

FORMULATION AND EVALUATION OF A FIXED DOSE COMBINATION OF CEFTRIAXONE DISODIUM AND ORNIDAZOLE

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ABSTRACT

Objective: The overall objective of this study was to develop a formulation of the antibacterial agents, Ceftriaxone disodium and Ornidazole in a fixed dose to be dispensed as sterile dry powders, to evaluate the antimicrobial efficacy and to develop a precise, accurate, rapid and economical spectrophotometric method based on simultaneous equation to quantify the two drugs in the formulation.

Methods: Preliminary studies such as solubility, study of physical compatibility and antimicrobial efficacy using the agar well-diffusion assay method was carried out to determine the dose for combining the two agents. Spectral characteristics of the two drugs were determined to develop a spectrophotometric analytical method for the simultaneous estimation of the two drugs. The method was then employed during stability testing for the assay of the drugs after its validation statistically.

Results: FT-IR studies and other preliminary studies indicated no interaction between the drugs either physically or chemically when combined in different ratios. Antibiotic susceptibility tests showed an increase in antimicrobial efficacy with an increase in concentration of the two drugs. The wavelengths selected for analysis were 241.0 nm (λ_{\max} of Ceftriaxone) and 319.0 nm (λ_{\max} of Ornidazole) respectively in water. The linearity was obtained in the concentration range of 5-25 $\mu\text{g/ml}$ for Ceftriaxone and 3-15 $\mu\text{g/ml}$ for Ornidazole. The three formulations containing Ceftriaxone disodium and Ornidazole in the ratio of 2:1, 3:1 and 4:1 were found to be stable in all the commonly used intravenous diluents.

Conclusion: The combination of Ceftriaxone disodium and Ornidazole in fixed dose has a good potential not only for use against anaerobic and aerobic bacterial infections but also against those infections caused by protozoa.

Keywords: Ceftriaxone Disodium, Ornidazole, Antibiotic resistance, Aerobic bacteria, Anaerobic bacteria, Protozoal infections, Simultaneous equation method, Stability analysis.

INTRODUCTION

A number of antimicrobial agents are available today to treat infectious diseases. The use of antibiotics either individually or in fixed dose combination in the treatment of bacterial infections has achieved fabulous success [1].

Antibiotics such as penicillins and cephalosporins are inhibitors of bacterial cell wall biosynthesis. The bacterial cell wall differs from the mammalian cells in physical and chemical composition, so selective chemotherapy of bacterial infections can be achieved. Differences also exist in the cell walls of gram positive and negative organisms [2]. As a result not all antibiotics can penetrate gram negative bacterial cell walls, thus their use is limited for the treatment of infections caused by gram positive bacteria only.

Resistance is a major problem encountered with antibiotic therapy and involves mechanisms such as decreased cellular uptake of the drug due to known penetration barriers, lowered binding affinity to penicillin binding proteins and elaboration of the enzyme β -lactamase by micro-organisms which inactivate the antibiotic to penicilloic acid by causing hydrolysis of β -lactam bond. Allergic reactions to penicillins and cephalosporins in case of hypersensitive patients need to be taken into account before any therapy with these agents is initiated. Allergic reactions are likely to occur with the drugs commonly used like ampicillin and amoxicillin. In such cases, one often needs alternate and effective choices for therapy. Cephalosporin can be administered to patients experiencing allergenicity to penicillins.

Although antibiotics like aminoglycosides have broad antimicrobial spectra, their use is limited because of toxicity. Macrolide antibiotics have advantages like lower and less frequent dosing owing to their lipophilicity, but resistance is developed of an active efflux mechanism which expels the drug from the cell. Tetracycline also has resistance problems and troublesome side effects which may limit their use in therapy. The use of cyclic polypeptide antibiotics like vancomycin and bacitracin are restricted to gram positive organisms. Mupirocin has a broad spectrum of activity but can be used only topically for skin infections.

