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Market Surveillance of Anti-TB Drugs Used In Senegal: Assay and *In Vitro* Dissolution Profiles of Separated and Combined Formulations

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ABSTRACT

Objective: The main objective of the present study was to control the quality of drugs used against tuberculosis in Senegal. Methods: The assay and dissolution tests were performed according to the methods described in the United States Pharmacopoeia. The dissolution profiles of the medicines were compared using the independent model approach with a similarity factor f_2 and difference factor f_1 to meet the official requirements. Results: All the molecules analyzed met the requirements for assay (90%-110%) and dissolution (≥80%) tests. As regards the comparison of the dissolution profiles, isoniazid and pyrazinamide in simple and combined forms showed similarity (more than 85 % dissolved in 15 min), while those of ethambutol and rifampicin showed no similarity with f_2<50 in an independent model approach. Conclusion: Except formulation D, all the molecules analyzed in other formulations exhibited a dissolution rate higher than 85% in 30 min.

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1. INTRODUCTION

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis*. It is one of the major public health problems in developing countries. For 2012, an estimated 8.6 million people contracted the disease and 1.3 million died. The number of TB deaths is considerable. All the countries are affected but most cases (85%) occur in Africa (30%) and Asia (55%). Tuberculosis is the third leading cause of death after HIV/AIDS and ischemic heart disease among people between 15 and 59 years old [1].

Indeed, one of the biggest hindrances facing to fight against TB is to ensure that the patients observe the complete treatment. Thus, the World Health Organization (WHO) and the International Union Against Tuberculosis and Lung Disease (IUATLD) recommend the introduction and use of fixed-dose combinations (FDCs) of TB essential drugs under the expanded DOTS strategy (directly observed treatment, short-course) to reduce the risk of drug resistance. This includes avoiding monotherapy, but also to simplify the drugs administration by reducing the number of pills a patient takes each day and decreasing the risk of incorrect prescriptions [2]. These associations contain up to four active ingredients and provide in one tablet 2, 3 or 4 essential first-line drugs. With the correct dosage, they allow easy adoption of regimens recommended by WHO [3].

In resources-limited countries, the treatment against tuberculosis using poor quality drugs is not only the cause of treatment failures but also facilitates the drug resistance. This situation induces a serious public health problem particularly in Africa. Also, the quality, safety and efficacy of all TB drugs including FDCs used by National Tuberculosis Program (NTP) are of the utmost importance to eradicate the disease [2].

Previous studies revealed that despite a carefully controlled manufacturing, absorption of rifampicin component may be incomplete. This weak absorption may seriously compromise the results of the treatment and induce the development of drug resistance [5].

Indeed, in a recent study [4], it was proved that only one FDC formulation among four tested had a rifampicin dissolution profile comparable to that for the corresponding free combinations and hence passed the bioequivalence test. Another study [5], showed a low rate of dissolution for rifampicin in some formulations.

For this reason, the IUATLD and WHO recommend the use of fixed-dose combinations, but only when their bioavailability is well established [6].

In this context, this study aimed at determining the quality of TB drugs distributed in Senegal both in the private and public sectors.

2. MATERIALS AND METHODS

2.1 Test samples

The samples of isoniazid tablets (100mg), pyrazinamide tablets (400mg), ethambutol HCl tablets (400mg), Rifampicin/Isoniazid tablets (150/75mg), Rifampicin /Isoniazid /Pyrazinamide /Ethambutol HCl tablets (150/75/400/275mg) were supplied by the Senegalese National Program against Tuberculosis. The Rifampicin capsules (300mg) were purchased at pharmacy in Dakar (Senegal). Six formulations were available and coded with the letters A to F.

2.2. Instrumentation and conditions

The uniformity of mass was investigated according to the European Pharmacopoeia [7] using an analytical balance (Sartorius analytical balance model LA230S, serial N° 12310278).

A Perkin Elmer series 200 High Performance Liquid Chromatography (HPLC) system connected with Diode Array Detector was used. Data acquisition and treatment were performed with Totalchrom software version 4.0.

The chromatographic analysis for assay and dissolution was carried out using a USP L10 (CN) column (250x4.6mm, 5µm particle size) for ethambutol and a USP L1 (C18) column (150 x 4.6mm, 5µm particle size) isoniazid and pyrazinamide [8,9]. The UV detection was carried out at 200 and 238nm and TCNav software for data acquisition. The spectrophotometric measurements were performed using a Jasco V-570 UV-visible spectrophotometer with matched 1cm quartz cells (Jasco instruments, Tokyo, Japan). The instrument is interfaced to an IBM computer loaded with Spectra Manager software and connected to a Lexmark Z2420 printer.

