Tuberculosis treatment in paediatrics: liquid pharmaceutical forms

D. Mercadé Frutos, I. Madrid Hidalgo, JM. Suñé Negre

Service Development of Medicines (SDM). Department of Pharmacy and Pharmaceutical Technology, and Physical chemistry. Faculty of Pharmacy and Food Science. University of Barcelona.

Summary

Tuberculosis is one of the ten main causes of death in the world, infecting more than one million children every year. The treatment of the disease consists of using combination therapy in fixed-dose anti-tuberculosis drug formulations. However, there are no commercial presentations in liquid form that combine the main drugs used for treatment due to the physical and chemical incompatibilities they present in this medium. Health organisations and research groups have carried out the necessary research to obtain a liquid formulation indicated for treating tuberculosis in patients who require such preparations. In 2018 the research group of the Drug Development Service (SDM) of the Faculty of Pharmacy and Food Sciences at the University of Barcelona, successfully developed a liquid formulation for oral administration and an extemporaneous preparation with three of the main anti-tuberculosis agents (rifampicin, isoniazid and pyrazinamide), achieving a preparation that remains stable for 27 days under refrigeration. In spite of the good results obtained, it is still true that this new formulation has been marketed to the end patient, mainly because tuberculosis is a minority disease in first-world countries, and therefore of little commercial interest.

Key words:
Tuberculosis. Liquid formulations. Paediatrics. Rifampicin. Isoniazid. Pyrazinamide.

Tratamiento de la tuberculosis en pediatría: formas farmacéuticas líquidas

Resumen

La tuberculosis es una de las diez principales causas de muerte en el mundo produciendo cada año la infección de más de 1 millón de niños. El tratamiento de la enfermedad consiste en utilizar terapia de combinación en formulaciones de dosis fija de antituberculosos. Sin embargo, no existen presentaciones comercializadas en forma líquida en todo el mundo que combinen los principales fármacos utilizados como tratamiento debido a las incompatibilidades físico-químicas que presentan en este medio. Organizaciones de salud y grupos de investigación han realizado la investigación necesaria para obtener una formulación líquida indicada para tratar la tuberculosis en pacientes susceptibles de tomar este tipo de preparados. El año 2018 el grupo de investigación del Servicio de Desarrollo del Medicamento (SDM) de la Facultad de Farmacia y Ciencias de la Alimentación de la Universidad de Barcelona, elaboró con éxito una formulación líquida de administración oral y preparación extemporánea con tres los principios activos antituberculosos considerados de primera línea (rifampicina, isoniazida y pirazinamida), logrando un preparado estable durante el período de 27 días bajo condiciones de refrigeración. A pesar de los buenos resultados obtenidos, aún hoy en día es todo un reto que esta nueva formulación llegue comercializada al paciente final, debido principalmente a que la tuberculosis es en los países del primer mundo, es una enfermedad minoritaria y, por tanto, de bajo interés comercial.

Palabras clave:
Tuberculosis. Formulaciones líquidas. Pediatría. Rifampicina. Isoniazida. Pirazinamida.

Correspondencia: Debora Mercadé Frutos
E-mail: debora.mercade@ub.edu

Introduction

Tuberculosis (TB) is a bacterial infection caused by a *mycobacterium* called *Mycobacterium tuberculosis*. The bacterium usually attacks the tissue of the lungs, but it can also spread to different parts of the body. The infection is transmitted from person to person through airborne bacilli, and only a small amount of inhaled bacilli is enough for a person to become infected. It is estimated that a quarter of the world's population has "latent tuberculosis", i.e., they are infected with the bacillus, but have not yet developed the disease or do not transmit the infection. Between 5 and 10% of the latent population risks ending up with the disease during their lifetime. When the disease develops, and therefore the first symptoms appear (cough, fever, night sweats, etc.), known as "active tuberculosis", if it is not treated, it can persist for many years and can even lead to chronic disease.