A number of combinations of antibiotics are available in the market. Combination therapy of ampicillin/amoxicillin with clavulanic acid (irreversible β -lactamase inhibitor) enhances the potency against β -lactamase producing strains of bacteria. Ticarcillin and potassium clavulanate have more potent antipseudomonad activity. A more clinically popular combination is that of ampicillin and sulbactam/piperacillin and tazobactam. Both sulbactam and tazobactam are β -lactamase inhibitors. The problem that surfaces here is that not all β -lactamase can be inhibited by clavulanic acid/tazobactam/sulbactam. The β -lactamase inhibitors have little or no antibacterial activity. An alternative to the use of penicillin's in order to overcome resistance by β -lactamase is cephalosporin. Allergic reactions are less common and less severe when administered to patients which are a big advantage over penicillins. These agents also have an extended spectrum of antibacterial activity for example; cefmetazole and cefoxitin can be used against anaerobes as well. A few of these agents possess oral activity too. Several of cephalosporins are used in combination with β -lactamase inhibitors like Sulbactam/Tazobactam thereby providing greater therapeutic benefit.

One noteworthy combination of cephalosporins is administration with a nitro-imidazole like Ornidazole. A combination of Cefixime and Ornidazole can be administered orally, but cefixime does not have significant activity against gram negative bacteria and staphylococci. On account of these problems the present research work has been undertaken to combine Ceftriaxone, a third-generation cephalosporin with a nitro-imidazole such as Ornidazole to overcome some problems with the use of other antibiotics like allergenicity and narrow antimicrobial spectrum of activity.

Ceftriaxone is a long acting, broad spectrum parenteral cephalosporin. Structurally it has a C-3 side chain consisting of thiotriazinedione which is acidic and metabolically not vulnerable in addition to a C-7 methoxy imino group [3]. It is useful for severe infections prophylactically and post surgery. It also has anti-gram negative activity and is useful in meningitis and gonorrhoea infections. It inhibits the synthesis of bacterial cell wall leading to its death.

Ornidazole is a 5-nitro imidazole derivative which is effective against protozoal diseases and anaerobic infections [4]. It is also used for the prophylaxis of post-operative Crohn's disease. Its action is due to reduction of nitro group to an amine that attack microbial DNA, inhibiting its further synthesis and causing degradation of existing DNA.

Literature survey suggests that Ceftriaxone can be administered in combination with Metronidazole/Ornidazole and no significant physical or chemical incompatibilities have been reported. Ornidazole is preferable over Metronidazole because of longer half-life and fewer side-effects. At present no such fixed dose combination of Ceftriaxone with Ornidazole is available in the market. The proposed fixed dose combination will have a prolonged serum half life (~13 hours). Frequency of administration can be minimized (once a day dosing), thus reducing the work for nursing staff [5-7]. The combination will also reduce treatment costs due to reduced hospital stay and will provide additional protective effect against anaerobes and protozoal infections [8-14].

Therefore the objective of this work is to develop a fixed dose combination of Ceftriaxone disodium and Ornidazole to be given as an intravenous infusion/injection which will provide an alternative to the combinations of antibiotics already existing in the market and the therapy of which can be initiated at the institutional level, to evaluate the combination to prove its efficacy against combinations of Ceftriaxone with other antibacterial agents like tazobactam/sulbactam and to develop a rapid, economical and accurate analytical method for determining the concentration of the two drugs in solution.

MATERIALS AND METHODS

Ceftriaxone disodium and Ornidazole were procured as gift samples from Koprana Pharma, Mumbai and Elder Pharmaceuticals, Navi Mumbai respectively. Dextrose monohydrate and sodium chloride were purchased from Loba Chemie, Mumbai. Other chemicals and reagents were of analytical grade.

Study of Physical Interaction between Ceftriaxone and Ornidazole

Infrared spectrum was recorded by scanning the samples of pure drugs individually and that of the combination over a wave number range of 4000 to 400 cm^{-1} using Fourier Transform infrared spectrophotometer (FT-IR 4100, JASCO) to investigate the physical interaction between the two drugs. The two drugs were combined in various proportions like 1:1, 2:1, 3:1 and 4:1 to determine the physical compatibility in the solid state.

Solubility analysis

Both Ceftriaxone disodium and Ornidazole are readily soluble in water at room temperature. Ornidazole is highly soluble in methanol but Ceftriaxone disodium is only sparingly soluble. The solubility of both the drugs in water being quite high, this was used as a solvent system to dissolve both the drugs for preparing the infusion. Solubility was also determined in 0.9 % sodium chloride solution and in 5 % and 10 % dextrose monohydrate solutions [15-17].

