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SUPRAMOLECULAR COMPLEX OF NORFLOXACIN AND SULFAMETHOXAZOLE: SYNTHESIS, CHARACTERIZATION AND EVALUATION OF THE ANTIBACTERIAL ACTIVITY

Avila CD[1], Ayala Gómez R,[1] Becerra MC[2], Mazzieri MR[2], Pinto Vitorino G[1].

[1]Depto. Farmacia, CRIDECIT, FCNyCS, UNPSJB, Km. 4, (9000), Comodoro Rivadavia, Chubut. [2]Depto. de Ciencias Farmacéuticas, [3]IMBIV-CONICET, FCQ, UNC. (5000) Córdoba.

gpinto@unpata.edu.ar

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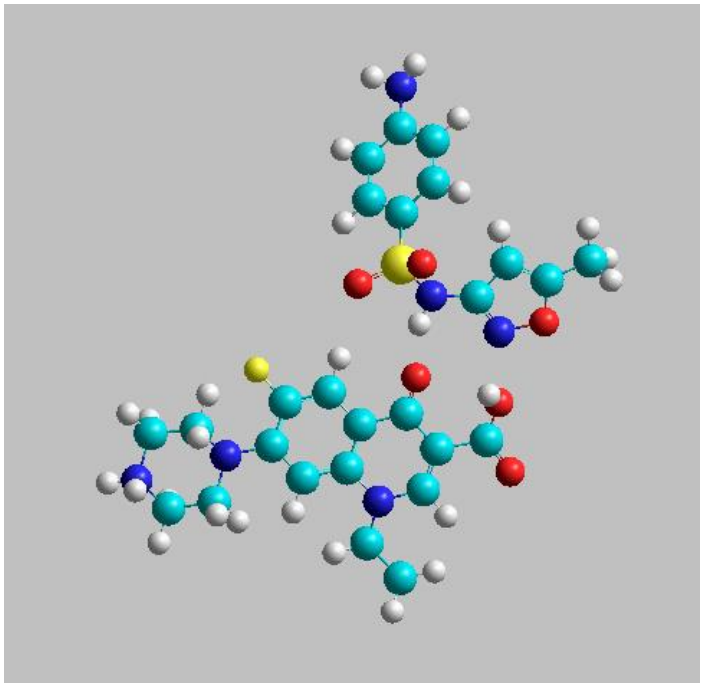


Fluorquinolones (FQs) and sulfonamides (SAs) are antibacterial compounds widely used in therapeutics. The combination of antibacterials is a strategy commonly employed to overcome resistance and to improve pharmacokinetic properties. So, the aims of this study were to design, prepare and characterize a multicomponent solid formed by the FQ norfloxacin (NOR) and the SA sulfamethoxazole (SMX), and to evaluate the *in vitro* antibacterial activity of their combinations. SMX and NOR were ground with acetonitrile. The obtained solid was analyzed by melting point (m.p), ^{1}H MNR, Hot Stage Microscopy, Differential Scanning Calorimetry (DSC), Thermogravimetry, Powder X-ray Diffraction (PXRD) and DRIFT.[1] The antibacterial effect of NOR, SMX and NOR-SMX was evaluated against *Escherichia coli* ATCC 25922 using the macrodilution method and checkerboard. The minimum inhibitory concentration (MIC) and the fractional inhibitory concentration (CIF) were determined.[2] The solid obtained showed m.p. and DSC extrapolated onset temperatures different from NOR or SMX, and both components were present in the solid in a 1:1 ratio. The PXRD patterns demonstrated that NOR and SMX form a new solid phase. Results of the checkerboard method showed synergistic effect, with a FIC index of 0.40.[3] These results are very promising so we continue developing new biopharmaceutical assays.

Keywords: norfloxacin, sulfamethoxazole, supramolecular complex, synergism.

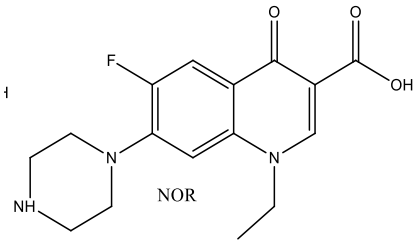
INTRODUCTION

Crystal engineering constitutes a potentially fruitful approach to improve the solubility, dissolution rate and bioavailability of poorly water soluble active pharmaceutical ingredients (APIs)

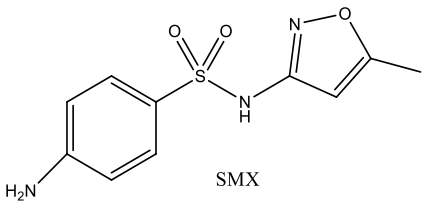
Fluorquinolones (FQs) and sulfonamides (SAs) are antibacterial compounds widely used in therapeutics. The combination of antibacterials is a strategy commonly employed to overcome resistance and to improve pharmacokinetic properties.

