Studying of the physical and chemical characteristics for two formulations of Co-Trimoxazole and Trimethoprim oily injections

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Keywords: TMP oily injection, PABA, Dihydrofolate reductase, sulfa drugs.

Received (August) **, Accepted** (June)

Abstract

Over the past century there has been a dramatic increase in the utilization of the combination of Sulfamethoxazole (SMX) and Trimethoprim (TMP) solution as IV injection (Cotrimoxazole injection) as antibacterial agent in human and Trimethoprim Oily injection (IM) for sheep, goats . The aim of this study was to determine some physical and chemical properties for the above two formulations (appearance, pH, contents, sterility) as well as histopathological studying for trimethoprim oily injection in sheep,where both two formulations have pH = 9.8 , and the content of sulfamethoxazole and trimethoprim was 95.7% , 94.3% respectively for cotrimoxazole , while trimethoprim content in trimethoprim oily injection is 101% according to B.P. .

دراسة الخواص الفيزيائية والكيميائية لمستحضرين من حقن كوترايموكسازول والترايميثوبريم الزيتي

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كلمات مفتاحية: حقن ترايميثوبريم الزيتي, بارا امينو بنزوك اسد، انزيم داي هايدرو فوليت ردكتيز، ادوية السلفا

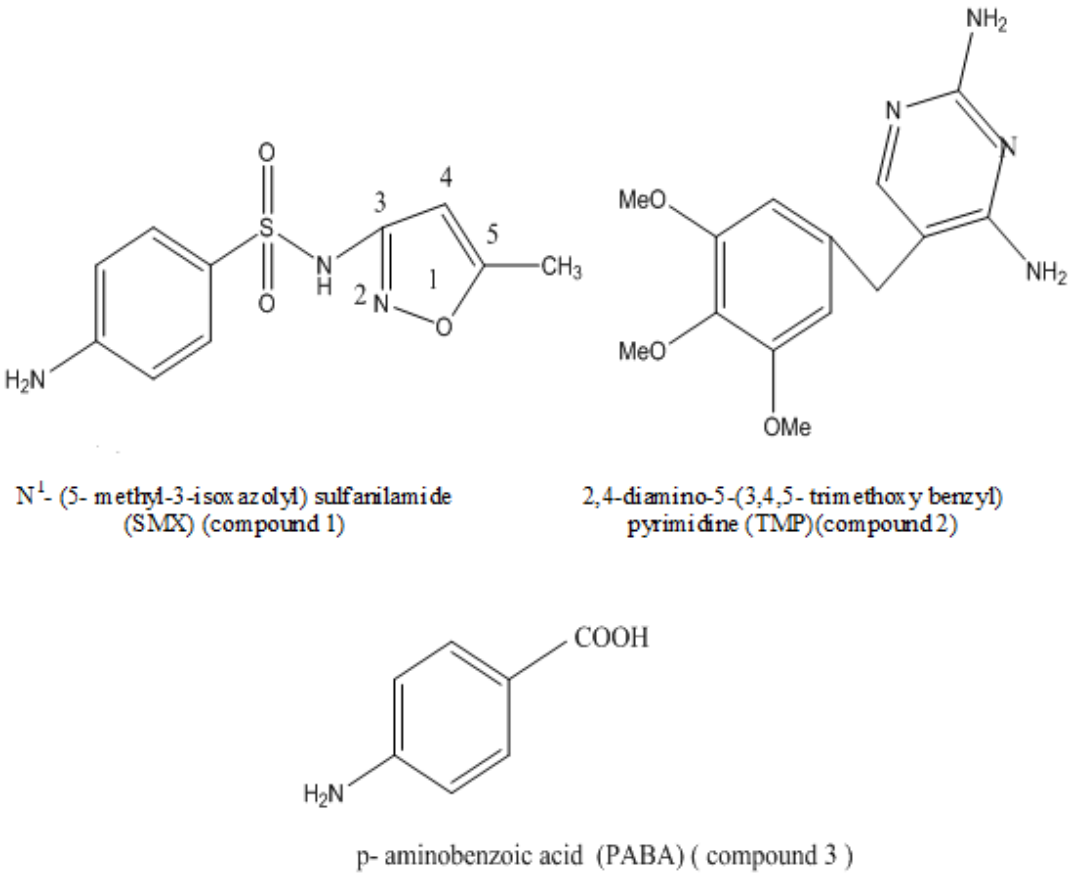
الملخص

منذ القرن الماضي وهنالك استخدام متزيدا لخليط من مركبات سلفاميثوكسازول وترايميثوبريم الحقن الوريدي (حقن كوترايموكسازول) كمضادات للبكتريا في الانسان وحقن ترايميثوبريم الزيتي للحقن العضلي للأغنام والماعز. أن الهدف من هذه الدراسة هو تعيين بعض الخواص الفيزيائية والكيميائية للتركيبتين اعلاه من حيث (المظهر ، درجة الاس الحامضي ، المحتوى ، العقامة) بالإضافة الى دراسة التشريح المرضي للأنسجة في الاغنام لمستحضر حقن الترايميثوبريم الزيتي.حيث ان درجة الاس الحامضي لكلا التركيبتين هي 9,8 ,وان المحتوى لمادتي سلفاميثوكسازول والترايميثوبريم كانت 95,7% , 94,3% على التوالي لحقن كوترايموكسازول

, بينما محتوى الترايميثوبريم في حقن الترايميثوبريم الزيتي هو 101% وكلها ضمن المدى لدستور الادويه البريطاني .

Introduction

An old effective combination drug therapy used as antibacterial activity containing Sulfmathoxazole (SMX) N^1 –[5-methyl-3-isoxazolyl] sulfanilamide (compound 1) and Trimethopirm (TMP) 2,4-diamino-5- (3,4,5- trimethoxy benzyl)pyrimidine (compound 2) known as cotrimoxazole which also called by its brand names (Septrin ,Bactrim) , and used for treat many infections in human such as pneumonia , urinary tract and intestinal infections. (1-3).