Determination of Spectral Characteristics

Solutions of Ceftriaxone disodium and Ornidazole (10 $\mu\text{g}/\text{ml}$, each) were prepared separately by appropriate dilution of standard stock solution (0.5 mg/ml) in double distilled water. A double-beam Lab India-UV 3000+UV- Visible spectrophotometer with a spectral band width of 2nm and a pair of 1 cm matched quartz cells was used to measure the absorbance of the resulting solutions. Both the solutions were scanned in the spectrum mode from 380 nm to 200 nm. Overlay absorption spectra was recorded.

Antimicrobial activity of Ceftriaxone and Ornidazole in combination

Agar-well diffusion assay method was used to determine the susceptibility of the two drugs in combination on selected bacterial strains like *Staphylococcus aureus* (gram positive aerobe), *Escherichia coli* (gram negative aerobe) and *Klebsiella pneumoniae* (gram negative aerobe).

20 ml of sterile agar suspension was poured in to sterile Petri plates so as to keep the thickness of agar gel uniform in each plate. The bacterial suspension was spread on to the surface of the agar by a sterile spreader. A single well of a suitable diameter was cut in to each plate with a sterile borer (5 mm). Different concentrations of the mixture of the two drugs in solution were applied to each of the plates (100 μl). The activity was compared to Standard Ceftriaxone and Ornidazole solutions having concentrations 10 mg/ml and 6.25 mg/ml respectively which served as positive control. Negative control was water. The results were then compared with marketed formulations of Ceftriaxone in combination with other antimicrobial agents like tazobactam /sulbactam.

The synergistic effect of the two drug substances was studied by the strip method on agar plates by placing filter papers soaked with the drug solution perpendicular to each other and measuring the distance of overlapping of the two filter papers. Incubation of the plates was done at 37°C for 24 hrs. A clear zone of inhibition for each of the plates was observed indicating that bacteria have been adversely affected by the anti bacterial agents. The size of the inhibition zone was measured in each of the plates of different concentrations of the formulation using a transparent ruler in millimeters. All concentrations were tested in duplicates. The well variant of the diffusion method was used as it was more sensitive than the disc variant method and gave reproducible results.

Formulation Development

The results of the agar well diffusion assay method was used to formulate the fixed dose combination of Ceftriaxone and Ornidazole having different concentrations to be administered as intravenous infusion/injection after reconstituting the dry powders in different IV diluents. The formulations were then subjected to stability analysis.

RESULTS AND DISCUSSION

FTIR studies for Ceftriaxone showed characteristic peaks at 3432.7 cm^{-1} (N-H stretching mode of H-bonded amide group), 1741 cm^{-1} (β -lactam C=O stretching vibrations) and 1592 cm^{-1} (oxime C=N stretching vibrations). The spectra for Ornidazole revealed peaks at 3258.14 cm^{-1} (O-H stretching vibrations), 1536 cm^{-1} (asymmetric NO_2 stretching vibrations) and the C=O stretching mode at 1741 cm^{-1} . No significant change in the appearance of the characteristic peaks of both the drugs when combined was observed in the IR spectrum (figure 1, 2 and 3). No change in the colour of the mixture from its original white or no caking and liquefaction of the mixture was observed when kept in amber coloured glass vials with rubber closures at room temperature and in the refrigerator for a period of 3 months. This indicates that there is no physical interaction between the two drugs when combined.

Solution of Ceftriaxone disodium in water exhibited a λ_{max} at 241 nm with an absorbance value of 0.475 as shown in figure 4 whereas Ornidazole solution exhibited a λ_{max} at 319.2 nm with an absorbance value of 0.403 as shown in figure 5. The isobestic point as found from the overlain spectra was 289.6 nm with an absorbance value of 0.370 (figure 6).