THE AIMS

- To design, prepare and characterize a multicomponent solid formed by the FQ norfloxacin (NOR) and the SA sulfamethoxazole (SMX).
- To evaluate the *in vitro* antibacterial activity of their combinations.



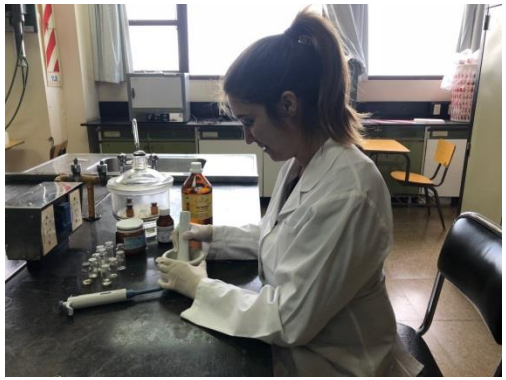
NOR



SMX

RESULTS AND DISCUSSION

With the aim to generate the complexes, equimolecular quantities of SMX and NOR were ground with a mortar and pestle, with the addition of acetonitrile during 30 min.



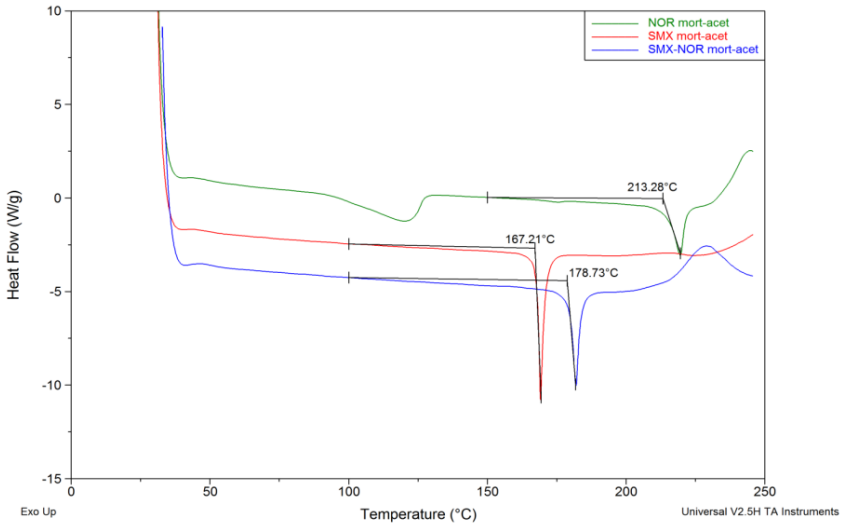
The obtained solid was analyzed by:

- melting point (m.p)
- ^{1}H MNR,
- Hot Stage Microscopy
- Thermogravimetry
- Differential Scanning Calorimetry (DSC)
- Powder X-ray Diffraction (PXRD)
- DRIFT

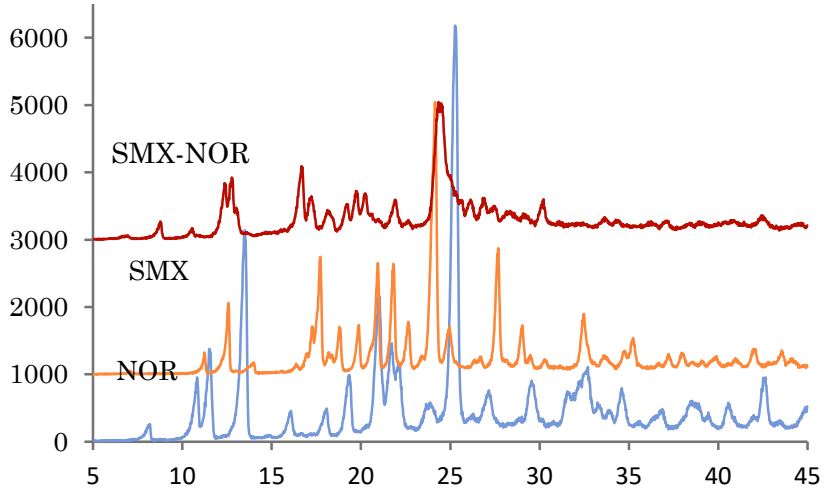
RESULTS AND DISCUSSION

The solids obtained by grinding SMX with NOR showed melting points and DSC extrapolated onset temperatures (T_{onset}) different from those of the starting APIs.

