

*IN-VITRO COMPARATIVE EVALUATION OF
DIFFERENT CO-TRIMOXAZOLE TABLET
PRODUCTS OBTAINED FROM DRUG RETAIL
OUTLETS IN ADDIS ABABA*

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ACRONYMS

AIDS= Acquired Immunodeficiency Syndrome

BP= British Pharmacopoeia

HIV= Human Immunodeficiency Virus

RS= Reference Standard

SMZ= Sulphamethoxazole

TMP= Trimethoprim

T₅₀%= Time for 50% Drug Release

T₉₀%= Time for 90% Drug Release

USP= United States Pharmacopoeia

ABSTRACT

Evaluation studies provide a means of identifying quality differences between same products obtained from various manufacturers and quality evaluations are crucial in the era of increasing resistance to antibacterial agents

The introduction of trimethoprim in combination with sulphamethoxazole constitutes an important advance in the development of clinically effective antimicrobial agents. In much of the world the combination of sulphamethoxazole with trimethoprim in the proportion of 5 to 1 is known as Co-trimoxazole. Co-trimoxazole has been used in a diverse range of infections due to sensitive bacteria and is widely prescribed for various indications. By virtue of sequential blockade of microbial folic acid synthesis the antimicrobial combination has excellent *in vitro* inhibitory activity against many common respiratory and urinary tract pathogens, as well as many nosocomial-infecting strains. In patients infected with the human immunodeficiency virus (HIV), trimethoprim-sulphamethoxazole provides prophylactic and therapeutic potency against *Pneumocystis carinii* but at the risk of side effects. Trimethoprim-sulphamethoxazole (TMP-SMX) is also used for treatment of pulmonary and disseminated nocardiosis and some forms of Wagener's granulomatosis, as well as for prophylaxis of spontaneous bacterial peritonitis.

Bacterial resistance to trimethoprim-sulphamethoxazole is a rapidly increasing problem and is exacerbated by use of substandard products. In this work, it is aimed to evaluate the physical properties and the dissolution profiles of trimethoprim-sulphamethoxazole tablets produced by ten different manufacturers and are obtained from drug outlets in Addis Ababa.

Accordingly, the different tablets were evaluated for physical properties (Diameter, thickness, shape, hardness, friability and disintegration time) and their dissolution profiles were compared by the USP XXVI paddle method.

The tablets investigated in this study could roughly be grouped as those, which exhibited, delayed drug release and those, which released the drug contained immediately. The tablets evaluated in this study differed in many of their physical properties. The average weights ranged from 502.41mg (Bactrim) to 709.98mg (Cotrimol), 480mg being the expected strength of the combination. The mean disintegration times ranged from 0.17 (± 0) minutes (Cotrimoxazole) to 13.05 (± 5.24) minutes (Lagatrim).

Nine of the tested products gave an assay value above the lowest limit (93%-107%) specified in the pharmacopoeia. Assay values greater than the upper limit for sulphamethoxazole was obtained with four of the evaluated products. One product gave an assay value greater than the upper limit for both sulphamethoxazole and trimethoprim.

Most of the tablets released the drug contained in an increasing fashion from the 5th minute to the 60th minute with varying proportion of increment. Products obtained from Europe released most of the drug within the first 5 to 10 minutes. More than 90 % of trimethoprim is released within 5 minutes from Bactrim and Septrin and with in 10 minutes from Lagatrim and Deprim. While Lagatrim and Septrin released more than 90 % of their Sulphamethoxazole within 10 minutes, Bactrim and Deprim released their Sulphamethoxazole within 20 minutes. However the European

and the non-European products differed significantly in their sulphamethoxazole release. Both products showed a comparable trimethoprim release pattern. All of the tested products released more than 90% of the trimethoprim after one hour, ranging from 92.81%-Cotreich to 111.77%-Septrin.

The very long $t_{90\%}$ values of Cotreich and Cotrimol (60 & 45minutes respectively) for trimethoprim, the very long $t_{50\%}$ & $t_{90\%}$ values of Cotreich (38 and >60minutes respectively) for trimethoprim and sulphamethoxazole, and the very long $t_{90\%}$ values of Cotreich, Cotrimol and Kanprim (>60 minutes) for sulphamethoxazole indicate that these products could result in lower rate and extent of bioavailability in the body. Similar problems could be encountered with the relatively long $t_{90\%}$ values of Cotrimoxazole (45minutes) and Oriprim (40minutes) for Sulphamethoxazole. The smaller amount of sulphamethoxazole and trimethoprim released from products with delayed release could compromise the *in vivo* efficacy of these tablets.

1.1 Therapeutic Success and Quality of Pharmaceuticals

There is a growing concern about the availability of substandard pharmaceutical products to the general public in developing countries. Such products have therapeutic as well as social and economic implications. There is little data available which points to the reasons for products being substandard but the majority of literature reports contain anecdotal evidence and assume the products to be counterfeit. There are, however, other reasons for products being substandard, such as poor quality control during manufacture or decomposition of the active ingredient(s) [1].

The quality of drugs in less-developed settings is inadequate, although evidence is largely anecdotal. Reasons for poor quality include the widespread counterfeiting of medicines, decomposition of the active ingredient in drugs due to high temperature and humidity of storage, and poor quality assurance during the manufacture of medicinal products [2].

The Counterfeit Intelligence Bureau of the United States of America estimated that in 1991, 5% of the world's trade was counterfeit. This percentage is likely to be higher for pharmaceuticals that are easily transported and are in great demand [3]. Such counterfeit products have bypassed regulatory controls for quality assurance, thus potentially resulting in medications that contain too little or none, or even entirely different active ingredients [4].

In countries like Ethiopia where the drug control is weak the quality of marketed drug products cannot be guaranteed. Evaluation of some of the marketed products could give an insight as to the quality of products sold & consumed and could lay basis for future corrective measures.

1.1.1 Quality and Trading in Pharmaceuticals

Two medications are by no means identical because they contain a chemically equivalent active ingredient. Differences in the other components used, differences in the manufacturing procedures, differences in the methods and degree of quality control and the stages at which it is exercised, can result in a great difference in products and their therapeutic effect. In spite of the best efforts, there is often some deterioration and the total amount of drug present in a preparation goes down with time [5].

Medicines must be safe, effective and of acceptable quality and should be used rationally in order to produce the desired effect. They can be dangerous if there is no adequate control over their manufacture, storage and distribution or their use by the patient. During the past few decades, tremendous advances have been made in pharmaceutical technology and science. As a result, a large number of preventive and curative medicines are now available to fight diseases. Similarly, sophisticated and highly sensitive methods have been developed to ensure the quality of drugs. Unfortunately, however, despite all the advances made, concern about the quality of drugs has not abated [6].

In the past few years, the number of pharmaceutical manufacturers and distribution channels has proliferated. The export of pharmaceutical products, which used to be directed from a manufacturing country to an importing country, is now taking place from stocks held in one or more intermediate countries or through trading houses via duty-free ports/zones. The activities in intermediate countries or trading houses may some times involve repackaging and/or relabelling

which may be carried out without any controls and under the conditions that do not comply with good manufacturing practices (GMP) requirements. This situation, coupled with ineffective drug regulation in many countries, has facilitated the appearance of diverse problems, one of which is the counterfeiting of pharmaceutical products. According to the World Health Organization (WHO) definition, counterfeit products may include products with correct ingredients, wrong ingredients, without active ingredients, with the incorrect quantity of active ingredient or with fake packaging [6].

In developing countries, the two top-ranking classes of medicines reported to be counterfeited are anti-infectives and anti-parasites, whereas in industrialized countries anabolic steroids and dermatological products accounted for the majority of counterfeit products [6]. A survey of the quality of drugs moving in the markets of three African countries, carried out between 1991 and 1993, showed 18% of the tested samples to be substandard [7].

1.2 Sulphonamides

The sulphonamide drugs were the first effective chemotherapeutic agents to be employed systemically for the prevention and cure of bacterial infections in human beings. The considerable medical and public health importance of their discovery and their subsequent widespread use were quickly reflected in the sharp decline of morbidity and mortality figures for treatable infectious diseases. The advent of penicillin and subsequently of other antibiotics had diminished the usefulness of the sulphonamides, and they presently occupy relatively smaller place in the therapeutic armamentarium of the physician. However, the introduction in the mid-

1970s of the combination of trimethoprim and sulphamethoxazole has resulted in increased use of sulphonamides for the prophylaxis and/or treatment of specific microbial infections [8].

Sulphonamides are an important class of antimicrobial drugs used in medicine and veterinary practice. They are rapidly absorbed, establishing a therapeutic range of 30-150 µg/ml in plasma and 500-1000 µg/ml in urine following an 800 mg daily dose. The major metabolite, the N⁴-acetylated sulfonamide, has no antimicrobial activity, but retains the toxicity of the parent compound [9].

1.2.1. Sulphamethoxazole

Sulphamethoxazole belongs to the sulphonamide group of chemo-therapeutics. Despite the availability of numerous antibiotics, sulphonamide is still an important drug for therapeutic use, particularly in the treatment of acute urinary tract infection (UTI). Sulphamethoxazole is an ingredient of co-trimoxazole. Chemically sulphamethoxazole is N'- (5-Methylisoxazol-3-yl) sulfonilamide (see Fig.1.1) [10].

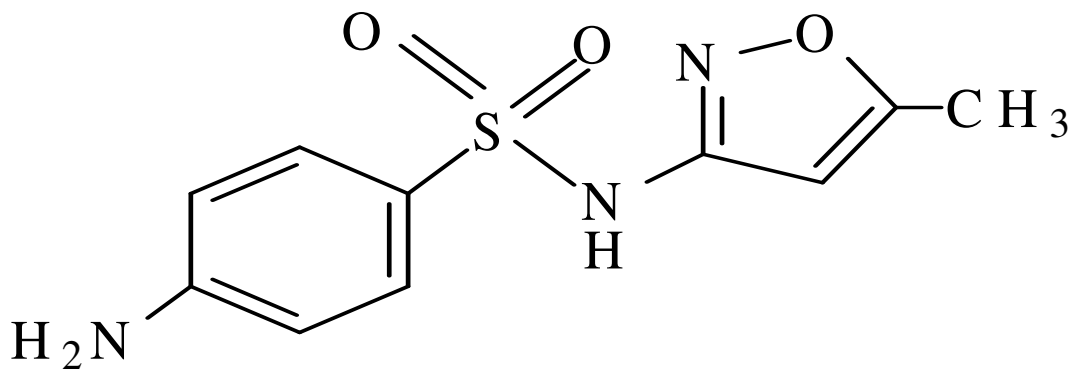


Figure 1.1 Structural Formula of Sulphamethoxazole ($C_{10}H_{11}N_3O_3S$).

According to the specifications of BP (2000), sulphamethoxazole (Molecular Weight 253.3) is a white or almost white, crystalline powder, practically insoluble in water, freely soluble in acetone, sparingly soluble in alcohol, slightly soluble in ether. It dissolves in dilute solutions of sodium hydroxide. Sulphamethoxazole shows amphoteric properties and consequently has two dissociation constants. At $25^{\circ}C$ with pK_{a1} of 1.76 and pK_{a2} 5.81. Research data indicate that sulphamethoxazole obeys Noyes-Whitney relationship where dissolution rate is directly proportional to saturation solubility [11].

1.2.2 Synergists of Sulphonamides

The use of sulphonamides has increased greatly with the introduction of trimethoprim-sulphamethoxazole mixtures, which represent a synergistic combination of antimicrobial agents. Sulphonamides are marketed either alone or in combination with other agents. Phenazopyridine,

erythromycin ethylsuccinate and trimethoprim are examples of drugs that could be combined with sulphonamides. Trimethoprim is one of the most active agents that exerts a synergistic effect when used with a sulphonamide [12,13]. Trimethoprim was specifically developed in the late 1960s as a sulphonamide potentiator and was launched in combination with sulphamethoxazole as co-trimoxazole. Trimethoprim is an inhibitor of dihydrofolate reductase, which potentiates the activity of sulphonamides against a wide variety of bacterial species [14]. Chemically, trimethoprim is 5-(3,4,5-trimethoxybenzyl) pyrimidine-2, 4-diyldiamine (see Fig.1.2) [10].

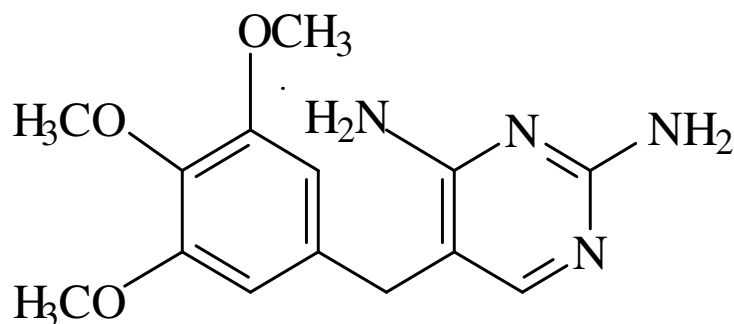


Figure1.2 Structural Formula of Trimethoprim (C₁₄H₁₈N₄O₃).

According to the specifications of BP (2000), trimethoprim (Molecular Weight 290.3) is a white or yellowish-white powder, very slightly soluble in water, slightly soluble in alcohol. It shows polymorphism. It is a weak difunctional base with both basic groups titrating almost simultaneously with a pK_a of 7.2. A study showed trimethoprim to be absorbed well and highly distributed in both dog and man [15].

Although trimethoprim is a relatively stable drug it is susceptible to degradation after prolonged storage or after being subjected to severe conditions of heat or sunlight [16].

1.2.3 Solubility of Sulphamethoxazole and Trimethoprim

A study indicated marked variation in solubilities of sulphamethoxazole and trimethoprim with pH. On both molar basis and mg/ml, the solubility of trimethoprim is approximately twice that of sulphamethoxazole at pH 1.0. At pH 5.0, trimethoprim is approximately twenty-fold more soluble than sulphamethoxazole. Hence although these drugs are formulated with five fold more sulphamethoxazole than trimethoprim, solubilities and the conversion of trimethoprim to trimethoprim H⁺ and different volumes of distribution complicate the behaviour of these drugs in man. The dissolution rates of these drugs may be pH dependent in man and this may contribute to the variable absorption rates of these drugs [11].

1.2.4 Trimethoprim-Sulphamethoxazole Combination

The introduction of trimethoprim in combination with sulphamethoxazole constitutes an important advance in the development of clinically effective antimicrobial agents and represents the practical application of a theoretical consideration; that is, if two drugs act on sequential steps in the pathway of an obligate enzymatic reaction in bacteria, the result of their combination will be synergetic. In much of the world the combination is known as Co-trimoxazole [13]. Co-trimoxazole is a mixture of the sulphonamide sulphamethoxazole with trimethoprim, in the proportion of 5 to 1 [17].

Several workers have reported that the activity of trimethoprim-sulphonamide combination is largely dependent upon the activity of trimethoprim alone, but its use alone is not preferred because of the emergence of increasingly resistance strains to trimethoprim. The advocated administration of the established dosage schedule of twelve hourly administration of 960 mg of co-trimoxazole would produce therapeutic concentrations to be effective in generalized as well as tissue infections [18,19].

1.2.5 Antibacterial Spectrum of the Combination

Chemotherapeutic agents play an important role in the treatment of acute infections and co-trimoxazole a combination of trimethoprim with sulphamethoxazole has been used in a diverse range of infections due to sensitive bacteria [20]. After 33 years of use in the United States [21] and 42 years of use in the United Kingdom [22] trimethoprim-sulphamethoxazole is widely prescribed for various indications.

Biogenesis of tetrahydrofolate cofactors for bacterial growth and survival is blocked by sulphamethoxazole-trimethoprim [23]. By virtue of sequential blockade of microbial folic acid synthesis the antimicrobial combination has excellent *in vitro* inhibitory activity against many common respiratory and urinary tract pathogens, as well as many nosocomial-infecting strains. In patients infected with the human immunodeficiency virus, trimethoprim-sulphamethoxazole provides prophylactic and therapeutic potency against *Pneumocystis carinii* but at the risk of side effects. Trimethoprim-sulphamethoxazole is also used for treatment of pulmonary and

disseminated nocardiosis and some forms of Wegener's granulomatosis, as well as for prophylaxis of spontaneous bacterial peritonitis [21].

The antibacterial spectrum of trimethoprim is similar to that of sulphamethoxazole, although the former drug is usually 20 to 100 times more potent than the latter. Most gram-negative and gram-positive microorganisms are sensitive to trimethoprim, but resistance can develop when the drug is used alone [24]. *Pseudomonas aeruginosa*, *Bacteroides fragilis*, and *Enterococci* usually are resistant to trimethoprim-sulphamethoxazole. *Chlamydia diphtheria* and *Neisseria meningitides* are susceptible to trimethoprim-sulphamethoxazole. Although most *Staphylococcus pneumoniae* are susceptible, there has been a disturbing increase in resistance. From 50% to 95% of strains of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus pyogenes*, the *viridans* group of *Streptococci*, *Escherichia coli*, *Proteus mirabilis*, *Proteus morgani*, *Proteus rettgeri*, *Enterobacter* species, *Salmonella*, *Shigella*, *Pseudomonas pseudomallei*, *Serratia*, and *Alcaligenes* species are inhibited by trimethoprim-sulphamethoxazole. Also sensitive are *Klebsiella* species, *Brucella abortus*, *Pasteurella haemolytic*, *Yersinia pseudotuberculosis*, *Yersinia enterocolitica*, and *Nocardia asteroides*. Methicillin-resistant strains of *Staphylococcus aureus*, although also resistant to trimethoprim or sulphamethoxazole alone, may be susceptible to the combination [8,25,26].

1.2.6 Mechanism of Action of the Combination

The antimicrobial activity of the combination of trimethoprim and sulphamethoxazole results from its actions on two steps of the enzymatic pathway for the synthesis of tetrahydrofolic acid.

Sulphonamide inhibits the incorporation of para-aminobenzoic acid (PABA) into folic acid, and trimethoprim prevents the reduction of dihydrofolate to tetrahydrofolate. The synergistic interaction between sulphonamide and trimethoprim is predictable from their respective mechanisms. There is an optimal ratio of the concentrations of the two agents for synergism, and this is equal to the ratio of the minimal inhibitory concentrations of the drugs independently. While this ratio varies for different bacteria, the most effective ratio for the greatest number of microorganisms is 20 parts of sulphamethoxazole to 1 part of trimethoprim. The combination is thus formulated to achieve a sulphamethoxazole concentration *in vivo* 20 times greater than that of trimethoprim [12,13].

1.2.7 Pharmacokinetic Profile of the Combination

The pharmacokinetic profiles of sulphamethoxazole and trimethoprim are closely but not perfectly matched to achieve a constant ratio of 20:1 in their concentrations in blood and tissues. The ratio in blood is often greater than 20:1 and that in tissues is frequently less [27]. After a single oral dose of the combined preparation, trimethoprim is absorbed more rapidly than sulphamethoxazole. The concurrent administration of the drugs appears to slow the absorption of sulphamethoxazole. Peak blood concentrations of trimethoprim usually occur by 2 hours in most patients, while peak concentrations of sulphamethoxazole occur by 4 hours after a single oral dose. The half-lives of trimethoprim and sulphamethoxazole are approximately 11 and 10 hours, respectively [28].

When 800 mg of sulphamethoxazole is given with 160 mg of trimethoprim (the conventional 5:1 ratio) twice daily, the peak concentrations of the drugs in plasma are approximately 40 and 2 µg/ml, the optimal ratio [28]. Trimethoprim is rapidly distributed and concentrated in tissues, and about 40% is bound to plasma protein in the presence of sulphamethoxazole. The volume of distribution of trimethoprim is almost nine times that of sulphamethoxazole [29]. The drug readily enters cerebrospinal fluids and sputum [30]. High concentrations of each component of the mixture also are found in bile [31,32]. About 65% of sulphamethoxazole is bound to plasma protein. Sulphamethoxazole undergoes conjugation mainly in the liver, chiefly to the inactive N⁴-acetyl derivative; this metabolite represents about 15% of the total amount of sulphamethoxazole in the blood. About 10 to 20% of trimethoprim is metabolized in the liver and small amounts are excreted in the faeces via the bile, but most is excreted in urine, predominantly as unchanged drug. About 60% of administered trimethoprim and from 25% to 50% of administered sulphamethoxazole are excreted in the urine in 24 hours [8].

1.3 Antimicrobial Resistance and Drug Quality

1.3.1 The Global Scenario

Resistance to antimicrobials is a natural biological phenomenon. The introduction of every antimicrobial agent into clinical practice has been followed by the detection in the laboratory of strains of microorganisms that are resistant, i.e., able to multiply in the presence of drug concentrations higher than the concentrations in humans receiving therapeutic doses. Such resistance may either be a characteristic associated with the entire species or emerge in strains of

a normally susceptible species through mutation or gene transfer. Resistance genes encode various mechanisms, which allow microorganisms to resist the inhibitory effects of specific antimicrobials. These mechanisms offer resistance to other antimicrobials of the same class and sometimes to several different antimicrobial classes [33]. All antimicrobial agents have the potential to select drug-resistant subpopulations of microorganisms [34].

