ORIGINAL ARTICLE

SYNTHESIS, CHARACTERIZATION AND MICROBIOLOGICAL ACTIVITY EVALUATION OF NOVEL HARD GELATINE CAPSULES WITH CEFACLOR AND PIROXICAM

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Abstract

Cefaclor and piroxicam combination is relevant for the pharmaceutical field due to synergistic bactericidal and anti-inflammatory potential, provided by the second generation cephalosporin and first generation NSAID. In order to obtain new products with higher pharmacological properties, we developed hard gelatine capsules with cefaclor and piroxicam, in a fixed-dose combination. A multi-analytical approach was carried out by Fourier Transform-Infrared Spectroscopy (FT-IR) and Thin Layer Chromatography (TLC). The screening of the antimicrobial activity of the capsules was performed by determination of the minimal inhibitory concentration (MIC) through microtiter broth dilution method.

Rezumat

Abordarea industriei farmaceutice vizează crearea de medicamente cu potențial dual, sinergic, capabile să satisfacă cerințele pacienților, la un preț de cost scăzut și, în cazul asocierii lor, având eficacitate identică cu a substanțelor singulare. Scopul acestui studiu a fost formularea unor capsule gelatinoase tari cu cefaclor și piroxicam, în combinație fixă și abordarea lor din punct de vedere analitic, folosind spectroscopia de absorbție în infraroșu (FT-IR) și cromatografia în strat subțire (CSS). Evaluarea activității antimicrobiene s-a realizat prin determinarea concentrației minime inhibitorii (CMI), folosind metoda diluțiilor.

Keywords: cefaclor monohydrate, piroxicam, hard gelatine capsule, analytical methods

Introduction

Cephalosporins are a group of bactericidal beta-lactam antibiotics, which contains four generations of compounds, classified according to clinical and microbiological criteria [13]. Second generation of cephalosporins has a broader spectrum, commonly used in infection caused by *Enterobacter, Serratia*, indole-positive *Proteus, Haemophilus influenza, Neisseria gonorrhoeae,* and *Neisseria meningitides. In vitro* activity is above penicillins or other beta-lactams for *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis* [9, 10, 17].

Fixed-dose combination therapy (FDC) offers a method of simplifying complex regimens. Efficacy and tolerability appear to be similar between FDC and treatment with individual agents. In addition, FDC can enhance adherence and improved adherence may result in improved antibacterial control. The availability of FDCs containing at least one antibacterial substance seems to be at odds with a strategy of prudent antibacterial use. In some cases, there may be improved adherence because of the convenience of taking fewer pills, as specified by the European Agency of Medicine in 2009. In a few cases, there may be a pharmacological rationale of combinations of synergistically acting substances.

The WHO List of Essential Medicines contains two pairs: amoxicillin and clavulanic acid (β-lactamase inhibitor) and trimethoprim and sulphamethoxazole (combination of two folic acid synthesis pathway inhibitors). In addition, for the short-term therapy of eradication of Helicobacter pylori, a combination of two antibacterials with a proton pump inhibitor has been recommended. [19]. Thus, the development of oral formulations containing a combination of a number of therapeutic agents acting synergistically is a necessity, because it is a more effective therapeutic alternative in comparison with the conventional forms. Such a dosage form reduces the dosing frequency, improves the ratio cost-effectiveness of treatment and patient's compliance [8]. Also, in the majority of the ear-nose-throat (ENT) infections, along with the superior respiratory tract and urinary infections, the pharmacological treatment contains an antimicrobial and anti-inflammatory drug. We have chosen the two active substances because they are worldwide used (correlated with the Biopharmaceutics Classification System [3]) and are, by means of chemical structure, representative for each belonged class of therapeutics. We developed hard gelatine capsules, taking into account that, after tablets, represent the most utilized pharmaceutical form, due to its multiple advantages: simple formulation, including the content, that does not require excipient, thus eliminating possible incompatibilities between components, but including powders, pellets, microtablets, microcapsules or a mixture of thereof; superior bioequivalence and bioavailability to other oral solid preparations; the dissolution profiles are independent on dose strength, and generally display a rapid and complete release, generally according to compendial specifications [7].

