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# STUDY OF ANALYTICAL METHOD ON EFFICACY OF EXPIRED DRUGS (DILOXANIDE FUORATE)

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ABSTRACT:

The present investigation aimed to ascertain the analysis of diloxanide fuorate, a medication that had expired. The goal of this study was to evaluate the practice regarding the potency of diloxanide fumarate after its expiration. It is crucial to determine the drug's efficacy and potency because there are several adverse effects that can arise from infections, including dry mouth, nausea, and headaches. The analysis of expired drugs can be conducted by the use of combination theory, β-cyclodextrin, enzymatic hydrolysis method, and pilot study. By carefully analyzing the body of current research, court decisions, and real-world situations, we want to significantly advance the continuing discussion on pharmaceutical safety and the administration of diloxanide furoate beyond its expiration date. By increasing knowledge of the detrimental effects of utilizing expired pharmaceuticals and enhancing patient well-being, the study's findings may have an impact on healthcare policy and regulatory procedures.

Keywords: combination theory, β-cyclodextrin, enzymatic hydrolysis , analysis, medications and pilot study.

## Introduction:

"Drug expiration" describes a date on which a medication may no longer be deemed safe for ingestion in its produced form. The shelf life of a medication can be found by referring to the expiration date printed on the pharmaceutical container. After the container is first opened, the majority of drugs' efficacy With the exception of certain medications like nitroglycerine, insulin, epinephrine, and tetracycline, stays at least 70–80% potent for a period of one to two years after the expiration date as long as it is stored properly [1]. Depending on the kind of product, a drug's expiration date can be found after the production process or after the medication is dispensed. It should be highlighted, nonetheless, that drug deterioration rates are not always the same because different medications deteriorate over time at varied rates. In contrast, medications can be divided into a number of categories that are synthetic or formulated, each with a specific shelf life. The World Health Organization (WHO) states that every pharmaceutical product should come with a package leaflet that offers detailed information on a number of topics, such as the medication's indications, possible side effects, potential interactions, and the expiration date [2].

Identification of novel medications, evaluation of their affinity and specificity, characterization of their molecular structures, and in vitro and in vivo efficacy testing are all included in the process of drug analysis [3]. Pharmaceutical analysis is limited to drug analyses in pharmaceutical formulations and raw materials; it may also incorporate clinical evaluations in some cases. In addition to determining the active components, pharmaceutical analyses also include the assessment of contaminants, excipients, the stability of the active components (as well as the intermediates or end products of their degradation), and other criteria like content uniformity, solubility, and dissolving rate [4].

The expiration date means the final day that the manufacturer guarantees the full safety and potency of a drug. medicine expiration dates exist on most drug labels, including prescription, over the counter (OTC) and dietary(herbal) supplements. A proper storehouse of specifics may help to extend their potency. The restroom and drug press aren't ideal places to store specifics due to heat and moisture and shouldn't be left in a hot car. Medications may remain stable in most of the dry, cool spaces away from light. Keep the prescription bottle caps tightly closed and always keep specifics out of reach of children. The potency of drugs gradually decreases from the starting moment of its manufacture. This process isn't in any way spontaneous after the expiry date. Expired medicines haven't necessarily lost their potency and efficacy. Solid lozenge forms, like tablets and capsules, are most stable past their expiration date (4). Medicines that exist in solution or as a reconstituted suspension may not have the required potency if used when outdated [5].

In 1979, the U.S. The Food and Drug Administration started requiring that over the counter and prescription medications have an expiration date. When determining whether a product is safe to use and will function as intended, the expiration date is an important consideration. The expiration date is sometimes printed after "EXP" on the label or stamped onto the bottle or carton. It's critical to understand and stick to the medicine's expiration date. It is dangerous and possibly harmful to your health to use expired medical supplies [6].