The Dissolution tests were carried out using a Hanson dissolution apparatus SR8 plus model (Hanson Research Corporation, USA). These tests followed the USP 36 guidelines [8, 9] for each of the investigated molecules. The dissolution assays were carried out using six vessels,

each containing 900 mL of the dissolution media. The temperature of the media was maintained to 37±0.5 °C. Ten milliliter of samples released were withdrawn at 5, 10, 15, 30 and 45 min. After each withdrawal, 10 mL of the media was replaced in the vessels. The concentration and quantity of the active pharmaceutical ingredients (API) of each sample were determined using liquid chromatography (LC) for ethambutol hydrochloride and combined forms, using also spectrophotometer UV/Vis for single forms except ethambutol hydrochloride.

Before any instrumental analysis, a system suitability test was conducted [10].

2.3. Reagents and chemicals

All reagents and chemicals used in these investigations were of the highest purity available. They included Pyrazinamide reference standard (RS), Isoniazid RS, (Rifampicin, Ethambutol obtained from USP (Rockville, USA).

Methanol and acetonitrile HPLC grade, orthophosphoric acid 85%, chlorhydric acid 37% and dibasic anhydrous phosphate were purchased from Sharlau (Barcelona, Spain). Ultrapure water was prepared using Milli-Q water system (Millipore, Molsheim, France) and used to prepare the eluent and to dissolve standards.

2.4. Statistical analysis

Microsoft Excel 2007 professional Edition was used to calculate the percentage of APIs released for 06 individual tablets. All experiments were performed at least in triplicate and the results are expressed as mean values standard deviations (SD) and/or relative standard deviations (RSD).

The similarity factor, f_2, was used to compare the dissolution profiles of the different products as required [11]. The difference factor (f_1) determines the percent (%) difference between two dissolution curves at each time point and is a measurement of the relative error between the two curves. The mathematical formula of f_1 and f_2 are above:

$$f_1=\{[\sum_{t=1}^{n}|R_t - T_t|]/[\sum_{t=1}^{n} R_t]\}.100$$

$$f_2=50.\log\{[1+(1/n)\sum_{t=1}^{n}(R_t - T_t)^2]^{-0,5}.100\}$$

Where n is the number of time points, R_t is the dissolution value of the reference batch at time t, and T_t is the dissolution value of the sample test at time t. The similarity factor (f_2) is a

logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percent (%) dissolution between the two curves. For curves to be considered similar, f_2 values should be close to 0, and f_1 values should be close to 100. Generally, f_1 values up to 15 (0-15) and f_2 values greater than 50 (50-100) ensure sameness or equivalence of the two curves and, thus, of the performance of the test and reference products [11].

3. RESULTS

3.1. Samples characteristics

The information related to the samples collected are presented in Table 1.

Table 1: Samples characteristics

Code Formulation	Designation	Batch N°	Manufacturing date	Expiry date	Manufacturing laboratory
Formulation A	Rifadine® Rifampicin 300mg capsules	A3193	-	06/2016	Sanofi Aventis France
Formulation B	Isoniazid tablets BP 100mg	EIV224 A	12/2012	11/2016	Macleods pharmaceuticals ltd India
Formulation C	Pyrazinamide tablets BP 400mg	EPA82 06A	04/2012	03/2015	Macleods pharmaceuticals ltd India
Formulation D	Ethambutol HCl tablets BP 400mg	ETA00 21	12/2010	11/2014	Cadila pharmaceuticals ltd India
Formulation E	Rifampicin 150mg/ Isoniazid75mg tablets	KRC31 2B	02/2013	01/2015	Macleods pharmaceuticals ltd India
Formulation F	Rifampicin 150mg, Isoniazid 75mg, Pyrazinamide 400mg, Ethambutol HCl 275mg tablets	KRF32 9A	03/2013	02/2015	Macleods pharmaceuticals ltd India

All formulations were from the same manufacturer except Rifampicin capsules and ethambutol tablets.