Both Ceftriaxone and Ornidazole were found to absorb at the λ_{max} of the other. Therefore the technique of simultaneous equation (Vierodt's method) was developed to determine the concentrations of two drugs for the prepared formulations and also for the assay in the stability studies. The simultaneous spectrophotometric method based on Vierodt's method was validated statistically for parameters like linearity and range, precision, accuracy and specificity [18-20]. For linearity determination, from a stock solution of 1 mg/ml for Ceftriaxone and 0.5 mg/ml for Ornidazole, series of solutions were prepared containing Ceftriaxone 5,10,15,20,25 $\mu\text{g}/\text{ml}$ and Ornidazole 3,6,9,12,15 $\mu\text{g}/\text{ml}$ by diluting the stock standard solution with water in standard volumetric flasks of 25 ml. After scanning all the dilutions in the wavelength range of 200-400 nm for assuring the λ_{max} , the absorbance of each solution was measured at 241 nm and 319 nm. Standard absorptivity values were calculated. Mixed sample solutions of different concentrations were used and the absorbance of these solutions was measured at selected

wavelengths as A_1 and A_2 . The concentration of two drugs in each solution was calculated using simultaneous equations:

$$A_1 = ax_1C_x + ay_1C_y \dots\dots\dots 241 \text{ nm}$$

$$A_2 = ax_2C_x + ay_2C_y \dots\dots\dots 319 \text{ nm}$$

Where ax_1 and ax_2 are absorptivity of Ceftriaxone at 241 and 319 nm.

ay_1 and ay_2 are absorptivity of Ornidazole at 241 and 319 nm.

A_1 and A_2 are absorbance values of sample solutions at 241 and 319 nm.

C_x and C_y are concentrations of Ceftriaxone and Ornidazole respectively.

Statistical parameters of linearity studies are reported in Table 1.

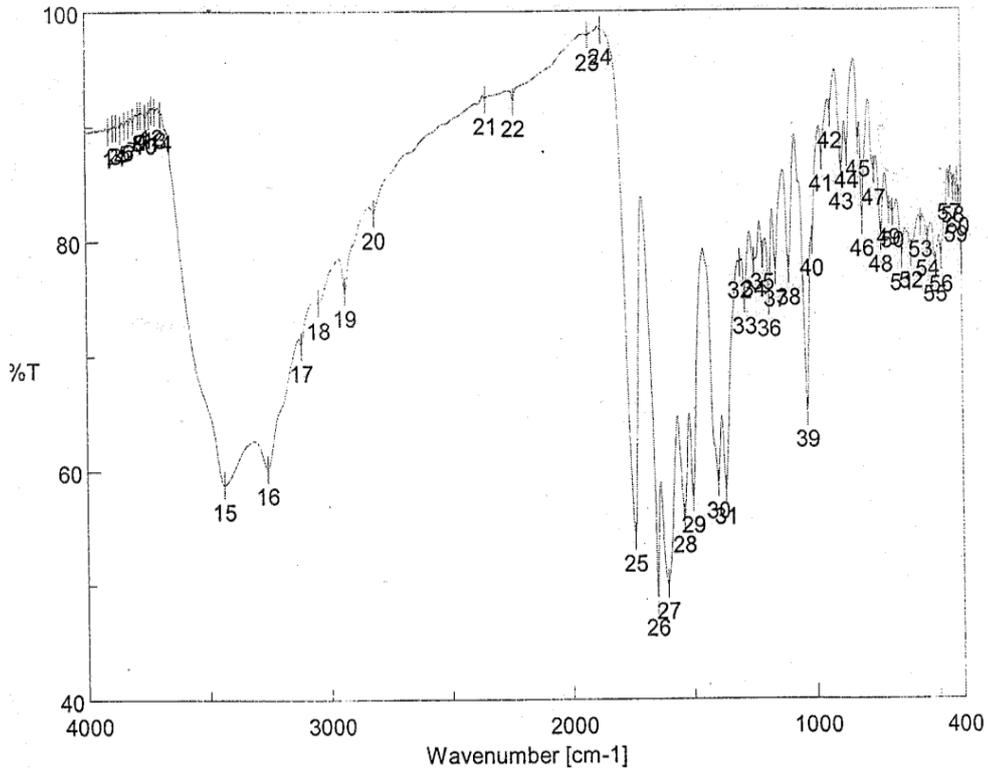


Fig. 1: FT-IR Spectrum of Ceftriaxone disodium.