Over time, the chemical makeup of medicines can change, potentially leading to a decrease in their effectiveness. And when you take expired specifics, it can cause-

- Contamination of medication
- Susceptibility to antibiotic resistance
- Increased risk of infections

- Allergies
- Changes in metabolism and bodily functions

When it comes to products that can grow bacteria, like medications, the expiration date matters a lot. Medicines can get contaminated with bacteria after expiration, therefore, leaving a consumer susceptible to infections. Also, taking expired medicines increases the threat of antibiotic resistance. Antibiotics that are expired or have lost some of their potency will not effectively treat infections. The bacteria will change, grow, and reappear in large numbers because the medication was unable destroy them completely. The bacteria will become resistant to the antibiotic's effects when you take it again. Also, Expired medicines can cause health complications, giving rise to other conditions. In worst- case scripts, they can cause harm to the kidneys and liver. After taking expired medications, there is a risk of allergies, lowered immunity, or altered metabolism. You can't tell for sure whether an expired medicine is still potent or not, but it's better to be safe than sorry. It's better to stock your drug cabinet with fresh medicines and dispose of the expired ones [7].

## The Benefits of Using Expiration Dated Drugs:

Losing outdated prescription drugs has several drawbacks.

Many middle-class and developing nations struggle to afford pharmaceuticals, and they suffer from a severe lack of some essential medications, including antibiotics. Redirecting expired medication that has been shown to be safe for use to other nations would be a more moral decision, saving money and preventing waste. It might be possible to demonstrate goodwill and solidarity by using the donor's expired medicines and sending new batches to nations that are short on supplies or in need of medicine. As a result, adding months or years to the shelf life can increase the accessibility of certain medications and make them affordable for people with limited resources. For these groups, these medications represent hope. Health authorities and insurance providers can also benefit from this, as it reduces costs and the lack of certain prescriptions. However, since they may be supplied more quickly than making new batches, expired medications that have been shown to be safe and Efficiency may be very useful in an emergency [8].

## Pharmaceutical Companies' Financial Interests：

The majority of manufacturers are aware that their products have a longer shelf life and that their expiration date can be moved forward by several months or even years. If they carried out more research, they could do this, but they do nothing. They take advantage of the lack of legislation or regulation on shelf-life extensions, which allows them to avoid feeling obligated to make such claims. They can also argue that they do not wish to jeopardize patient safety, even though it is highly likely that neither safety nor confidentiality would be jeopardized. It is expensive and time-consuming to do extension research, therefore there is no financial incentive to look into this further Long- or short-term stability may not come at a high cost, but there are other aspects that could drive up costs, like human, logistical . Pharmaceutical companies are biased against taking steps to extend the usable life of drugs because it is in their interest when medications are thrown away as "expired" despite their safety and fundamental effectiveness; if a drug is thrown away, a replacement product will need to be purchased. A lot of drug corporations would rather concentrate on increasing sales by using the orbit to create new medications. The requirement of extending the expiration date may hinder research and development efforts and have a detrimental effect on both their output and the pharmaceutical sector [9].

Pharmaceutical products may become more expensive and harder to obtain as a result of this.

## The Shelf-Life Extension Program's Solution:

Following an Air Force request, the FDA and the US Department of Defence (DoD) decided to prolong the shelf life of several medications to reduce replacement costs in the late 1980s. Consequently, the first Shelf-Life Extension Program (SLEP) was established. Every year, pharmaceuticals from stocks are chosen based on their worth and impending expiration, and their end dates are examined in batches to see if they can be safely postponed. Experience has shown that many medications have a true shelf life that is far longer than their stated expiration date. After use, no one has been harmed by any expired medications in the research. Occasionally, such as stockpiler initiated. The Emergency Use Authorization (EUA) is only permitted by the FDA for Chemical, Biological, Radiological, Nuclear, and Explosive (CBRN) situations if an expired product is deemed unapproved [10].