3.2. Quality control results

Table 2: Visual and physical inspection

Samples	Visual and physical inspection
Formulation A	Red capsules printed, red fine powder
Formulation B	White round tablets, scored
Formulation D	White round tablets, coated
Formulation C	White round tablets, easily friable
Formulation E	Film-coated , round tablets , red
Formulation F	Oval film-coated tablets, light orange

All samples complied with the visual and physical inspection requirements, as none of them was damaged upon receipt except pyrazinamide 400mg (Formulation C) which appeared very friable.

Table 3: Uniformity of mass

Drugs (tablets)	Mean of individual weight	Standard deviation	Sum	Weight max	Weight min	RSD (%)	Standard European pharmacopoeia [7]
Formulation B	0.1859g	0.0034g	3.666g	0.186g	0.179g	1.82%	±7.5%
Formulation D	0.5124g	0.0071g	10.313g	0.525g	0.507g	1.39%	±5%
Formulation C	0.4499g	0.0040g	9.012g	0.458g	0.443g	0.88%	±5%
Formulation E	0.3041g	0.0057g	6.119g	0.313g	0.293g	1.89%	±5%
Formulation F	1.0669g	0.0142g	21.267g	1.085g	1.040g	1.33%	±5%
Capsules	**Filled capsules's Weight**	**Empty capsules's Weight**	**Powder's Weight**	**Mean of individual powder's Weight**	**Standard deviation**	**RSD %**	**Standard European pharmacopoeia [7]**
Formulation A	4.4014g	0.7813g	3.6201g	0.3620g	0.0021g	0.59%	±7.5%

All samples were compliant with the mass uniformity specifications of the European Pharmacopoeia [7].

Table 4: Results of assay by HPLC

Drugs		% Assay	RSD (%)	Specifications USP 36 [8,9]	Conclusion
Formulation A		93.2%	3.3%	[90.0%-110.0%]	Compliant
Formulation B		94.7%	1.4%	[90.0%-110.0%]	Compliant
Formulation C		93.5%	6%	[90.0%-110.0%]	Compliant
Formulation D		93.9%	3.2%	[90.0%-110.0%]	Compliant
Formulation E	Rifampicin	102.5%	4.5%	[90.0%-110.0%]	Compliant
	Isoniazid	94.8%	2.2%	[90.0%-110.0%]	
Formulation F	Rifampicin	99.0%	1.9%	[90.0%-110.0%]	Compliant
	Isoniazid	97.7%	1,7%	[90.0%-110.0%]	
	Pyrazinamide	90.6%	0.6%	[90.0%-110.0%]	
	Ethambutol	94.9%	1.2%	[90.0%-110.0%]	

For the assay test, 3 test samples were made for each sample. All samples analyzed were in accordance with the specifications of USP 36 [8, 9] and exhibited RSDs≤10%.

Table 5: Results of dissolution test

Drugs		% of Dissolution at 45 min	RSD (%)	Standard USP [8,9] after 45min ≥	Conclusion
Formulation A		88.67%	2.60%	80%	Compliant
Formulation B		110.77%	3.69%	85%	Compliant
Formulation C		91.09%	3.48%	80%	Compliant
Formulation D		89.81%	8.02%	80%	Compliant
Formulation E	Rifampicin	105.5%	2.70%	80%	Compliant
	Isoniazid	94.8%	5.51%	80%	
Formulation F	Rifampicin	90.7%	4.41%	80%	Compliant
	Isoniazid	90,2%	2.51%	80%	
	Pyrazinamide	101.3%	2.07%	80%	
	Ethambutol	100.6%	7.34%	80%	

For the dissolution test all analyzed samples were in accordance with the USP specifications [8,9]. The percent relative standard deviation (% RSD) for all time points fulfilled all

requirements of FDA [11] (=20% for 15 min, =10% for other time points), so the results are valid.

Dissolution profiles comparison

The fig. 1 shows the dissolution profiles of Rifampicin in three formulations.

A low dissolution rate for rifampicin in Formulation A compared to E and F was noted.

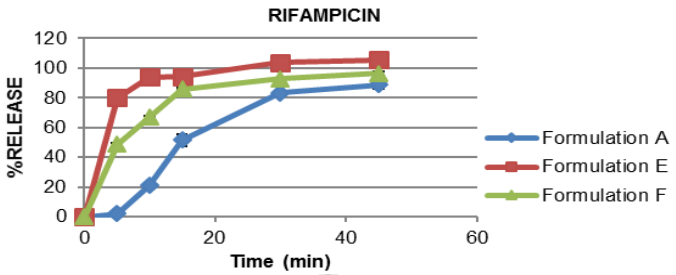

Figure 1: Dissolution profiles of Rifampicin in single and combined forms

The fig. 2 shows the dissolution profiles of Isoniazid in three formulations.