N^1- (5- methyl-3-isoxazolyl) sulfanilamide (SMX) (compound 1)

2,4-diamino-5-(3,4,5- trimethoxy benzyl) pyrimidine (TMP)(compound 2)

p- aminobenzoic acid (PABA) (compound 3)

Figure 1: chemical structures of SMX, TMP, and PABA respectively

The mechanism of action is sequential double blockage of bacteria . Sulfamethoxazole (1) inhibit bacterial synthesis of dihydrofolic acid

which is necessary for bacterial growth by competing with p-aminobenzoic acid (PABA) (compound 3), these compounds are listed in the figure 1. While, trimethoprim block of tetrahydrofolic acid in form dihydrofolic acid by inhibiting reversibly the dihydrofolate reductase.(4).

There are many pharmaceutical preparations of Cotrimoxazole such as tablet, syrup, and (IV) injection .Trimethoprim oily (I.M) injection used only for veterinary uses (sheep, goats & cows).(5) Trimethoprim is currently being investigated as definitive therapy for a wide range of infections, including bacterial exacerbations of chronic bronchitis, bacterial pneumonia, and typhoid fever. Initial reports are encouraging.(5)

The aim of this study was designated to determine the physical , chemical properties of both Cotrimoxazole and Trimethoprim oily injections , as well as the quantitative determination of the active ingredients (Sulfamethoxazole and Trimethoprim) contents in the Cotrimoxazole and the content of Trimethoprim in Trimethoprim oily injection using spectrophotometric methods.

Experimental part

Materials

Sulfamethoxazole (B.P.) &Trimethoprim (B.P.)were obtain from Sigma. The Co-Trimoxazole and Trimethoprim oily injection . Absolute ethanol 99.7%, standard buffers (pH 4, pH7& pH9), Hydrochloric acid, Sodium nitrate, Sodium hydroxide, Acetic acid (from BDH and Fluka companies) & double distill water .

Instruments

Oven, autoclave, pH–meter model pH 3510, Jenway, UK, Electronic balance, Denver instrument Germany TP- 214, UV-VIS 160 SHIMADZU, Japan, Ultraviolet- Visible spectrophotometer, using 1 cm quartz cells.

Biological tests

-The microbiological test are for two samples were done.

-histopathological studies test for trimethoprim oily injected in sheep dialy for three consecutive days.

Procedures

Assay of Sulfamethoxazole in Co-Trimoxazole injection (6&7)

To a solution volume equivalent to (0.00158mole,0.4g) Sulfamethoxazole add 60ml distilled water with 10ml of hydrochloric acid and carry out the method of amperometric titration . Each one ml of the 0.1M Sodium nitrate equivalent to 0.02533g of $C_{10}H_{11}N_3O_3S$ as shown in table 1.