In all cases, the disease is treated when in its active form, based on the joint administration of different anti-tuberculosis drugs. The established dosage schedule includes a 6- to 9-month treatment period, which, together with other factors such as adverse effects, the limitation of pharmaceutical forms in commercial presentations or daily dosage complexity, requires high patient therapeutic adherence. This is one of the main approaches to eradicating this pathology, not only by obtaining anti-tuberculosis medicines, but also by achieving good therapeutic adherence by patients.

Despite the advances in the methods used to prevent and treat the disease, at present, TB is one of the main causes of death in the world and the main cause of death among the HIV-positive population. It is estimated that in 2018, of the 10 million people infected, 1.1 million children died of TB[1].

Pharmacological treatment

TB can be treated and cured. Treatment against the active form, which is sensitive to antibiotics, is with a standard combination of four antimicrobial drugs; rifampicin (R), isoniazid (H), pyrazinamide (Z) and ethambutol (E), administered for a minimum of six months. Support to the patient, by means of informing and assistance throughout treatment, is essential in order to guarantee therapeutic compliance and, consequently, total remission of the disease.

Between 2000 and 2018, it is estimated that 58 million lives were saved thanks to the diagnosis and effective treatment of TB[1].

The use of fixed-dose combination therapy (FDC) in a standardised system is the fundamental strategy proposed jointly by the World Health Organisation (WHO) and the International Union against Tuberculosis and Lung Disease (UICTER) for the treatment of TB. This type of therapy is defined as treatment with two or more drugs. This combination of Active Pharmaceutical Ingredients (APIs) has proven to be successful in the treatment of infectious diseases such as TB and its resistant forms.

Among the different treatments to combat TB, there are first-line treatments, which are chosen when the disease has been diagnosed, and second-line treatments, used when resistance or complications arise during the administration of first-line treatments. R, H and Z are considered first-line, and the combination of the three is standard for most health organisations in the world[2,3]. Updates to the guidelines for the pharmacological treatment of TB also include E in first-line therapy, advising its withdrawal in the event of obtaining an antibiogram with sensitivity to R, H and Z[2].

Among the treatments considered as second-line therapy are APIs such as streptomycin (S), amikacin (Am), capreomycin (Cm), kanamycin (Km) or those belonging to the family of fluoroquinolones (mainly levofloxacin (Lfx), moxifloxacin (Mfx) and gatyphloxacin (Gfx)[2].

Although some patients present TB that is ultra-resistant or pharmaco-resistant to second-line anti-tuberculosis drugs, in this case it is recommended to use longer-term treatments with drugs such as bedaquilin and delamanide[1,2].

At present, anti-tuberculosis presentations exist on the market nationwide and include the principles considered as first-line treatment in the same presentation. Some of these active ingredients are also presented individually.

Table 1 shows the presentations of R, H, Z, and E alone or in combination that exist in Spain[4].

Table 1. National presentations of rifampicin, isoniazid, pyrazinamide and ethambutol[4].

Active ingredient(s)	Pharmaceutical form
Rifampicin	Capsules Oral Suspension Recovered tablets Injectables
Isoniazid	Injectables Tablets
Pyrazinamide	Tablets
Ethambutol	Recovered tablets
Rifampicin + Isoniazid	Recovered tablets
Rifampicin + Isoniazid +Pyrazinamide	Recovered tablets
Rifampicin+Isoniazid+Pyrazinamide +Ethambutol	Tablets

Table 2. International presentations of rifampicin, isoniazid, pyrazinamide and ethambutol[5].

Active ingredient(s)	Pharmaceutical form
Rifampicin	Capsules Injectables Oral Suspension
Isoniazid	Tablets Injectables
Pyrazinamide	Tablets
Ethambutol	Tablets Recovered tablets
Rifampicin + Isoniazid	Tablets Recovered tablets Film-coated tablets
Rifampicin + Isoniazid + Pyrazinamide	Tablets Film-coated tablets

Similarly, on an international level, presentations of the APIs considered first-line treatments exist individually or in combination in the same presentation. Table 2 shows the presentations of R, H, Z, and E alone or in combination that exist in the international market[5].