With the wide spread use of antimicrobials, the prevalence of resistance to each new drug has increased. The prevalence of resistance varies between geographical regions and overtime, but sooner or latter resistance emerges to every antimicrobial. While much evidence supports the view that the total consumption of antimicrobials is the critical factor in selecting resistance, the relationship between use and resistance is not a simple correlation. In particular, the relative contribution of mode of use (dose, duration of therapy, route of administration, dosage interval) as opposed to total consumption is poorly understood. Paradoxically, under use through lack of access, inadequate dosing, poor adherence and sub-standard antimicrobials may play as important a role as overuse [34].

Bacterial resistance to trimethoprim-sulphamethoxazole is a rapidly increasing problem, although resistance is lower than it is to either of the agents alone. Resistance often is due to the acquisition of a plasmid that codes for an altered dihydrofolate reductase. The development of resistance is a problem for treatment of many different bacterial infections. For example, 50% of *Shigella sonnei* isolates from Netherlands were resistant to trimethoprim-sulphamethoxazole [35]. Emergence of trimethoprim-sulphamethoxazole resistant *Staphylococcus aureus* and *Enterobacteriaceae* is a special problem in AIDS patients receiving the drug for prophylaxis of *Pneumocystis carinii* pneumonia [36].

Deaths from acute respiratory infections, diarrhoeal diseases, measles, AIDS, malaria and tuberculosis account for more than 85% of the mortality from infection worldwide [37]. Resistance to first line drugs in the pathogens causing these diseases ranges from zero (0) to almost hundred (100%). In some instances resistance to second–and third-line agents is seriously compromising treatment outcome. While rich countries, to a large extent, are still able to rely on the latest antimicrobials to treat resistant infections, access to these life-saving drugs is often limited or totally absent in many parts of the world [34]. The relentless emergence of antimicrobial resistance has an impact on the cost of health care worldwide. Ineffective therapy due to antimicrobial resistance is associated with increased human suffering, lost productivity and often death .The emergence of antimicrobial resistance is regarded as a major future trait to the security and political stability of some regions [38].

Resistance to antimicrobial drugs is becoming more serious throughout the world [39,40]. The widespread use of these compounds is thought to encourage the emergence of antimicrobial resistance [3]. Long treatment duration, sub-therapeutic or sub-optimal dosage [41,42] and inadequate amounts of active ingredients or their absence in dosage forms [43] have been correlated with antimicrobials resistance development.

1.3.2 Antimicrobial Resistance Pattern in Ethiopia

The Ethiopian antibacterial resistance scenario is not an exception. It was shown that many bacterial isolates (*Salmonella typhi*, *Campylobacter jejuni*, *Yersinia enterocolitica*, *Vibrio*

cholerae, *Aeromonas hydrophila* and *Vibrio parahaemolyticus*) were found to be resistant to one or more of the classical antimicrobial agents (amoxicillin, sulphamethoxazole, trimethoprim, tetracycline, and chloramphenicol) [44,45]. *Campylobacter spp.* showed a pronounced resistance to co-trimoxazole (58.8%) and other commonly used antibiotics [46]. Resistance to antimicrobials has been shown to be increasing steadily in Tikur Anbessa teaching hospital, Addis Ababa University and elsewhere in the country. In a two year study conducted at Tikur Anbessa hospital, Addis Ababa, bacteria showed a high rate of resistance to the common antimicrobials; ampicillin (85.9%), chloramphenicol (70.4%), trimethoprim-sulphamethoxazole (68.4%)[47].

Resistance for ampicillin and co-trimoxazole, the two most commonly used antimicrobials, was found to be high in Gondar [48] and Jimma [49] hospitals. A seven-year interval (1989-1995) study in Addis Ababa showed that ampicillin and co-trimoxazole accounted for a third of all the antibiotics prescribed. In the same study three trade names (Mezil, Bactrim and Augmentin) made up 69% of all antimicrobial trade names [50]. In a study conducted in northwest Ethiopia (Gondar, Bahir Dar and Debre Tabor Hospitals), co-trimoxazole was found to be the second most prescribed antibiotic [51]. In the literature irrational drug use is highly correlated with development of drug resistance [46,50]. And as the tablet dosage form is the most commonly used, many of these antibiotics are presented as tablets. Release of an active ingredient from a tablet involves two distinct processes; disintegration of the tablet and dissolution of the active ingredients.

1.4 Tablet Properties

Conventional tablets in general should have a certain amount of hardness, resistance to friability to withstand the rigors of mechanical shocks encountered during their production, packaging, transportation and handling prior to use. These tablet properties together with uniformity of weight, disintegration and dissolution depend on tableting conditions employed, on size and distribution of the granules and predominantly on the formulation (granulation binding agent, moisture content)[52]. The type and concentration of binder used in the granulation step influence the corresponding tablet properties [53]. Drying time, compression force, size of particles and moisture content of wet granules influence tablet properties, like formation and disintegration [54].

1.4.1 Weight Variation and Content Uniformity

The United States pharmacopoeia (USP) provides criterion for tablet weight variation of intact dosage units. The uniformity of dosage units method for determining tablet uniformity is based on assessment of tablet weight variation. Each of the ten tablets must contain between 85% and 115% of the drug the label claims to be present, and the standard deviation (SD) of the batch should not be greater than 6% [17].

1.4.2 Hardness and Friability

Hardness and friability are the most common measures used to evaluate tablet strength. Factors that may alter tablet hardness are alterations in machine speed, changes in particle size, distribution of the granulation mix and lubricants. Dies filled with large particles of low density granules (light fill) will produce a softer tablet than dies filled with small particles of high density (heavy fill) granules. Tablet hardness will be significantly affected if lubricants are used in too high concentration or mixed for too long [52]. It was also reported that the duration of lubricant mixing significantly changed the apparent bulk volume of the mix, ejection force during tableting, hardness, disintegration and dissolution properties of tablets [55].

1.4.3 Tensile Strength

The strength of a tablet may also be expressed as a tensile strength (breaking stress of a solid unit cross section in kg/cm^2). Tablet tensile strength, measured using the diametrical compression test, allows the dimensions of the tablets to be taken into account while tablet hardness is only a measure of the force at which the tablet breaks. Since the radial tensile strength measurements considers the thickness of a tablet, and only tensile stress and axial tensile strength express the strength in the direction in which capping may occur, the tensile strength characterizes the strength of a tablet more completely than hardness.

The radial tensile strength is expressed as:

$$\sigma_x = \frac{2F}{Dt\pi} \quad (1.1)$$

Where:-

F = Crushing Load

D = Diameter

t = thickness of the tablet

The axial tensile strength is expressed as:

$$\sigma_z = \frac{4F}{D^2\pi} \quad (1.2)$$

Reports indicate that flat-faced tablets have slightly higher tensile strength than deep biconcave tablets compressed to the same packing fraction [56]. Moreover, increasing the compression speed generally decreased the tensile strength of tablets. In common compressed tablets, the number of contact points between particles plays important role in tablet tensile strength. With decreases in tablet porosity, the number of contact points increases, and tensile strength of the tablets shows a higher value. The tensile strength was found to increase linearly with the log of porosity because more solid bridges are formed between particles [54].

Maximum tensile strength occurs when the tablet contained moisture between 2.5% and 4.5%, which is the optimum moisture. The increase in table hardness with increase in water content up to the optimum range could be attributed to the lubricating effect of water. Increasing the water

content above the optimum range caused a reduction in tablet crushing strength due to the hydrodynamic resistance to consolidation [56,57].

1.4.4 Disintegration

The importance of tablet disintegration was recognized as early as 1879 when a patent recommended that pills be perforated to admit gastric juice for better disintegration [58,59]. Before a tablet goes into solution it must breakdown into smaller particles or granules. Complete tablet disintegration is defined as that state in which any residue of the tablet, except fragments of insoluble coating, remaining on the screen of the test apparatus is a soft mass having no palpably firm core. Often the disintegration of a tablet is a limiting factor of drug dissolution, particularly for drugs with low aqueous solubility [52].

For tablets to be disintegrated, it is necessary to overcome the cohesive strength introduced into the mass by compression and by any binder present. It is therefore usual practice to incorporate a disintegrant, which will induce this process.

Studies show that porosity, hydrophilicity, swelling ability of particles and interparticle forces are important factors for tablet disintegration. Tablet porosity is clearly related to water absorption, which is a very important step of the disintegration process. For conventional tablets, when tablet porosity is high, water can be absorbed easily and destruction of tablets is not very difficult. Disintegration is hardly affected by tablet formulation. However, when tablet porosity is not high, disintegration will be influenced by the properties of the excipients used [59]. The

disintegration time of tablets were found to increase linearly with the log of porosity [54,60]. Generally, disintegration time increases with increasing compression force.

The wettability of the formulation plays a vital role in the process of disintegration and dissolution, which lead to release of the drug into the blood stream. Wetting is closely related to the inner structure of tablets and to the hydrophilicity of excipients [60]. The wetting and subsequent penetration of liquid into the capillary structure of the tablets are controlled, respectively, by the contact angle of the liquid on the solid surface θ , and by its surface tension γ and the pore radius. The adhesion tension ($\gamma\cos\theta$), which is a measure of tablet wettability, could be useful in providing information about the wetting and disintegration characteristics of tablet formulations [61,62].

Although disintegration is frequently considered a prerequisite for drug dissolution, it in no manner assures that a drug will dissolve and hence have the potential for satisfactory bioavailability [63].

1.4.5 Dissolution

1.4.5.1 What is Dissolution?

Dissolution is defined as the process by which a solid substance enters in the solvent to yield a solution. Stated simply, dissolution is the process by which a solid substance dissolves. Fundamentally, it is controlled by the affinity between the solid substance and the solvent. The

following scheme is proposed by Carstensen to illustrate the processes involved in the dissolution of solid dosage forms [64].

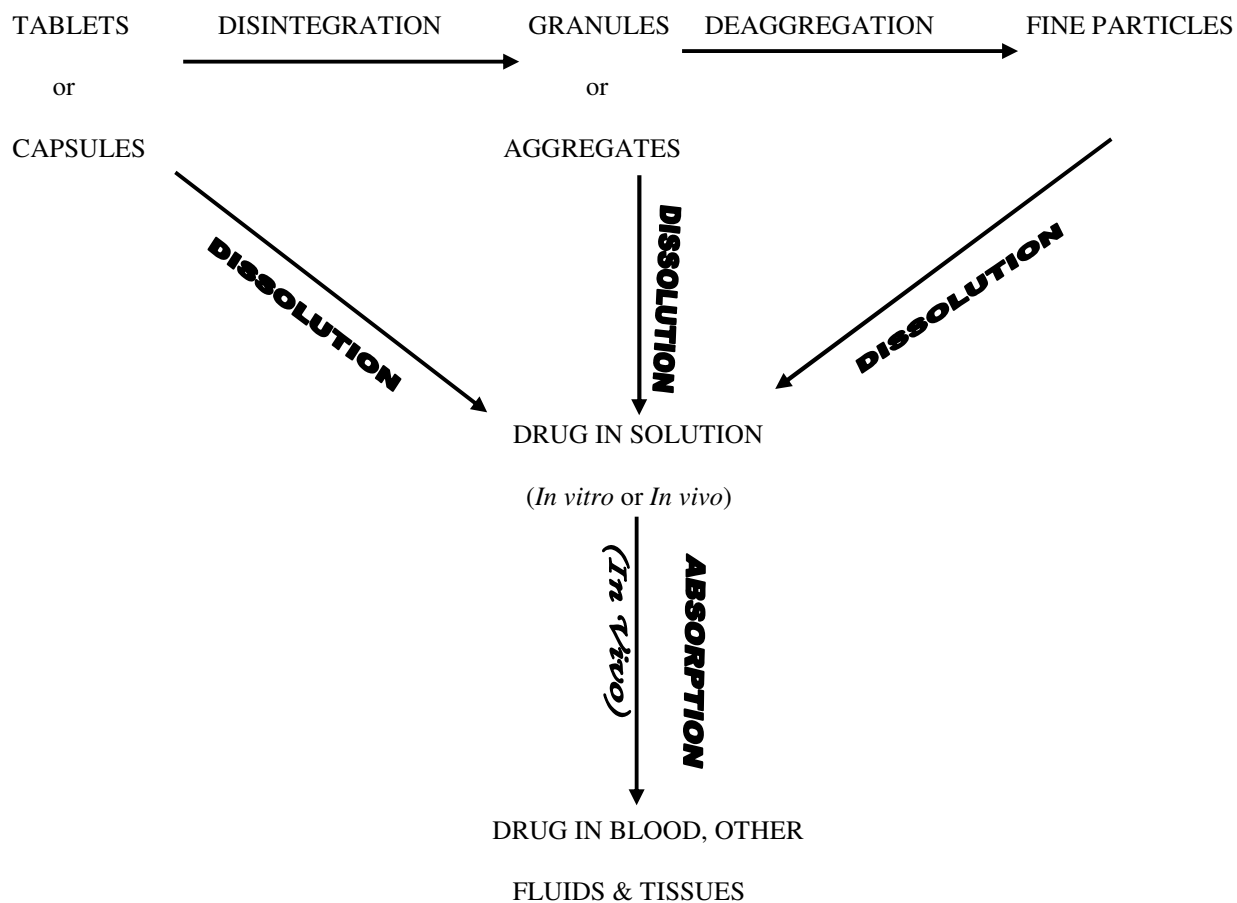


Fig. 1.3 Schematic Illustration of Dissolution Process of solid dosage forms [Adopted From Ref. 65].

This scheme incorporates the following sequences: initial mechanical lay, witting of the dosage form, penetration of the dissolution medium into the dosage form, disintegration, deaggregation of the dosage form and dislodgment of the granules, dissolution and occlusion of some particles of the drug. The rate of dissolution of a drug from the solid state is defined as the amount of drug substance that goes into solution per unit time under standardized conditions of liquid/solid interface temperature, and solvent composition. It is apparent from Fig. 1.3 that the rate of dissolution of the drug can become the rate-limiting step before the drug appears in blood [64].

An equation known as the Noyes-Whitney equation was developed to define the dissolution from a single spherical particle. According to this equation, the rate of mass transfer of solute molecules or ions through a static diffusion layer (dm/dt) is directly proportion to the area available for molecular or ionic migration (A), the concentration difference (ΔC) across the boundary layer, and is proportional to the thickness of the boundary layer (h) [66]. Stated simply,

$$\frac{dm}{dt} = \frac{k_1 A (C_s - C)}{h} \quad (1.3)$$

where:-

k_1 = Dissolution rate constant

$C_s - C = \Delta C$

C_s = Solubility of solid in dissolution medium

C = Concentration of solute in solution at time t

1.4.5.2 Factors Influencing Dissolution Rates

Factors affecting dissolution rates may be derived from consideration of the terms that appear in the Noyes -Whitney equation (Equ. 1.3) and knowledge of the factors that in turn affect these terms.

- Surface area of undissolved solid (A)

Surface area in turn is affected by size of solid particles, dispersibility of powdered solid in dissolution medium and porosity of solid particles.

- Solubility of solid in dissolution medium (C_s)

Several factors affect the solubility of a solid in a dissolution medium. These factors include temperature, nature of dissolution medium, molecular structure of solute, crystalline form of solid and presence of other compounds.

- Concentration of solute in solution at time t (C)

Any process that removes dissolved solute from the dissolution medium and volume of the dissolution medium affects concentration of solute in solution at time t .

- Dissolution rate constant (K_1)

This constant is affected by thickness of the boundary layer and the diffusion coefficient of the solute in the dissolution medium [65].

1.4.5.3 Factors Influencing Tablet Dissolution

The process of dissolution of an active ingredient from solid pharmaceutical dosage forms particularly tablets involves several intermediate physicochemical steps, such as wetting, swelling, capillarity, solubility, and diffusion. Among the most significant factors that control the process of dissolution are the type and nature of the dosage form within which the active ingredient is contained. All factors that influence the physicochemical properties of the dosage form will influence the dissolution. There are primarily two pathways via which the drug entities made available to the dissolution medium from tablets, either the tablet disintegrates thereby exposing the drug contents to the medium or the dissolution process continues without the disintegration of the tablet [52].

The rate of dissolution of a drug substance in solid form from a granule or a tablet depends to a large extent on its solubility in the solvents phase and its concentration in that phase. Factors such as wetting characteristics of the solid dosage forms, the penetration ability of the dissolution medium into the dosage forms, the swelling process, disintegration and deaggregation are critical during the dissolution testing of tablets. Surface area of tablets will change during the dissolution process. The change in surface area will alter the fluid flow dynamics involved in the dissolution rate constant [52].

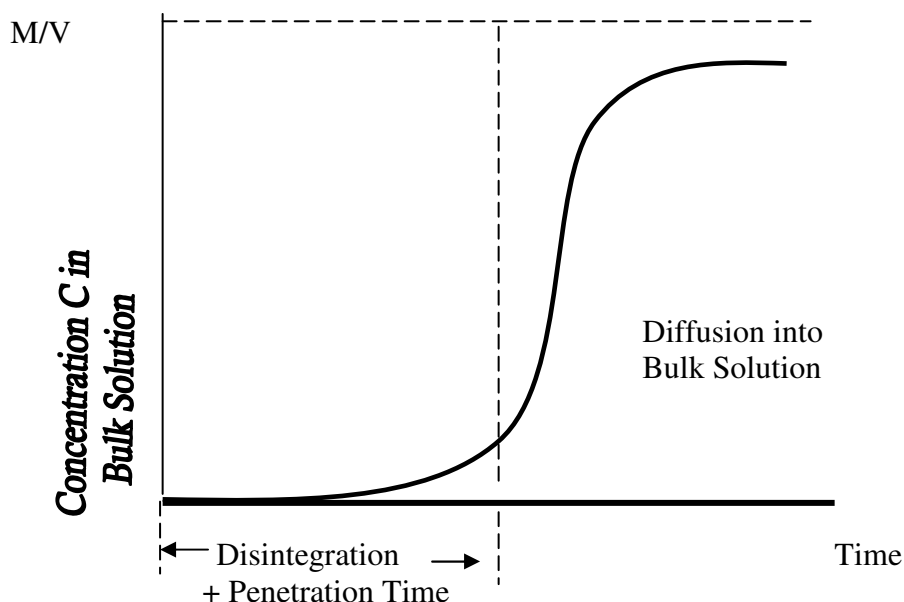


Fig. 1.4 Dissolution-rate curve for a wet granulated tablet [Adopted from Ref.52]

The rate of shear of fresh dissolution medium in contact with the surface area of the solid varies with particle size, shape and density. Usually, the dissolution rate increases with decrease in particle size. However, in certain instances there may be mutual interference in the particulate motion, changes in electrical potential between particles, molecular layers of solvent tightly bound around particles, and other retarding influences, including greater influence of

hydrophobic properties imparted to the liquid-solid interface by various means. In such cases, smaller particles may exhibit slower dissolution rates in actuality [64].

1.4.5.4 Formulation and Processing Variables Affecting Dissolution

Tableting of a medicinal substance allows the introduction of several variables during the manufacture of the dosage form. These could be grouped as process and formulation variables. These variables influence disintegration and dissolution of tablets and leads to variation in release profiles of products of different manufacturers. Processing and formulation variables which influence dissolution include; nature of diluents, process of mixing, granule size and distribution, nature of disintegrant, nature and concentration of lubricant, age of finished tablets, presence or absence of surface-active agent, physical properties of the drug, flow of granulation through hopper and into dies and compressional force in production [52].

Quite a number of processing and formulation variables are reported to have a significant effect on the dissolution rates of solid dosage forms [66]. Several authors reported that an increase in binder concentration resulted in a decrease in dissolution rates of tablets [67-69]. It was shown that the type of binder used in the formulation of tablets affected their dissolution rates [70]. The dissolution rates of tablets were found to increase with decreasing granule size and increasing disintegrant concentration [71,56]. The type of disintegrant used in tablet formulation and its mode of incorporation were also shown to influence dissolution rates [72-74]. It has been reported that a hydrophobic lubricant (magnesium stearate) retarded dissolution, while a water-soluble lubricant (Sodium lauryl sulphate) enhanced dissolution rate. An increase in the mixing time of a hydrophobic lubricant was found to increase the disintegration times of compressed

tablets by decreasing their wettability [75]. This clearly could decrease the dissolution rates of the tablets. Studies [75-76, 55] have indicated that drug and some excipients interact with one another when thoroughly mixed. Specific particle-particle interactions involving a drug, a diluent, a disintegrant, a lubricant may result in incomplete dissolution and/or a decrease in the drug dissolution rate. It was shown that these interactions and their adverse effect on drug dissolution could be avoided by carefully evaluating and selecting the proper excipients. Formulation and processing changes may directly influence the dissolution and bioavailability of a pharmaceutical formulation during development, manufacture, and product optimization. The process of scale-up may also alter dissolution and bioavailability [78-80]. As a tableting process is moved from one tablet press to another in a scale-up or process transfer, tablets may change with respect to hardness, friability, disintegration, dissolution and other properties. Granulation properties, drying time, mode of drying, speed of compression machine, and related changes that occur during scale up were shown to affect tablet properties [81-85]. Formulations of different brands have different types and/or amount of adhesives, disintegrants, lubricants, or other excipients, as well as different compression forces, which affect the disintegration and dissolution rate of a given formulation. Substantial related research has been published such as Ibrahim who studied the influence of compression forces on the dissolution profile of Hydrochlorothiazide and Phenylbutazone. It was proven that dissolution is positive proportional to the logarithm of compression forces [86]. Tablet intensity is also functionally related with compression forces, so it is feasible to establish the relationship of tablet intensity and dissolution rate. Desai documented reduced dissolution rate of Hydrochlorothiazide formulation containing sodium starch glycolate without which the dissolution would be unacted. Given different disintegrating and dissolution rates, formulations will give lower drug effect and absorption *in vivo*. Bioavailability and bioequivalence problem are involved [87].

