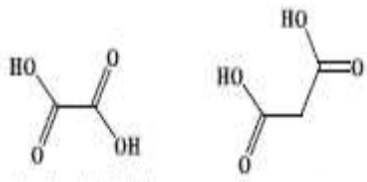


Sparfloxacin (Spx)



Oxalic acid (oxa) Malonic acid (MLO)

Figure. 1. Structure of active pharmaceutical ingredients used in this study.

2.3. FTIR analysis and X ray diffraction

IR spectra were collected in the 400–4000 cm−1 region with KBr pellet by Shimadzu 8400 FTIR spectrometer. X-ray powder diffraction (XRD) was conducted using a Rigaku D/ max 2500 V PC diffractometer at 40 kV and 200 mA, with Cu Kα radiation in a low background holder. The date was collected at room temperature with a scanning speed of 10°/min and the 2 range of 5–50°.

2.4. Thermogravimetric analyses (TGA)

Thermogravimetric analyses (TGA) were carried out by a perkin-Elmer TGA7 (PerkinElmer, USA). Approximately 5–10 mg of samples was placed in Al_2O_3 crucibles and instrument under the nitrogen atmosphere at a heating rate of 10 °C/min and in a range of 20–500 °C (Dai et al., 2016).

2.7. The antimicrobial assay

The antimicrobial activity of SPX-OXA and SPX-MLO and the corresponding parent compounds has been tested against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* by using the Kirby-Bauer agar diffusion method.

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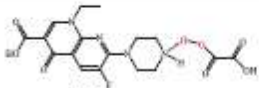
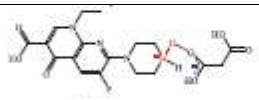
Result and discussion

In SPX-OXA, one enoxacin molecule and one oxalic acid molecule form a building unit through O4-H4···N2 and C3-H3A···O6 intermolecular hydrogen bonds. The adjacent building units are connected through water bridges via O7-H102···O6 and O7-H101···O3 interactions to form a new pattern of supramolecular synthon. Meanwhile the adjacent supramolecular through water bridges via O5-H103···O4, O5-H104···O4 and π-π interactions to result in simile to azipper motif. Adjacent 1D bands are connected through oxalic acid bridges via O5-H103···O4 and O5-H104···O4 to result in a 2D layered structure. In SPX-MLO, one proton is transferred from malonic acid to the piperazidine N atom of EX, and the MLO anion and EX cation form a building unit via Charge assisted hydrogen bonds (CAHBs) (N4-H4B•••O3 and N4-H4B•••O5). Through C13-H13A•••O2 hydrogen bonds, the neighboring building units are further connected to form a novel pattern of supramolecular synthon. In the meantime, π-π interactions link neighboring building units to form a the azipper motif. The comparison of SPX-OXA and SPX-MLO have been shown in table 1.

Table 1: The comparison of SPX-OXA and SPX-MLO

Compounds	**Structure**	**CAHBs**	**Distance of hydrogen bond (Å)**
SPX-OXA		No	2739
SPX-MLO		Yes	2767

CAHBs: charge assisted hydrogen bonds.

FTIR analysis XRD

The IR spectra of SPX-OXA and SPX-MLO was compared with literature. According to the literature, the N-H stretching of the piperazinyl ring in fluoroquinolones displays a characteristic IR absorption at 2500 cm^{-1}. Piperazinyl N-H stretching for compounds SPX-OXA, SPX-MLO are attributed 2500 cm^{-1}, 2523 cm−1 and 2514 cm^{-1}, respectively, implying the protonation of the SPX-MLO piperazinyl ring N atom (NH^{2+}). In the meantime, three salts/cocrystals of SPX-OXA, SPX-MLO corresponds to the characteristic peaks of carboxylic acid C = O at 1708 cm^{-1}, 1669 cm^{-1} and 1669 cm^{-1}, respectively, but SPX-MLO also have the characteristic peaks of protonation of carboxylic acid C=O at 1548 cm^{-1} and 1534 cm^{-1}, which indicates carboxylic acid

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moiety on oxalic acid molecule remains in its neutral state but carboxylic acid moiety on malonic acid molecule is protonation. Salification of EX-MLO is further confirmed by the IR spectra. Further, The IR spectra also provide key information about three EX salts/cocrystals. For SPX-OXA, the broad absorption at 3420 cm^{-1} corresponds to the O-H stretching from water molecules. The absorption peaks at 1440 cm^{-1} and 1269 cm^{-1} for SPX-OXA, 1445 cm^{-1} and 1281 cm^{-1} for SPX-MLO and 1448 cm−1 can be ascribed to ν(C-N) and ν(C-F) (Joshi et al., 2018; Liu et al., 2020).

XRD analysis

The cocrystals' purity in the crystalline phase was examined using the XRD. The X-ray powder diffraction was applied to check the crystalline phase purity of EX-OXA, EX-MLO. The findings demonstrate that the three salts'/cocrystals' patterns differ from those of SPX and coformers. Furthermore, every peak seen in the measured patterns nearly corresponds to every peak shown in the simulated patterns created from diffraction data from a single crystal. In summary, novel SPX-OXA and MLO single phases were generated.

Thermal analysis:

The outcomes of EX-OXA and EX-MLO thermal (TG) analyses showed three stages of weight loss in the thermo behavioral analysis of SPX-OXA. The water molecule may have been released during the initial, slow weight loss between 42 and 115 degrees Celsius (theoretical weight loss of 10.0%, observed weight loss of 9.42%). The second weight loss, which was 16.82% and happened between 229 and 262 degrees Celsius, might be attributed to the oxalic acid part's breakdown (theoretical weight loss of 16.22%). Further heating causes the SPX component of SPX-OXA to break down when the coformer is lost. The thermobehavior of two phases of weight loss is displayed in the SPX-MLO TG curve. In the temperature range of 128 to 180 °C, the first weight loss is 23.98%, which may be due to the malonic acid part's breakdown (theoretical weight loss of 24.28%). Further heating causes the EX component of SPX-MLO to break down when the coformer is lost.