Clearly there is a lack of high-quality liquid formulations for anti-tuberculosis products, either alone or in combination with each other. Only R is marketed nationally and internationally in oral suspension form, leaving H, Z and E aside, in this type of preparation, despite being considered a first-line therapy.

It is important to note that most of the medicines used to treat TB are designed for adults. They can be counterproductive when administered to the paediatric population, since manipulating them for administering to children usually leads to inaccurate dosage, which can lead to a reduction in effectiveness due to under- or over-dosing. It should be noted that children are not small adults, and therefore, the commercial presentations for adults are not designed for their physiological and metabolic characteristics. The dosage must be adjusted to take the patient's, age, weight and body surface area into account[6,7].

However, the scientific community takes these differential aspects and characteristics of this population group very much into account, and at the level of the Regulatory Agencies it tries to promote research by creating new regulations that promote the stimulation of research in the areas where knowledge is scarce and making more medicines available for children[8,9]. There is still growing concern about the development of medicines for paediatric patients due to the existence of a number of drawbacks, such as low commercial interest and/or the existence of a possible conflict in the implementation of clinical services, which obviously conditions the development of specific medicines for paediatrics[7-9].

The fact that paediatric patients take medicines whose established dosage and pharmaceutical form are designed for the adult population means that therapeutic compliance remains low and, consequently, so do the success of treatment and the chances of cure. Therefore, one of the keys to achieving good therapeutic adherence is to obtain an additional pharmaceutical form that is adapted to the needs of the paediatric population, given the physiological heterogeneity of this group.

Furthermore, it should be considered that the paediatric dosage is established according to weight, and this hinders treating with tablets or capsules marketed in specific doses[10]. The use of medicines in the form of solutions or oral suspensions enables adapting the dosage for administration according to the patient's weight. The WHO[11] dosage guidelines indicated for R, H and Z are set out in Table 3.

However, as can be seen in Tables 1 and 2, there is still no medicine that combines the three APIs in the appropriate form for paediatric administration. In terms of formula development, masking the unpleasant taste of a liquid formulation is not an easy task, and colourings and flavourings can trigger allergic reactions in children. It should also be noted that the stability and bioavailability of the formulated medicines may not be satisfactory and that the excipients may exacerbate the conditions previously mentioned[3,8]. It is important to define the chemical, physical and technological characteristics of the three APIs in order to be able to produce a robust and appropriate design for some of the pharmaceutical forms used in paediatrics.

Liquid formulations

Whenever possible, oral administration is preferable. This is the most physiological, the safest and the easiest route to use[12]. Liquid formulations are part of the various formulations that allow the administration of drugs orally. The Royal Spanish Pharmacopoeia defines liquid formulations for oral use as solutions, emulsions or suspensions containing one or more APIs in an additional vehicle, normally water[13]. For its part, the latest revision of the European Pharmacopoeia indicates that liquid pharmaceutical forms are usually solutions, emulsions or suspensions containing one or more active substances in a suitable vehicle. In addition, liquid preparations, as extemporaneous preparations, are powders or granules that should be reconstituted before administering with a suitable vehicle. The vehicle for any preparation for oral use is chosen considering the nature of the active substance(s) and to provide the appropriate organoleptic characteristics for the intended use of the preparation[14]. This type of preparation is suitable for selected groups of patients, such as the paediatric population, the general population, people with

Table 3. Dosage guidelines for rifampicin, isoniazid and pyrazinamide[11].

Antitubercular	Dose (mg/kg/day)	Positive trend	Rang therapeutic (mg/kg/day)	Maximum dose (mg/day)	Observations
Rifampicin	15	Delivery in once or twice per day	10-20	600	_____-
Isoniazid	10	Once per day 30 minutes before meals or 2 hours later	10-15	300	In combination with rifampicin do not exceed 10 mg/kg/day for hepatotoxicity problems
Pyrazinamide	25	Once per day	20-30	2000	_____-

swallowing problems and users of enteral nutrition probes, so as to allow the simple, safe and effective administration of the medicine in question. In addition, they allow adjusting the exact dose according to the weight and characteristics of the specific patient, providing the adequate quantity of liquid medication in each case.