Cefaclor monohydrate (CAS 56238-63-2) is an orally administered second generation cephalosporin, available since 1979, that has been widely used throughout the world for the management of bacterial infections of the respiratory and urinary tracts, the skin and associated structures [4, 5]. Despite its ready availability, cefaclor remained effective clinically after its first decade of use in the USA and other countries [Moellering, 1988] and continued to show good activity against a range of common pathogens, including *Haemophilus influenza*, *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Escherichia coli* [5, 15]. An advanced modified release formulation has become available for the use as a twice a day antibiotic and show good potential for treating infection of the respiratory tract, the urinary tract and skin [2]. It has been shown that low-dose cefaclor is an effective long-term prophylactic treatment for recurrent urinary tract infection. When compared with other antimicrobials used commonly for treating urinary tract infection, only ciprofloxacin showed greater activity, though cefaclor showed significantly greater *in vitro* activity than cephalexin, ampicillin and trimethoprim [4]. It binds to specific penicillin-binding proteins located inside the bacterial cell wall and inhibits the final stage of bacterial cell wall synthesis. As a consequence of the defective cell walls, the bacteria cells are autolysed by autolysins, autolytic enzymes. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins.

Piroxicam (CAS 36322-90-4) is one the most potent nonsteroidal anti-inflammatory agent used for the treatment of acute and chronic rheumatoid arthritis or osteoarthritis, traumatic contusions and different regional inflammatory disorders such as muscle pain or low back pain [16]. Its non-selective inhibition of the cyclooxygenase (COX) is also responsible for the digestive adverse effects (gastric irritation, nausea, gastrointestinal ulcer, gastric bleeding and perforation) [14, 15], associated with long term administration of oral immediate-release dosage forms [6, 16].

The main objective of our paper was to develop hard gelatine capsules with cefaclor and piroxicam, followed by their characterization using several methods: FT-IR, TLC [1] and screening of the antibacterial activity.

Materials and Methods

Chemicals and Reagents. Cefaclor monohydrate and potassium bromide used in this study were obtained from Sigma Aldrich (Germany) and piroxicam from Nantong (China). The sodium croscarmellose was provided from Spectrum Chemical Manufacturing Corp. (China) and dimethicone and magnesium stearate from SC Medchim TM (Romania). All substances were of pharmaceutical grade. Solvents: methanol (Loba Chemie, India), ethyl acetate, toluene, 80% formic acid (Chimopar SA, Romania). All chemicals were of analytical reagent grade. Aqueous solutions of the compounds were prepared by using double distilled water. The hard gelatine capsules, 0 size, Capsugel® were provided from Capsugel, France.

The capsules output. With the purpose of obtaining a homogenous mixture, with compact characteristics, that could integrate ranged quantities between 65 mg and 1 g of powders, with free flowing, but insuring the mass uniformity of the capsules and having a 30 minutes time of disintegration, according to the compendial references, 0 size Capsugel® were used, along with sodium croscarmellose, dimethicone and magnesium stearate as excipients. The qualitative composition is presented in Table I.

Table I

Qualitative composition of the experimental formulation

	Amount (g/capsule)
Crude material	
Cefaclor monohydrate (corresponding to cefaclor)	0.2623 (0.2500)
Piroxicam	0.0200
Excipients	
Sodium croscarmellose	0.0207
Dimethicone 350CST	0.0090
Magnesium Stearate	0.0030
Shell capsules	
Gelatine	0.8742
Quinolin yellow E104	0.00755
Sunset yellow E110	0.00025
Titanium dioxide	0.0180

Thin layer chromatography was performed using a stock standard solution prepared by dissolving accurately weighed powders (0.00807 g piroxicam and 0.10055 g cefaclor) in methanol, in a 250 mL volumetric flask. The sample solution was prepared by dissolving the powder from an in-house capsule in methanol, using a 500 mL volumetric flask. The samples were applied on TLC plate using a semiautomatic sampler with the help of nitrogen gas, using a plan presented in Table II. The plate was developed in a vertical chamber to a distance of 5 cm, dried with a stream of hot air. Chromatography was performed on precoated silica gel 60 F254 plates (Fluka). The mobile phase used in these experiments was made of methanol-toluene-ethyl acetate-80% formic acid 5/20/65/10 (v/v/v/v). The purity and identity of the analyte spots were determined by scanning in absorbance–reflectance mode from λ = 200 to 400 nm.