Antimicrobial assay:

At a concentration of 0.5 mg/mL SPX-OXA complex and SPX MLO complex revealed significant anti-bacterial activity against tested strain *Escherichia coli* with the Zone of Inhibition ranging from 30±0.5 mm and 32±0.5 mm respectively in comparison to Sparfloxacin 29±0.5. The results were comparable with standard Sparfloxacin (25 ±0.5mm) (figure 2) and the SPX-OXA complex and SPX MLO complex, 22±0.5 mm and 28±0.5 respectively against *Staphylococcus aureus*, antifungal activity was found 14 ±0.5 mm and 12±0.5 mm respectively against *Candida albicans* in comparison to standard Sparfloxacin 10±0.5mm (Table 2; figure 3 & 4).

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Table 2: Antibacterial activities of Sparfloxacin and its two new salts SPX-OXA and SPX-MLO.

Bacterial Strain	**Sparfloxacin (0.5 mg/ml)**	**SPX-OXA (0.5 mg/ml)**	**SPX-MLO (0.5 mg/ml)**
	Zone (mm) at conc.		
Escherichia coli	29 ±0.5	30±0.5	32±0.5
Staphylococcus aureus	25 ±0.5	22 ±0.5	28 ±0.5
Candida albicans	10 ±0.5	14 ±0.5	12 ±0.5

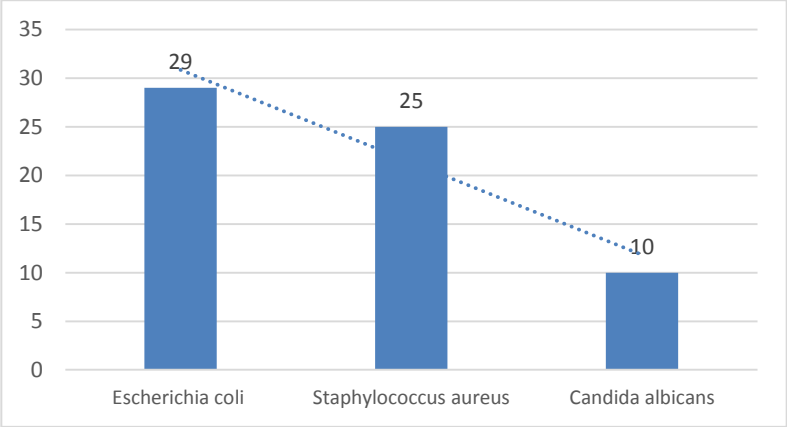

Figure 2: Antimicrobial activity of Sparfloxacin (0.5 mg/ml)

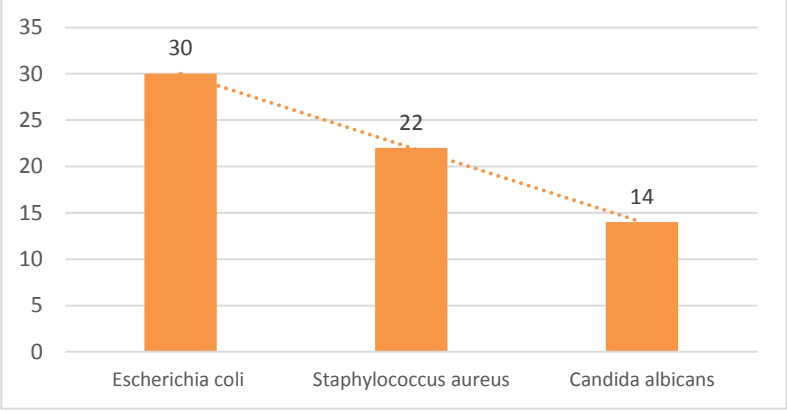

Figure 3: Antimicrobial activity of SPX-OXA (0.5 mg/ml)

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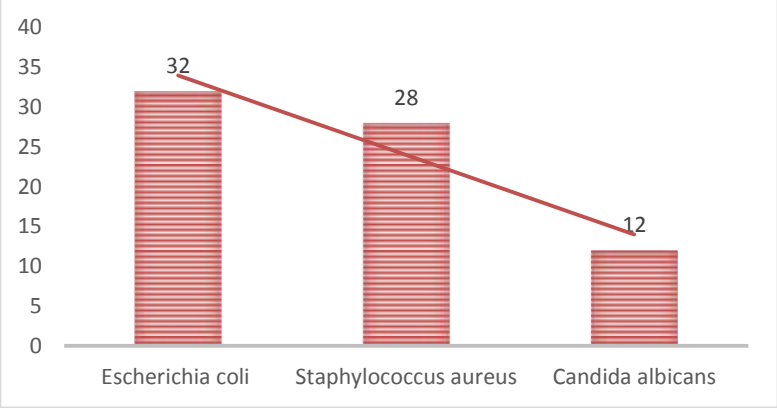

Figure 4: Antimicrobial activity of SPX-MLO (0.5 mg/ml)

Conclusions:

An advanced technique that can be used to increase an antibiotic's antibacterial efficacy is co-crystallization with dicarboxylic acid, such as malonic acid and oxalic acid. Such compounds like the sparfloxacin co-crystals SPX OXA and SPX MLO can be commercialized in place of sparfloxacin alone.

CRediT authorship contribution statement: None

Data availability: All datasets generated or analyzed during this study are included in the manuscript.

Declaration of competing interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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