1.4.5.5 Dissolution and Bioavailability/Bioequivalence Related

As early as 1955, Parrott and co-workers stressed that the release of a drug from the primary particle and its subsequent availability to the body is governed by the dissolution rate of the particle. In 1969, Poole added that the properties of the dosage form that modify the dissolution rate must of necessity influence the blood levels of the drug, and thus may function as the controlling factor in determining the magnitude of the pharmacological response elicited and sometimes even of determining whether or not such a response is exhibited at all [64].

The pharmaceutical and medical literature is replete with reports showing variability in clinical response among orally administered drug products that contain chemically equivalent amounts of a drug and the variation has generally been attributed to difference in their rate of dissolution. There is adequate evidence to conclude that the rate at which a drug dissolves (dissolution rate) from its intact or fragmented dosage forms in the human gastrointestinal tract, often partially or completely control the rate at which the drug appears in blood (absorption rate). Additionally, adequate evidence alludes to the fact that in many instances *in vitro* dissolution rate test results can be employed to "explain" observed differences in results obtained in animals and humans [64].

The physical and chemical characteristics of a drug as well as its formulation are of prime importance in bioavailability because they can affect not only the absorption characteristics of the drug but also its stability. Since a drug must be dissolved to be absorbed, its rate of dissolution from a given product must influence its rate of absorption [87]. There are numerous reports of the effects of formulation and processing variables on the dissolution of active ingredients from drug

products; an apparently inert ingredient may affect drug absorption. For example, magnesium stearate, a lubricant, commonly used in tablet and capsule formulations, is water-insoluble and water-repellent. Its hydrophobic nature tends to retard drug dissolution by preventing contact between the solid drug and the aqueous gastrointestinal fluids. Thus, increasing the amount of magnesium stearate in the formulation results in a slower dissolution rate of the drug, and decreased bioavailability [88].

The bioavailability of active ingredients from per oral dosage forms is dependent on product formulation and production control. This is particularly true of tablets, because the rate of dissolution has been correlated closely with the bioavailability [53,89]. In a number of instances, poor tablet formulation has been shown to cause a significant reduction of physiologic availability of the active ingredient and impairment of clinical responses [90]. Certain tablet formulation and processing factors apparently affect the dissolution rate of drugs contained in tablets, since it has been found that generically identical tablet products made by different manufacturers exhibit significant differences in dissolution rate of active ingredient [71].

In vitro dissolution rate screening has been used as a sensitive quality control measure to show changes in drug release for products undergoing variable storage conditions. It is also used to warn of poor bioavailability of drugs from dosage forms that show erratic release patterns in comparative studies [52]. Over the past 30 years, dissolution testing has not only been recognized as a valuable quality control test but also proved itself as a useful indicator of differences in bioavailability. This is due to the fact that drug absorption after oral administration depends on the release of the drug substance from the drug product, the dissolution or solubilization of the drug under physiological conditions and the permeability across the gastrointestinal tract [91].

The influence of dissolution rate on the bioavailability of a drug is well documented, and in most cases, dissolution rate of per oral solid dosage forms is the rate-limiting step in the absorption process [67]. When ever a significant difference in bioavailability has been found among supposedly identical products, the dissolution test most of the time has been able to discriminate among these products. In fact, dissolution is so sensitive to formulation factors that bioequivalent formulations sometimes show differences in their dissolution profiles [17,78, 92].

Pharmacodynamics concerns the relationship of formulations and pharmacological effects; especially how solid dosage forms are absorbed *in vivo*. Complicated factors are involved, among which, drug disintegration and dissolution are very important ones [66].

Drug dissolution experiments were first mentioned in U.S. Pharmacopoeia XVII, and experimental items started to be covered in detail in USP XVIII, in order to assure that drugs have pharmaceutical equivalence in different batches or different brands with the same ingredients. Subsequently, dissolution tests for six products were introduced into the USP XVIII. This has increased to 481 tests in the USP XXII [93].

It is not easy and remains in doubt to establish the correlation of *in vivo* bioavailability and bioequivalence depending on the facts of *in vitro* dissolution experiments. Even if we fail to establish the bioavailability and bioequivalence relationship of *in vivo* and *in vitro*, it is still meaningful for comparison of the formulation quality of different brands [78, 94-95].

On several occasions evaluation studies [96-103] that compared various brands and generics of a given product yield statistically significant differences in physical properties, (hardness, friability

and weight variation) disintegration time, dissolution rate and drug content and some products do not even meet the criterion for which they are investigated.

1.5 Methods of Assessing Bioavailability

Bioavailability testing is a means of predicting the clinical efficacy of a drug; the estimation of the bioavailability of a drug in a given dosage form is direct evidence of the efficiency with which a dosage form performs its intended therapeutic function. The bioavailability of a drug substance formulated into a pharmaceutical product is fundamental to the goals of dosage form design and essential for the clinical efficacy of the medication. Thus, bioavailability testing, which measures the rate and extent of drug absorption, is a way to obtain evidence of the therapeutic utility of a drug product. Bioavailability determinations are performed by drug manufacturers to ensure that a given drug product will get the therapeutic agent to its site of action in an adequate concentration. Bioavailability studies are also carried out to compare the availability of a drug substance from different dosage forms or from the same dosage form produced by different manufacturers [104].

1.5.1 *In-Vivo* Methods

One method for assessing the bioavailability of a drug product is through the demonstration of a clinically significant effect. However, such clinical studies are complex, expensive, time-consuming and require a sensitive and quantitative measure of the desired response. Further, response is often quite variable, requiring a large test population. Practical considerations,

therefore, preclude the use of this method except in initial stages of development while proving the efficacy of a new chemical entity [104].

Quantification of pharmacologic effect is another possible way to assess a drug's bioavailability. This method is based on the assumption that a given intensity of response is associated with a particular drug concentration at the site of action; e.g., variation of miotic response intensity can be directly related to the oral dose of chlorpromazine. However, monitoring of pharmacologic data is often difficult, precision and reproducibility are difficult to establish, and there are only a limited number of pharmacologic effects (e.g. heart rate, body temperature, blood sugar levels) that are applicable to this method. Because of these limitations, alternative methods have been developed to predict the therapeutic potential of a drug. The current method to assess the clinical performance of a drug involves measurement of the drug concentrations in the blood or urine [105].

1.5.2 *In-vitro* Methods

1.5.2.1 Disintegration Test

The early attempts to establish an indicator of drug bioavailability focused on disintegration as the most pertinent *in-vitro* parameter. The first official disintegration test appeared in the United States Pharmacopoeia (USP) in 1950 [64]. However, while it is true that a solid dosage form must disintegrate before significant dissolution and absorption can occur, meeting the disintegration test requirement only ensures that the dosage form (tablet) will break up into sufficiently small particles in a specified length of time. It does not ensure that the rate of solution

of the drug is adequate to produce suitable blood levels of the active ingredient. Therefore, while the test for tablet disintegration is very useful for quality control purposes in manufacturing, it is a poor index of bioavailability [105].

1.5.2.2 Dissolution Test

To date *in vitro* dissolution tests seem to be the most sensitive and reliable predictors of *in vivo* availability. Since dissolution of a dosage form *in vivo* is often the rate-limiting factor determining the physiologic availability of a drug, measurement of the *in vitro* dissolution rate or a related parameter is more likely to offer a meaningful indication of physiologic availability [64].

Dissolution testing has been recognized as a relatively fast and inexpensive *in vitro* technique that can be utilized in the assessment of the release characteristics of dosage forms under investigation. Over the past 30 years it has been established that dissolution testing is probably the most important *in vitro* test that can be used to assess and control variables associated with formulation excipients, design, and manufacturing, which may alter the release characteristics of the active moiety from the formulation. Currently dissolution testing is implemented in the assessment and evaluation of the release rates and bioavailability of a variety of conventional tablet and capsule dosage forms. Though it is difficult to say whether the *in vitro* dissolution of a tablet actually predicts the *in vivo* dissolution, it is believed that if a tablet does not dissolve properly in *in vitro* test, it certainly will not do so in *in vivo* tests for dissolution [64].

Since a drug must go into solution before it can be absorbed, and since the rate at which a drug dissolves from a dosage form often determines its rate and/or extent of absorption, attention has been directed at the dissolution rate. It is currently considered to be the most sensitive *in-vitro* parameter most likely to correlate with bioavailability [106-107].

Pharmaceutical scientists have for many years been attempting to establish a correlation between some physicochemical property of a dosage form and the biological availability of the drug from that dosage form. The term commonly used to describe this relationship is "correlation" [65]. Specifically, it is felt that if such a correlation could be established, it would be possible to use *in-vitro* data to predict a drug's *in-vivo* bioavailability. This would drastically reduce, or in some cases, completely eliminate the need for bioavailability tests. The desirability for this becomes clear when one considers the cost and time involved in bioavailability studies as well as the safety issues involved in administering drugs to healthy subjects or patients. It would certainly be preferable to be able to substitute a quick, inexpensive *in-vitro* test for *in-vivo* bioavailability studies [108].

1.5.2.2.1 Official Dissolution Tests

Dissolution tests are an extremely valuable tool in ensuring the quality of a drug product. Generally, product-to-product variations are due to formulation factors, such as particle size differences, excessive amounts of lubricant and coatings. These factors are reactive to dissolution testing. Thus, dissolution tests are very effective in discriminating between and within batches of drug product(s). The dissolution test, in addition, can exclude definitively any unacceptable

product. Thus the *in vitro* test may be a quick method of ensuring *in vivo* performance [109]. There are two official USP dissolution methods: Apparatus 1, (basket method), and Apparatus 2 (paddle method) [64].

1.5.2.2.2 Limitations of Dissolution Tests

There are, however, problems with *in-vitro* dissolution testing, which should be noted - problems which make correlation with *in- vivo* availability difficult. The first is related to instrument variance and the absence of a standard method. The tests described in the USP are but a few of the large number of dissolution methods proposed to predict bioavailability. Since the dissolution rate of a dosage form is dependent on the methodology used in the dissolution test, changes in the apparatus, dissolution medium, etc., can dramatically modify the results [106-107].

In this work it is aimed to evaluate the physical properties and *in vitro* dissolution profiles of nine different brands and one generic co-trimoxazole tablets obtained from various drug outlets in Addis Ababa.

1.6 Objectives

1.6.1 General Objective

The main objective of this work is evaluation of different brand and generic co-trimoxazole tablets obtained from various drug retail outlets in Addis Ababa with respect to physical properties and dissolution profiles.

1.6.2 Specific Objectives

The specific objectives of the study are

- ◆ To compare the physical properties (Thickness, Diameter, Hardness and shape) of the ten different co-trimoxazole tablets;
- ◆ To compare the weight variation and content uniformity of the ten different tablet products;
- ◆ To compare the disintegration times of the ten different tablet products;
- ◆ To compare the assay results of the ten different tablet products, and
- ◆ To compare the dissolution profiles of the ten different tablet products.

2.1 Materials and Methods

2.1.1 Materials

Different brand and generic co-trimoxazole (480mg) tablets were purchased from various drug retail outlets in Addis Ababa.

HPLC grade Methanol (Hiper Solv™ for HPLC. BDH Laboratory Supplies, Poole, BD15 1TD, England. 99.8%), HPLC grade Acetonitril (Hiper Solv™ for HPLC. BDH Laboratory Supplies, Poole, BD15 1TD, England, 99.9%), Triethylamine (MERCK-Schuchardt, Germany. 99%). Hydrochloric acid (Riedel-de Haën, Germany), Glacial Acetic acid (Anala R®, BDH Laboratory Supplies, Poole, BD15 1TD, England. 100 %), Cellulose Nitrate Filters (Pore Size: 0.45µm, Sartorius, Germany), and Column (HICHROM. KR100-5C18-6136. Hichrom Ltd., UK) were used as received. Sulphamthoxazole and trimethoprim USP reference standards were obtained from Ethiopian Pharmaceuticals manufacturing Share Company. All other chemicals used were analytical grade.

Analytical balance (SCALTEC®, SBC 31, Germany), friability tester (SOTAX®, F2 Friabilator USP, Switzerland), integrated hardness, thickness and diameter tester (Pharma Test®, PTB 311, Germany), disintegration apparatus (ERWEKA, ZT 3, Germany), dissolution apparatus (ERWEKA, DT 700 HH, Germany) and high performance liquid chromatography (Total Chrom Work Station, Perkin Elmer Series 200 Diode Array Detector, UV/ VIS Detector, Pump, Auto Sampler, Vacuum Degasser, Perkin Elmer Network Chromatography Interface (NCI) 900. Perkin

Elmer Instruments, Norwalk, CT 06859 USA) were used for the study.

2.1.2 Methods

2.1.2.1 Comparison of Physical Properties of Co-trimoxazole Tablets

The different tablets were tested for tablet weight variation, friability, hardness, thickness, diameter, and disintegration time.

2.1.2.1.1 Tablet Weight Variation

The weights of 20 tablets from each brand were determined individually and the percentage weight variations and standard deviations were calculated.

2.1.2.1.2 Dosage-Unit Uniformity

10 tablets from each brand were accurately weighed individually. From the result of the assay, the content of active ingredient in each of the 10 tablets is calculated.

2.1.2.1.3 Friability Testing

Twenty tablets from each brand were dusted and weighed on the analytical balance (SCALTEC®, SBC 31, Germany). The tablets were placed in the drum of the friability tester

(SOTAX®, F2 Friabilator USP, Switzerland) and rotated at 25 rpm for four minutes (100 times). The tablets from each brand were re-dusted and re-weighed. According to the USP, the tablets should not lose more than 1% of their total weight.

2.1.2.1.4 Hardness, Thickness and Diameter Testing

Using forceps, ten tablets were individually placed between the platens of integrated hardness, thickness and diameter tester (Pharma Test®, PTB 311, Germany). The test button was pushed and the resulting visual readings of tablet hardness, thickness and diameter were recorded.

2.1.2.1.5 Disintegration

The disintegration tests of all brands were carried out using (ERWEKA, ZT 3, Germany) disintegration apparatus. The disintegration media for all the brands comprised of distilled water maintained at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$. Tablets were considered completely disintegrated when all particles passed through the wire mesh.

2.1.2.2 High Performance Liquid Chromatography (HPLC) System

2.1.2.2.1 Chromatographic System

A High-pressure liquid Chromatograph (Total Chrom Work Station, Perkin Elmer Series 200 Diode Array Detector, UV/ VIS Detector, Pump, Auto Sampler, Vacuum Degasser, Perkin Elmer

Network Chromatography Interface (NCI) 900. Perkin Elmer Instruments, Norwalk, CT 06859 USA) equipped with a diode array detector and a stainless steel column (25 cm x 4mm i.d.) was used. Absorbance readings were taken at 254nm. The column packing was porous silica particles with an octadecylsilane-bonded coating. Flow rate of the mobile phase was maintained at 1.5 ml per minute with a column pressure of ≈ 3800 psi. Standards and Samples were injected by an autosampler equipped with a 20 μ l loop, and all analyses were performed at ambient temperature. The standard preparation was chromatographed, and printouts of the peak responses were collected. The resolution, R, between sulphamethoxazole and trimethoprim was not less than 5.0 and the relative standard deviation for replicate injections was not more than 2.0%. Relative retention times are 3.5 minute for trimethoprim and 8 minute for sulphamethoxazole.

2.1.2.2.2 Mobile Phase Preparation

1400 ml of distilled water was mixed with 400 ml of acetonitril and 2 ml of triethylamine in a volumetric flask. This was allowed to equilibrate to room temperature and adjusted to pH 5.7 ± 0.1 with dilute glacial acetic acid (1 in 100). The volume was adjusted to 2 liters with water and filtered through a 0.45 μ m cellulose acetate membrane filter [17].

2.1.2.2.3 Standard Preparation

8 mg of USP RS trimethoprim and 40 mg USP RS sulphamethoxazole were dissolved in about 10 ml of methanol, sonicated for 5 minutes and diluted to 25 ml with methanol to obtain a solution containing, in each ml, about 0.32 mg of trimethoprim and 1.6 mg of sulphamethoxazole,

respectively. 5 ml of this solution was transferred to a 50 ml volumetric flask and diluted to volume with mobile phase, and mixed to obtain a standard preparation having a known concentrations of about 0.032 mg of USP trimethoprim RS per ml and 0.16 mg of sulphamethoxazole RS per ml.

2.1.2.2.4 Assay Method

The tablets were assayed for their content of sulphamethoxazole and trimethoprim according to the method described in the USP [17]. Based on this method, twenty (20) sulphamethoxazole and trimethoprim (Co-trimoxazole) tablets were weighed and finely powdered. An accurately weighed powder equivalent to 80 mg of sulphamethoxazole was transferred to a 50 ml volumetric flask. About 25 ml of methanol was added and sonicated with intermittent shaking for 5 minutes. This was allowed to equilibrate to room temperature, diluted with methanol to volume, and mixed. 5.0 ml of this solution was transferred to a 50 ml volumetric flask and diluted with mobile phase to volume, mixed and filtered with 0.45 µm cellulose acetate membrane filter.

2.1.2.2.5 Dissolution Study

The dissolution tests of all the brands were carried out according to the USP XXVI specifications using an 8-Flask Bath dissolution apparatus, Paddle method or type II (ERWEKA, DT 700 HH, Germany). The dissolution media for all the brands comprised of 900 ml of 0.1 N HCl (pH= 1). The rotational speed of the apparatus was held constant at 75 rpm and temperature of the dissolution media was always maintained at 37 ± 0.5 °C. Samples of 9 ml were withdrawn from

the dissolution media at predetermined time intervals, i.e., at 5, 10, 20, 30, 45 and 60 minutes. After the necessary dilution each solution was filtered using cellulose acetate membrane filter (0.45 μm). The sample solutions were chromatographed (analysed) for trimethoprim and sulphamethoxazole using Perkin Elmer High Pressure Liquid Chromatograph at the λ max (254 nm). Sample volumes were replaced with equal volumes of dissolution media maintained at 37 $^{\circ}\text{C}$. All dissolution tests were done in triplicate. Necessary corrections for dilution were made when calculating amount of drug released at each sampling time. Not less than 70% (Q) of the labeled amounts of sulphamethoxazole ($\text{C}_{10}\text{H}_{11}\text{N}_3\text{O}_3\text{S}$) and trimethoprim ($\text{C}_{14}\text{H}_{18}\text{N}_4\text{O}_3$) are expected to dissolve in 60 minutes.

2.1.2.1.6 Assay and Dissolution Study Procedure

Equal volumes of (about 20 μl) of the standard preparation and the assay preparation were injected into the chromatograph by the auto sampler and responses for the peaks were presented as a print out. The quantities, in mg, of trimethoprim ($\text{C}_{14}\text{H}_{18}\text{N}_4\text{O}_3$) and sulphamethoxazole ($\text{C}_{10}\text{H}_{11}\text{N}_3\text{O}_3\text{S}$) in the portion of tablets taken (or dissolution solution) were calculated by the formula:

$$1000C(r_u/r_s),$$

Where C is the concentration, in mg per ml, of the appropriate USP reference standard in the standard preparation, r_u is the response of the corresponding analyte obtained from the assay preparation (or dissolution solution) and r_s is the response of the standard preparation.

2.2 Construction of Calibration Curve

Various concentrations of the reference standards of trimethoprim and sulphamethoxazole were prepared and the respective peak areas were determined chromatographically at 254 nm. Peak areas were determined at 3.2 µg/ml, 6.4 µg/ml, 16 µg/ml, 32 µg/ml, 64 µg/ml and 160 µg/ml for trimethoprim and 16µg/ml, 32µg/ml, 80µg/ml, 160µg/ml, 320µg/ml, and 800µg/ml for sulphamethoxazole. Then concentrations of trimethoprim and sulphamethoxazole against peak area were plotted to obtain the Beer–Lambert standard calibration curves. The linear regression equations were also derived for each as shown in Figure 2.1 and Figure 2.2.