Table II

The plan used for applying the standard and sample solutions on the plates

Corridor	Solution	Applied amount of solution (µL)	Piroxicam/ Cefaclor (µg/ µL)
1	Standard	1.0	0.080 / 1.000
2	Sample	1.0	0.032 / 0.402
3	Standard	1.4	0.112 / 1.400
4	Sample	2.0	0.064 / 0.804
5	Standard	1.8	0.144 / 1.800
6	Sample	2.6	0.083 / 1.045
7	Standard	2.2	0.176 / 2.200
8	Sample	1.0	0.032 / 0.402
9	Standard	2.6	0.208 / 2.600
10	Sample	2.0	0.064 / 0.804
11	Standard	1.0	0.080 / 1.000
12	Sample	2.6	0.083 / 1.045
13	Standard	1.4	0.112 / 1.400
14	Standard	1.8	0.144 / 1.800
15	Standard	2.2	0.176 / 2.200
16	Standard	2.6	0.208 / 2.600

The spectrophotometric determination was performed using two methods: the potassium bromide pellets technique and the attenuated total reflection (ATR) technique. The KBr pellets were prepared by mixing the clay sample with KBr powder (1:100) and pressed in a hydraulic press (10 tonnes). All the clay samples were dried at 120ºC for 6 h and cooled under *vacuum* before recording the spectra.

Apparatus. For the TLC analysis, we used a glass chromatographic tank with a flat bottom and a size suitable for the plates, a Scanner 3 Camag TLC densitometer (Switzerland), equipped with a 5 Linomat (Camag 5) semiautomatic staining system under nitrogen atmosphere and another one for the visualisation of the plates, using UV lamps at 254 and 365 nm (Switzerland) and WinCats manger ver. 1.4.4 software. FT-IR spectra were recorded on a FT-IR Bio-Rad FTS 115 spectrometer (United Kingdom) with samples prepared as KBr pellets in the range of 4000 - 400 cm^{-1} and processed using Omnic V.6 software. For the ATR technique, a FT/IR Jasco 4200 spectrometer with an ATR PRO450-S diamond optical accessory (Germany) was used. Sterilization of materials and equipments was carried out using Raypa steam sterilizer Autoclave. Microbiology samples were incubated at 37°C using WTC binder incubator. 96-Flat bottom microplates were used in the conduction of broth dilution test. ELx 800 UV universal microplate reader, Biotek instrument was used to determine the turbidity in the wells.

Microbiological studies

Antibacterial activity screening. The antibacterial activity of the synthetized hard gelatine capsules was analysed on 7 bacterial strains, two Gram positive (*Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778) and five Gram

negative (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* NCTC 2091, *Salmonella enteritidis* IC 10871). All these strains were obtained from the National Collection of National Research Institute for Development, Microbiology and Immunology "Cantacuzino", Bucharest, Romania. *Agar well diffusion method* [20] was used to find out and compare the antibacterial activity of the hard gelatine capsules and standard drugs used. Nutrient media was prepared, sterilized and poured into sterilized Petri plates, cautiously covered and left for 24 hours at 37ºC to check contamination. Prepared Petri plates were inoculated with 100 µL bacterial species from broth culture; wells were prepared and filled with powder content of the capsule and pure active substance, in order to make a comparison between the antibacterial activity of synthesized mixture of the pharmaceutical product and the standard. Sterile filter disks impregnated with 50 µL of sample solution were placed in inoculated Petri plates with the help of sterile forceps. The plates were then incubated at 37°C for 24 hours. Afterwards, inhibition zones were measured and means were recorded. Zones of inhibition were measured and recorded for each species and after that their means were measured and compared by applying Pearson's test at 5% level of significance.

Minimum inhibitory concentration (MIC) assay [21]. MICs were determined by using the broth microdilution method recommended by the NCCLS with some modifications. In order to evaluate the antimicrobial activity, serial dilutions of the capsules content (1 - 1024 µg/mL) were prepared in 96-well microtiter plates to a final volume of 25 µL. Thus, 10.24 mg from each solution was dissolved in 10 mL of 24% dimethylsulfoxide (DMSO), The 24% DMSO was required in order to obtain a maximum accepted value of 3% DMSO in final volume [18]. From the stock solution, 25 µL were added in the first well of the microtiter plate, and from this we performed the binary dilutions; on the last well the complex concentration was 0.06 µg/mL. The turbidity of the inoculum was adjusted to a concentration of 0.5 McFarland. From this, 10 µL were mixed with 9990 µL Muller-Hinton broth, and 175 µL were inoculated into the corresponding wells. The plates were incubated at 35°C for 24 hours. For all samples, the minimum inhibitory concentration (MIC) was interpreted at the last dilution which presented no bacterial growth. Working inoculums (0.1 mL) were added to the microtiter plates, which were incubated in a humid environment at 35ºC for 24 h. Uninoculated medium (200 µL) was included as a sterility control. In addition, growth controls (medium with inoculums but without antibiotics or the compounds) were also included. The growth in each well was compared with that of the growth in the control well. MICs were visually determined and defined as the lowest concentration of the compounds produced ≥ 95% growth reduction compared with the growth in the control well. Each experiment was performed in triplicate.