The liquid preparations used in the case of TB are basically intended to treat the paediatric patient but can also be used to treat patients who are susceptible to overmedication in liquid form. They are very useful for the paediatric population as they are better adapted to the patient's characteristics, facilitate drug administration, and allow greater dosage flexibility to adapt to the patient's weight and age. However, from a galenic point of view, oral liquid formulations present several disadvantages, such as a shorter shelf life and less stability than solid oral forms, physico-chemical incompatibility problems, microbial contamination and, in many cases, an unpleasant taste. Therefore, it is not surprising that pre-formulation studies play a decisive role when preparing this type of preparation, using compatible vehicles that facilitate the administration of the drugs and the addition of excipients that improve not only the organoleptic characteristics but also the conservation and stability of the final formula[15].

In the clinical practice, however, there is a need to administer anti-tuberculosis drugs in FDCs (fixed-dose combination) using a liquid vehicle to ensure correct dosage and good therapeutic adherence. Therefore, it is not surprising that in this type of presentation, there are many differences in the liquid form of the main API considered to be of prime importance (especially in the case of R, H and Z).

It should be noted that the order of sensitivity to the degradation of the three API in a liquid environment is R > H > Z, which can be considered as a practically stable API. Moreover, with regard to FDC products, the stability of R is still lower when compared to the presence of the other APIs, since the combination of the three compounds directly affects their stability. The degradation of R is generally insignificant when alone or in combination with Z, but when combined with H it causes this phenomenon to occur at a very high level[16].

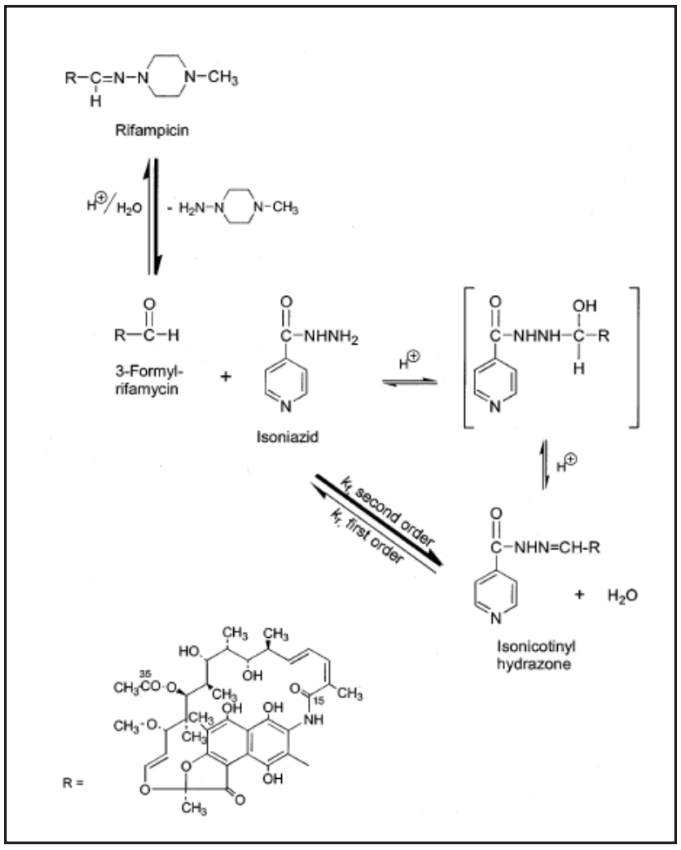
In terms of stability, the results obtained from *in-vitro* and *in-vivo* studies show that the presence of H increases the decomposition of R in mid-acid[17,18]. Furthermore, under *in-vivo* conditions, it should be noted that this process of degradation is favoured in fasting subjects, where the pH of the stomach is between 1.4 and 2.1[16].