The science and practices related to the detection, assessment, understanding, and prevention of side effects or any other potential medication-related issues are known as pharmacovigilance. Among the methods most frequently used to generate safety data is the spontaneous reporting of adverse events and adverse drug reactions. Safety data generated from clinical trials is incapable of identifying infrequent or late onset adverse drug reactions. When a new drug is marketed only limited information regarding its safety in children is available.

The FDA's Shelf Life Extension Program (SLEP), run for the Department of Defense, provides the greatest evidence that some medications can be used past their expiration date [11].

The SLEP program was initially designed to save government money as well as ascertain the true duration of military drugs that were stored for potential usage.In the SLEP program, more than 3000 lots representing 122 distinct medicinal items were evaluated. Evaluations were done for potency, pH, water content, dissolution, physical appearance, and impurity presence. The expiration dates of 88% of the lots were extended by an average of 66 months beyond their original expiration date, according to stability data. Roughly 12% of these lots continued to be steady four years or longer after the expiration date. Merely 18% of the 2652 lots were terminated owing to failure. Common pharmaceutical medicines like amoxicillin, ciprofloxacin, diphenhydramine, and morphine sulphate injection were examined and shown to be successful.10 These goods' drug expiration extensions ranged from 12 months to 184 months (more than 15 years).3,10 The SLEP program does not cover biologics. In the SLEP study, potassium iodide, which has been stored in the US in case of radiation emergencies, did not significantly deteriorate during a 20-year period [12].

The FDA announced in June 2020 that, under certain circumstances and for emergency usage in specific states, the expiration dates of some stockpiled influenza antivirals, including Tamiflu (oseltamivir) 75 mg capsules and Relenza (zanamivir), may be extended. It is possible to extend the expiration dates of Tamiflu by 15 years and Relenza by 10 years. furthermore, studies published in The Medical Letter revealed that several drugs maintained their potency for decades after they were supposed to expire. The authors point out that no reports of human harm resulting from the consumption, injection, or topical use of an existing medication formulation beyond its expiration date have been published. The potency of medication starts to diminish as soon as it is manufactured. After the expiration date, this process is not in any way spontaneous. Drugs that have expired do not always lose their effectiveness and potency. The labelled potency is guaranteed to last at least until the expiration date, but that is all. According to ongoing research, many medications maintain 90% of their potency when stored under ideal conditions for at least five years—and occasionally even longer—after the indicated expiration date. Many medicines maintain a large portion of their initial potency even ten years after their expiration date [13].

## Potency and Efficacy in relation to expired drugs:

Medication's effectiveness gradually decreases from the moment it is manufactured. Following the process, there is no indication of spontaneity in its occurring.
date of expiration. It is possible that the effectiveness and potency of expired drugs are not harmed. The product's stated potency will remain active until the stipulated expiration date, as guaranteed by the date of designation. According to current studies, many medications retain 90% of their efficacy when stored under optimum conditions for at least five years after the product's specified expiration date, and sometimes even longer. Occasionally, medications retain a significant portion of their original effectiveness for up to ten years after they expire. The effectiveness of medication can be impacted by psychological variables.

As a result, it is imperative to stress that the expiration date serves as evidence of the stability of drug quality and appropriateness [14-15]. While the American Medical Association

Since neither the Food and Drug Administration nor the Association currently offer guidelines for the administration of expired pharmaceuticals, it is critical to have a conversation about this issue in order to learn more about the potential risks associated with some expired medications and to support future research [16].

## Adverse drug reactions (ADR)of expired drugs :-

An unpleasant and unintentional reaction to a medication that happens at dosages typically used in humans for disease prophylaxis, diagnosis, treatment, or alteration of physiological function An intervention related to the use of a medicinal product that results in a noticeably harmful or unpleasant reaction and that indicates a risk from further administration and calls for prevention, targeted treatment, changing the dosage schedule, or stopping the product altogether. Any unexpected, unwanted, excessive, or undesired reaction to a medication that necessitates stopping the medication (for therapeutic or diagnostic purposes), altering the drug therapy, changing the dose (apart from small dose adjustments), requiring hospital admission, extending hospital stay, requiring supportive treatment, significantly complicating diagnosis, adversely affecting prognosis, or causing temporary or permanent harm, disability, or death.