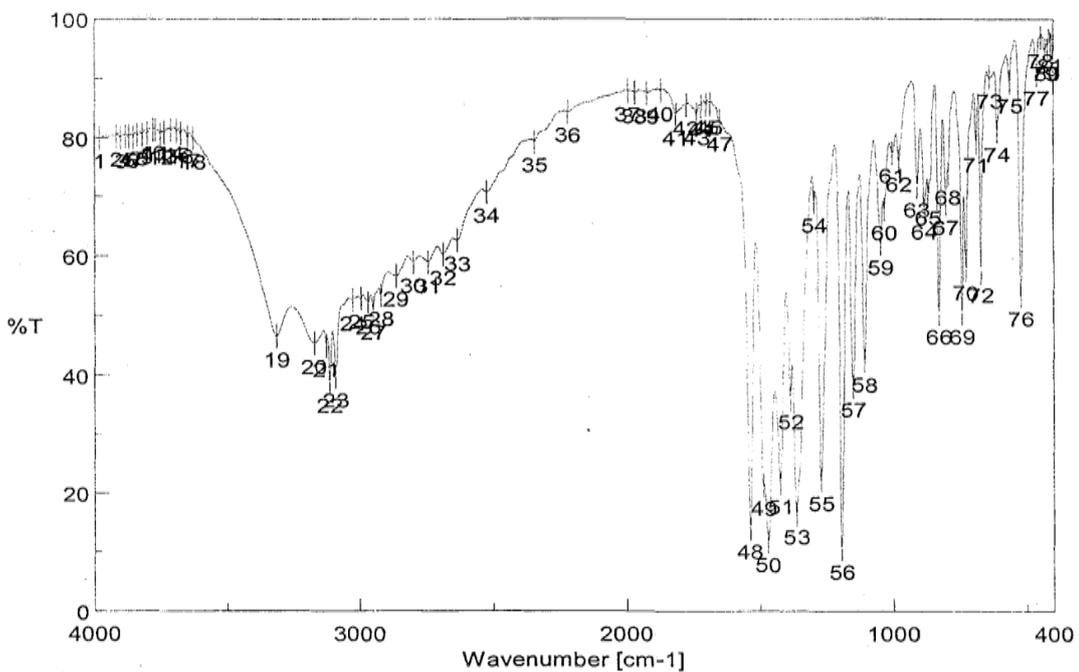


Fig. 2: FT-IR Spectrum of Ornidazole.

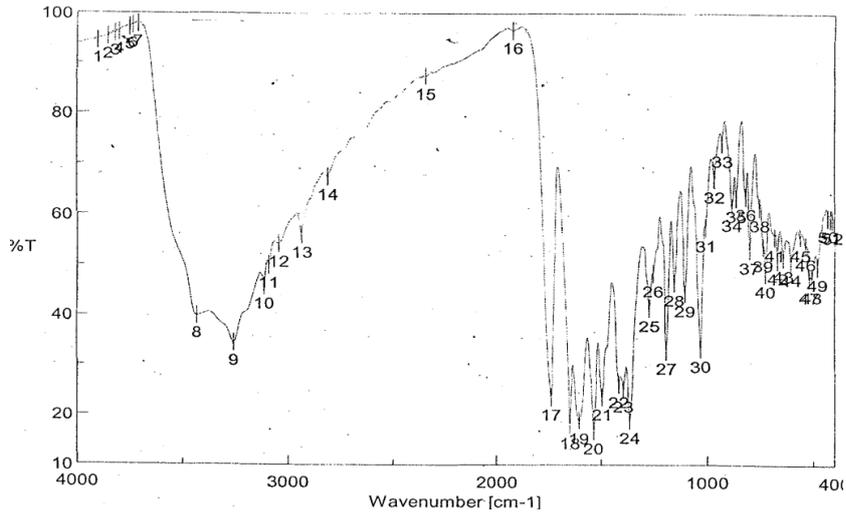


Fig. 3: FT-IR Spectrum of Ceftriaxone disodium and Ornidazole in combination.