Results and Discussion

Capsules manufacturing. Although obtaining hard gelatine capsules involves rather a few technological operations, it is a challenging issue. Surveilling the scientific literature, we have added the minimum necessary excipients (a disintegrator to allow granules a faster dissolution that may occur during the preparation/conditioning, a lubricant to assist in the flow of the powder in order to facilitate the preparation, a solubilizing agent to allow faster dissolution of the active substances in liquid medium, taking into account the particularity of piroxicam, which is sparingly soluble in water) to have an accurate picture on the physical, physico-chemical and even bacteriological profiles of the innovative mixture of cefaclor and piroxicam. We decided for this formulation to use active substances in a ratio 12.5 to 1 (cefaclor: piroxicam, 250.00 mg: 20.00 mg, w/w), thus being therapeutic doses. We prepared powders mixture as recommended in the literature, we selected capsules (size 0) to ensure an easier filling and an accurate one for the patient administration, and filled capsules using the capsule filler, as accepted method of literature for producing micro-gelatine capsules. The innovative cefaclor and piroxicam capsules are shaped in the form of elongated gelatine cylinders countries, rounded at the ends, to be closed by joint and are yellow. The capsule content is a powder, visually homogeneous, white to light yellow, without smell and having the characteristic taste of the components, bitter weak (Figure 1).

Figure 1.
The in-house hard gelatine capsules with cefaclor and piroxicam (250 mg + 20 mg)

FT-IR spectroscopy analysis. The FT-IR spectrum of standard drugs (cefaclor and piroxicam) was compared to those of the powders mixture from the capsules, in order to confirm a large number of

overlapped bands and a relatively small number of specific bands for the two substances (Figure 2). Thus, in the first spectral region, at 3856 cm^{-1} the specific band for the NH group (ν_{NH}) of the piroxicam it occurs. At 3840.5 cm^{-1} a mild band of the piroxicam appears, overlaid on the same band (ν_{NH}) characteristic for cefaclor and at 3737.4 cm^{-1}, another band of piroxicam superposed over the cefaclor band (ν_{NH}) shows.

At 3336.3 cm^{-1}, a distinct band for piroxicam, appropriate to the group NH (ν_{NH}) appears and at 3033.0 cm^{-1} it appears the piroxicam band overlapped the cefaclor one (ν_{CN}). At 2089.5 cm^{-1}, a distinct band of piroxicam shows (ν_{CH}) and at 2132.0 cm^{-1} a distinct band for cefaclor (ν_{CH}). In the second spectral region, a large number of overlapping bands, most of the two substances, appeared. Thus, at 1774.2 cm^{-1}, the specific band for piroxicam appears superposed over the corresponding band of cefaclor (δ_{NH}) and at 1696.1 the piroxicam band superposed on that of the same group C=C from the cefaclor structure (ν_{CC}). At 1626.7 cm^{-1} it can be observed the distinct band corresponding to the C=C group (ν_{CC}) characteristic for piroxicam. At 1606.4 cm^{-1} the piroxicam band appears, superposed over the cefaclor one, and at 1574.4 cm^{-1} the spectrum revealed a distinct band of piroxicam (ν_{CO}). At 1574.6 cm^{-1}, a specific band appears overlaid on the cefaclor band (ν_{CO}), at 1524.5 cm^{-1} appeared a second band of the group C=O (ν_{CO}) superposed on the corresponding band of cefaclor (ν_{CO}), and at 1432.9 cm^{-1} appeared as a distinct band the one corresponding to the CS group of piroxicam. At 1348.0 cm^{-1} it appears very distinctive the band of the asymmetric vibration corresponding to the group SO_2 from piroxicam ($\nu_{as\text{-}SO2}$). At 1297.9 cm^{-1}, the appropriate piroxicam OH group band appears superposed on the same of cefaclor (ν_{OH}). At 1266.1 cm^{-1}, 1214.0 cm^{-1} and 1179.3 cm^{-1}, three characteristic bands of the group CH (δ_{CH}) of piroxicam superposed on the three characteristic bands for cefaclor appeared. At 1147.4 cm^{-1}, the appropriate band of piroxicam for the distinct group SO_2 (δ_{SO2}) appeared. At 1181.5 cm^{-1} and 1093.0 cm^{-1} two bands corresponding to the bending vibrations plan of the group NH (δ_{NH}) characteristic for piroxicam, overlapped to the corresponding bands of cefaclor, were revealed. At 1065.5 cm^{-1} and 1037.5 cm^{-1}, the bands corresponding to the bending vibrations outside the plane of the group NH (γ_{NH}) of piroxicam and overlaid to the cefaclor corresponding bands and at 961.3 cm^{-1} the band corresponding to bending vibrations outside the plane of the group CH (γ_{CH}) superposed the corresponding band of cefaclor are observed in the spectrum. At 938.4 cm^{-1}, it appears the appropriate bending vibration band in the plane (δ_{SN}) of the SN group and at 875.5 cm^{-1}, the band corresponding to the bending vibration occurs outside the plane (γ_{CH}), both distinct from cefaclor. At 770.4 cm^{-1} and at 730.9 cm^{-1}, appropriate bands, corresponding to the rocking vibrations appear out of the plane for the group NH (γ_{NH}), superposed over those of cefaclor and at 690.4 cm^{-1} and 618.1 cm^{-1}, the CC group of piroxicam bands appear superposed over those of cefaclor. 581.4 cm^{-1} and 557.3 cm^{-1} are two bands corresponding to the deformation vibrations in the plane (δ_{SO2}) of SO_2 group separately in the piroxicam molecule, and 455.1 cm^{-1} and 410.8 cm^{-1} are two bands corresponding to the deformation vibrations outside the plane of the C=C group (γ_{CC}), clearly defined for piroxicam. By using two different techniques, spectra recorded showed similarities (Figures 3 – 6), also straightened by Pearson correlation coefficient, calculated at 0.989.