- **Types A Effects (Augmented) (Dose related):**
  1. Due to pharmacological effects.
  2. Are dose related–may often be avoided by using doses which are appropriate to they individual patient.
  3. Example: Hypoglycaemia with Insulin, hypotension by beta blockers, NSAID's induced gastric ulcer.
- **Types B Effects (Bizarre):**
  1. Generally rare and unpredictable.
  2. occur in patients who are predisposed to intolerance; rare genetic polymorphisms and allergic reactions may account for this.
  3. Example: Penicillin hypersensitivity, malignant hyperthermia.
- **Types C Effects (Chronic):**
  1. Adverse reactions after long term therapy.
  2. In many cases, there is no suggestive temporal relationship, and establishing the connection may be extremely challenging. The frequency of "spontaneous" disease is increased when a drug is used.
  3. Example: Hypothalamic pituitary adrenal axis suppression by corticosteroids.
- **Types D Effects (Delayed):**
  1. Adverse effects may be presented years after a drug was used.
  2. Example: Teratogenesis, Carcinogenesis like clear cell cancer of female reproductive tract.
- **Types E Effects (End of treatment effect):**
  1. occurs due to sudden discontinuation of a drug after long term therapy.
  2. Example: corticosteroids in asthma treatment [17].

## Importance of analysis of expired drugs:

Perhaps the most important role in a life science company is pharmacovigilance. Companies that develop, produce, and market drugs are subject to stringent regulations. Many of these rules will focus on the safety of the patient and any additional benefits the medication may provide. This summarizes the goal of drug safety and demonstrates why it is such a crucial field in the pharmaceutical industry. Patient safety and continuous vigilance By definition, medication safety makes sure that a patient's health and safety are protected at every stage of the medication development process, even after the medication is widely accessible on the market. In fact, medications are constantly checked on patients for any additional side effects, and new data is gathered and submitted to health authorities on a regular basis. No other department has such a sharp focus on patient safety as an endpoint, while other areas strive to improve patient lives in everything they do. Power and authority Because of this continuing scrutiny, senior executives in a drug safety team have the power and obligation to advise that a drug be taken off the market or that a development process be stopped along with other business leaders.

Drug safety plays a crucial role in this process, as evidenced by the significance of EU QPPVs. Keeping it moving Drug safety plays a major role in keeping a pharmaceutical company operating smoothly. Drug safety operates on a highly cross-functional basis due to its nature. As a result, the division can have a huge impact and add significant value to other business areas [18].

## Aim & objectives:

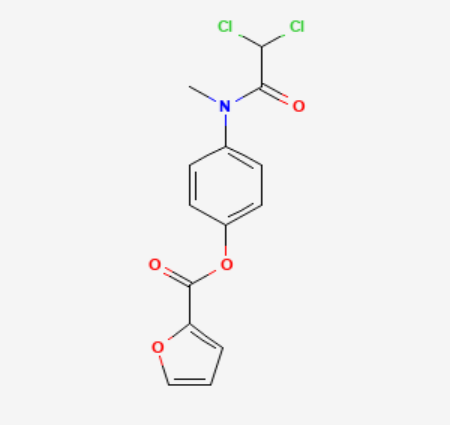
Aim: To study the effect of expired drug by using analysis.

OBJECTIVES:

- To analyse minute concentration of any [diloxanide furoate] drug
- To know the impurity with other substances.
- To know the peak value of desired drugs.
- To check the purity of desired drugs.