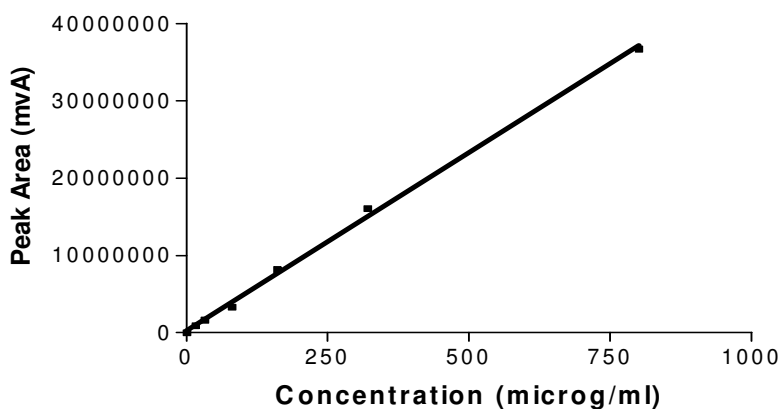


Figure 2.1 Standard Beer-Lambert calibration curve of pure sulphamethoxazole in the concentration ranges of 16 µg/ml to 800 µg/ml (The regression equation is: $y = 0.2809 + 0.23045x$, where y is the peak response in mVA and x is the concentration in µg per ml; $R^2 = 0.998$).

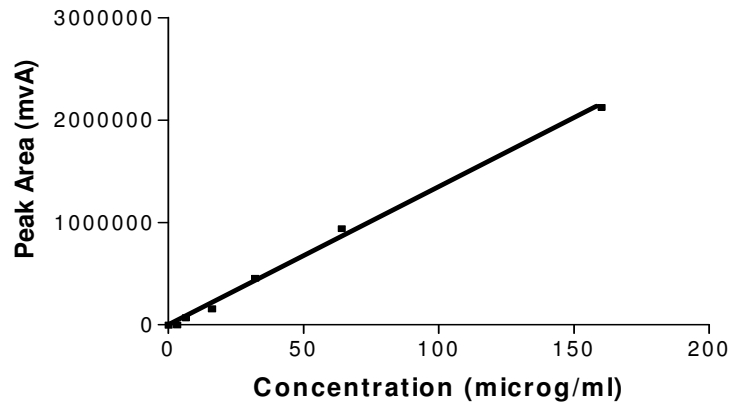


Figure 2.2 Standard Beer-Lambert calibration curve of pure trimethoprim in the concentration range of 3.2 $\mu\text{g/ml}$ to 160 $\mu\text{g/ml}$ (The regression equation is: $y = -0.007103 + 0.01351x$, where y is the peak response in mvA and x is the concentration in μg per ml; $R^2 = 0.997$).

3.1 Tablets Included in the Study

The tablets included in this study originated from three different continents: Europe, Asia and Africa. Two of the tablets are from Switzerland, (Bactrim and Lagatrim). Septrin is from England and Deprim from Cyprus. Bisepton, Cotreich, Cotrimol and oriprim are manufactured in India. Kanprim is South Korean in origin. Only one product included in this study is manufactured locally: Cotrimoxazole. 50 % of the tablets studied are from Asia (4 from India and 1 from Korea). 40 % of the tablets are European in origin. Of the evaluated products, eight are imported by different private businesses where as one product namely Cotrimol is imported by government agency. All of the ten products are presented in a blister pack. Table 3.1 shows the details of tablets evaluated in this study.

3.2 Evaluation of Physical Properties of Tablets

The results for the physical properties of the products tested in this study are shown on table 3.2 and table 3.3.

3.2.1 Friability

Tablets require a certain degree of strength, or crushing strength and resistance to friability and abrasion, to withstand mechanical shocks of handling during manufacturing, packaging and shipping. Adequate tablet hardness as well as reasonable friability are requisites for consumer acceptance. Conventional compressed tablets that have crushing strength greater than 50N and that lose less than 1% of their weight after friability tests are generally considered acceptable

[67]. Percent friability of the ten products is listed on table 3.2. Only one product (Cotreich-India) gave a friability value above the specified limit (1.88%). The rest of the products are below the specified 1% maximum limit. The innovator product has a friability value of 0.72%. The smallest friability value is registered by Lagatrim-a normal concave tablet. Five of the products are below 0.5% limit (Bisepton, Cotrimol, Kanprim, Lagatrim and Oripim). Except cotreich all the Indian products have a friability value less than 0.5%. Normal Concave shaped tablets gave relatively higher friability values Bactrim-0.72% and Septrin-0.70%. With the exception of Cotreich all flat shaped tablets gave the smallest friability values.

3.2.2 Weight Variation

As shown on table 3.2, the average weight of the tablets ranges from 502.41 mg (Bactrim) to 709.98 mg (Cotrimol). The expected strength of co-trimoxazole is 480 mg. The variation in average weights of the tablets studied reveals that different manufacturers use different kinds of excipients in varying proportions in their products. The innovator product (Bactrim) has about 22.41 mg various excipients in it. The tablet with the highest average weight (Cotrimol) has about 230 mg of various excipients in it. Cotrimol tablets have about 10 times more excipient in them than Bactrim tablets. As can be seen from table 1.1 the nine tablet products obtained from various manufacturers have excipients in excess of the innovator product. It is a well known fact that the amount of excipients added, their mode of addition, and processing conditions associated with their incorporation affect both the physical properties and the drug release profiles (both *in vitro* and *in vivo*) of a tablet product.

Product	Manufacturer	Batch Number	Manufacturing Date	Expiration Date	Mode of Packaging
Bactrim	La Hoffman La Roche Ltd. (Switzerland)	1082	Nov-01	Nov-06	20tab/Box
Bisepton	Flamingo Pharmaceuticals Ltd (India)	199	Feb-02	Mar-05	100tab/Box
Cotreich	Smithkline Beecham Pharmaceuticals (India)	1017	Sep-01	Aug-05	1000tab/Box
Cotrimol	IPCA Laboratories Ltd. (India)	2001	Jan-02	Feb-07	1000tab/Box
Cotrimoxazole	Addis Pharmaceuticals Factory (Ethiopia)	220129E	-	Dec-03	1008tab/Box
Deprim	Remedical Limited (Cyprus)	16133	May-01	May-06	20tab/Box
Kanprim	Kwang Myung Pharmaceuticals Company Limited (Korea)	203	Jun-02	Jun-07	100tab/Box
Lagatrim	Lagap SA Pharmaceuticals (Switzerland)	6380	Jan-02	Jan-07	20tab/Box
Oriprim	Cadila Health Care Limited (India)	00057	Nov-00	Dec-04	1000tab/Box
Septin	Wellcome (England)	A047975	Jun-01	Jun-06	1000tab/Box

Table 3.1 Details of different sulphamethoxazole-trimethoprim (Co-trimoxazole) tablets evaluated in this study .

Drug Product	Average Weight (g)	Friability (%)	Mean Disintegration time, Minute (±SD)
Bactrim (Switzerland)	0.5008(0.005)	0.72	0.33(±0.06)
Bisepton (India)	0.5335 (0.006)	0.34	1.57 (±0.25)
Cotreich (India)	0.5260(0.004)	1.88	8.72 (±2.32)
Cotrimol (India)	0.7064 (0.010)	0.31	8.10 (±1.36)
Cotrimoxazole (Ethiopia)	0.5675 (0.007)	0.62	0.17 (±0)
Deprim (Cyprus)	0.6261(0.017)	0.53	2.39(±0.64)
Kanprim (Korea)	0.5031(0.010)	0.35	11.98(±3.58)
Lagatrim (Switzerland)	0.6043(0.002)	0.27	13.05(±5.24)
Oriprim (India)	0.5252(0.006)	0.31	3.85(±1.4)
Septin (England)	0.5066(0.006)	0.70	1.2(±0.15)

Table 3.2 Summary of physical properties of co-trimoxazole tablets of ten different manufacturers. Values in parentheses are standard deviations.

Drug Product	Average Thickness (mm)	Average Diameter (mm)	Average Hardness (N)
Bactrim (Switzerland)	5.738(0.127)	11.661(0.109)	72.4(0.24)
Bisepton (India)	4.219(0.057)	12.667(0.020)	97(0.20)
Cotreich (India)	4.656(0.030)	12.135(0.017)	68.47(0.15)
Cotrimol (India)	4.777(0.056)	13.268(0.019)	87.56(0.10)
Cotrimoxazole (Ethiopia)	7.833(0.037)	12.507(0.015)	80.07(0.12)
Deprim (Cyprus)	4.396(0.039)	13.191(0.011)	88.84(0.11)
Kanprim (Korea)	4.227(0.106)	12.651(0.007)	87.72(0.15)
Lagatrim (Switzerland)	6.317(0.047)	12.146(0.030)	95.96(0.04)
Oriprim (India)	4.39(0.043)	12.515(0.015)	86.03(0.19)
Septin (England)	5.811(0.058)	11.512(0.008)	100.94(0.07)

Table 3.3 Physical properties of co-trimoxazole tablets of ten different manufacturers. Values in parentheses are standard deviations. Except in case of hardness where it is coefficient of variation.

The % mean deviation for the products tested is in the range of 0.40%(Lagatrim) to 1.15%(Oriprim). In other words the weight of all the tablets tested deviated from the average by less than 5% implying that all the tablets comply to the USP XXVI specifications for weight variation. The relatively small weight variations of the tablets observed in this study demonstrate that the manufacturers of the tested tablet products have reasonably controlled most of the

variables (i.e. granulation flow properties, granulation particle size and distribution, die fill, punch lengths, etc.) that could be responsible for weight variation.

3.2.3 Dosage-Unit Uniformity

The uniformity of dosage units can be demonstrated by either of two methods, weight variation or content uniformity. As co-trimoxazole tablets contain more than 50 mg of sulphamethoxazole and trimethoprim, the weight variation method is applied. According to the USP (2003), the requirements for dosage uniformity are met if the amount of the active ingredient in each of the ten tablets lies within the range of 85.0% to 115.0% of the label claim and the relative standard deviation is less than or equal to 6.0%. Calculation of the amount of sulphamethoxazole and trimethoprim in the 10 tablets of each of the products tested in this study shows that, every tablet met the requirement for dosage unit uniformity. All the tablets lied in the range of 85.0% to 115.0% and all products gave a relative standard deviation value less than 6.0% for both sulphamethoxazole and trimethoprim. The highest value obtained with sulphamethoxazole was 113.95% (Deprim) while the lowest being 97.02% (Bactrim). For trimethoprim the highest value was 112.35% (Cotrimol) while the lowest was 94.15% (Bactrim). The relative standard deviations (RSD) ranged from 1.69% (Oripim) to 0.36% (Bactrim) for sulphamethoxazole and 1.69% (Oripim) to 0.36% (Lagatrim) for trimethoprim. Hence all the products passed the requirements for dosage-unit uniformity.

3.2.4 Shape

The ten different tablets investigated in this study have two principal shapes. 70% of the tablets have flat beveled edge (Bisepton, Cotreich, Cotrimol, Cotrimoxazole, Deprim, Kanprim, and Oriprim). Of these five have breaking lines to facilitate fractioned dosing and to obtain a well-consolidated tablet with reasonable hardness (Bisepton, Cotrimol, Cotrimoxazole, Deprim and Kanprim). Two of the flat shaped products do not have breaking lines (Cotreich and Oriprim). The remaining 30% of the tablets have normal concave shape with breaking lines (Bactrim, Lagatrim and Septrin). Lagatrim has a unique deep breaking line unlike the rest of the tablets. 70% of the tablets are embossed with various inscriptions. Bactrim (ROCHE), Bisepton (COTRI 480), Cotreich (COTRIM 480 on one side and SB on the other) , Cotrimoxazole (APF), Kanprim (KPR), Oriprim (CO-TRI 480), and Septrin (WELL COME Y2B). Cotrimol, Deprim and Lagatrim are not embossed. All Asian, the African and one European tablets have flat beveled edge. Tablets originating from the most industrialized countries have normal concave shape (Switzerland and England).

In addition to tablet thickness and diameter, tablet shape tells us something about tablet tooling used by different manufacturers and the ease with which the tablets are manufactured. From the manufacturing point of view, flat bevelled edge tablets are easier to manufacture than normal concave or deep concave shaped tablets. The fact that both Asian, African and eastern European manufacturers chose the flat shape suggests the technological and technical knowledge differences between the developed countries and the poorer ones. As tablet shape departs from flatness, the difficulty faced while processing increases. And ease of manufacture reflects its light

on the cost of products. Selection of the flat shape implies the technical development level of a specified manufacturer.

All the ten products are white in colour. One product from India (Cotrimol) has a black discoloration on its side when produced from the blister. This could be due to degradation of some of the tablet components or due to processing conditions during the manufacture of the tablets.

3.2.5 Thickness

As shown on table 3.3 the mean thickness of the ten products tested in this study ranges from 4.22 mm to 6.32 mm. Most of the tablets (70%) have a thickness in the range of 4.22 mm to 4.83 mm, and 60% these tablets are manufactured either in Asia or Africa. All the thicker tablets are manufactured in Europe: 6.32 mm (Lagatrim), 5.81mm (Septrin) and 5.74mm (Bactrim). Deprim from Cyprus is 4.4 mm thick. Given variation in weight tablets could differ in their thickness.

3.2.6 Diameter

As shown on table 3.3 the mean diameter of the ten tablet products tested in this study ranges from 11.51mm (Septrin) to 13.27mm (Cotrimol). As can be seen from the mean thickness and mean diameter values on table 1.1, the thicker a tablet the wider is it's diameter. For the same strength product, thickness should be inversely related to diameter or otherwise the imbalance due to improper thickness and diameter should be compensated with tablet weight. Examination

of tablet thickness and diameter gives insight as to the tablet tooling (Upper Punch, Lower Punch and Die) used by the various manufacturers.

3.2.7 Hardness

As shown on table 3.3 the mean hardness of the ten products tested in this study ranges from 68.47 N (Cotreich) to 100.94 N (Septrin). Unusually higher and lower hardness values are registered by some products. Two Bisepton tablets gave a value of 111.5N and 146.9N. Of the tested tablets one Bactrim tablet and one Oripriam tablet gave a value close to the lower limit; i.e., 50.9 N and 50.7N respectively. One Cotreich tablet and one Bactrim tablet gave a value below 50 N; 45.5N and 46.2N respectively.

Most of the tested tablets gave a hardness value in the range of 60 N to 100 N. As can be seen from table 3.3. Two of the European tablets showed a mean hardness close to 100N, 100.94N (Septrin) and 95.96N (Lagatrim). Asian and the African tablet showed hardness in the range of 68.47N (Cotreich) to 87.72N (Kanprim). The innovator product has a hardness of 72.4N.

3.2.8 Disintegration Time

The disintegration time of tablets has to be limited so that it will not significantly alter their rate of dissolution. The mean disintegration times of the tested products is depicted on table 3.2. The maximum specified pharmacopoeial disintegration time of co-trimoxazole is 15 minutes. The highest disintegration time observed in this study is 13.05 (± 5.24) minutes with Lagatrim where

as the lowest is 0.17 (± 0) minutes with Cotrimoxazole. Four of the tested products disintegrated in less than 5 minutes- Bactrim- 0.33 ± 0.06 , Cotrimoxazole- 0.17 ± 0 , Bisepton- 1.57 ± 0.25 , Deprim 2.39 ± 0.64 , Oriprim- 3.85 ± 1.4 and Septrin 1.2 ± 1.5 . Of these Bactrim and Cotrimoxazole disintegrated in less than 1 minute. Two of the products needed more than 10 minutes to disintegrate, Kanprim- 11.98 ± 3.58 and Lagatrim 13.05 ± 5.24 . And two of the products disintegrated between 5 and 10 minutes- Cotreich 8.72 ± 2.32 and Cotrimol 8.10 ± 1.36 .

3.3 *In- Vitro* Evaluation of Tablets

3.3.1 Assay of Tablets

An assay value of the ten different tablet products evaluated in this study is shown on table 3.4. According to USP (2003) [17] sulphamethoxazole and trimethoprim tablets contain not less than 93.0 percent and not more than 107.0 percent of the labelled amounts of sulphamethoxazole and trimethoprim.

Name of Product	Assay Results (%)	
	Sulphamethoxazole	Trimethoprim
Bactrim	98.80	95.88
Bisepton	107.72	104.85
Cotreich	107.27	106.11
Cotrimol	112.27	110.81
Cotrimoxazole	109.40	107.00
Deprim	109.90	102.90
Kanprim	110.41	106.05
Lagatrim	107.23	105.10
Oriprim	103.81	101.06
Septin	110.93	107.80

Table 3.4 Assay results of co-trimoxazole tablets of ten different manufacturers.

As can be seen from table 3.4, five of the tested tablets (Bactrim, Bisepton, Cotreich, Lagatrim and Oriprim) met the requirement for assay as specified in the pharmacopoeia for both sulphamethoxazole and trimethoprim. Four of the tested products gave a higher assay value for sulphamethoxazole (Cotrimoxazole, Deprim, Kanprim and Septin). One Indian product (Cotrimol) gave an assay value higher than the pharmacopoeial specification for both sulphamethoxazole and trimethoprim.

Some manufacturers face dissolution problems with their products and enough amount of drug is not released within the specified time. Unofficial sources indicate that, some manufacturers use

an amount of the active ingredient in excess of that specified in the formulation to compensate for the deficit. Such practices could result in assay values higher than that specified as happens with some of the products in this study (Cotrimoxazole, from Addis Pharmaceutical Manufacturing).

3.3.2 Dissolution Studies

Percent of sulphamethoxazole and trimethoprim released from the ten products is presented on tables 3.5 & 3.6 and the dissolution profile of each tablet is depicted on figures 3.1-3.10. Also percent of sulphamethoxazole and trimethoprim released from the ten products is depicted on figures 3.11 and 3.12. As co-trimoxazole is a combination product, amounts of sulphamethoxazole and trimethoprim released with time are plotted individually.

According to their dissolution profiles (Drug release pattern), tablets investigated in this study could be grouped as those which released the drug contained immediately (Bactrim, Bisepton, Deprim, Lagatrim and Septrin-see Fig.3.1, 3.2,3.6,3.8 and 3.10) i.e. with no lag time and those with delayed release (Cotreich, Cotrimol, Cotrimoxazole, Oripirim and Kanprim-See Fig. 3.3, 3.4, 3.5, 3.7 and 3.9). Most of the tablets released the drug contained in an increasing fashion from the 5th minute to the 60th minute with varying proportion of increment. Products with out lag time released most of the drug within the first 5 to 10 minutes. Cotreich and Cotrimol released both trimethoprim and sulphamethoxazole very slowly. In case of Cotrimoxazole, Oripirim and Kanprim the sulphamethoxazole release was delayed where as trimethoprim release was fairly rapid and comparable to tablets with no lag time. Unlike all the tested products, Lagatrim

exhibited a superimposable dissolution profile for both trimethoprim and sulphamethoxazole (See Fig.3.8) despite the solubility difference between the two drugs.

Table 3.5 Percent of sulphamethoxazole released from the ten products with time.

Time, Min.	Percent Released (%)									
	Bactrim	Bisepton	Cotreich	Cotrimol	Cotrimoxazole	Deprim	Kanprim	Lagatrim	Oriprim	Septin
5	63.98	74.6	4.61	20.29	30.24	63.57	30.12	54.83	29.81	78.67
10	82.3	90.51	17.13	40.05	45.43	80.57	35.89	94.88	51.52	96.04
20	92.14	97.41	36.05	56.48	63.79	92.69	50.94	102.29	75.57	106.21
30	103.28	101.29	48.6	66.28	78.76	94.46	57.92	102.09	84.24	105.83
45	104.58	101.85	60.13	73.49	91.27	98.98	68.66	100.71	94.48	104.21
60	104.22	101.88	68.99	78.19	95.98	101.82	72.22	100.04	99.42	103.24

Table 3.6 Percent of trimethoprim released from the ten products with time.

Time, Min.	Percent Released (%)									
	Bactrim	Bisepton	Cotreich	Cotrimol	Cotrimoxazole	Deprim	Kanprim	Lagatrim	Oriprim	Septin
5	97.18	87.07	28.23	34.13	88.44	89.44	45.66	60.54	88.41	112.87
10	100.97	93.99	52.75	75.19	100.33	97	84.25	97.42	97.09	111.03
20	100.54	95.7	75.22	83.97	100.24	98.51	98.6	102.7	102.8	113.89
30	100.45	97.78	82.25	89.09	98.31	95.75	96.72	102	101.42	109.83
45	100.68	96.55	88.91	89.5	98.31	96.03	98.95	100.35	104.59	111.5
60	100.31	96.69	92.81	93.15	96.72	96.94	94.7	99.75	106.22	111.77

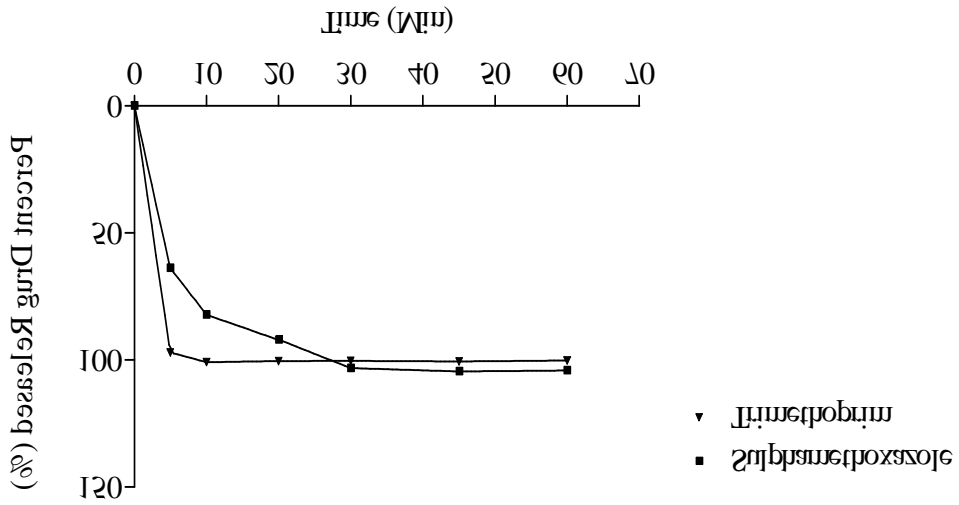


Figure3. 1 Dissolution profile of Bactrim (480mg) Tablets Using USP Paddle Method.

