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New Formulation of Trimethoprim Injectable Solution for Veterinary Use

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Summary

The aim of this study was prepared new formulation of trimethoprim injectable solution in the Physiology and pharmacology department /College ofVeterinary Medicine, University of Baghdad.Trimethoprim injection formulation is antibacterial and used against a number of bacteria, protozoa and Rickettsia. Data was collected about the materials used in the preparation of the formula from the well-known pharmacopeia, including the specification, physical and chemical properties of active ingredient, the additive and preservative that must be used. Three pilot formulae were prepared from analar chemical ingredient from which one formula was chosen and tested to approve its quantitative and qualitative specification. Quantitative evaluation, stability of the formula was also tested under different storage environmental condition of low and high temperature at different periods through and one year and through the questionnaire field. Questionnaire proved the product, stability and therapeutic efficiency. The composition obtained a certificate of acceptance from the Veterinary State Company and Veterinary Drug Research and production Centre as new preparation Formula of Trimethoprim Injectable Solution for Veterinary use.

***Key words*: Trimethoprim, Veterinary, therapeutic, bacteria, protozoa, Rickettsia**

تركيبة جديدة للترايمثوبريم محلول معد للحقن البيطـــري

عروبة محمد سعيد إبراهيم دريد عبد الهادي عباس
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الخلاصة

الهدف من هذه الدراسة هو تحضير تركيبة جديدة من الترايمثوبريم كمحلول معد للحقن قد تم تحضيره من قبل الباحثين في فرع الفسلجة والأدوية / شعبة الأدوية / كلية الطب البيطري - جامعة بغداد . مستحضر ترايميثوبريم/ حقن من المضادات البكتيرية ويستخدم ضد عدد من البكتريا والبروتوزوا والركتسيا. تم جمع بيانات حول المواد المستخدمة في إعداد الصيغة الدوائية المعرفة في دساتير الأدوية العالمية، بما في ذلك المواصفات والخصائص الفيزيائية الكيميائية للمواد الفعالة، فضلا عن إضافة المواد الحافظة. وقد أعدت ثلاث تركيبات تجريبية من المكونات الكيميائية والغرض منها الوصول إلى التركيبة النهائية المعتمدة والتي تم اختيارها بعد اختبارها وموافقتها للمواصفات الكمية والنوعية. التقييم الكمي تم اختباره مع استقرار الصيغة التركيبية تحت ظروف التخزين البيئية المختلفة من درجات الحرارة المنخفضة والعالية وفي فترات مختلفة خلال عام واحد. ومن خلال الاستبيان الحقلي الذي أجري على المستحضربرهن ثباتيته وكفاءته العلاجية. وقد حصلت التركيبة على شهادة القبول من الشركة العامة للبيطرة ومركز بحوث إنتاج الأودية البيطرية كمستحضر تركيبي جديد للترايمثوبريم للحقن وللاستعمال البيطري.

كلمات مفتاحية : ترايمثبريم، بيطري، علاجي، بكتريا، بروتوزا، ريكتسيا.

Introduction

Trimethoprim is a synthetic antibacterial combination product.That considered as bacteriostatic antibiotic, it belongs to the class of chemotherapeutic agents known as dihydrofolatereductase inhibitor. Trimethoprim acts by interfering with the action of bacterial dihydrofolate reductase, inhibiting synthesis oftetrahydrofolic acid. Which is an essential precursor in the *de novo* synthesis of the intermediate Thymidine monophosphate (dTMP), a precursor of DNA metaboliteThymidine triphosphate(1)

Bacteria are unable to take up folic acid from the environment and are thus dependent on their own synthesis. Inhibition of the enzyme deprivesthe bacteria of nucleotides which are necessary for DNA replication causing, in certain circumstances, cell lethality.Trimethoprim combination with Sulphadimidine antibiotic at 1:5 ratioinhibits an earlier step in the folate synthesis pathway **(figure 1)**