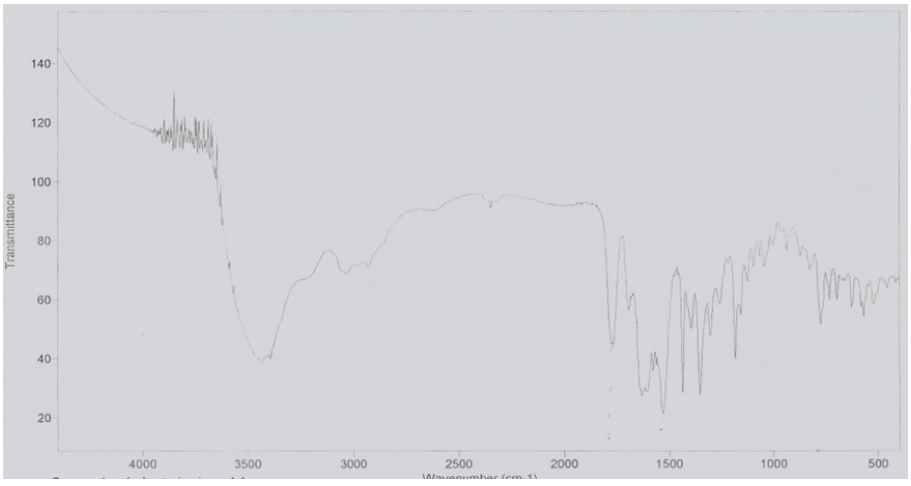


Figure 2.
The IR spectrum of the drugs mixture using the KBr pellets tehnique

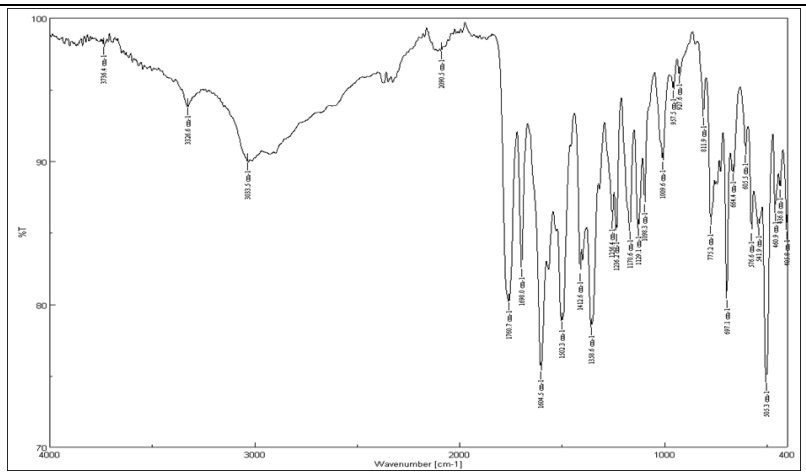


Figure 3.
The IR spectrum of the standard cefaclor using attenuated total reflection (ATR) technique