Muestra	pf (˚C)	T_{onset} (˚C)
NOR	202,7-216,5	213,3
SMX	164,4-168,7	167,2
SMX-NOR	176,0-182,1	178,7

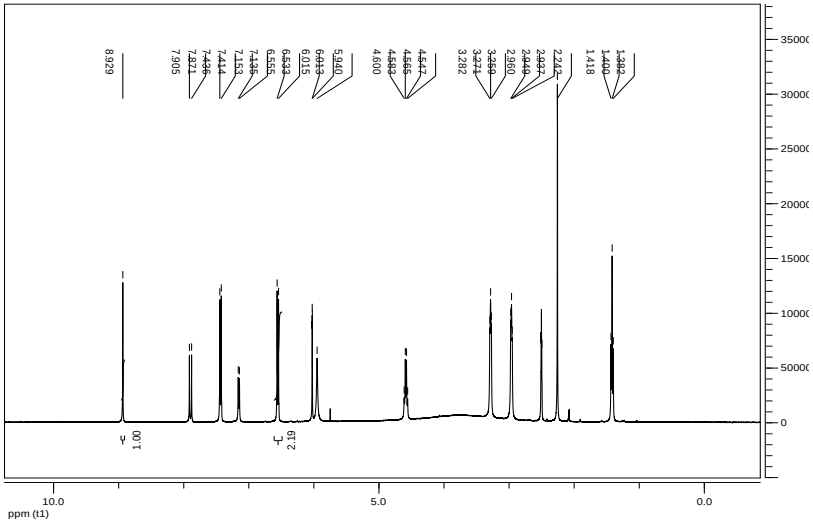


RESULTS AND DISCUSSION



The PXRD patterns demonstrated that the obtained sample was new solid phase.

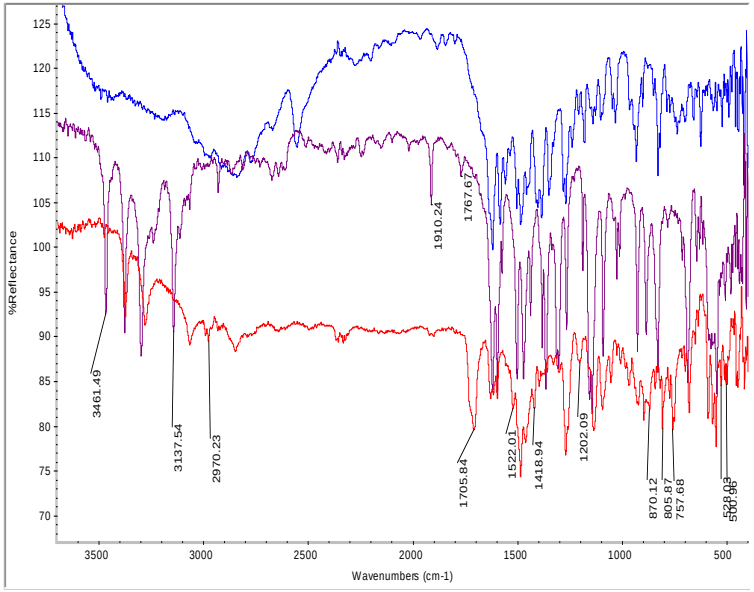
According to TLC results, both components are present in each solid, and ^{1}H MNR data confirmed a 1:1 ratio between them



RESULTS AND DISCUSSION

By comparing the DRIFT spectra of SMX-NOR (red) with those of the pure NOR (blue) and SMX (purple), the major differences were:

a) a new peak at 1705 cm^{-1} attributable to the presence of the COOH group of NOR and the lack of the peaks at 1590 cm^{-1} (COO^-) and 2550 cm^{-1} (NH_2),
b) the absence of the peak at 3461 cm^{-1} attributable to the NH stretching of SMX.



This results indicate that NOR pure predominate in the zwitterionic form and in the complexes is present as neutral specie, probably forming hydrogen bonding with the H atom of the SO_2NH group of the SA.