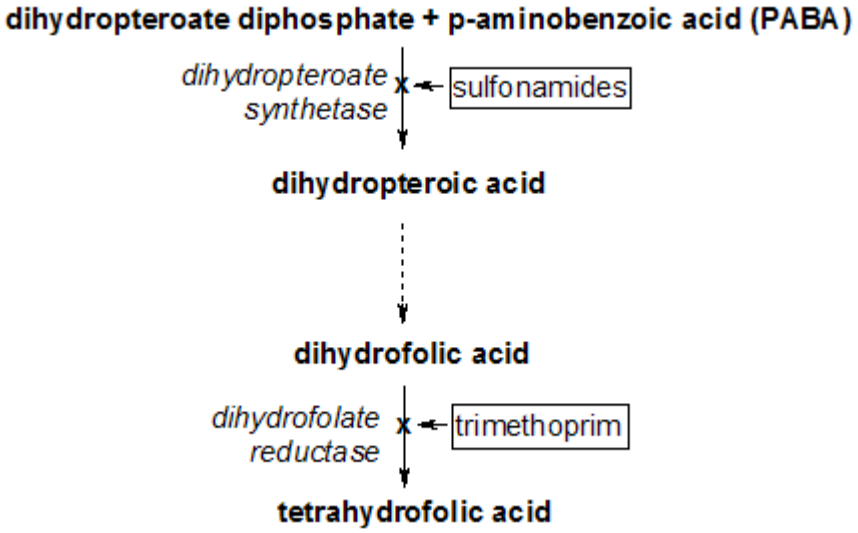

Figure1. Mechanism of action of trimethoprim

This combination, results in an in-vitrosynergistic antibacterial effect by inhibiting successive steps in folate synthesis. This claimed benefit was not seen in general clinical use (2). The combinations use has been declining due to reports of sulphadimidine or sulfamethoxazolebone marrow toxicity, resistance and lack of greater efficacy in treating common urine and chest infections, and side effects of antibacterial sulfonamides (3).

Sulphadimidine /trimethoprim injection is effective against susceptible strains of *Streptococcus pneumoniae, Escherichia coli, Haemophilusinfluenzae, Morganellamorganii, Proteus mirabilis,*indole-positive Proteus species (eg, *Proteus vulgaris*), Klebsiella species, Enterobacter species, *Shigellaflexnerii*, and*Shigellasonnei*. These agent is labeled for the treatment of *Pneumocystis jiroveci* (formerly *P. carinii*) pneumonia; enteritis caused by *S. flexneri* or *S. sonnei*; and severe or complicated urinary tract infections caused by *E. coli*, *M. morganii*, *Klebsiella* species, Enterobacter species, or Proteus species when oral therapy is infeasible and the organism is not susceptible to single antimicrobial agents (4).

The combination of Sulphadimidin and Trimethoprim acts synergistic and usually bactericidal against many Gram-positive and Gram-negative bacteria like *E. coli, Haemophilus, Pasteurella, Salmonella, Staphylococcus* and *Streptococcus spp.*

Gastrointestinal, respiratory and urinary tract infections caused by TMP and Sulphadimidin sensitive bacteria, *E. coli, Haemophilus, Pasteurella, Salmonella, Toxoplasmosis , Staphylococcus* and *Streptococcus* spp., in horse, foal, calves, cattle, pig (5).

Trimethoprim (Bp.): Trimethoprim, BW 56.72.2, 4-Diamino -5- (3,4.5-Trimethoxyben zyl) pyramidin. (14H18N4O3=290.3).A white odourless powder with a very bitter taste. M.P. about 200 soluble 1 in 2500 of water, 1 in 300 of alcohol, 1 in 55 of chloroform and 1 in 80 of methyl alcohol, practically in soluble in ether. A 1% suspension in water has a pH of 7.5 to 8.5.