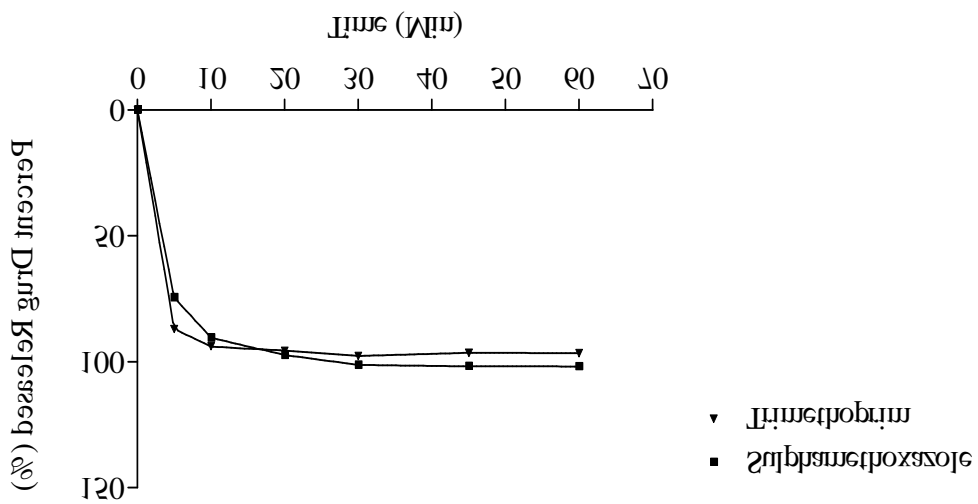


Figure 3. 2 Dissolution profile of Bisepton(480mg) Tablets Using USP Paddle Method.

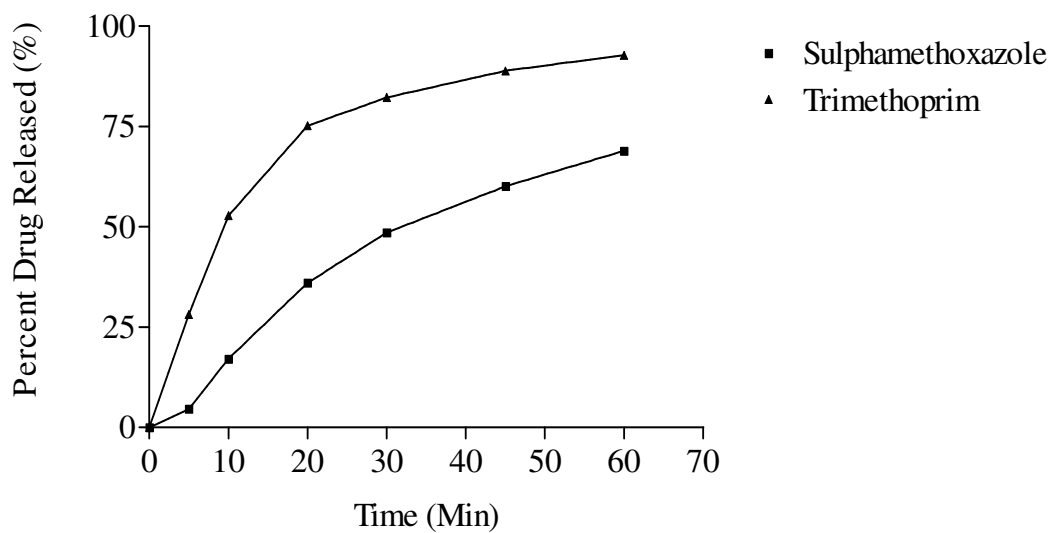


Figure 3.3 Dissolution profile of Cotreich(480mg) Tablets Using USP Paddle Method.

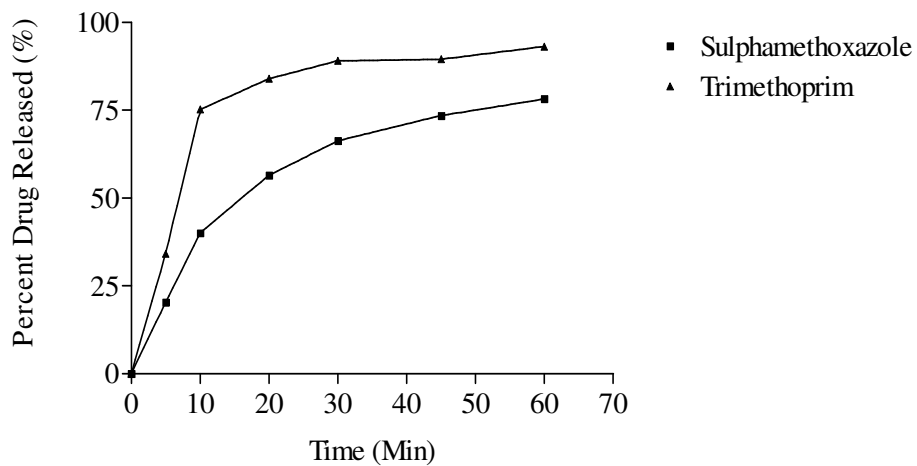


Figure 3.4 Dissolution profile of Cotrimol(480mg) Tablets Using USP Paddle Method.

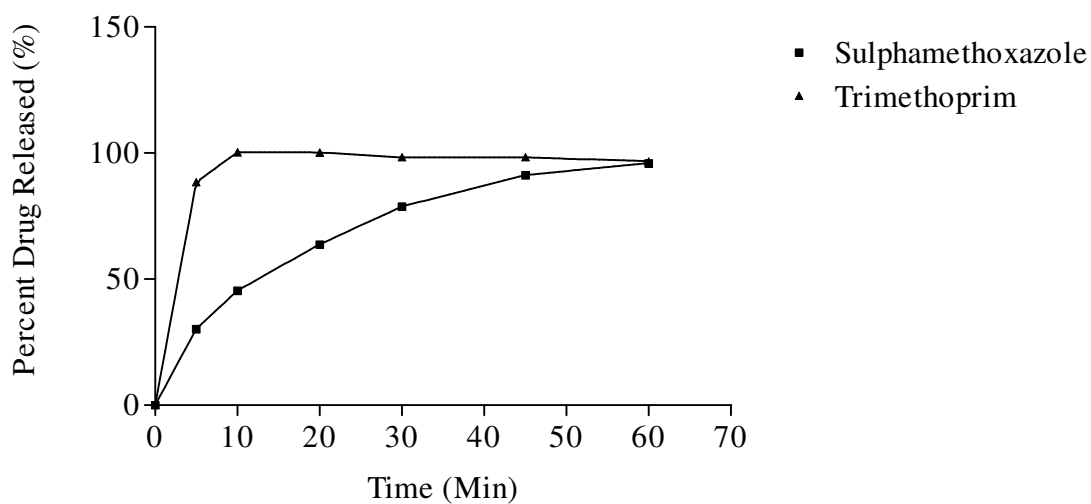


Figure 3. 5 Dissolution profile of Cotrimoxazole(480mg) Tablets Using USP Paddle Method.

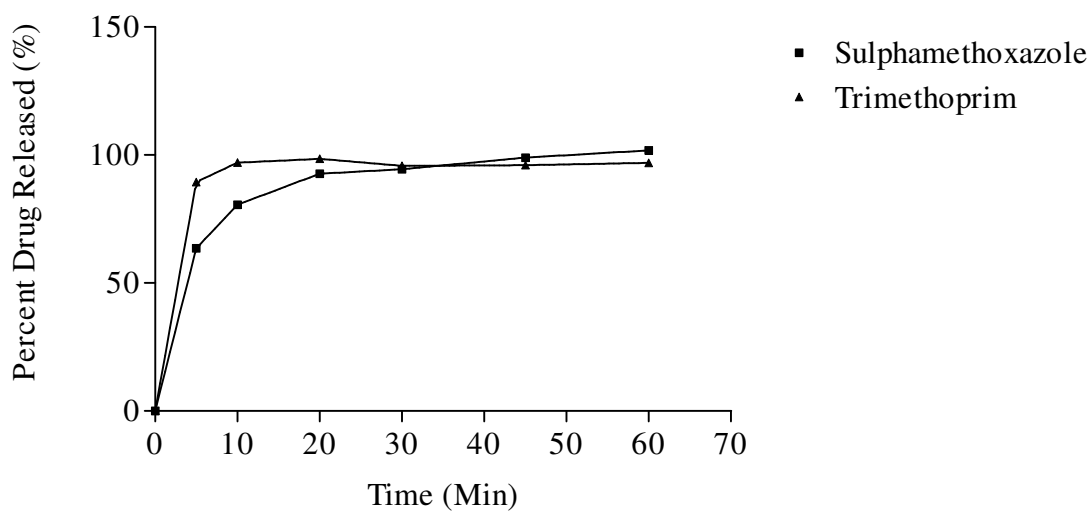


Figure 3. 6 Dissolution profile of Deprim(480mg) Tablets Using USP Paddle Method.

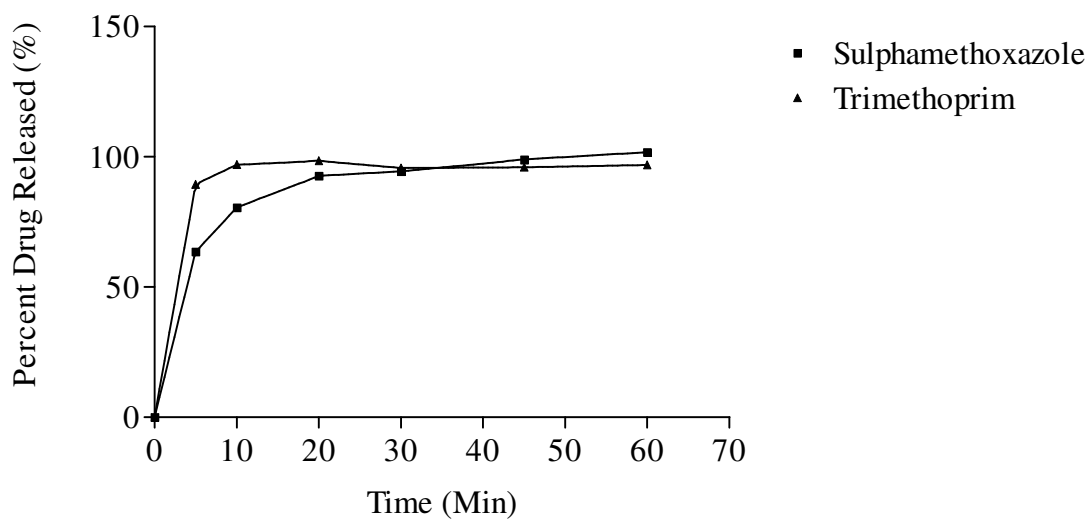


Figure 3.7 Dissolution profile of Kanprim(480mg) Tablets Using USP Paddle Method.

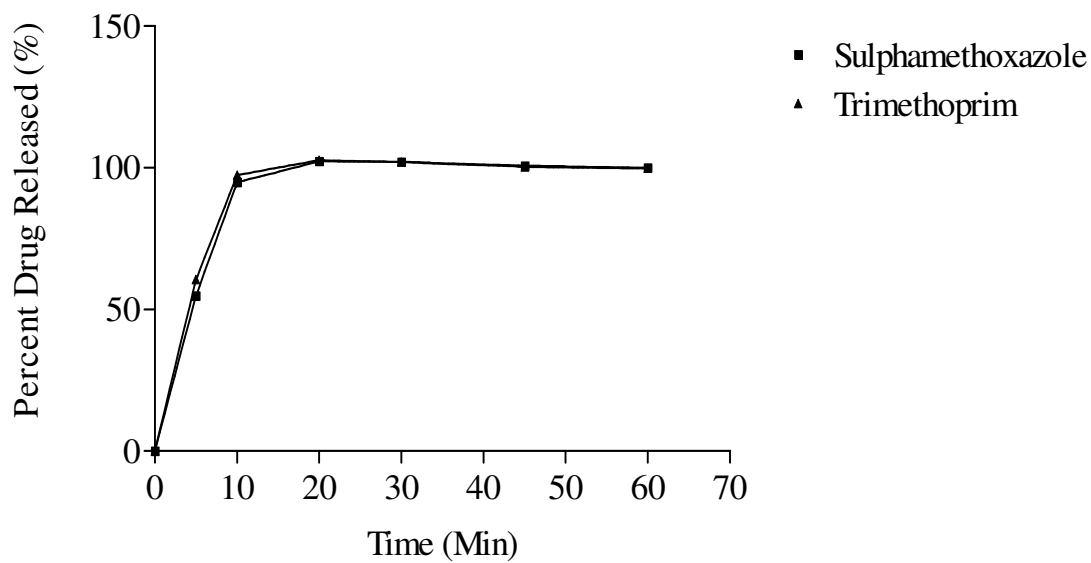


Figure 3. 8 Dissolution profile of Lagatrim(480mg) Tablets Using USP Paddle Method.

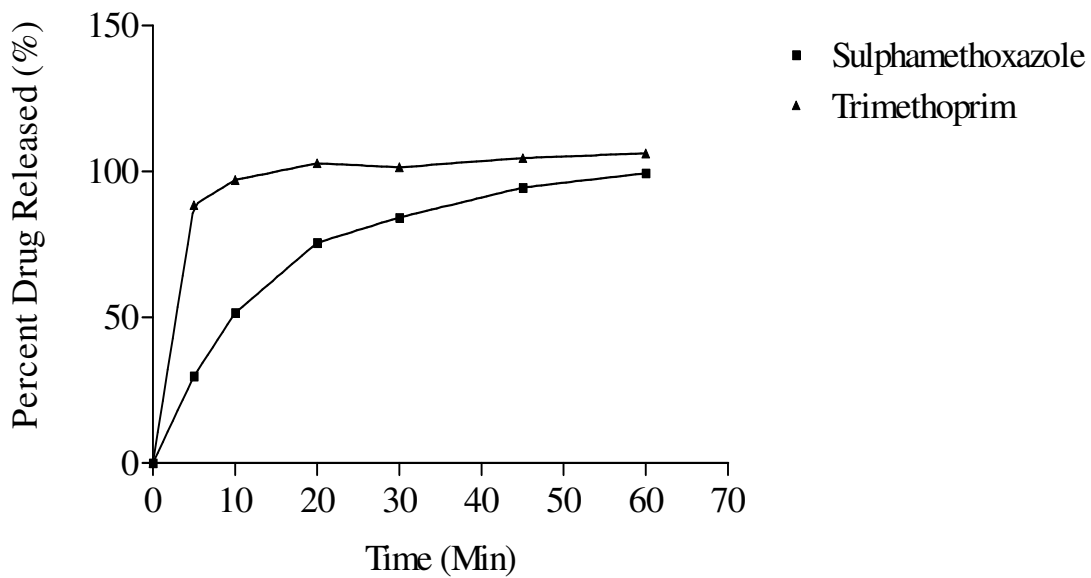


Figure 3.9 Dissolution profile of Oriprim(480mg) Tablets Using USP Paddle Method.

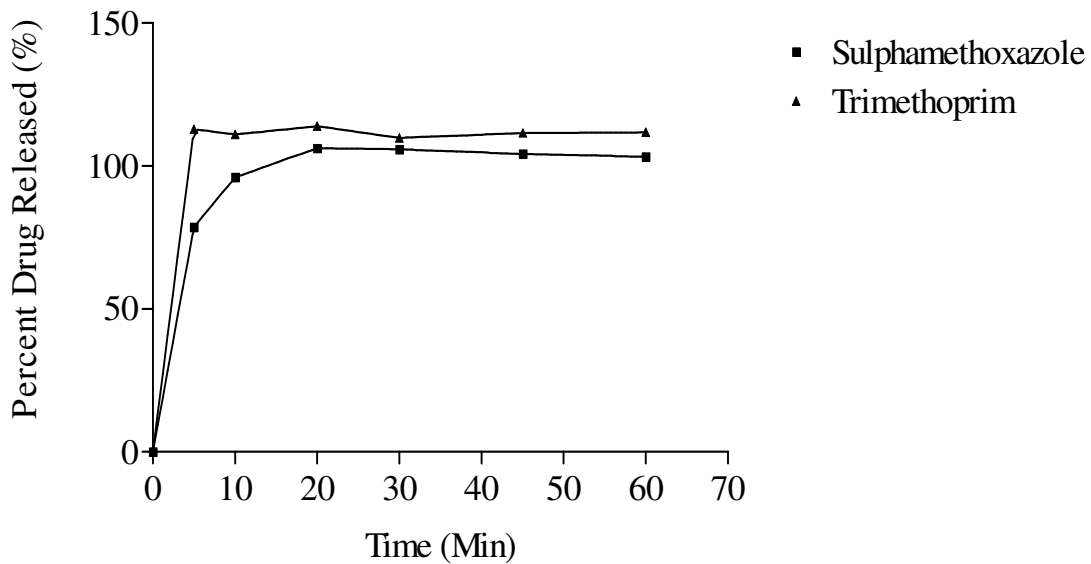


Figure 3.10 Dissolution profile of Septrin (480mg) Tablets Using USP Paddle Method.

The delayed release observed with these products could be attributed to formulation components (example, hydrophobic components) used in the tablets as well as processing conditions

(example, mixing time) [66,74]. Trimethoprim has better aqueous solubility compared to sulphamethoxazole (2x) [11]. Dissolution from the surface of the tablet as well as from disintegrated granules is possible for trimethoprim. And this could be the reason why larger amount of trimethoprim was released from all the tested tablets compared to sulphamethoxazole released, including tablets with lag time. The care exercised in selecting the various formulation components could also affect the solubility and hence the release profile. A reputable manufacturer tries to control all the factors that could affect the release and hence manufacture a product with good attributes.

Furthermore the relatively higher crushing strength of these tablets (Cotreich: 68.47N, Cotrimol: 87.56N, Cotimoxazole: 80.07N, Oripriam: 86.03N and Kanprim: 87.72N) could contribute to the delay in release observed. And also the relatively long disintegration time of these products (Kanprim: 11:98, Cotreich: 8:72, Cotrimol: 8:10 and Oripriam: 3:85 minutes) with the exception of Cotrimoxazole (0.17minutes) might have impeded the dissolution.

It is believed that harder tablets take longer time to disintegrate and go in to solution (dissolve). Though tablets investigated in this study had a crushing strength in the range of 68.47N to 100.94N all of them disintegrated before the 15minute (BP) or 30minute (USP) compendial limit for immediate release tablets.

Even if tablets with no lag time had higher crushing strength (Bactrim: 72.4N, Deprim: 88.84N, Bisepton: 97.0N, Lagatrim: 95.96N, and Septrin: 100.94N) than tablets with delayed release, they disintegrated in less than 5 minutes with the exception of Lagatrim that disintegrated in 13.05 minutes. As can be seen from figures 3.11 and 3.12 the dissolution rate of these tablets is fast.

This could result from the type of disintegrant used and its mode of incorporation, the type and concentration of the lubricant and its mixing time and/or the type and concentration of the binder used in the formulation of the tablets.

Tablets with longer disintegration time are expected to exhibit delayed dissolution profiles. As can be seen from table 3.2 six of the tested products disintegrated in less than 5minutes. All of these tablets released more than 70% trimethoprim within 5minutes. Whereas four of these tablets (Bactrim, Septrin, Bisepton and Deprim) released more than 60% Sulphamthoxazole and two (Cotrimoxazole and Oripim) released about 30% Sulphamethoxazole within 5minutes.

The disintegration time of four of the tested products (Cotreich, Cotrimol, Kanprim and Lagatrim) was more than 5minutes. However all of the tested products disintegrated within the pharmacopoeial specified time (i.e. 15minutes). Despite the disintegration time being within the specified limit, tablets with more than 5minutes disintegration time exhibited significant delay in dissolution (Cotreich, Cotrimol and Kanprim). Consistent with the expectation, tablets with relatively longer disintegration time released smaller amounts of trimethoprim and sulphamethoxazole initially (5th minute). One product (Cotreich) released only 4.61% of sulphamethoxazole within 5minutes.