RESULTS AND DISCUSSION

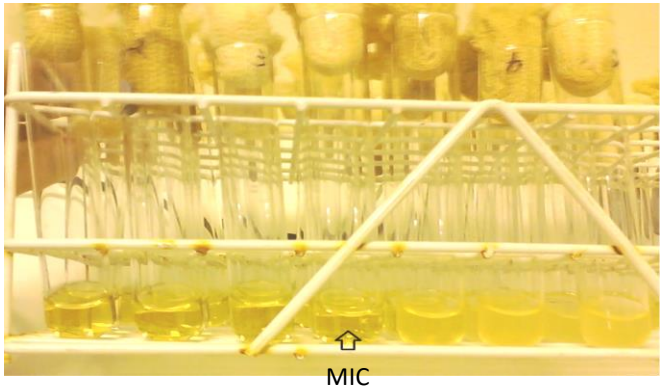
MICROBIOLOGICAL ASSAYS

MIC

For antimicrobial activity determinations against *Escherichia coli* ATCC 25922, MIC values of NOR and SMX were determined by using the macrodilution method with Mueller–Hinton broth.

MICs were measured by triplicate and determined according to CLSI recommendations.

The MIC was defined as the lowest concentration of antibiotic that inhibited all visible growth.



RESULTS AND DISCUSSION

FIC INDEX

To evaluate the synergistic effect of the combination by checkerboard, the Fractional Inhibitory Concentration (FIC) was calculated for the NOR-SMX combination as:

$$\text{FIC index} = \frac{MIC\ of\ NOR\ in\ combination}{MIC\ of\ NOR\ alone} + \frac{MIC\ of\ SMX\ in\ combination}{MIC\ of\ SMX\ alone}$$

RESULTS AND DISCUSSION

The minimum FIC between all of the calculated FICs was defined as FIC index. The obtained values of the FIC index were assigned according to the following table 1.

ANTIBACTERIAL EFECT	FIC INDEX
synergism	<0.5
partial synergism	0,5-1
indifference	1-4
Antagonism	>4

The results obtained from the MIC and FIC index are expressed in the table 2 :

ANTIBIOTICS	ANTIBACTERIAL CLASS	TARGET	MIC (µg/mL)	NOR-SMX FIC INDEX
NOR	Fluorquinolone	Enzymes: DNA gyrase and Topoisomerase IV	0,1 -0,06	0,4
SMX	Sulfonamide	Enzyme: dihydrofolate reductase	250-125	

These results are very promising, considering that CIF values lower than 0.5 indicate that the *in vitro* antibacterial activity of the mixture is better than each ATB individually.

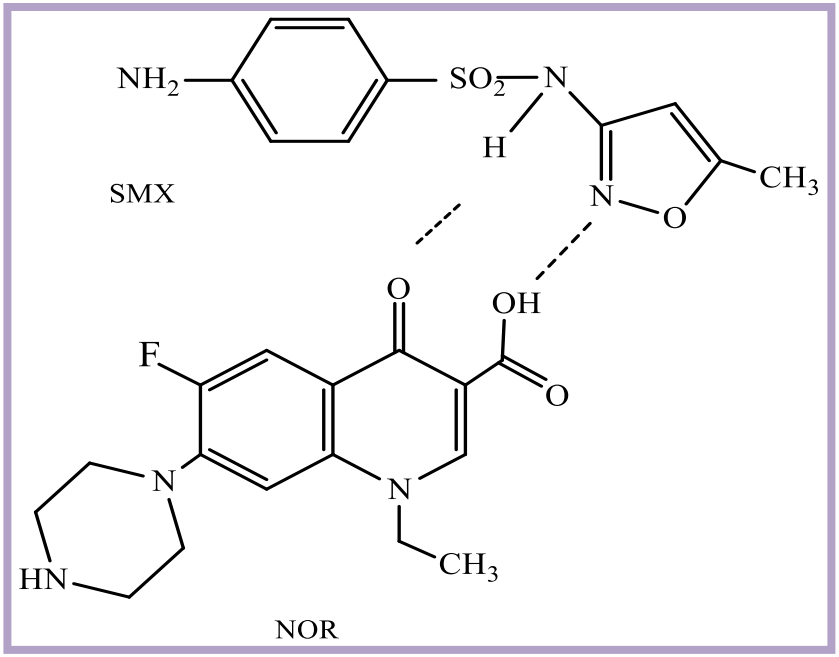
CONCLUSIONS

❖ By liquid assisted grinding, it was possible to obtain a new molecular complexes originated from the interaction in the solid state between NOR and SMX.

❖ The *in vitro* antibacterial activity of the combination NOR-SMX against *E. coli* 25922 was synergistic. There are several possible mechanisms to generate synergistic effect, one of which is that both groups of ATB acts at different levels of DNA synthesis, exerting a cooperative effect.

CONCLUSIONS

- The complex presented an 1:1 stoichiometry. According to DRIFT data, the interaction would occur between the COOH of NOR and the SO_2NH group of the sulfonamide (the methoxazol of SMX could be involved as well).



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