Trimethoprim affects the nucleoprotein metabolism of cells similarly to pyrimethamine by interference in the folic – folinic acid systems. Its effects are

considerably greater on the cells of microorganism than on mammalian cells. Sulfonamides act on the same biological pathway at a different point and the effects of Trimethoprim and sulfonamides are synergistic. Trimethoprim has been used in the treatment of malaria and may be of value in infection caused by strains of malaria parasites resistant to pyrimethamine although. It is less effective than pyrimethamine against sensitive strain, for human it may be given alone usually in dose of up to 1.5gm. daily for 7 days. Or in conjunction with Sulfonamides, when a single 500mg dose. (6)

Sulphadimidine (BP.): Sulphadimidine (I.P); Sulphadimethyl pyrimidine, Sulphamethazine, Sulphamerthazine (U.S.P.) Sulphadimerazine, Sulphadimezium. (7)

Materials and Methods

-Materials and Chemical compounds:

Trimethoprim, Sulphadimidine, Sodium Hydroxide, Sodium Sulphite, Methylparaben, Propylparaben, Sodium Nitrite, Hydrochloric acid Chloroform, Anhydrous sodium sulphate, Prechloric acid, Crystal violet, and Glacial Acetic

- Apparatus :

Sensitive balance, Magnetic stirrer, Millipore filtration, pH meter, Different glassware (Separator funnel, Volumetric Beaker, Cylinder, volumetric flask, Conical flask, Stirrer, ptri-dish, brown color vial) Starch – Iodine paper Autoclave and Incubator

Procedure: Weighing the material to produce 100 ml. injectable solution formula. Trimethoprim 4.0gm., Sulphadimidin 20.0gm., Sod. hydroxide 4.5gm., Sod. sulphite 0.1 gm., Methylparaben 0.07 gm., Propylparaben 0.03gm., Ethylalcohol 99% 10 ml. ,and Distilled water add to 100 ml.

Adding distilled water in volume beaker up to 50 ml adding the above materials as following:

4.5 gm Sod. hydroxide with continuous mixing , 0.1gm Sod. sulphite with continuous mixing, 20gmSulphadimidinwith continuous mixing.

Mixing distilled water and ethanol up to 50 ml in volume beaker and then step wise add (4 gm) Trimethoprim with continuous mixing, mixing the step (b) with (c) until clear solution, Adjusting the pH by using the buffer, Filtration the final product by using paper (0.2)M., packing the final product in volume bottle of 100 ml., Send sample to the quality control for estimation.(8,9,10,11,12). Table (1,2)

N.B. all step done under complete sterilized technique and under U.V light cabinet.

Composition: Each l00 ml contain

-Trimethoprim 4 gm

- Sulphadimidine 20 gm

Table 1: Quality Standard of Sulphadimidin / Trimethoprim Injection 15 February 2009

Quality Standard according to BP2002	
Moisture	**≤2%**
PH	**10.0~11.0**
Color of Solution	**≤Y_4**
Related Matters	**≤0.5%**

Table 2: Specification of Sulphadimidine / Trimethoprim Injection

Description	**clear brown solution**
Acidity or alkalinity	**pH 10 – 11(Quality Standard BP2002)**
Package	**l00 ml**
Storage	**under 25C^{o}**
Content of trimethoprime	**(95 –110) %**
Content of Sulphadimidin	**(95 –110) %**

Dosage: For intramuscular administration:
Horse, cattle : 0.15 ml/ kg body weight,
Calves, goats, sheep, foal and swine: 0.2ml / kg body weight
Dog and cat : 0.3ml / kg body weight
Once or Twice daily for 2-3 days
Withdrawal times: For meat: 12 days, For milk: 4 days.
Packaging: 10 ml/vial, 50 ml/vial and 100 ml/vial.
Assay:
1. Sulphadimidine:- The following steps was done

keep the temperature below 15C^{o}during all the procedure. Using volume equivalent to 0.5g. of Sulphadimidin in a conical flask. Add 75 ml, of water, and 10 ml, Conc. HCl, then mix and titrate with 0.1 M. Sod. nitrite until dark blue colour appears immediately after the addition drop of solution on starch–iodine paper. Each ml of 0.1).Sod.nitrite is equivalent to (0.02783)gm. of $C_{12}H_{14}N_4O_2S$ (7)

2. Trimethoprim:- The following steps was done

Transfering 10 ml, injectable solution to separator funnel and adding 5 ml, of 20% NaOH, that extracted three time with 30 ml, chloroform. Filter each extract chloroform layer through anhydrous sod. sulphate and collecting in 250 , conical flask. That keep for evaporate to dryness. Then dissolve it in 40 ml, glacial acetic and titrate with 0.1 N prechloric acid using crystal violet as indicator to the green – blue end point. Each ml. Of 0.1 N prechloric acid is equivalent to (0.0293)g mtrimethoprime(8).