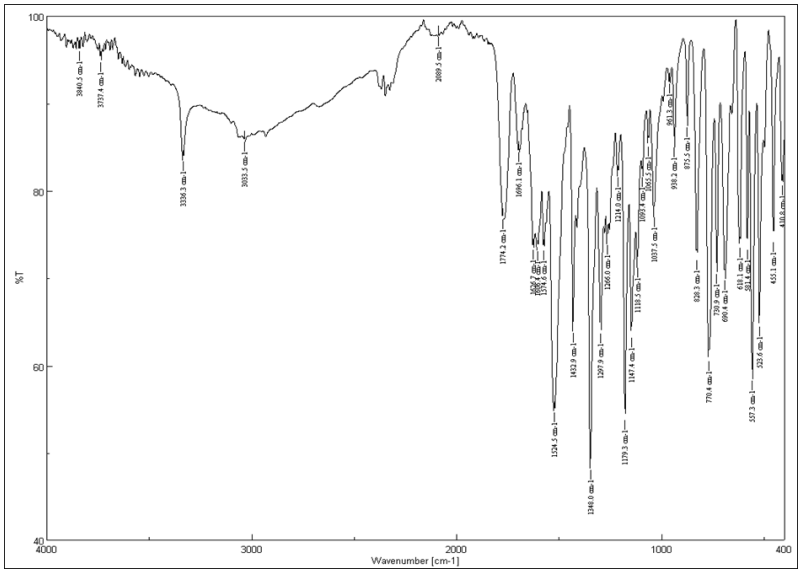


Figure 4.
The IR spectrum of the standard piroxicam using attenuated total reflection (ATR) technique

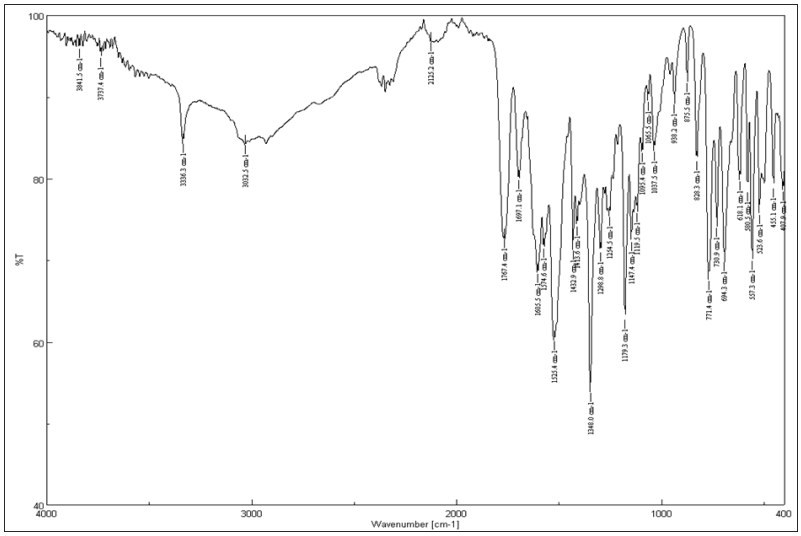


Figure 5.
The IR spectrum of the drugs mixture using attenuated total reflection (ATR) technique

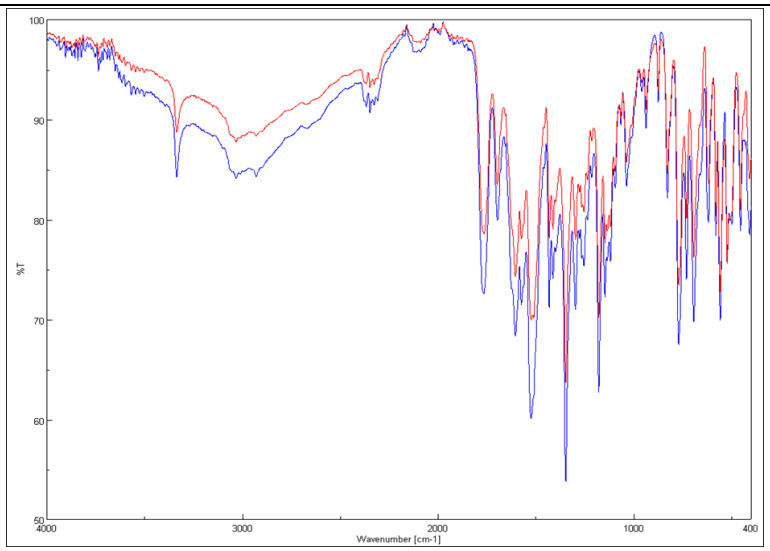


Figure 6.
The overlaid IR spectra for the standard drugs mixture using ATR technique (in red colour) and the drugs mixture from the capsules (in blue colour)

TLC analysis. The densitograms obtained after analysing the migration corridor under the UV light, at 254 nm, for a plate, are presented in Figure 7.