Assay of Trimethoprim in Co-Trimoxazole injection (6&7)

To a volume equivalent to (0.000166mole,0.048g) of Trimethoprim add 30 ml of 0.1M Sodium hydroxide and extract with 4 quantities each of 50 ml of chloroform, washing each extract with 10ml 0.1M Sodium hydroxide. The combine chloroform extract are shaken well and extract with four quantities each of 50ml 1M acetic acid, using 5ml of chloroform to wash the combined extract and dilute the aqueous extract to 250ml by 1M acetic acid.

To 10ml from the above dilute solution add 10ml of 1M acetic acid shake well and add enough distill water to produce 100ml and measure the absorbance of the resulting solution at the maximum wavelength at λ_{max} =271nm. Estimate the content of $C_{14}H_{18}N_4O_3$ taking 204 as the value of A (1%) at cell length 1cm with the maximum wavelength at about 271nm as shown in table 1.

Table 1: Physical and chemical properties of Co-Trimoxazole IV injection

Type of Test	Specification of	Specification	Results

	Co-Trimoxazole IV injection	according to B.P	
Liquid description	Colorless liquid	Colorless liquid to fait yellow	Complied
Clarity	Clear	Clear	No particulate
pH	9.8	9.5- 10.5	Narrow pH range, complied
Percentage of (SMX) content	95.7%	90- 110 %	Complied
Percentage of (TMP) content	94.3%	90- 110%	Complied
Sterility	Sterile	Sterile	(-)ve growth bacteria

Assay of Trimethoprim Oily injection (for Veterinary uses)(6)

Non- aqueous titration was carried out for determination the exact content of Trimethoprim in Trimethoprim Oily injection. The content of trimethoprim $C_{14}H_{18}N_4O_5$ was determined potentiometrically as shown in Table 2.

Table 2: Physical , chemical &biological properties of Trimethoprim oily injection

Types of Test	Specification of Trimethoprim	Specification according to B.P. & Veterinary Pharm.&	Results

	oily injection	Biological 82/ 83	
Liquid description	Colorless liquid	Colorless liquid	Complied
Clarity	Clear	Clear	No particulate
pH	9.8	8.5-10.5	Narrow pH rang, Complied
Content of Trimethoprim	101%	95 – 105 %	Only one active ingredient , compiled
Sterility	Sterile	Sterile	(-)ve growth bacteria
Histopathological studies	Not tissue damage	Not tissue damage	5 ml (I.M) was injected in sheep daily for 3 consecutive days.

Results & Discussion

Co-Trimoxazole also known as (Septrin , Bactrim) is a broad spectrum synthetic antibacterial in parenteral form for use when the patient is unable to accept oral therapy (Tablets, Syrup) , when initiation of treatment is particular urgent(2). Two synthetic drugs found in the Cotrimoxazole are Sulfamethoxazaole (SMX) and Trimethoprim and indicate in many cases i) Severe or complicated infections when oral therapy is not feasible ii) Treatment and prophylaxis of pneumocystis carinii pneumonia (PCP) in immunocompromised patients.

In the Cotrimoxazole injection Sulfamethoxazole was found as Sodium salt and adjusted their pH by addition of Sodium hydroxide as shown in equation