The Stability of trimethoprim injectable solution (formula was stable) after storage at room temperatures and temperatures 50C^{o} with different time periods. Quantitative analysis was studied using (HPLC) high-performance liquid chromatography**.** Samples were also examined visually for color change or precipitation. More than 98% of the initial concentration remained after storage (9) (table 3)

Table (3): Chemical analysis of Active ingredient *Trimethoprim injectable solution formula*

Type of analysis	Temperature	pH	Active ingredient %	Date
Fix methods Trimethoprim Sulphadimidine	RT	10.2	90.96% 99.03%	
Zero time Trimethoprime Sulphadimidin	RT	10.4	100.0 % 98.03 %	14.1.2007
After 1 month Trimethoprim Sulphadimidine	RT 50 c^o	10.0	100.1 % 98.05 %	14.2.2007
After 6 months Trimethoprim Sulphadimidine	RT 50 c^o	10.0	100.5% 98.08 %	14.8.2007
After over 1 year Trimethoprim Sulphadimidine	RT 50 c^o	10.0	101.0 % 99.0 %	14.1.2008

RT: Room Temperature

Antimicrobial activity of trimethoprim injectable solution (Formula)

Measurement of antimicrobial activity:

The antimicrobial activity of trimethoprim formula has been measured by the use of agar well diffusion techniques. This technique done by takes plates of agar mixed with cultures of particular bacteria, *Staphylococcus aureus* being the most commonly used, *Corynbacterium, Clostridia,.E.coli, Klebsella, Shigella, Salmonella, Proteus, Brucella* and *Pasteurella*. Wells are cut into the agar and solutions of the test material applied at varying concentrations of trimethoprim formula, the diameter of the zone of bacterial growth inhibition is measured after incubation of the plates at 37C^o for 24 hrs.(13, 14) (Table 4) .

Table (4): Diameter Zone inhibition (mm) of Trimethoprimat different concentration (ug/ml) against different microorganism

Microorganism	Concentration ug/ml				
	1.25	2.5	5	10	20
S. aureus	10 ± 0.2	12 ± 0.12	14 ± 0.3	18 ± 0.4	21 ± 0.12
Corynbacterium	9 ± 0.12	11 ± 0.02	13 ± 0.04	19 ± 0.4	23 ± 0.03
Clostridia	8 ± 0.2	10 ± 0.11	12 ± 0.11	18 ± 0.11	21 ± 0.04
E.coli	12 ± 0.3	13 ± 0.12	15 ± 0.3	19 ± 0.03	23.5± 0.20
Klebsella	10 ± 0.2	12 ± 0.02	14 ± 0.02	18 ± 0.04	20 ± 0.11
Shigella	11 ± 0.13	13 ± 0.10	15 ± 0.3	19.5 ± 0.4	23 ± 0.22
Salmonella	12 ± 0.4	14 ± 0.11	17 ± 0.01	20.5± 0.14	24 ± 0.02
Proteus	10 ± 0.2	12 ± 0.02	14 ± 0.3	18 ± 0.01	21 ± 0.01
Brucella	11 ± 0.11	13 ± 0.10	16 ± 0.02	21 ± 0.02	22 ± 0.11
Pasteurella	9 ± 0.10	10 ± 0.11	12 ± 0.3	16.5± 0.01	19 ± 0.02

While the Specification of final product of Trimethoprim, Sulphadimidin, Sod. Hydroxide and Sod. Sulphite showed in table (5)