Under acid conditions, 3-formyl rifampicin is formed, which interacts with H by giving a second-order reaction, a hydrazone. This hydrazone, when unstable in an acid environment, regenerates H and 3-formyl rifampicin through a pseudo-first-order reaction. As the second-order reaction is faster than the initial one (R with 3- formyl rifampicin) and the first-order reaction (hydrazone with H and 3- formyl rifampicin), the overall reaction favours the formation of the hydrazone (Figure 1). As a result, the degradation of R and its consequent decrease in stability and bioavailability is observed[16].

Furthermore, the addition of Z to a formulation containing the other two active ingredients increases the degradation of R because Z catalyses the reaction that occurs to form the hydrazone. This catalytic effect is due to the fact that it intervenes directly at intramolecular proton transfer level[16,19,20].

Taking into account the physico-chemical alterations that occur between the active ingredients, the enteric coating of one of the compounds could be considered as a possible solution. It is advisable to coat R, as it is much more sensitive than H to acid degradation, which occurs even without having interacted with

Figure 1. Mechanism of rifampicin degradation in the presence of isoniazid[16].



any of the other components of the formulation. Another possible solution that could be considered is to raise gastric pH by adding an alkalising substance. In a basic environment R would be in its most insoluble form, which would produce less decomposition of the active ingredient. Sodium bicarbonate is recommended as an alkalising substance and the use of antacids containing adsorbent substances is not recommended as it would cause a decrease in the bioavailability of R[16].

Another way for consideration is to make a liquid pharmaceutical form where the vehicle for use is oily. Different articles and patents show good results at when developing liquid pharmaceutical forms using oily media with bitter active ingredients, susceptible to hydrolysis[21] or sensitive to acidic environments[22].

Vegetable oils (sesame or soybean oil) and/or medium-chain triglycerides (MCT) are basically used as oily vehicles, the latter being the most used due to their low caloric intake and low propensity to rancidity. This low sensitivity to oxidation is due to the fact that the fatty acids they contain are saturated, and therefore do not have double bonds that can react with oxygen to produce aldehydes, the agents that cause rancidity[21,22].

It is also important to consider certain factors when formulating. Studies performed at different pHs (2-10) and in the presence and absence of phosphate regulatory solution to determine the stability and solubility profile of the three active ingredients using different surfactants (poloxamer 188, poloxamer 407, and sorbitol), suspending agents (carbopol 934 and carbopol 974F) and cyclodextrins, conclude that the use of the agents mentioned for the manufacture of a drug containing R, H and Z is not recommended because they affect the stability of the active ingredients[3].

Another factor to bear in mind when formulating the drug is that although H is stable in an acidic medium, in the presence of reduced sugars such as galactose, lactose, glucose or mannose, it forms hydrazones, which are characterised by being poorly absorbed by the gastrointestinal tract[16].

Other studies recommend taking advantage of the hydrophilic nature of H and Z with respect to the lipophilic nature of R to make a drug that contains all three active ingredients stably. These studies propose the microencapsulation of H and Z in a hydrophilic environment using polymers for forming suspended micro-spheres in a lipidic phase which contains dissolved R. These micro-spheres prevent interaction and hydrolysis between the APIs[3].

In spite of the instability presented, especially by R and H individually and in liquid form, at hospital level, standardised master formulas of the APIs are prepared separately, in an attempt to fill the existing gaps. These preparations are made by the Hospital Pharmacy Service and mix a stable quantity of APIs in the form of powder with water preservative and 70% sorbitol in the case of H, with a simple, watery syrup in the case of E, and only with simple syrup in the case of Z[23]. In the cases of E and Z, simple syrup is used to mask the taste and, due to its properties, as an anti-microbial.