***About drug: -***

**Diloxanide Furoate**




- IUPAC Name: - [4-[2,2-dichloroacetyl)-methylamino] phenyl] furan-2-carboxlate
- Molecular formula: - C14H11Cl2NO4
- Molecular weight: - 328.1g/mol
- Dosage: - 500 mg Oral, 20 mg/kg per day in three divided doses for 10 days.
- Side effects: -

Severe skin rash and hives
Difficulty in breathing
Flatulence
Nausea or vomiting
Abdominal pain and cramps
Loss of appetite
Diarrhoea
Headache
Dry mouth
Stomach pain [19].

***Uses of Diloxanide Furoate*:**

Amoebiasis is a parasitic infection of the intestines caused by the protozoan Entamoeba histolytica. The symptoms of amebiasis include loose stool, abdominal cramping, and stomach pain. However, most people with amebiasis would not experience significant symptoms. Diloxanide Furoate is used to treat this condition.

**Pharmacokinetics:**

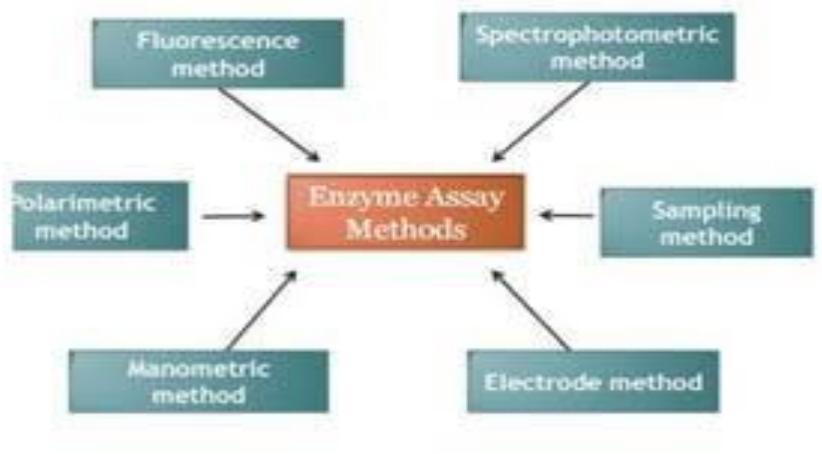
Human pharmacokinetic data is limited. Animal data show that diloxanide furoate is rapidly absorbed from the intestine. The furoate is hydrolysed in the gut, leaving high intraluminal concentrations of free diloxanide. About 75% is excreted via the kidney within 48 h, mostly as glucuronide.

**Pharmacodynamics:**

Diloxanide is a luminal amebicide, however the mechanism of action of diloxanide is unknown. Diloxanide destroys the trophozoites of E. histolytica that eventually form into cysts. The cysts are then excreted by persons infected with asymptomatic amebiasis. Diloxanide furoate is a prodrug and is hydrolysed in the gastrointestinal tract to produce diloxanide, the active Ingredient [20].

## Methods:

- Enzymatic method:




Drugs used for therapeutic and recreational purposes can be identified both qualitatively and quantitatively using the enzyme multiplied immunoassay technique (EMIT). Droplet compartmentalization offers a highly valuable tool that enables reliable measurements of low substance concentrations to be performed, avoiding diffusion of the product outside of the discrete drop volume. Cell-based enzymatic assays are frequently used in cell biology for drug screening. We will investigate the enzymatic hydrolysis technique of diloxanide furoate in the presence of β-cyclodextrin since the single study of the enzymatic method on diloxanide furoate is not available.