The dissolution profiles of isoniazid in Formulations B, E, F were comparable.

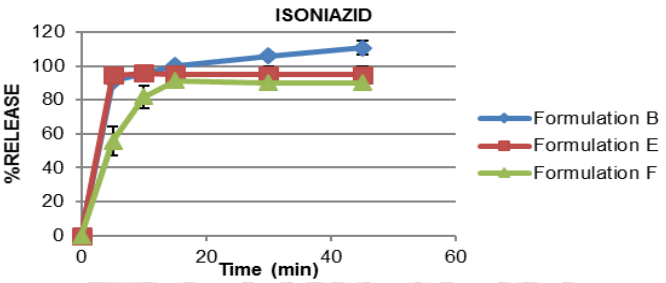

Figure 2: Dissolution profiles of Isoniazid in single and combined forms

The fig 3 shows the dissolution profiles of Pyrazinamide in two formulations.

The dissolution profiles of Formulations C and F were comparable for pyrazinamide.

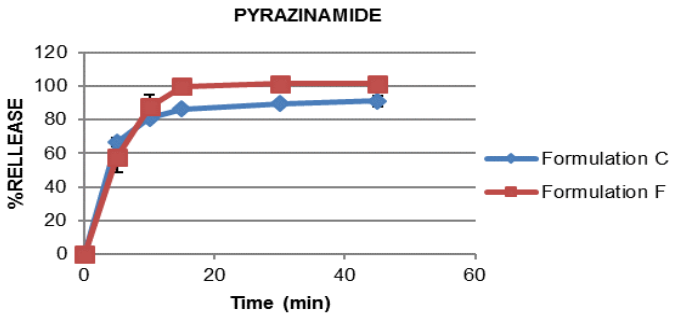

Figure 3: Dissolution profiles of Pyrazinamide in single and combined forms

The fig 4 shows the dissolution profiles of Ethambutol HCl in two formulations.

It was noted a low dissolution rate for ethambutol HCl in Formulation D compared to F.

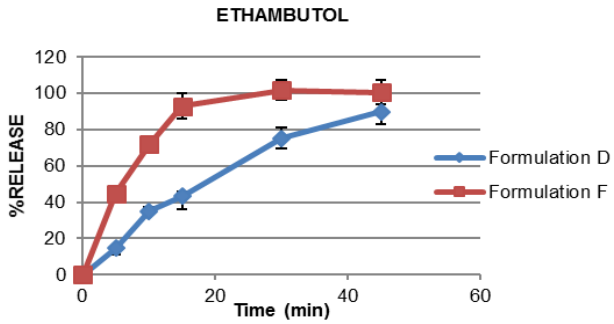

Figure 4: Dissolution profile of Ethambutol in single and combined forms

Table 6: Dissolution profiles of Rifampicin in Formulation A against Rifampicin in Formulation E

Time		Max%	Min%	Mean%	Time	R_t	T_t	(R_t-T_t)	$(R_t-T_t)^2$
5min	Rt	2.00	1.84	1.91	5min	1.91	79.87	77.96	6078.04
	Tt	82.14	75.61	79.87	10min	20.99	93.88	72.89	5312.26
10min	Rt	23.09	19.58	20.99	15min	51.45	94.34	42.89	1839.41
	Tt	103.61	88.28	93.88	30min	83.24	103.71	20.46	418.69
15min	Rt	56.14	47.20	51.45	45min	88.67	105.45	16.78	281.66
	Tt	98.53	88.73	94.34	Sum R_t			246.26	
30min	Rt	85.22	81.14	83.24	Sum (R_t-T_t)			230.98	
	Tt	112.24	99.06	103.71	Sum $(R_t-T_t)^2$			13930.07	
45min	Rt	92.48	85.25	88.67	**Similarity factor f_2**			**14**	
	Tt	109.93	101.36	105.45	**Difference factor f_1**			**94**	

$f_2<50$ and $f_1>15$ show a lack of similarity between the dissolution profiles of Rifampicin in Formulation A and Formulation E.