Table (5): Specification of final product

Substance name	Specification	Description	Solubility
Trimethoprim	**BP 20l0**	**White, odourless powder with a very bitter taste.**	**Soluble 1 in 2500 of water 1 in 300 of Alcohol 1 in 55 of chloroform 1 in 80 of methyl alcohol Practically soluble in ether**
Sulphadimidin	**BP 20l0**	**White or creamy-white crystals or powder, odourless or almost odourless**	**Very slightly soluble in water Soluble in 120 part of water**
Sod. Hydroxide	**BP 20l0**	**White sticks, pellets fused masses, scales, dry hard brittle and showing a crystalline**	**Completely or almost completely soluble in 7 part of water**
Sod. Sulphite	**BP 20l0**	**White, crystalline powder, odourless or almost odourless**	**soluble in water Practically soluble in ether**

Results and Discussion

The results of evaluation (table 1) of the effectiveness and stability of the formula product at room temperature and at time zero, immediately after the preparation without storage, indicated that the effectiveness was very good for active ingredient and was stable during the last six months of follow-up (table 3). When the product stored at room temperature and 50C^{o}, observed an increasing in the pharmacological activity of the preparation, this was noted when an older drug, and this determines the old of product at a temperature not exceeding 25C^{o} and that old of the product up to (1) year (15) . The stability and quantitative analysis of trimethoprim in this solution after storage at room temperatures and 50^{o}C also studied, by using high-performance liquid chromatography (table 3). Samples were also examined visually for signs of changing in color or precipitation. More than 98% of the initial concentration remained after storage at 50^{o}C, for 160 days. Examination of the stability data suggested that trimethoprim degradation was a zero-order process, although a first-order process could not be excluded.

Extrapolation of data from a logarithmic plot yielded a zero-order trimethoprim degradation rate constant at 25C^{o} of 0.0113 day-1. (16)The time for 25% trimethoprim degradation at 25C^{o} would be 845 days. No precipitation was observed, clear brown solution during storage. The extent of color change was associated with the degree of trimethoprim degradation. Trimethoprim, when prepared in the non aqueous solution, is stable at 25 C^{o}. and antimicrobial activity of trimethoprim after storage at room temperatures and 50 C^{o} was nearly stable for different time period. The preparation of trimethoprim injectable solution as antibacterial formula can be used against a number of species of bacteria, protozoa and Rickettsia in particular and other cases in general(10, 12, 17).

Trimethoprim (3,4,5-trimethoxybenzylpyrimidine) inhibits dihydrofolic acid reductase 50,000 times more efficiently in bacteria than in mammalian cells. This enzyme reduces dihydrofolic to tetrahydrofolic acid, a stage in the sequence leading to the synthesis of purines and ultimately of DNA. Sulfonamides and trimethoprim each can be used alone to inhibit bacterial growth. If used together, they produce sequential blocking, resulting in a marked enhancement (synergism) of activity. Such mixtures of sulfonamide

(five parts) plus trimethoprim (one part) have been used in the treatment of pneumocystis pneumonia, malaria, *shigella enteritis*, systemic salmonella infections, urinary tract infections, and many others. *Streptococci, Staphylococci, Corynbacterium, Clostridia, E. coli, klebsella, Salmonella, Proteus, Brucella and Pasteurlla* (11, 13, 18 and19).

Pyrimethamine also inhibits dihydrofolatereductase, but it is more active against the enzyme in mammalian cells and therefore is more toxic than trimethoprim. Pyrimethamine plus sulfonamide or clindamycin is the current treatment of choice in toxoplasmosis and some other protozoal infections (15, 16 and20,).

Sod. Hydroxide act to maintain the pH of the product. And prevent the degradation that gets during testing and after packing. The materials are part of a portfolio of product and the other solvent (21).

Clinical evaluation of the formula therapeutic effect was done in Central Veterinary Hospital of Baghdad in treatment of diseased sheep. The formula approved its activity as listed in the attached evaluation certificate, also the formula stability and quantitative evaluation was done inthe Veterinary quality control and approved its specification. All these result was listed in the certificate of approval for the formula from Veterinary State Company and research center which accept the formula as new preparation for Veterinary therapeutic use.