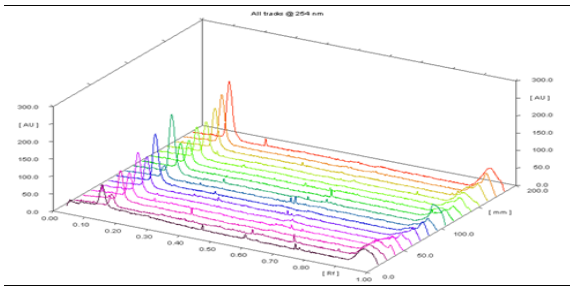


Figure 7.
The tridimensional representation of the densitograms recorded at 254 nm

We evaluated the retardation factors, as revealed by Table III.
Also, it can be observed that, for cefaclor, a small peak is notable, with a value for R_f reproducible (0.13 ± 0.004), different for piroxicam (R_f = 0.95 ± 0.010), the value being affected by the small amount of substance. Thus, the migration in the tested mobile phase system is very close to the solvent front, affecting the detection.

Table III
The retardation factors for piroxicam and cefaclor

Migration corridor	Solution	R_f cefaclor	R_f piroxicam
1	Standard	0.12	0.95
2	Sample	0.12	0.94
3	Standard	0.13	0.94
4	Sample	0.13	0.95
5	Standard	0.13	0.95
6	Sample	0.13	0.96
7	Standard	0.13	0.94
8	Sample	0.13	0.96
9	Standard	0.13	0.96
10	Sample	0.13	0.93
11	Standard	0.13	0.93
12	Sample	0.13	0.94
13	Standard	0.13	0.94
14	Standard	0.13	0.94
15	Standard	0.13	0.95
16	Standard	0.12	0.95

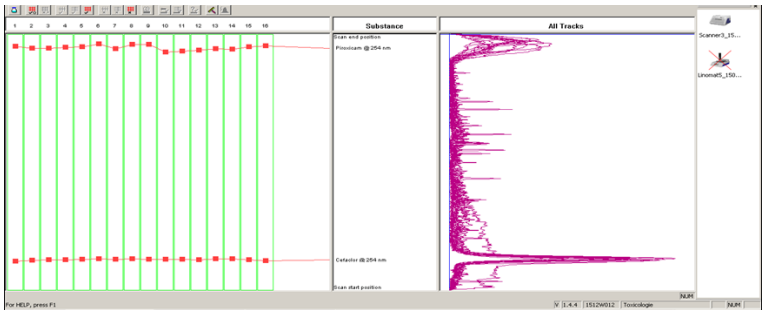
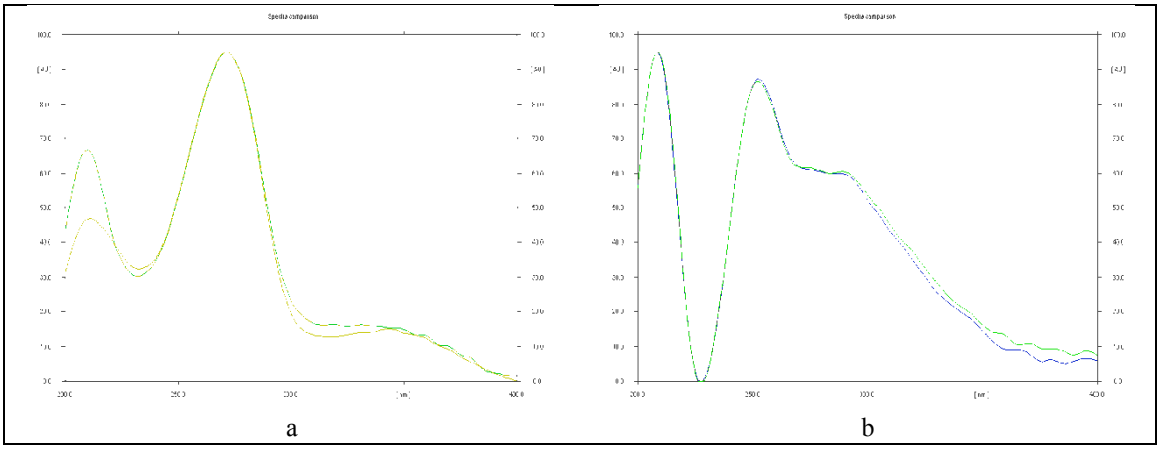