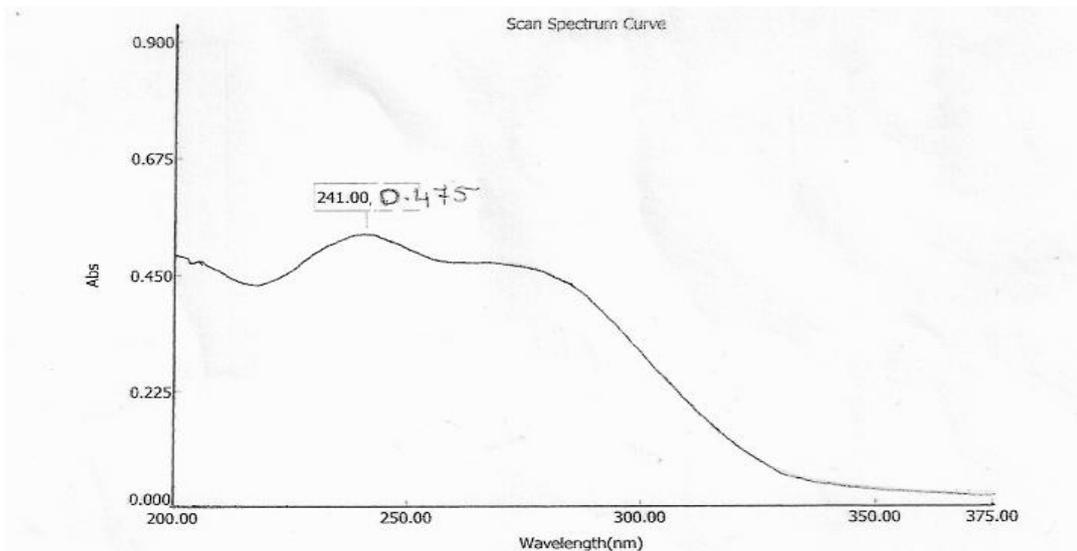


Fig. 4: UV Spectrum scan of Ceftriaxone disodium (10 µg/ml) in distilled water.

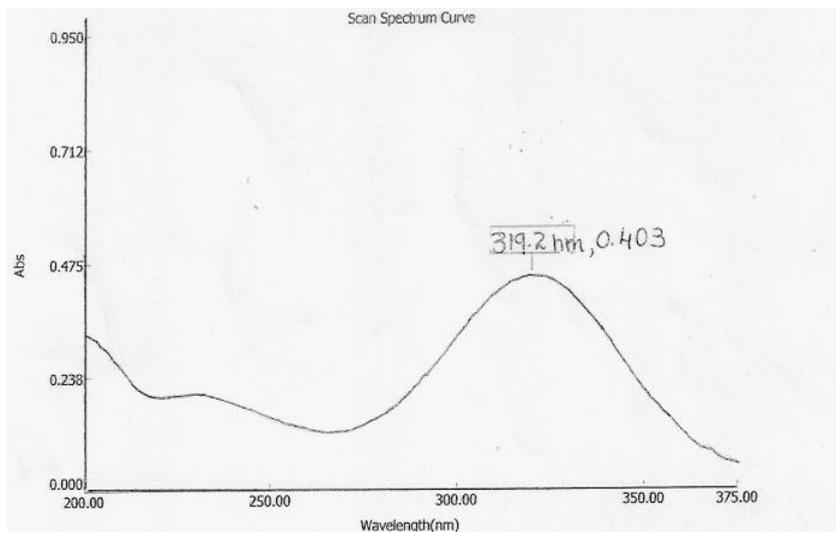


Fig. 5: UV Spectrum scan of Ornidazole (10 µg/ml) in distilled water.