Although the validity period is 30 days, in all cases it is indicated that the results of the physico-chemical stability studies[23] are pending, but considering the information obtained on the stability of these drugs in liquid medium, a decrease is to be expected in the richness of the master formulas within a few days due to the degradation of the APIs. Furthermore, when preparing a formula of this type, the solubility of the API must be taken into account in order to select an appropriate vehicle and, if a suspension is obtained, the corresponding pre-formulation studies must be carried out to select excipients with the required added viscosity, which facilitates the redistribution of the API and prevents the appearance of phenomena such as caking or creaming, as well as the addition of preservatives to prevent the growth of microorganisms and fungi and taste- and/or smell-masking agents. The

particle size of the APIs must also be taken into account when making a liquid pharmaceutical form, particularly in the case of suspensions, since they can greatly condition the final stability of the latter. If the resulting liquid preparation is not homogeneous, it can critically condition the correct dosage of the drug and, consequently, the cure or worsening of the disease, as it can increase the appearance of adverse effects due to overdosage.

In short, all that is proposed as a solution at hospital level is the development of standardised master formulae of different anti-tuberculosis agents to try to solve the problem existing at the time of administering them to the child population. We must bear in mind that there is still a long way to go for hospitals to obtain a medicine in liquid form from the three stable, safe, effective and quality-tasting drugs, without any pre-formulation studies to guarantee the correct bioavailability of the APIs.

In 2015, the WHO and UNICEF *(United Nations Children's Fund)* issued a joint statement for all national TB programmes to replace medicines for the adult population used in children weighing less than 25 kg with the dispersible TB vaccine developed by the WHO and the Global Alliance for TB Drug Development (TB Alliance). These new preparations will follow the dosage guidelines published in the WHO Guide to National TB Programmes on the Management of TB in Children[24,25].

Although there is great hope that the joint WHO/UNICEF publication will improve therapeutic adherence by the paediatric population and put an end to the problems caused by incorrect dosage, it is still not a reality that is suitable for the administration of dispersible FDC forms. Although the WHO proposal calls for the substitution of classic treatments with these new forms of administration, it is clear that their creation is mainly aimed at meeting the needs of underdeveloped countries, those most likely to suffer from TB, and those with a higher incidence of the disease. Therefore, there is still a lack of TB treatment in developed countries, particularly for children.

In fact, the research by Chen in 2000 rejected and considered the formulation of a liquid pharmaceutical form of the three drugs simultaneously for the treatment of TB unfeasible. The research carried out at Rhodes University (USA) studied the behaviour of the three APIs combined in solutions with different pHs (from 2 to 10) and the presence and absence of surfactants and cyclodextrins, suspension agents and phosphate regulatory solution, concluding that in all cases, the stability of the active ingredients is equally compromised and the addition of the proposed compounds to stabilise the solution[3].

Between 2015 and 2018, the research group of the Drug Development Service (SDM) of the Faculty of Pharmacy and Food Sciences at the University of Barcelona studied and finally obtained a liquid formulation for oral administration and an extemporaneous preparation with three of the main anti-tuberculosis agents, R, H and Z. This preparation contains all three phases in the same formulation and is stable for a period of 27 days under refrigeration (5 ºC ± 3 ºC). In order to develop this formula, a wide bibliographic survey was carried out, covering all the subjects related to obtaining the final preparation, from the properties of the APIs to the existing galenic and stability studies. Also, an in-depth study of the physical, chemical and technological characteristics of R, H and Z was carried out in order to develop a series of pre-formulation and formulation studies with different excipients, with the aim of obtaining results in the galenic field such as the solubilisation of the APIs, the stabilisation of the pharmaceutical form and/or obtaining a preparation with additional organoleptic characteristics, always with the final objective of achieving a preparation with all three APIs in a stable manner. During the formulation studies, difficulties arose mainly concerning the different physical and chemical properties, especially in terms of the solubility of the APIs and incompatibilities between them and between the different vehicles under study. The preparation of different formulations and the evaluation of different parameters of each of them allowed us to overcome the galenic and stability problems that arose and, through the use of analytical procedures that corroborated the viability of the final formulation. Pre-formulation and formulation studies, stability studies and the development and validation of the analytical method were carried out in order to obtain a new medicine that allows the combination of the three most used APIs for the treatment of TB in the paediatric population for administering in a simple, joint, individualised manner. This has been possible after exhaustive research, which has enabled proposing, as a new medicine, an anhydrous suspension of an extemporaneous preparation of oral administration of the drug in paediatric patients, with the advances that it has already made in terms of therapeutic adherence, of therapeutic compliance and, consequently, of curing the disease, as well as individualised dosage according to the characteristics of each patient, guaranteeing both the effectiveness of the treatment and the reduction of possible harmful effects derived from incorrect drug dosage[26].