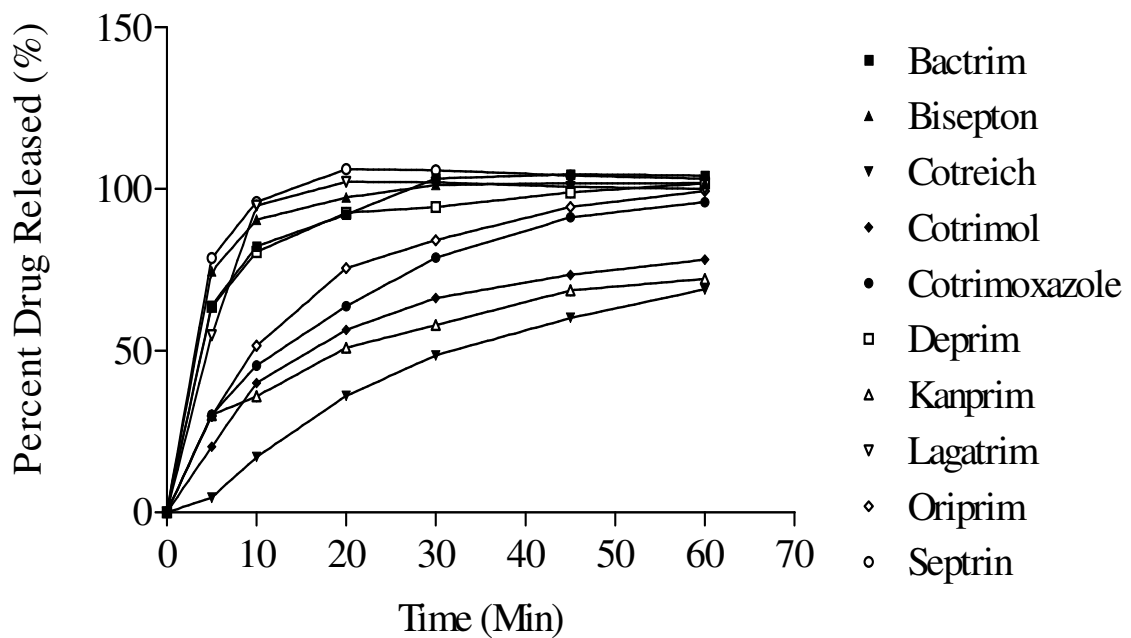


Figure 3.11 Percent of sulphamethoxazole released from ten different Co-trimoxazole tablets of different manufacturers.

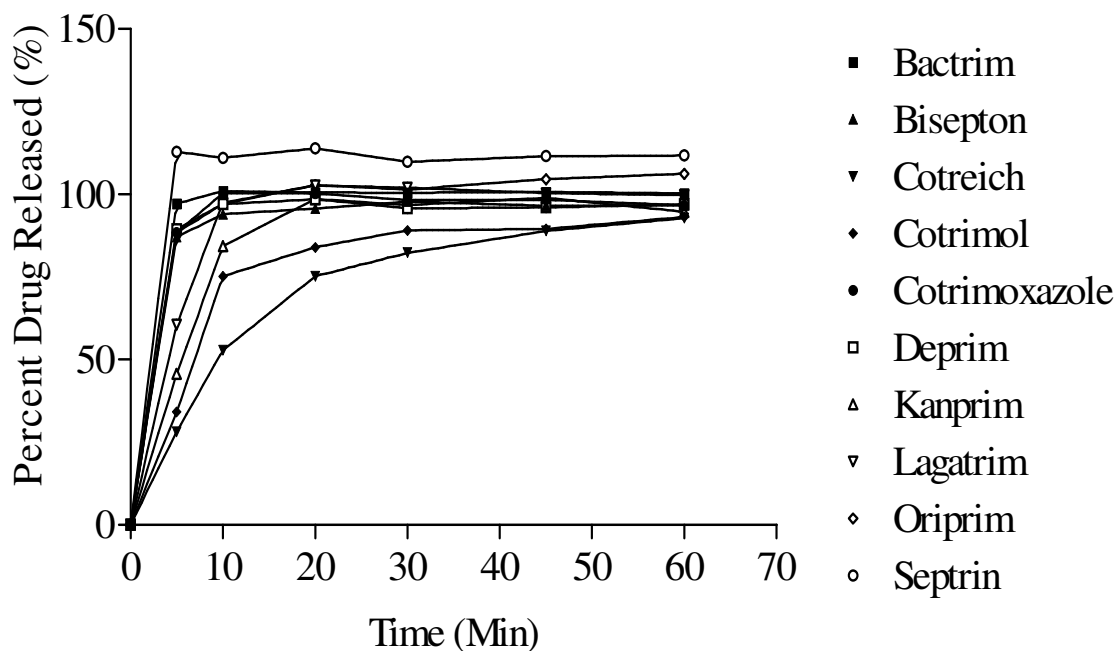


Figure 3.12 Percent of trimethoprim released from ten different co-trimoxazole tablets of different manufacturers.

As can be seen from figure 3.12 all the tested products released more than 90% trimethoprim after one hour, ranging from 92.81%-Cotreich to 111.77%-Septrin. Most products released more than 90% trimethoprim until the 20th minute whereas for products with lag time (Cotreich and Cotrimol) 90% is reached at the 60th minute.

However the release of sulphamethoxazole from tablets with delayed release and from those without lag time is significantly different. As can be seen from figure 3.11 tablets with no lag time released more than 90% sulphamethoxazole up to the 20th minute. Tablets with delayed release exhibited similar sulphamethoxazole release pattern and in most cases smaller amount is released at each sampling interval as compared to tablets with no lag time. Three of the tablets with delayed release failed to release 90% sulphamethoxazole at the end of the 60th minute. One

tablet (Cotreich) released as small as 4.61% of sulphamethoxazole after 5 minutes and only 68.99% is released after 60 minutes, which is less than the minimum compendial requirement (70%) where as Cotrimol and Kanprim released 78.19% and 72.22% respectively after 60 minutes. Even though Cotrimoxazole and Oripriam exhibited delayed sulphamethoxazole release initially, more than 90% was released at the 60th minute.

Product	Dissolution Parameters			
	Trimethoprim		Sulphamethoxazole	
	t _{50%} (min.)	t _{90%} (min)	t _{50%} (min)	t _{90%} (min)
Bactrim	1	3	3	18
Bisepton	2.5	8	2.5	10
Cotreich	9	60	38	> 60
Cotrimol	7	45	20	> 60
Cotrimoxazole	1.5	6	14	45
Deprim	1.5	5	3	18
Kanprim	7	15	20	> 60
Lagatrim	2.5	6	3.5	7
Oripriam	1.5	6	10	40
Septin	1	1	1	7

Table 3. 7 Dissolution parameters (t_{50%} and t_{90%}) of co-trimoxazole tablets of different manufacturers.

The t_{50%}(the time elapsed for 50% of the drug to be released from the tablet) and t_{90%}(time required for 90% release) of co-trimoxazole tablets from different manufacturers are shown on table 3. 7. Products with delayed release took longer time to release 50% and 90% of their

trimethoprim and sulphamethoxazole. The very long $t_{90\%}$ values of Cotreich and Cotrimol (60 & 45minutes respectively) for trimethoprim, the very long $t_{50\%}$ & $t_{90\%}$ values of Cotreich (38 and >60minutes respectively) for trimethoprim and sulphamethoxazole, and the very long $t_{90\%}$ values of Cotreich, Cotrimol and Kanprim (>60 minutes) for sulphamethoxazole indicate that these products could result in lower rate and extent of bioavailability in the body. Similar problems could be encountered with the relatively long $t_{90\%}$ values of Cotrimoxazole (45minutes) and Oriprim (40minutes) for Sulphamethoxazole. The most effective synergistic ratio for the greatest number of microorganisms is 20 parts of sulphamethoxazole to 1 part of trimethoprim. The combination is thus formulated to achieve a sulphamethoxazole concentration *in vivo* 20 times greater than that of trimethoprim [12,13]. However the smaller amount of sulphamethoxazole released from products with delayed release could compromise the *in vivo* efficacy of these tablets. And as Co-trimoxazole is an antibiotic, a drug level below the minimum inhibitory concentration (MIC) could lead to the emergence of resistant strains of microbes.

3.4 Are Cost and Quality Related?

Like many other commodities, there is a widely held belief that cost of pharmaceuticals is strongly correlated with their quality. The cheaper the price, the poorer the quality of the product is believed to be. However the validity of this argument needs to be ascertained with data derived from research.

In countries like Ethiopia where most of the drug products are imported from various countries and manufacturers, there could be variation in the price of the drug products and even between the same drug products emanating from different sources. Thought not supported by investigative

study (especially in Ethiopia), there is a widely held claim among the public and consumers that price is directly related (proportional) to quality. And also the origin of the products is strongly associated with quality, i.e., drugs originating from less developed countries are supposed to be of poorer quality.

The retail prices of the products evaluated in this study ranges from 2.10 Birr / 20 tablets to 71.80 Birr / 20 tablets at the time of purchase (November 2002). As can be expected from the difference in their origin, European products are more expensive than the Asian or the African. The innovator product (Bactrim) is the most expensive of them all. The two brands from Switzerland are the most expensive in the list of brands studied; Bactrim 71.80 Birr / 20 tablets and Lagatrim 54.00 Birr / 20 tablets. Septrin of England is the third in price 44.00 Birr / 20 tablets. Septrin is cheaper than the two because it is presented in a carton containing 100 blisters of 10 tablets (1000 tablets).

The cheapest product is from Ethiopia 2.10 Birr / 20 tablets. There is about a 270 % price difference between the most expensive brand and the cheapest brand. This much-exaggerated disparity in price could be associated with various reasons.

Different manufacturers of the same drug product use different kinds of formulation components (additives in particular) in their products. Additives could be obtained from different sources. With variation in source there could be: difference in packaging material used, level of purity. And these differences coupled with other factors could affect cost of the additives used and this in turn would affect the cost of the finished tablets. With emphasis on cost manufacturers could use additives that could have an implication on the cost of the finished product. For instance, a

manufacturer could select a cheaper kind of binder (starch over poly vinyl pyrrolidone-PVP) to minimize cost. Similar arguments holds true to the rest of the additives included in a tablet and this complicates the price differences between products of different manufacturers.

As much as the formulation components, production conditions also vary from manufacturer to manufacturer. As drug product manufacturers are distributed all over the planet (geographically), the cost of various in-puts for production differ from one country to the other depending on the socio-economic conditions of the country in question. Labour cost, utility (water, electricity, fuel, land and others) cost differ between countries and also policy differences depending on the existing government affect the cost of production in a given country. The mentioned sources of variation affect the cost of a finished product.

Three of the products (30%-Bactrim, Deprim & Lagatrim) in this study are presented in individual boxes containing 2 blisters of 20 tablets with enclosed usage instructions (leaflets). And in connection with this, Bactrim and Lagatrim are the most expensive of the ten products however Deprim is relatively cheap may be because it is an east European product. Bisepton, Cotrimol, kanprim and Oriprim are presented as 100 tablets per box (10 Blisters of 10 tablets) with one leaflet inside. Cotrich, Cotrimoxazole and Septrin are presented in a box of 1000 tablets (100 blisters of 10 tablets) with single leaflet inside. The most expensive tablet is presented as a box of 20 tablets where as the cheapest is presented in a box containing 1000 tablets. Though Septrin is presented in a box of 1000 tablets, it is the third in price may be because of its origin (Switzerland).

Patent rights, costs incurred for research and development, reputation of the manufacturer, distribution channels, market shares & access, transportation costs, promotional costs, managerial costs, taxation rates in various countries, government policy difference and other related aspects could also be mentioned as reasons that cause price differences.

Of the products investigated in this study, the cheaper ones exhibited delayed release during dissolution testing and they released smaller amount of drug compared to the others.

CONCLUSION

In summary, this study has attempted to make a comparative evaluation of the physical properties and *in vitro* bioequivalence of ten different Co-trimoxazole tablet products marketed in Addis Ababa. Proof of bioequivalence can involve *in vitro* dissolution studies, *in vivo* bioavailability studies, or combination of these. Carefully designed *in vitro* dissolution tests can be used as an index of the physiological availability of active constituents from orally administered formulations. In addition, in at least some situations, *in vitro* tests has been used to meet FDA requirements for proof of bioequivalence. However, *in vivo* dissolution and absorption tests would likely give a more complete evaluation of solid formulations than could *in vitro* test. Therefore, further *in vivo* studies should be conducted for obtaining a complete picture of bioequivalence of tablets originating from various manufacturers.

SUGGESTION FOR FURTHER WORK

- ◆ The *in-vivo* bioavailability of the ten products can be investigated and their therapeutic efficacy could be compared.
- ◆ The stability of these products can be investigated and related degradation products could be identified and quantified.
- ◆ Counterfeit products that entered the country through illegal channels could be assessed.
- ◆ Products collected from different regions in the country could be compared both *in-vitro* and *in-vivo*.

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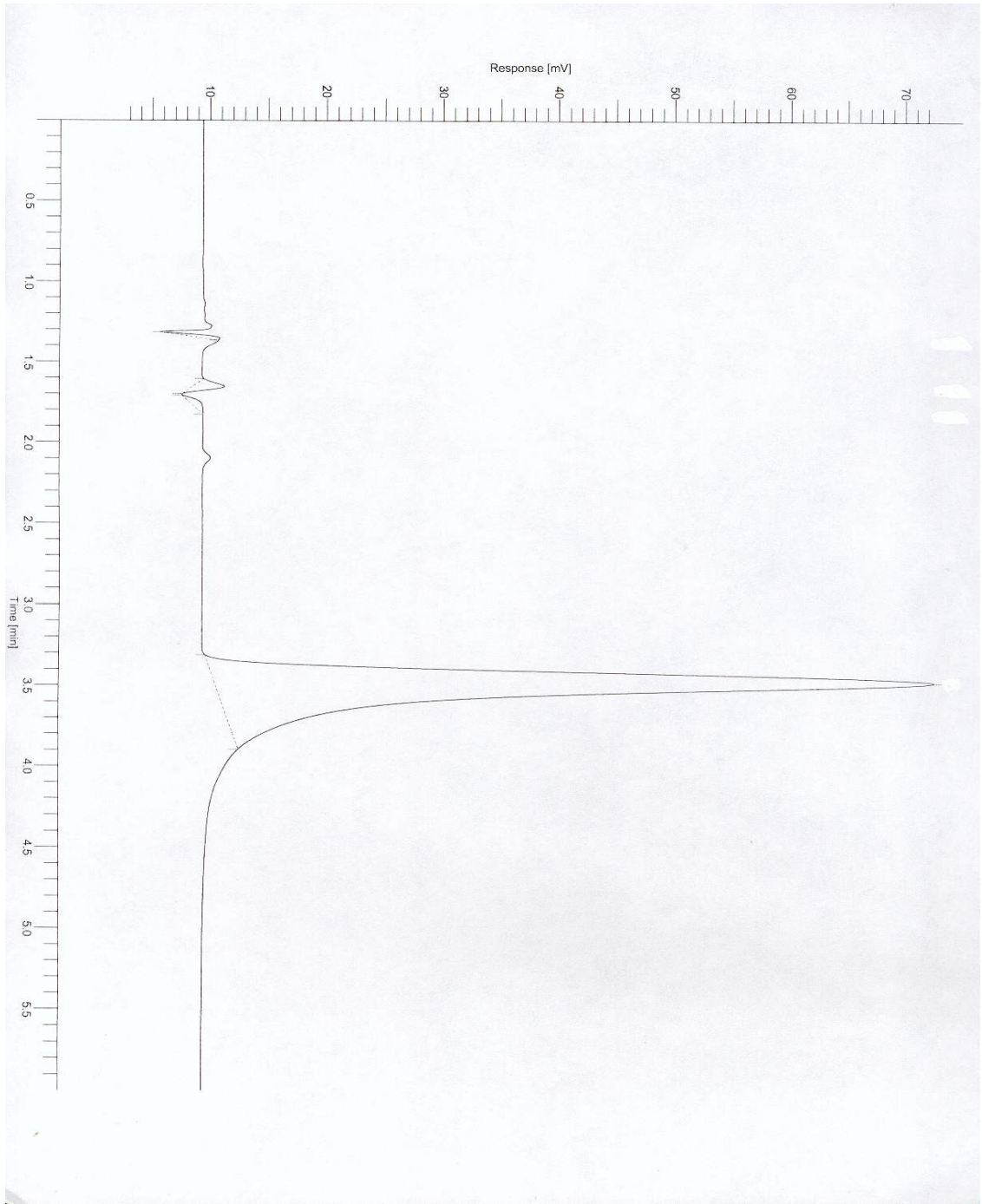
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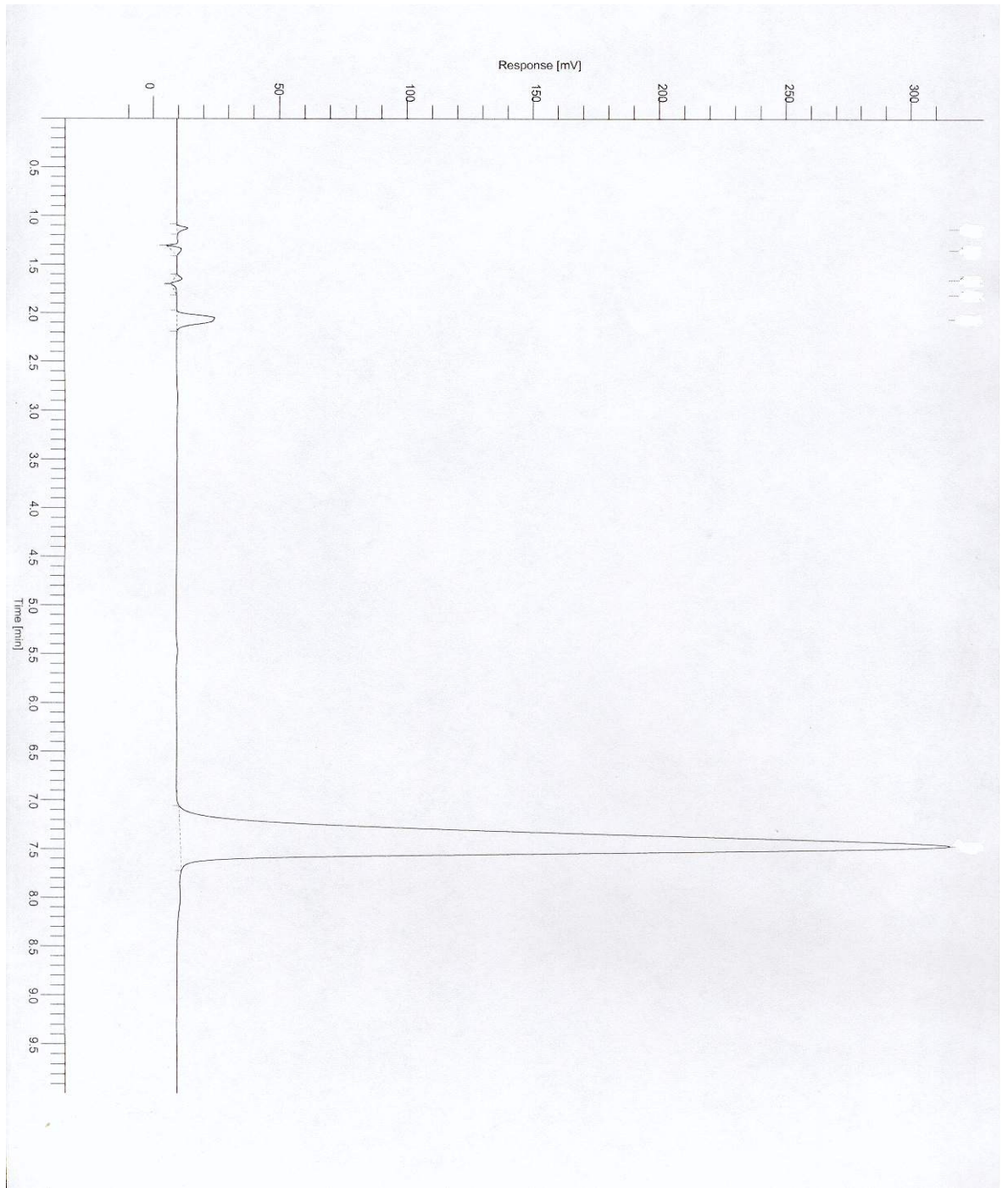
ANNEX

HPLC chromatograms of pure trimethoprim, sulphamethoxazole, trimethoprim-sulphamethoxazole combination and of the individual tablets at 30th minute is depicted below.