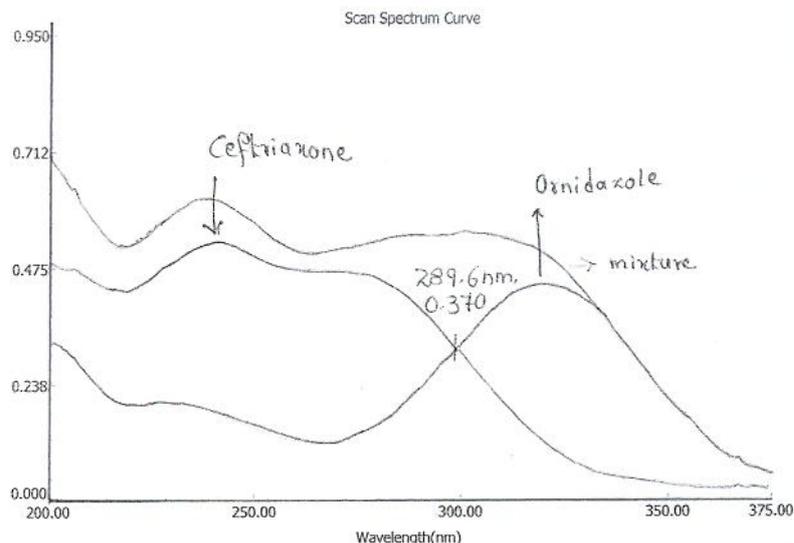


Fig. 6: Overlain spectra showing isobestic point.

Table 1: Results of Linearity data and Range

Parameters	UV - Simultaneous equation method			
	CEFT		ORN	
	241 nm	319 nm	241 nm	319 nm
Linearity Range ($\mu\text{g/ml}$)	5-25	5-25	3-15	3-15
Linear Regression equations	$y=0.048x+$ 0.022	$y=0.008x+$ 0.010	$y=0.014x+$ 0.001	$y=0.040x+$ 0.000
Std. Deviation	0.3697	0.0629	0.0657	0.1933
Coefficient of Variation	0.4938	0.4469	0.5155	0.5252
Correlation Coefficient	0.9990	0.991	0.9990	0.9998

CEFT and ORN stands for Ceftriaxone disodium and Ornidazole respectively.

Recovery studies were carried out for both the drugs to determine the accuracy of the proposed analytical method by spiking standard drug in the powdered formulations at the level of 80%, 100%, 120% amount of each dosage as per ICH guidelines. The results of the recovery analysis are shown in Table 2.

The reproducibility of the method was ascertained by performing the assay of mixed solutions at different time intervals on the same day (intraday precision) and on three different days (inter day precision). % RSD was found to be 0.868 (for Ceftriaxone) and 0.505 (for Ornidazole) for intraday precision. Inter day precision was

found to be 0.736 for Ceftriaxone and 0.892 for Ornidazole [21-23]. The results of the validation studies of the developed analytical method suggests that the concentration of two drugs in combination can be determined spectrophotometrically by simultaneous equation method with greater accuracy and precision and is simple, rapid and economical as well.

The anti bacterial activity of the formulations and the observed zone of inhibition values as determined against maintained bacterial strains *in vitro* by agar well diffusion assay method are reported in Table 3.

Table 2: Results for Recovery Studies

Formulation	mg/Dose taken		mg/Dose obtained		% Recovery \pm S.D.*
	CEFT	ORN	CEFT	ORN	
F1	250	125	251.28	126.31	100.51 \pm 1.78
F2	375	125	379.83	123.75	102.35 \pm 0.77
F3	500	125	495.62	124.09	99.23 \pm 1.705

* denotes n=6 average of six determinations. CEFT and ORN stands for Ceftriaxone disodium and Ornidazole respectively. F1= Formulation containing 250 mg of Ceftriaxone disodium and 125 mg of ornidazole. F2= Formulation containing 375 mg of Ceftriaxone disodium and 125 mg of Ornidazole. F3= Formulation containing 500 mg of Ceftriaxone disodium and 125 mg of Ornidazole.

Synergism is exhibited as the combined effects of two drugs against the test bacterial strain was found to be greater than the sum of the individual effects of each drug acting alone. The degree of antimicrobial activity varied with the concentration of the two drugs i.e., increase in activity was observed with increase in concentration. The combination was found to be more active against gram negative aerobes (*E. coli* and *K. pneumoniae*) than gram positive aerobe (*Staph. aureus*). The activity against *Staph. aureus* is due to Ceftriaxone as no activity was observed for Ornidazole. The maximum tolerant concentration for Ornidazole was found to be 12.5 mg/ml beyond which no significant increase in effect was observed. The activity against anaerobic bacteria could not be

determined as it was difficult to maintain the bacterial strain in the laboratory conditions. The antimicrobial properties of fixed dose combination of Ceftriaxone and Ornidazole injection/infusion evaluated in some aerobic and anaerobic bacteria suggest that the combination is superior and exhibits synergism as compared to Ceftriaxone and Ornidazole individually. It is already well established that Ornidazole has anti-protozoal activity and also has activity against anaerobic bacteria. Comparative antimicrobial activity of Ceftriaxone/ tazobactam and Ceftriaxone/sulbactam with the combination of Ceftriaxone and Ornidazole demonstrated that the proposed formulation is as effective as the marketed formulations of Ceftriaxone with β -lactamase inhibitors.