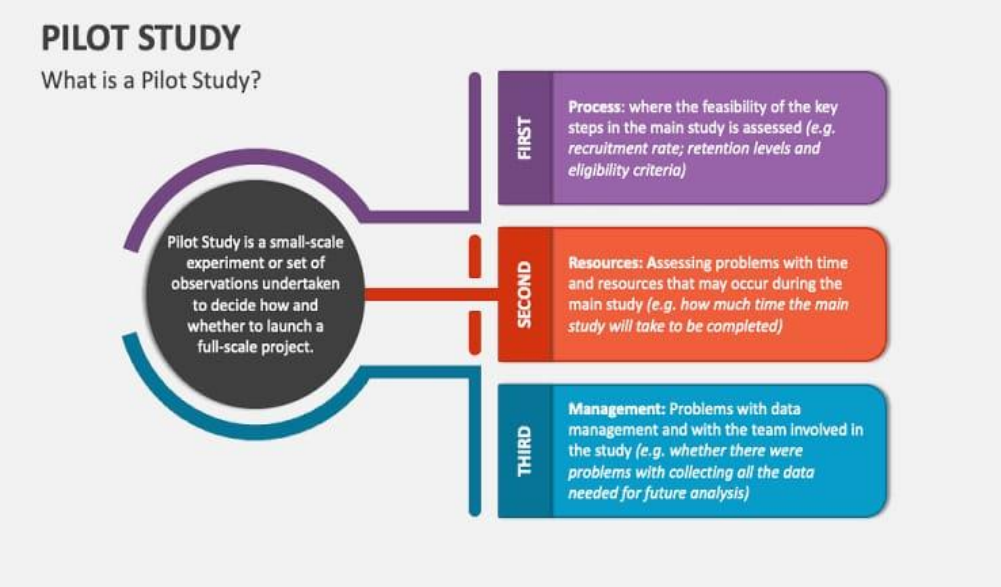
In this work, we examined the enzymatic and alkaline hydrolysis susceptibility of diloxanide furoate (DF) and its aqueous solution cyclodextrin inclusion complexes. β-cyclodextrin (β-CD), (2,6-di-O-methyl)-β-cyclodextrin (DM-β-CD), and (2,3,6-tri-O-methyl)-β-cyclodextrin (TM-β-CD) were the cyclodextrins (CDs) that were used. Diloxanide furoate hydrolysis was stabilized by all the cyclodextrins that were investigated. Without the enzyme, β-CD and TM-β-CD had a comparable impact on the stability of DF in alkaline hydrolysis (pH 10.75), with an inhibition factor of about 2.0. Conversely, the DM-β-CD, with an inhibition factor of about 8, offered a more noticeable stabilizing impact than the other two CDs.

The pH 7.0 range is where the enzyme was most active. With an order of 10 DF hydrolysis inhibition factor, all cyclodextrins had a same impact when enzymes were present. Nonetheless, the rate of hydrolysis against [CD] plot corresponded well with an equation derived from a model that takes the enzyme's connection with the CDs into account. Thus, it can be inferred that DF's stability results from both its cyclodextrin complex and the enzyme inhibition caused by cyclodextrin complexation [21].

- **COMBINATION THEORY:**

Combination therapies exploit the chances for better efficacy, decreased toxicity, and reduced development of drug resistance and owing to these advantages, have become a standard for the treatment of several diseases and continue to represent a promising approach in indications of unmet medical need. In this context, studying the effects of a combination of drugs to provide evidence of a significant superiority compared to the single agents is of particular interest. A combined formulation of diloxanide furoate and metronidazole was used to treat amoebiasis [22].

- **Pilot study:**




A feasibility study, sometimes referred to as a pilot study, is a small-scale preparatory investigation carried out in advance of the primary research to assess the viability or enhance the research design. It is also carried out to evaluate possible recruits and the safety of treatments or interventions; it looks at the randomization and blinding process; it broadens the researchers' experience with study techniques, medications, and therapies; and it provides estimates for sample size computation.