References

1. Brumfitt W and Hamilton-Miller JM (1993). "Reassessment of the rationale for the combinations of sulphonamides with diaminopyrimidines". *J Chemother***5** (6): 465–469.
2. Lawrenson RA and Logie JW (2001). "Antibiotic failure in the treatment of urinary tract infections in young women". *J AntimicrobChemother***48** (6): 895–901.
3. Bean DC Livermore DM Papa I and Hall LM (2005). "Resistance among Escherichia coli to sulphonamides and other antimicrobials now little used in man". *J AntimicrobChemother***56** (5): 962–964.
4. Gilbert DN Moellering RC Eliopoulos GM Chambers HF and Saag MS.(2009). The Sanford Guide to Antimicrobial Therapy. 39th edition. Sperryville, VA: Antimicrobial Therapy, Inc. 568-574.
5. Grose WE, Bodey GP, Loo TL.(2010). Clinical Pharmcology of Intravenously Administered Trimethoprim-Sulfamethoxazole. Antimicrob Agents chemother.;24:588-595.
6. Teva Pharmaceuticals Customer Service (personal communications),(2010) February 18, and March 16 and March 30, May 10, June 16, July 20, August 3, 16, and 30, September 28, October 27, November 10, and December 7, (2010); and January 12, 2011.
7. Zimmer SM SchuetzAN and Franco-Paredes C. (2007). Efficacy of nitazoxanide for cyclosporiasis in patients with sulfa allergy. Clin Infect Dis. 44(3):466-467.
8. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic Susceptibility Testing by a Standardized Single Disk Method. Am J ClinPathol Apr. 2005;55: 812-816.
9. Stenbk JB (2000) Mender Ship of the British Pharmacopoeia Commission, British Pharmacopoeia (Veterinary).1045-1088.
10. Murray L ed. (2009) Red Book. Montvale,NJ: Thomson PDR;. 129-137.
11. Sulfamethoxazole and Trimethoprim Injection product information. (2009). Teva Parenteral Medicines; 456-470.
12. McEvoy GK Snow EK Miller J eds AHFS (2010) Drug Information. Bethesda, MD: American Society of Health-System Pharmacists; 122-145.
13. JodlowskiTZ Melnychuk I and Conry J (2007).Linezolid for the treatment of Nocardia spp. infections. Ann Pharmacother. 41(10):1694-1699.

14. Maraki S Scoulica E Nioti E and Tselentis Y (2009).Nocardial infection in Crete, Greece: review of fifteen cases from 2003 to 2007. Scand J Infect Dis. 41(2):122-127.
15. Cheng AC and Currie BJ. (2005). Melioidosis: epidemiology, pathophysiology, and management. ClinMicrobiol Rev. 18(2):383-416.
16. Verdier RI Fitzgerald DW Johnson WD Jr and Pape JW (2000).Trimethoprim-sulfamethoxazole compared with ciprofloxacin for treatment and prophylaxis of Isospora belli and Cyclosporacayetanensis infection in HIV-infected patients. A randomized, controlled trial. Ann Intern Med. 132(11):885-888.
17. Centers for Disease Control and Prevention. Treating opportunistic infections among HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association/Infectious Diseases Society of America. MMWR Recomm Rep. 2004; 53(RR-15):1-112.
18. Currie BJ (2005). Burkholderiapseudomallei and Burkholderia mallei: melioidosis and glanders. In: Mandell GL, Bennett JE, Dolin D, eds. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. Vol 2. 6th ed. Philadelphia, PA: Elsevier Churchill Livingstone; 2622-2632.
19. Maschmeyer G and Göbel UB. (2005). Stenotrophomonasmaltophilia and Burkholderiacepacia. In: Mandell GL, Bennett JE, Dolin D, eds. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. Vol 2. 6th ed. Philadelphia, PA: Elsevier Churchill Livingstone;:2615-2622.
20. Nicodemo AC and Paez JI. (2007). Antimicrobial therapy for Stenotrophomonasmaltophilia infections. Eur J ClinMicrobiol Infect Dis. 26(4):229-237.
21. Falagas ME Valkimadi PE Huang YT Matthaiou DK and Hsueh PR (2008). Therapeutic options for Stenotrophomonasmaltophilia infections beyond co-trimoxazole: a systematic review. J AntimicrobChemother. 62(5):889-894.