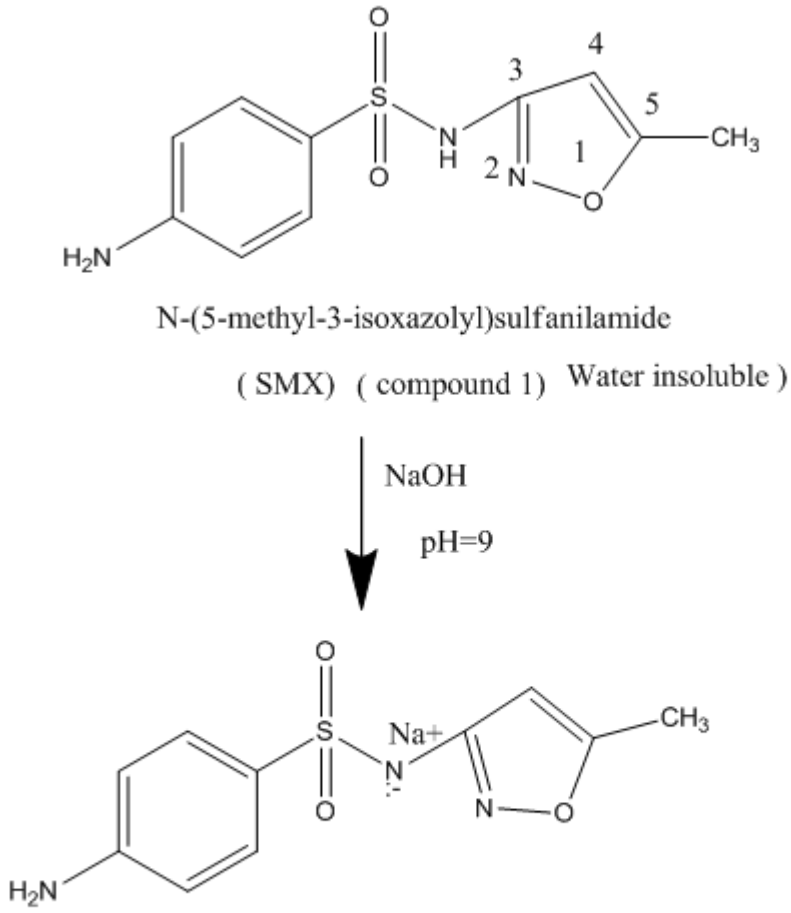


Figure 2 : Sodium salt formation of Sulfa compound (SMX - Na salt).

The Cotrimoxazole solution have specific properties , the basic pH relatively unstable for most injection leads to particles or precipitation of active ingredients, for this reason there is narrow pH range of the injection. Increasing or decreasing the solubility of a sulfa compound are two of the most important reasons for salt selection (Figure 2)(9). That is, the salts of the stronger acid will produce slurries with a lower pH, thus promoting the dissolution of the bases.

Also, this method of quantitative determination of the active ingredients was simple, cheap according to the British pharmacopeia (B.P.), and the Cotrimoxazole should be diluted before use , both two active ingredients are compiled (5), as well as the sterility. The activity of the two ingredients (SMX) and (TMP) in Cotrimoxazole injection in the folic acid synthesis in the bacteria by two consecutive steps. This drug

was very active but it is painful at site of injection as well as many unwanted effects . Also, the product was sterile and no growth of bacteria.(5)

Trimethoprim Oily injection was used as antibacterial agents for veterinary uses mainly for sheep and goats. The oily injectable formulations are administered l.M. injection providing a depot for sustained drug delivery. Trimethoprim is only the active ingredient was dissolve in a mixture of propylene glycol and absolute ethanol.(8) Trimethoprim Oily injection pH has wide range and stable as compare with Cotrimoxazole injection leads to increase stability at least three years , the content also was measured using potentiometric method and the contents about 16mg /ml(8&10) .

The histopathological studies were carried by Injection of 5 ml intramuscularly in sheep daily for 3 consecutive days was not associated with tissue damage at the site of injection.

Conclusions

Two different formulations of Trimethoprim injection were study their physical and chemical properties and quantitative determination of their contents. Cotrimoxazole injection less stable but broad spectrum of antibacterial as compare with Trimethoprim oily I.M. injection more stable but less effective and used for veterinary medications.

References

[1] Fishman JA.(1998) Treatment of infection due to pneumocystis carinii. Antimicrob. Agents Chemther , 42 : 1309.
[2] David A. Williams , (2012) Foyes Principle of Medicinal Chemistry.
[3] Coque TM, Singh KV, Weinstock GM, Murray BE,(1999) Characterization of dihydrofolate reductase genes from trimethoprim-susceptible & trimethoprim resistance strains of Enterococcus faecalis . Antimicrob. Agents Chemther, 43: 141.
[4] Katzung BG, Masters SB & Trevor AJ,(2014) Basic & Clinical Pharmacology 13 Ed. McGraw-Hill Education .
[5] US national Library of Medicine .Augest,(2010) Sulfamethoxazole & Trimethoprim injection. FDA approval proved product .

[6] Arooba M.S. Ibrahim and Duraid A. H. Abbas (2012) New Formulation of Trimethoprim Injectable Solution for Veterinary Use The Iraqi J. Vet. Med. 36 (1):137 – 144 .
[7] Teva Parenteral Medicines, (2009) Sulfamethoxazole and Trimethoprim Injection product information, 456-470 .
[8] Laurie Hess,(2014) The Merck Veterinary Manual .
[9] Danielle pavliv and Nyedra booker , (2016) National center for health research .
[10] Pradhan E , Bhandaris, Gilbert RE , Stanford M, (2016) Antibiotics versus no treatment for toxoplasma retinochoroiditis .Cochrane database syst rev. 5 .