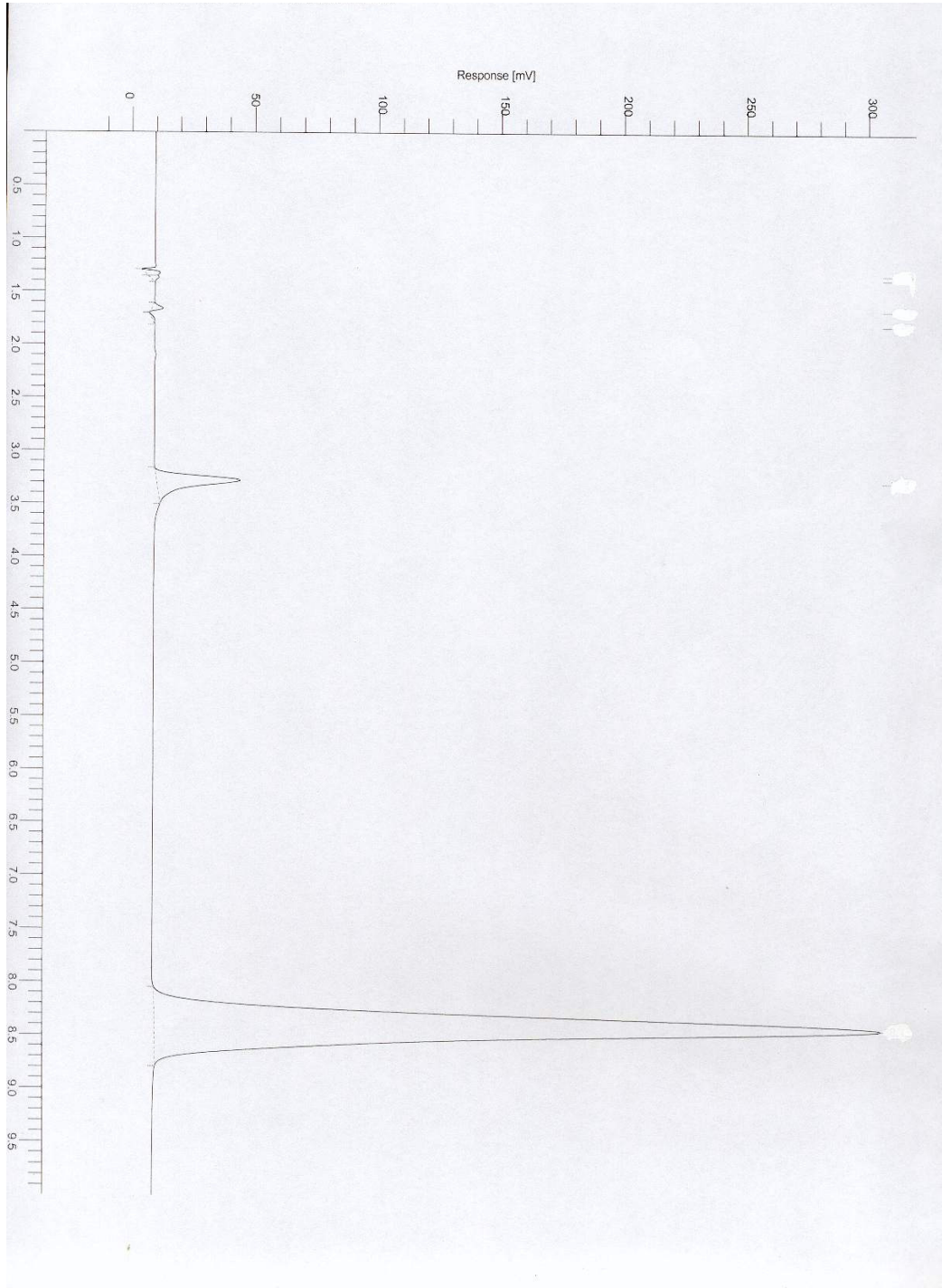
1. Pure trimethoprim.
2. Pure sulphamethoxazole.
3. Pure trimethoprim-sulphamethoxazole combination.
4. Bactrim at 30th minute.
5. Bisepton at 30th minute.
6. Cotreich at 30th minute.
7. Cotrimol at 30th minute.
8. Cotrimoxazole at 30th minute.
9. Deprim at 30th minute.
10. Kanprim at 30th minute.
11. Lagatrim at 30th minute.
12. Oriprim at 30th minute.
13. Septrin at 30th minute.



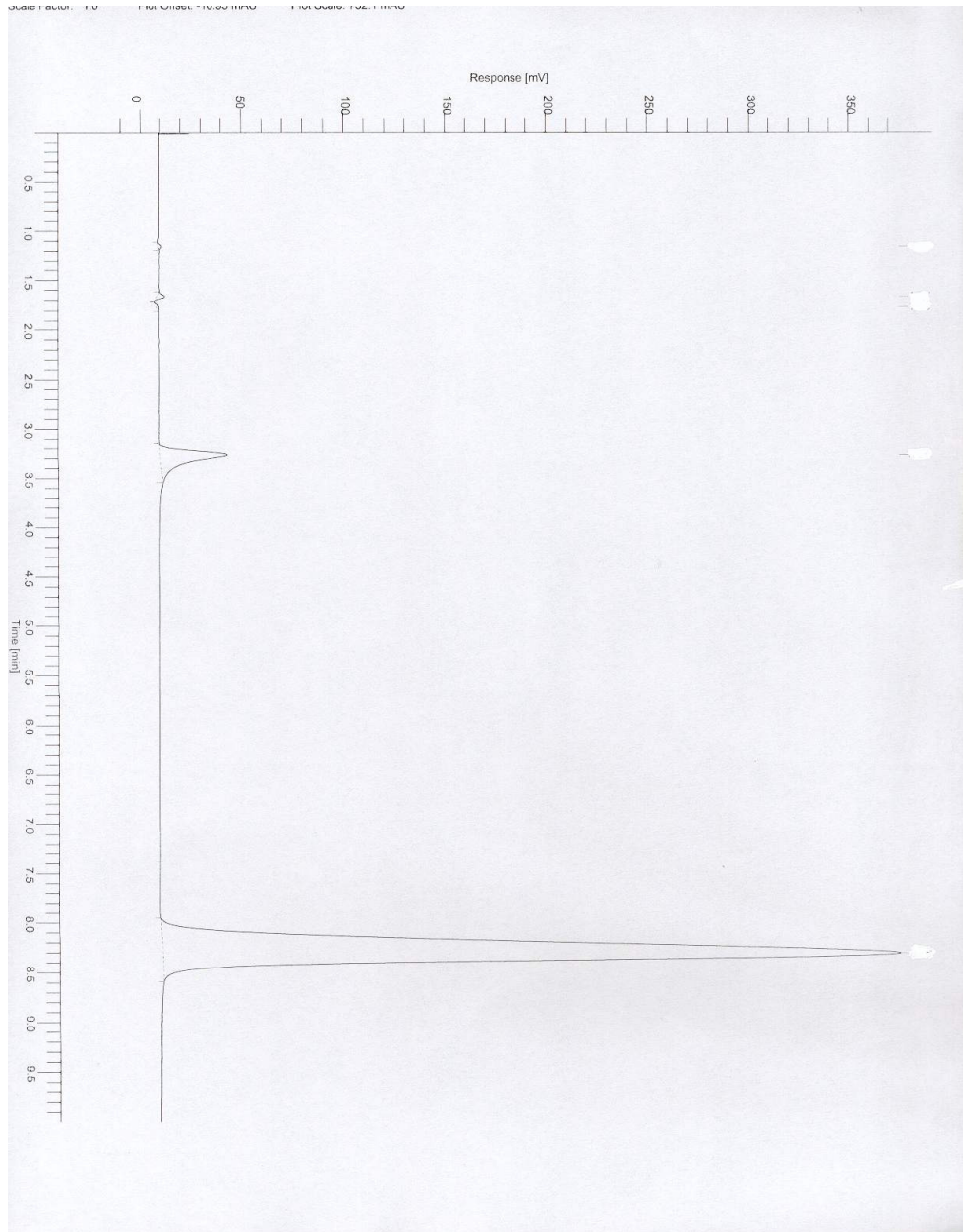
Annex 1 Chromatogram of Pure TMP



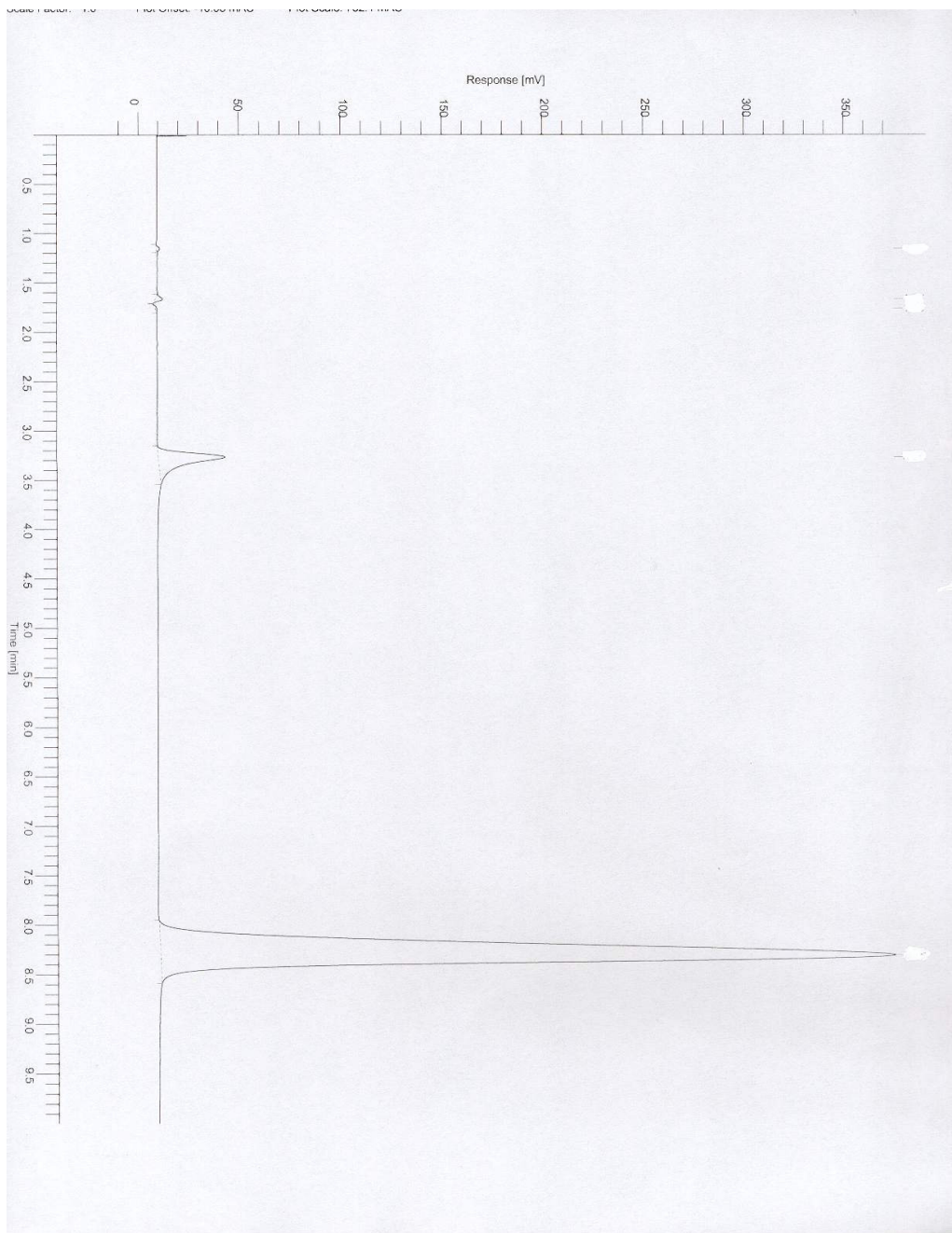
Annex 2 Chromatogram of Pure SMZ



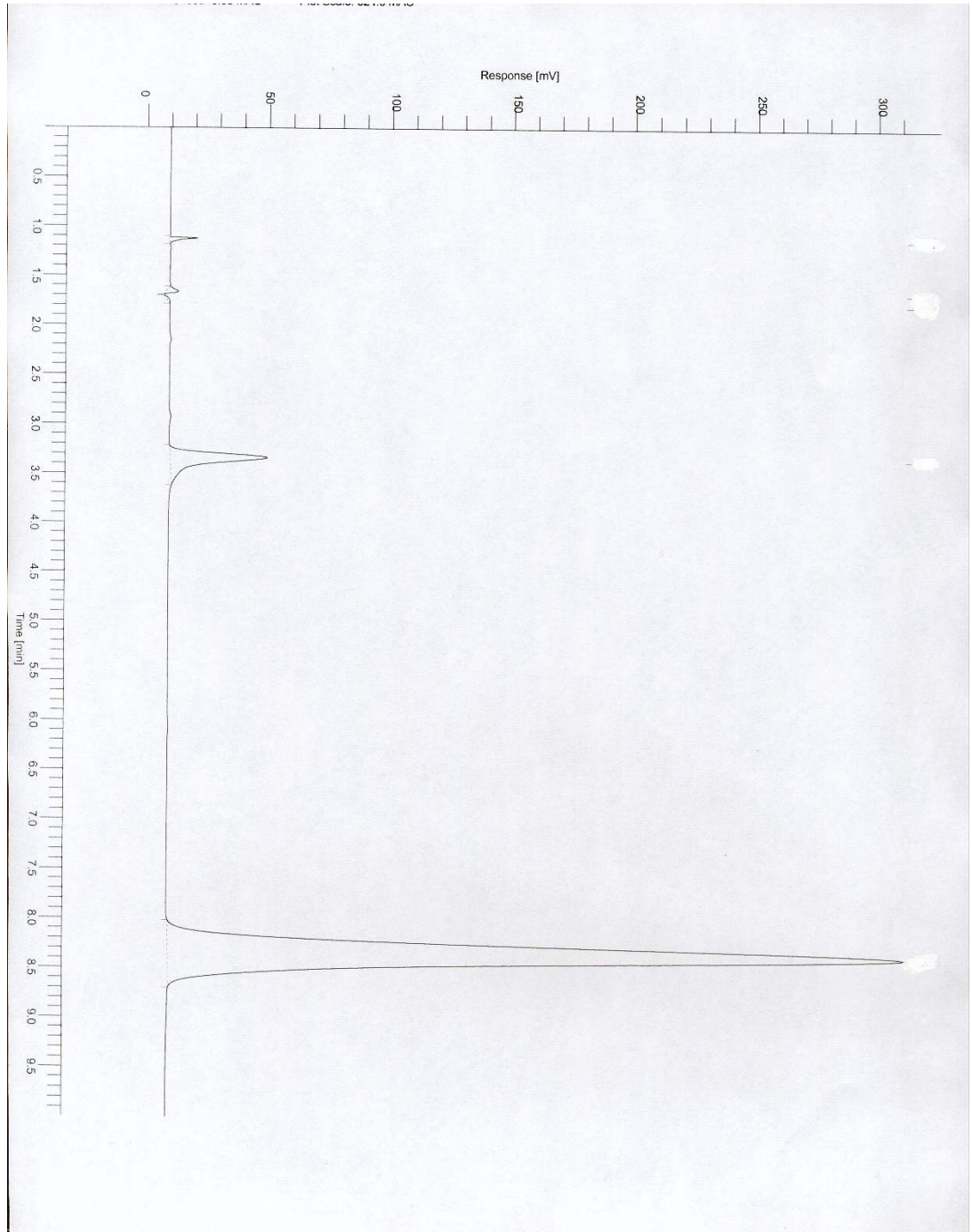
Annex 3 Chromatogram of Pure TMP-SMZ Combination



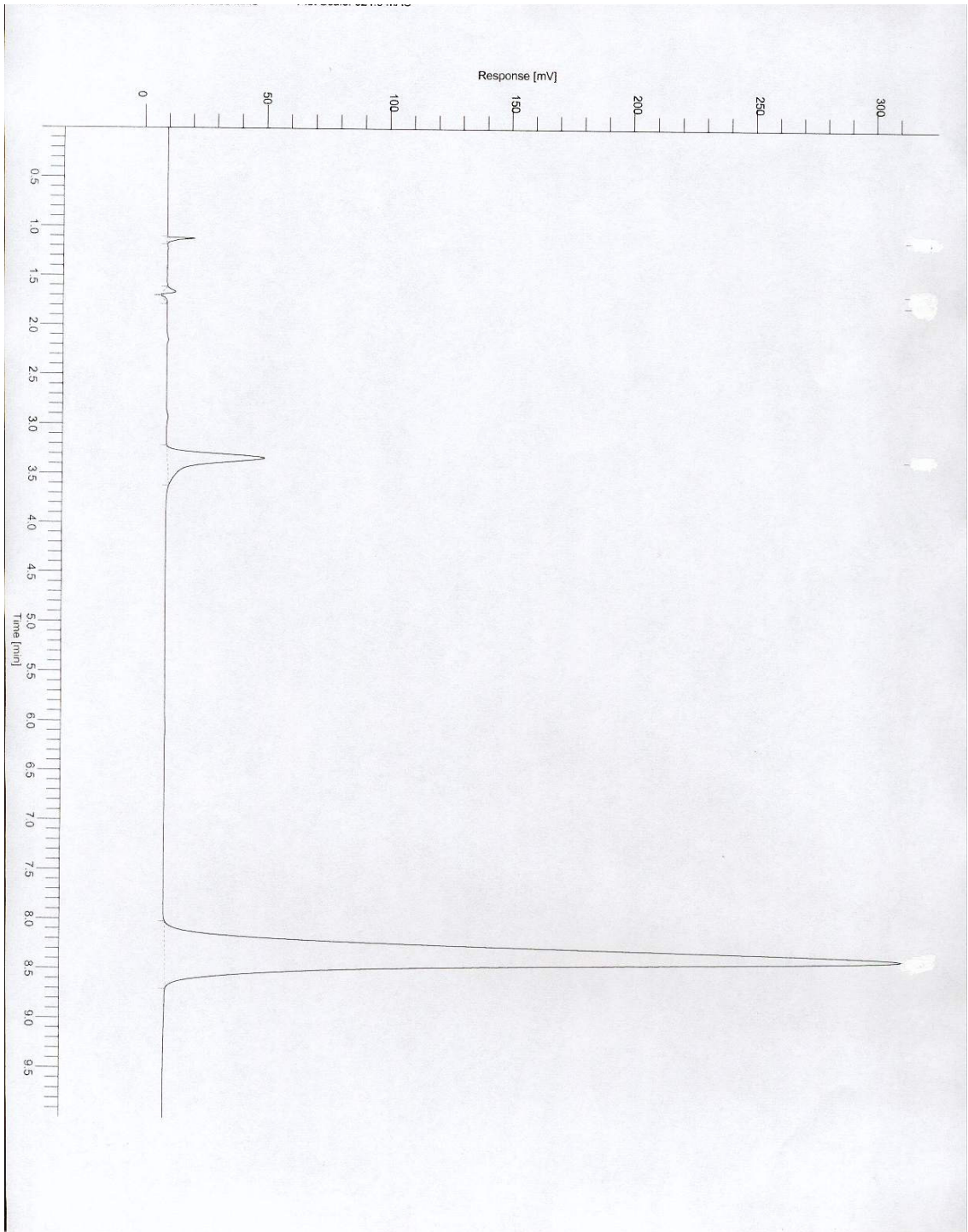
Annex 4 Chromatogram of Bactrim at 30th minute



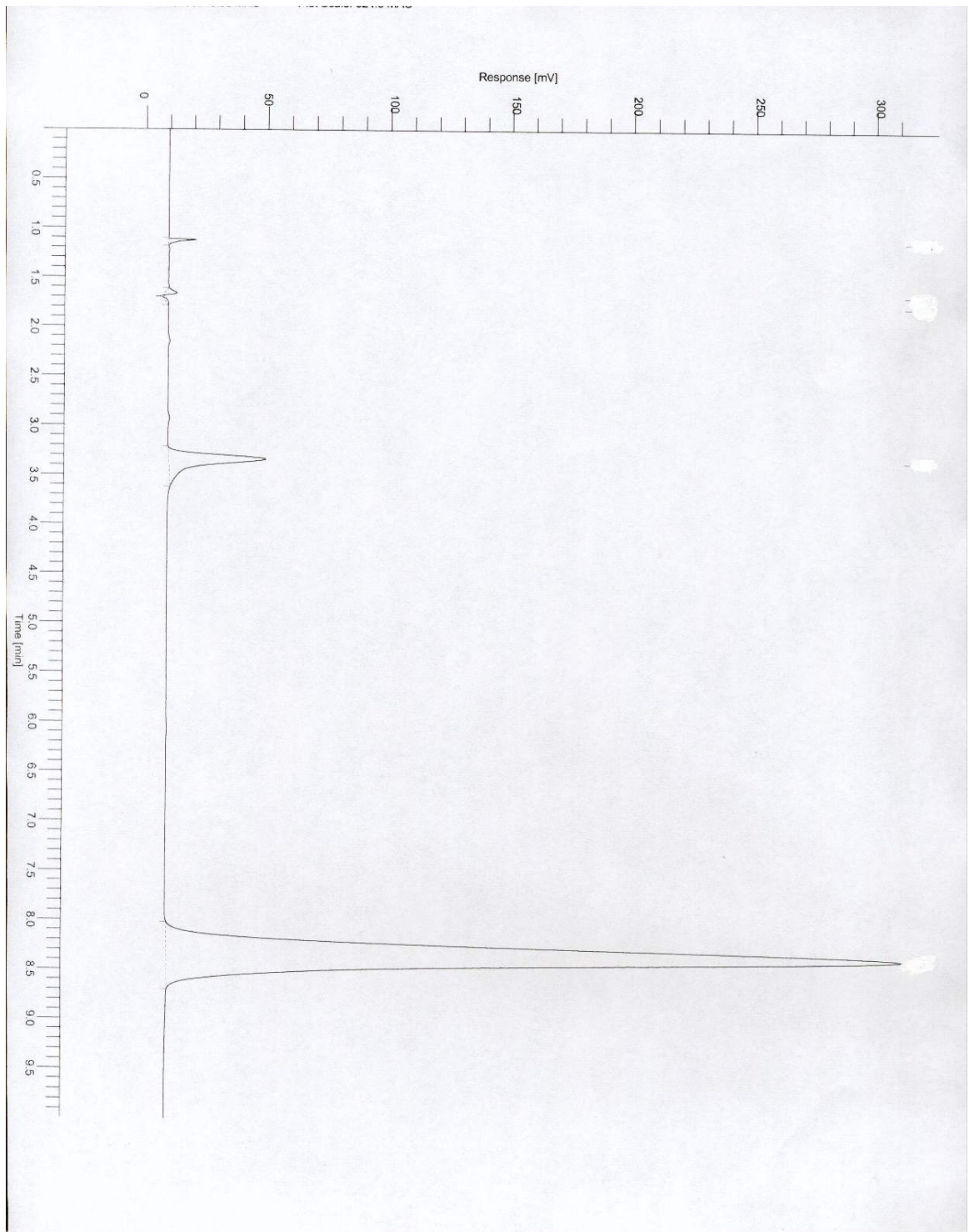
Annex 5 Chromatogram of Bisepton at 30th minute