Obtaining a liquid preparation with the three main anti-tuberculosis agents allows them to be administered together, simply and individually, to the paediatric population, adapting them at all times to the particularities and needs of each patient. In the treatment of TB in infants, this liquid formulation is, to date, the best solution in the field of therapeutics and pharmaceutical technology, and it allows solving the current problem of administering a TB treatment to the paediatric population. Despite the

results of the studies carried out by the SDM research group, this preparation has not yet reached the end patient, and although it would offer clear advantages for treating this type of disease, TB is now a minority disease in first world countries. As a research group, a series of -steps have been taken to help make the new product known in different areas where it could be of interest, from exposure in thematic forums for pharmaceutical laboratories to meetings with companies and associations related to the sector and to the pathology, without obtaining any positive response, due, principally to considering that the investment that must be made to bring this product to market is not going to see any satisfactory financial returns. Therefore, it is up to governments and the pharmaceutical industry to jointly commit to marketing this liquid formulation and ensure that all paediatric patients can benefit from this treatment, which would help to cure and eradicate the disease.

Conclusions

The combination of the three active ingredients most commonly used to treat tuberculosis (R, H and Z) via a common dosage form poses a challenge due to the physico-chemical instabilities that arise when mixed in an aqueous vehicle. Many studies have been conducted unsuccessfully in order to try to solve the problems of stability. In 2018, the research group of the Drug Development Service (SDM) of the Faculty of Pharmacy and Food Sciences at the University of Barcelona developed an anhydrous suspension containing the three active ingredients (R, H and Z) that remained stable for 27 days under refrigeration. This represents an improvement in the dosing regimen and treatment adherence in paediatric patients or patients that may have difficulty swallowing. Despite the clear benefits for dosing and for ensuring treatment compliance and curing the disease, it is still a challenge to get the preparation to the end patient, due to the little commercial interest aroused by this disease among the pharmaceutical industry and the little aid and/or scarce solutions proposed by governments to help it reach the patients who really need it.