Table 3: Observed zone of inhibition

Concentration of CEFT, ORN	Zone of Inhibition in mm		
	<i>Staph. aureus</i>	<i>E. coli</i>	<i>K. pneumonia</i>
10 , 6.25 mg/ml	15	25	23
12.5 ,6.25 mg/ml	17	28	25
12.5 ,12.5 mg/ml	19	31	25.1
25 , 12.5 mg/ml	22.2	36.2	30
37.5 ,12.5 mg/ml	25	39	33
50 , 12.5 mg/ml	29.3	42	36
10 mg /ml CEFT	16	16	18.1
6.25 mg/ml ORN	-	15.2	16
CEFT/ Sulbactam* (1000 mg , 500 mg)	17	29.3	25
CEFT/Tazobactam* (1000 mg , 125 mg)	23.3	26	24.3
Synergistic effect of CEFT 25 mg/ml and ORN 12.5 mg/ml	25	32	-

* Denotes marketed preparations. CEFT and ORN stands for Ceftriaxone disodium and Ornidazole respectively.

Solution state stability analysis of three different concentrations of the proposed formulation was studied at room temperature (25°C) and refrigerated temperature (4°C) when stored in amber coloured glass containers in the following diluents as depicted in Table 4. It was thus found that solutions of Ceftriaxone and Ornidazole remained stable at concentrations of 250 and 125 mg/10 ml, 375 and 125 mg/10 ml, 500 and 125 mg/10 ml in the commonly used

intravenous diluents. The loss of potency was found to be less than 10 %. The colour of the solutions ranged from light yellow to amber colour based on the time period of storage and the concentration. The solution state stability analysis data suggests that no significant degradation occurs for both the drugs when combined and the formulation after reconstitution in the commonly used IV diluents remains stable for sufficient time period.

Table 4: Stability analysis data

Serial No.	Diluents	Formulation	Refrigeration (4°C)	Room Temp. (25°C)
1.	Sterile Water for injection	F1	10 days	4 days
		F2	10 days	4 days
		F3	10 days	4 days
2.	0.9 % Sodium Chloride solution	F1	5 days	3 days
		F2	5 days	3 days
		F3	5 days	3 days
3.	5 % Dextrose	F1	5 days	2 days
		F2	5 days	2 days
		F3	5 days	2 days
4.	10 % Dextrose	F1	2 days	2 days
		F2	2 days	2 days
		F3	2 days	2 days
5.	5 % Dextrose and 0.45 % Sodium Chloride solution	F1	2 days	2 days
		F2	2 days	2 days
		F3	2 days	2 days
6.	5 % Dextrose and 0.9 % Sodium Chloride solution	F1	2 days	24 hours
		F2	2 days	24 hours
		F3	2 days	24 hours

F1= Formulation containing 250 mg of Ceftriaxone disodium and 125 mg of ornidazole. F2= Formulation containing 375 mg of Ceftriaxone disodium and 125 mg of Ornidazole. F3= Formulation containing 500 mg of Ceftriaxone disodium and 125 mg of Ornidazole.

CONCLUSIONS

As a result of decline in research in antibiotics and the problem of antibiotic resistance growing steadily, there is a need for newer and novel combinations of antibacterial agents to be administered in fixed dose. A regimen that is safe, efficacious and reduces the work for the nursing staff resulting in gained nursing time and also inexpensive is required. As the administration of Ceftriaxone and Ornidazole has been reported to have favorable pharmacokinetic properties, the new fixed dose combination of Ceftriaxone and Ornidazole has therefore, all the advantages needed for an effective antimicrobial therapy. Thus as discovered through this research, it may be concluded that the fixed dose combination of Ceftriaxone and Ornidazole is an alternative and effective option for use in many mixed microbial infections caused due to aerobic and anaerobic bacteria and pathogenic protozoa .

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