Figure 8.
The overlaid chromatograms, performed for the 16 migration corridors

The identity of the analyte spots were determined by scanning in absorbance–reflectance mode from λ = 200 to 400 nm. The spectra are perfectly overlaid and are identical for cefaclor and for piroxicam, as it can see in Figure 9.



a b

Figure 9.
The detailed spectra for cefaclor (a) and piroxicam (b)

Microbiological studies. The susceptibility of bacteria against tested capsules are presented in Table IV. Regarding the antibacterial activity against selected Gram negative and Gram positive strains, the hard gelatine capsules showed similar MIC values with cefaclor, although it was expected to exhibit lower antibacterial activity than the antibiotic himself, due to the piroxicam content. As the literature data confirms [11, 12], *in vitro* performed studies showed that piroxicam has strong activity against *Staphylococcus aureus* and *Brucella species*.

Table IV

Diameter of zone inhibition and minimum inhibitory concentrations for the tested capsules, cefaclor and piroxicam

No.	Bacterial strains	Cefaclor and piroxicam capsules		Cefaclor		Piroxicam	
		DD[a]	MIC[b]	DD[a]	MIC[b]	DD[a]	MIC[b]
1.	*Staphylococcus aureus*	22.00	1.86 ± 1.3	29.00 ± 0.76	2 ± 0.0	9.33	----
2.	*Escherichia coli*	20.33	8.14 ± 0.5	25.50	8 ± 0.0	5.67	----
3.	*Pseudomonas aeruginosa*	8.00	----	12.00	----	0.00	----
4.	*Klebsiella pneumoniae*	20.33	0.96 ± 0.1	29.17	1 ± 0.0	0.00	----
5.	*Proteus mirabilis*	9.67	8.35 ± 1.0	11.50	8 ± 0.1	0.00	0.02 ± 0.5
6.	*Bacillus cereus*	7.83	6.21 ± 0.3	21.50	8 ± 0.1	6.00	0.04 ± 0.1
7.	*Salmonella enterica*	16.33	6.86 ± 1.3	27.17	9.11 ± 0.0	15.17	----

Data is represented as Mean ± SD of triplicate determination of each extracts against each microbial strain. [a]Diameter of inhibition zone expressed in mm. [b]Minimum inhibitory concentration measured in µg/mL. (----) indicates no antimicrobial activity

As it can be seen, the aqueous solution of cefaclor was efficient against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Bacillus cereus* and *Salmonella enterica* and had no antimicrobial effect against *Pseudomonas aeruginosa* and *Proteus mirabilis*. The anti-inflammatory drug had no antibacterial effect, except for the activity against strain no. 7, *Salmonella enterica*, on which it was recorded an intermediate susceptibility. The same result was registered for the pharmaceutical product, who was efficient against strains no. 1, 2, 4, 6 and 7 and inefficient against strains no. 3 and 5. If this data is confirmed by the subsequent microbiological studies (involving yeast and fungi), it would be possible to demonstrate the effect of the preparation increased in the case of chronic infections with Gram-positive bacteria and some Gram-negative bacteria.

Conclusions

In our study, we developed hard gelatine capsules with cefaclor and piroxicam, in order to obtain a pharmaceutical product that could comply with the patient needs in terms of acceptance and compliance. Consequently the physico-chemical characterization and the qualitative assay using spectroscopic and chromatographic methods, we tested the microbiological activity of the hard gelatine capsules with cefaclor and piroxicam, in order to establish the pharmacological effect exerted on the pathogenic bacteria, selected as representative for the main groups of microorganisms. We also tested the antimicrobial

action of cefaclor and piroxicam. According to the literature data, the results yielded: cefaclor presented antibacterial effect above the selected bacterial strains, excepting *Pseudomonas aeruginosa* and piroxicam had antibacterial effect. The innovative formulation (250 mg cefaclor and 20 mg piroxicam capsules) presented similar activity against Gram positive and Gram negative selected bacteria, close to the cefaclor effect. We noticed a stronger antibacterial effect on diluted bacterial suspensions.

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