## Study protocol:

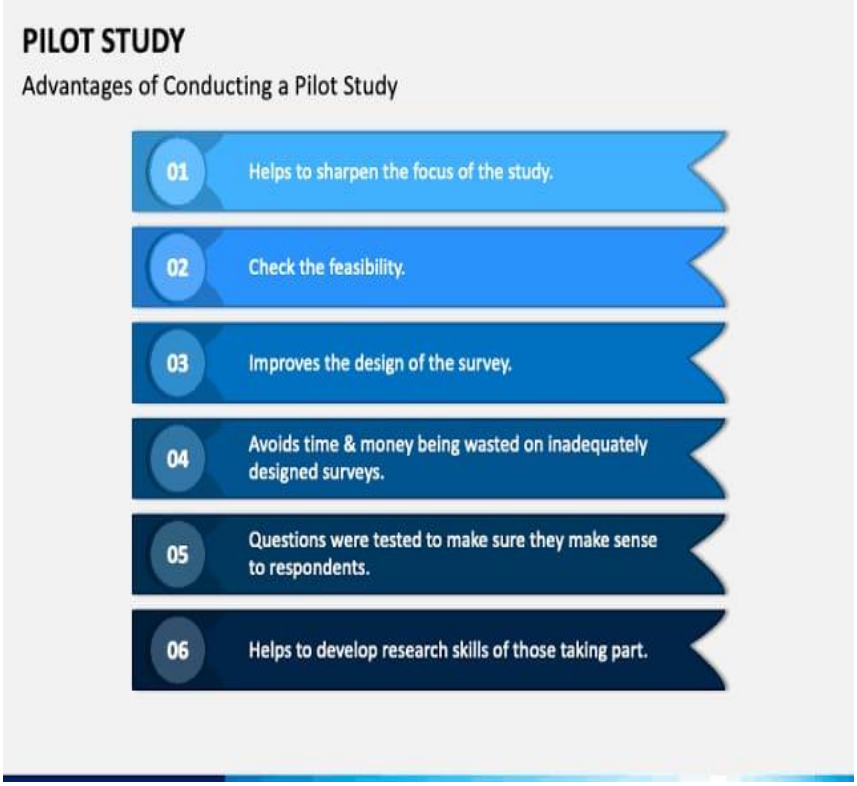
Prior to a 14-day course of treatment consisting of 500 mg of diloxanide furoate three times a day, 160/800 mg of trimethoprim/sulfamethoxazole twice a day, and 400 mg of carnidazole three times a day, a single baseline faecal specimen was obtained from each patient. Two more stool samples were collected: one 48 hours after stopping antibiotics, and the other 4 weeks later. At the beginning of the research, six weeks later, and following the completion of antibiotics, patients underwent clinical reviews. Every patient maintained a diary in which they noted their overall health (rated 1–10, from poor to excellent), the frequency of their daily bowel movements (number per day), and the consistency of their faces (scored: 1 = very hard, 2 = hard, 3 = formed, 4 = loose, and 5 = watery). Four weeks after stopping the antibiotics, routine electrolyte testing, liver function testing, and a full blood count were redone.

## Diagnostic method:

A straightforward unstained wet faecal smear, xenic in vitro culture (XIVC), and PCR were used to test each sample in parallel for the presence of Blastocystis spp. (confirmed as Blastocystis spp. by DNA sequencing). If any one of the tests came back positive, the patient was deemed to be positive.

## Parasitological methods:

Fresh, unpreserved stool samples from the patients were analysed using light microscopy at a 40× magnification. Blastocystis organism loads were categorized as light, medium, or heavy (less than five, five to ten, and more than ten organisms per high-powered field; O/HPF). Furthermore, Jones' culture medium was infected with 100 mg of fresh stool, and the mixture was cultured for 48 hours at 37°C. By using light microscopy, cultures were checked for the presence of blastocytes spp [23].




## Conclusion:

1.The efficacy of expired drugs has been determined by studying

- Enzymatic method
- combination theory
- pilot study

2.This method were validated according to ICH guidelines

3.This method indicated that diloxanide fuorate is well tolerated only side effect are occurring nausea, itching and hence it can be effective for few months but 70%-80%; it is possible only suitable storage conditions are maintained throughout.

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