Bibliography

1. World Health Organization (2020). Tuberculosis: facts and figures. WHO press centre. Available at https://www.who.int/es/news-room/fact-sheets/detail/tuberculosis [see 04/04/2020].
2. Ministry of Health and Consumer Affairs and Social Welfare (2020). Plan for the prevention and control of tuberculosis in Spain. Available at https://www.mscbs.gob.es/profesionales/saludPublica/prevPromocion/PlanTuberculosis/docs/PlanTB2019.pdf [see 18/04/2020].
3. Chen Y. The solubility enhancement and the stability assessment of Rifampicin, Isoniazid and Pyrazinamide in aqueous media. Thesis for the degree of Master of Science of Rhodes University, 2000.
4. AEMPS-CIMA Online Information Centre on Medicines (2020). Available at https://cima.aemps.es/cima/publico/home.html [see 18/04/2020].
5. Medicines and Healthcare Products Regulatory Agency (2020). Medicines: information about specific products. Available in https://products.mhra.gov.uk/substance/?query= [see 18/04/2020].
6. Lorenzo P, Moreno A, Lizascain I, Leza JC, Moro MA, Portoles A. *Basic and clinical pharmacology*. 18th edition. Pan-American Medicine. Madrid, 2008.
7. Bavdekar S.B. Paediatric clinical trials. *Perspectives in Clinical Research*. 2013;4(1):89-99.
8. Diana A, Riet-Nales V, Wang S, Saint-Raymond A, Robert JL. The EMA quality guideline on the pharmaceutical development of medicines for paediatric use. *International Journal of Pharmaceutics.* 2012;435(2):132-4.
9. Zuccotti G, Clementi E, Molteni M, Rocchi F, Tomasi P. The development of medicines for children. *Pharmacological Research*. 2011;64:169-75.
10. Mellado Peña MJ, Santiago García B, Baquero Artigao F, Moreno Pérez D, Piñeiro Pérez R, Méndez Echevarría A, *et al.* Antoni Noguera 384 J. Update on the treatment of tuberculosis in children. *Annals of Paediatrics*. 2018;88(1):52.e1-52.e12.
11. WHO. Fixed-dose combinations for tuberculosis: lessons learned from a clinical, formulation and regulatory perspective. Background documents complied by WHO. Available in http://www.who.int/publications/2003/ [see el 17/06/2020].
12. Vila Jato JL. *Pharmaceutical Technology*. Volume I and II Madrid: Summary; 1997.
13. Spanish Agency for Medicines and Healthcare Products. *Royal Spanish Pharmacopoeia*. 5th edition. Madrid: 2015.
14. European Pharmacopoeia. 10th edition. Liquid preparations for oral use. Disponible en https://pheur.edqm.eu/app/10-1/content/10-1/0672E.htm [see 03/09/2020].
15. Alonso Gonzalo AC. Oral liquid forms. In Vila Jato JL. *Pharmaceutical Technology.* 2nd edition. Ed. Synthesis. Madrid, 2001;2:25-54.
16. Saranjit S, Mariappan R, Sankar R, Sarda N, Singh B. A critical review of the probable reasons for the poor/variable bioavailability of rifampicin from anti-tubercular fixed-dose combination (FDC) products, and the likely solutions to the problem. International *Journal of Pharmaceutics.* 2001;238:5-17.
17. Shisho CJ, Shah SA, Rathod IS, Savale SS. Impaired bioavailability of rifampicin in presence of isoniazid from fixed dose combination (FDC) formulation. M.J. *International Journal of Pharmaceutics*. 2001;228(1-2):53-67.
18. Sosa M, Széliga ME, Fernández A, Bregni C. Rifampicin and bioavailability in combination formulation. *Ars Pharmaceutica.* 2005;46(4):353-64.
19. Seifart HL, Parkin DP, Donald PR. Stability of isoniazid, rifampicin and pyrazinamide in suspensions used for treatment of tuberculosis in children. *The paediatric infections disease Journal*. 1991;10(11):795-880.

20. Bhutani H, Singh S, Jindal KC, Chackrabuti AK. Mechanistic explanation to the catalysis by pyrazinamide and ethambutol of reaction between rifampicin and isoniazid in anti-TB FDCs. *Journal of Pharmaceutical and Biomedical Analysis.* 2005;39:892-9.

21. Roig Carreras M, TubauArino P, Julve Rubio J. 16 October 2004. LaboratoriosSalvat S.A. Liquid pharmaceutical composition for oral administration of bitter active substances susceptible to hydrolysis. Patent ES 2 215 665 T3, A61K 47/44, 2 215 665.

22. Cano Corral C, González Rodríguez ML, Pérez Martínez JI, Alarcón-Payer C, Martínez López I, Rabasco Álvarez A. Preparation and characterisation of an omeprazole oil suspension for administration in paediatrics. *Ars Pharmaceutica*. 2012;53(2):29-35.

23. Pharmacology. Informative bulletin. Volume 7. Nº 1 JANUARY - APRIL 2017. SEFH Pharmacists Working Group.

24. WHO (2020). Statement on the use of child-friendly fixed-dose combinations for the treatment of TB in children. Available https://www.who.int/tb/publications/ChildTbStatementFDCs/en/ [see 04/05/2020].

25. StopTB (2020). Global Drug Facility medicines catalogue. Availablein http://www.stoptb.org/gdf/drugsupply/product_catalog.asp [see 04/05/2020].

26. Mercadé Frutos D, SuñéNegre JM. 4 June 2018. University of Barcelona. Suspension Formulation for the Treatment of Tuberculosis. Patent EP18382385.5.