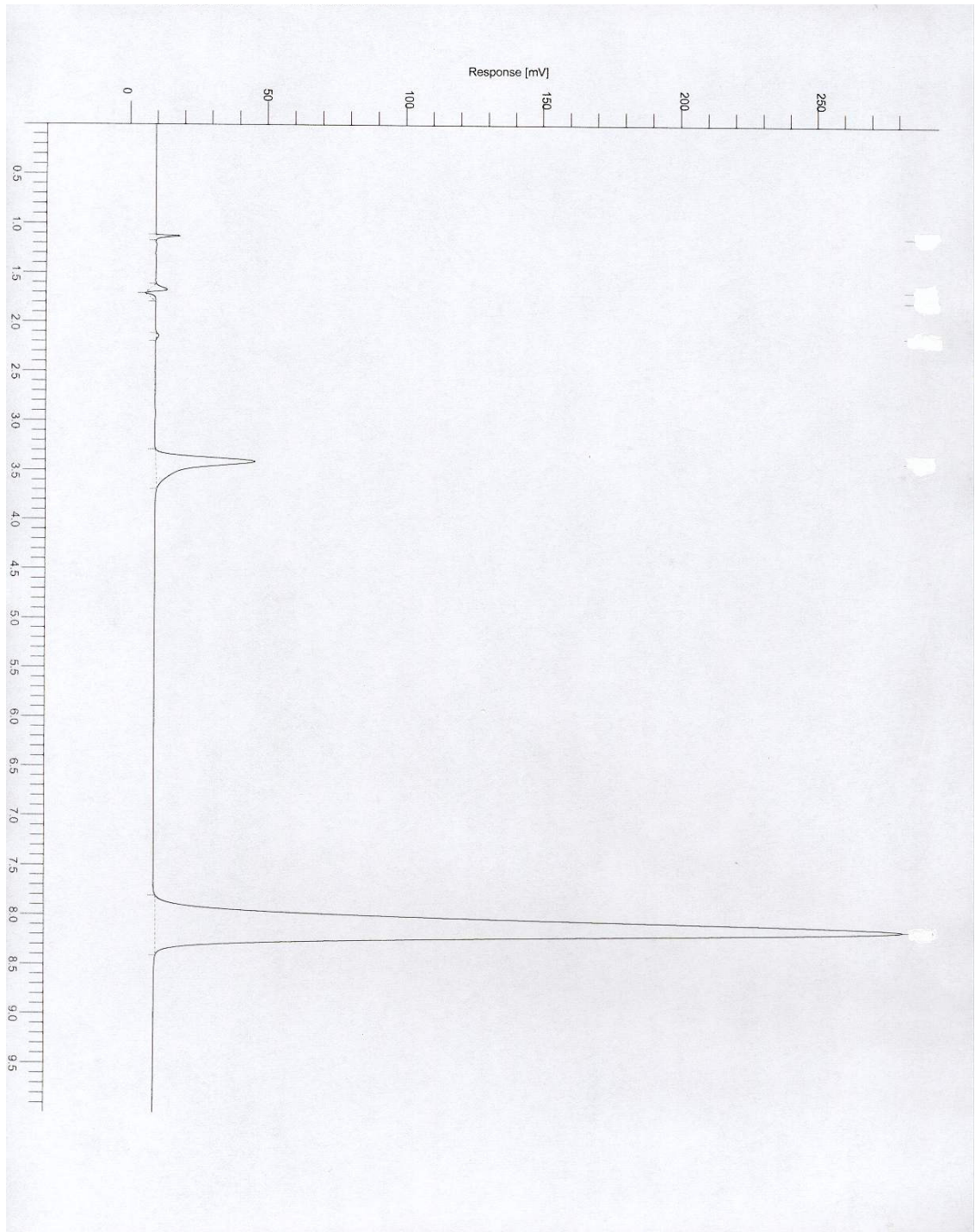
Annex 6 Chromatogram of Cotreich at 30th minute



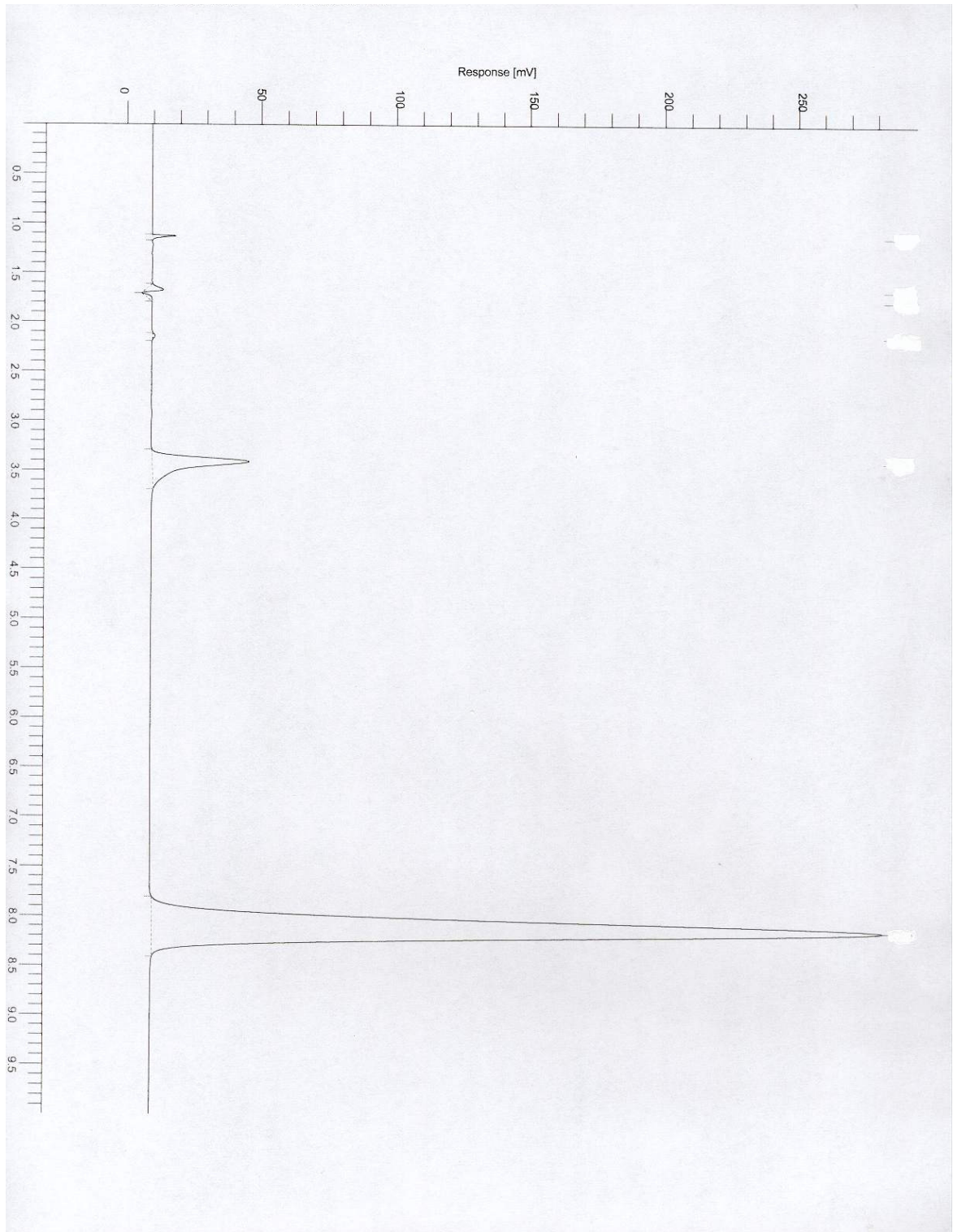
Annex 7 Chromatogram of Cotrimol at 30th minute



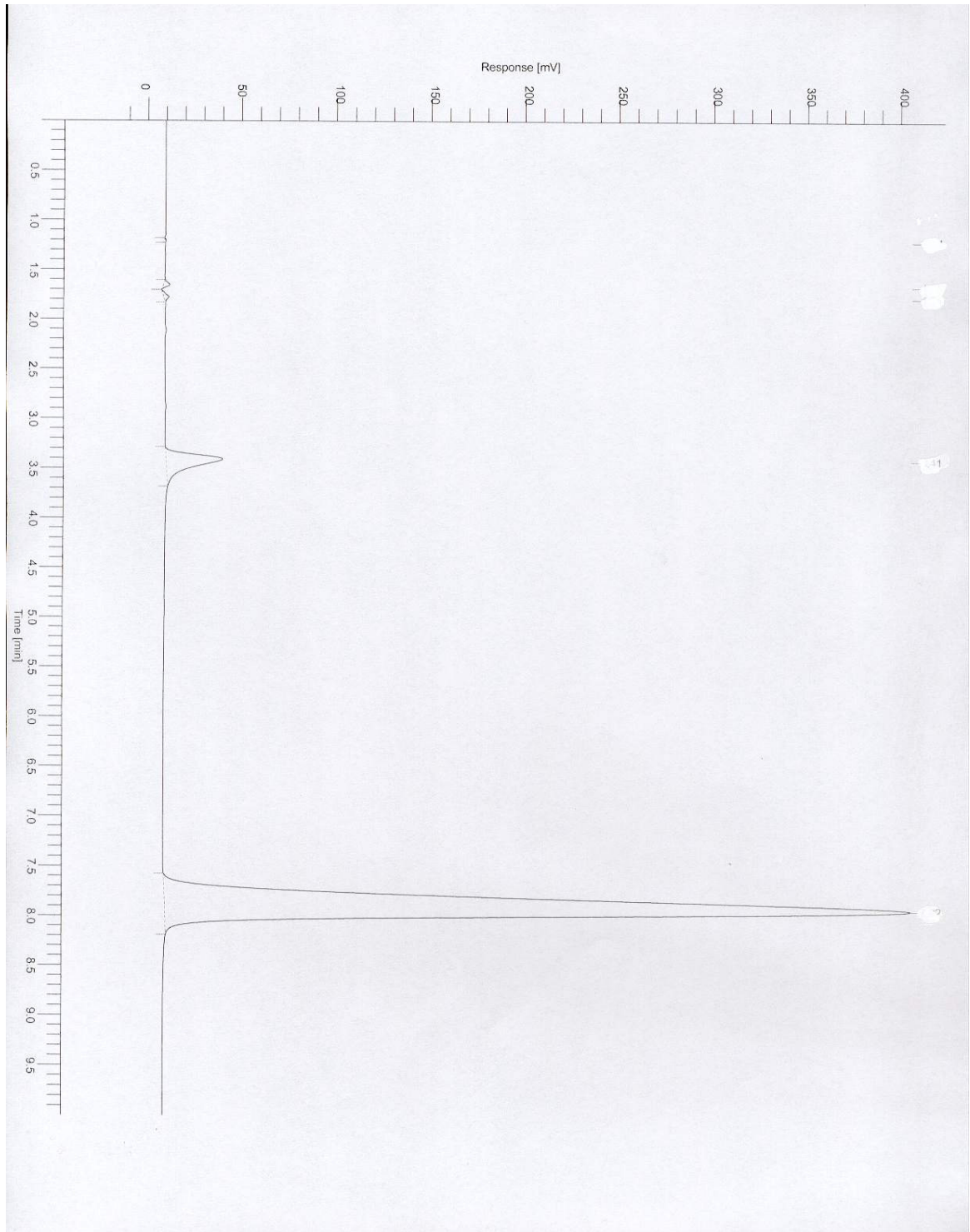
Annex 8 Chromatogram of Cotrimoxazole at 30th minute



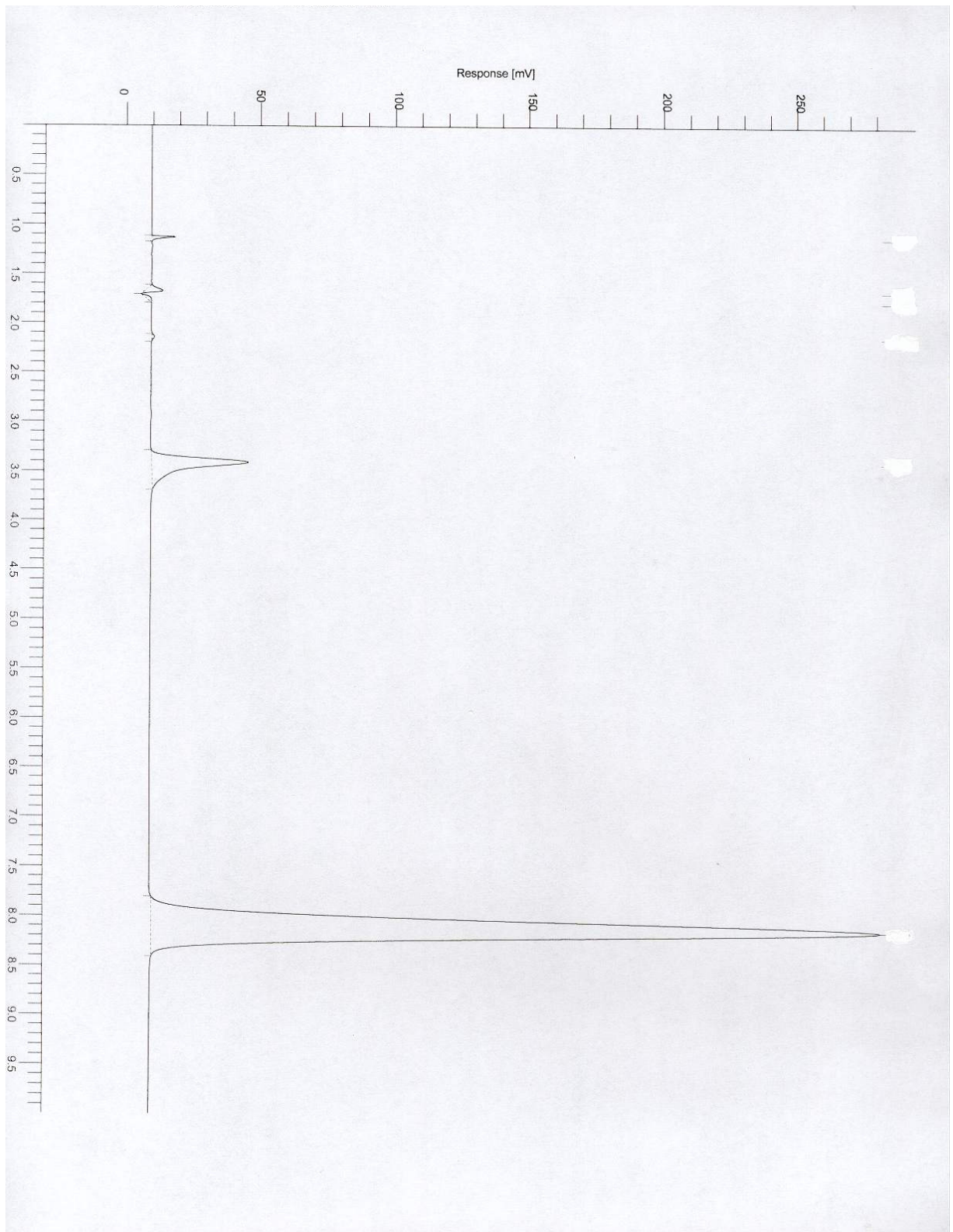
Annex 9 Chromatogram of Deprim at 30th minute



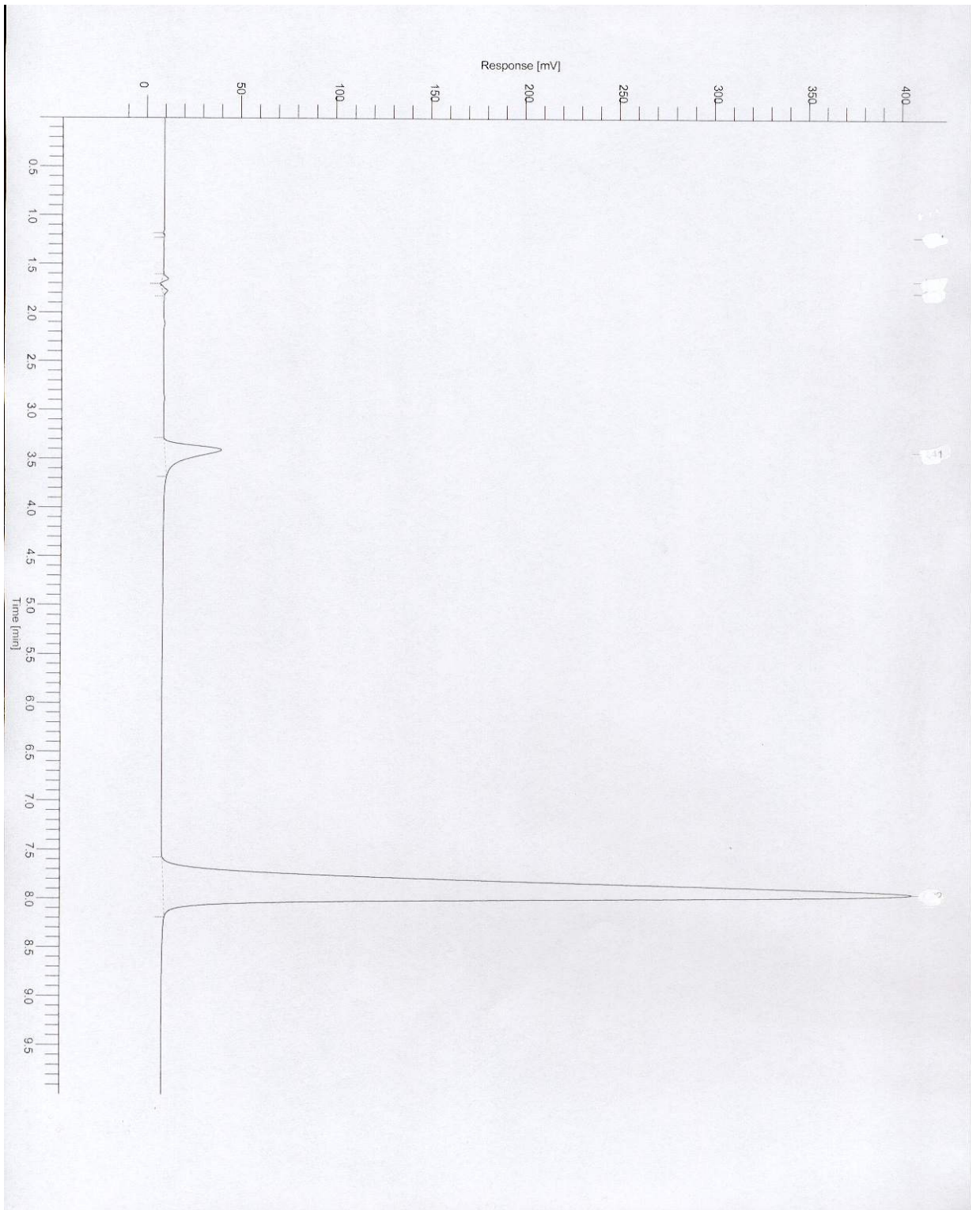
Annex 10 Chromatogram of kanprim at 30th minute



Annex 11 Chromatogram of Lagatrim at 30th minute



Annex 12 Chromatogram of Oripim at 30th minute



Annex 13 Chromatogram of Septrin at 30th minute