Table 7: Dissolution profiles of Rifampicin in Formulation A against Rifampicin in Formulation F

Time		**Max%**	**Min%**	**Mean%**	**Time**	**R_t**	**T_t**	**(R_t-T_t)**	**$(R_t-T_t)^2$**
5min	Rt	2.00	1.84	1.91	5min	1.91	48.65	46.74	2184.61
	Tt	57.64	40.61	48.65	10min	20.99	66.91	45.92	2108.48
10min	Rt	23.09	19.58	20.99	15min	51.45	86.01	34.56	1194.61
	Tt	85.47	63.50	66.91	30min	83.24	92.68	9.44	89.13
15min	Rt	56.14	47.20	51.45	45min	88.67	96.47	7.80	60.81
	Tt	90.40	79.14	86.01	Sum R_t		246.26		
30min	Rt	85.22	81.14	83.24	Sum (R_t-T_t)		144.46		
	Tt	101.27	86.94	92.68	Sum $(R_t-T_t)^2$		5637.63		
45min	Rt	92.48	85.25	88.67	**Similarity factor f_2**		**24**		
	Tt	102.34	91.84	96.47	**Difference factor f_1**		**59**		

$f_2<50$ and $f_1>15$ show a lack of similarity between the dissolution profiles of Rifampicin in Formulation A and Formulation F.

Table 8: Dissolution profiles of Isoniazid in Formulation B against Isoniazid in Formulation E

Time		**Max%**	**Min%**	**Mean%**		**Time**	**R_t**	**T_t**	**(R_t-T_t)**	**$(R_t-T_t)^2$**
5min	Rt	93.75	91.02	91,08		5min	91.08	94.51	3.43	11.78
	Tt	99.74	89.71	94,51		10min	95.90	95.68	0.22	0.05
10min	Rt	98.02	93.75	95,90		15min	100.32	95.32	5.00	24.97
	Tt	103.27	88.48	95,68		30min	105.97	95.04	10.93	119.45
15min	Rt	101.19	99.02	100,32		45min	110.77	94.81	15.96	254.80
	Tt	104.64	87.82	95,32		Sum R_t		504.05		
30min	Rt	108.17	102.93	105,97		Sum (R_t-T_t)		35.54		
	Tt	101.51	89.13	95,04		Sum $(R_t-T_t)^2$		411.05		
45min	Rt	114.90	103.50	110,77		**Similarity factor f_2**		**52**		
	Tt	103.24	88.05	94,81		**Difference factor f_1**		**7**		

$f_2>50$ and $f_1<15$ show a similarity between isoniazid dissolution profiles in Formulation B and Formulation E.

Table 9: Dissolution profiles of Isoniazid in Formulation B against Isoniazid in Formulation F

Time		Max%	Min%	Mean%	Time	R_t	T_t	(R_t-T_t)	$(R_t-T_t)^2$
5min	Rt	93.75	91.02	91.08	5min	91.08	55.66	35.42	1254.77
	Tt	67.20	45.71	55.66	10min	95.90	81.89	14.01	196.31
10min	Rt	98.02	93.75	95.90	15min	100.32	91.39	8.93	79.74
	Tt	90.88	74.88	81.89	30min	105.97	90.04	15.93	253.77
15min	Rt	101.19	99.02	100.32	45min	110.77	90.16	20.62	425.01
	Tt	93.49	87.85	91.39	Sum R_t		504.05		
30min	Rt	108.17	102.93	105.97	Sum (R_t-T_t)		94.91		
	Tt	92.02	87.68	90.04	Sum $(R_t-T_t)^2$		2209.60		
45min	Rt	114.90	103.50	110.77	**Similarity factor f_2**		**34**		
	Tt	92.87	86.56	90.16	**Difference factor f_1**		**19**		

f_2<50 and f_1>15 show a lack of similarity between the dissolution profiles of Formulation B and isoniazid in Formulation F. Also table 9 shows that the dissolution percent of the two formulations was higher than 85% at the 15th min.

Table 10: Dissolution profiles of Pyrazinamide in Formulation C against Pyrazinamide in Formulation F

Time		Max%	Min%	Mean%	Time	R_t	T_t	(R_t-T_t)	$(R_t-T_t)^2$
5min	Rt	70.73	63.86	66.57	5min	66.57	57.64	8.93	79.82
	Tt	68.64	46.08	57.64	10min	80.85	87.56	6.71	44.98
10min	Rt	83.75	77.85	80.85	15min	86.43	99.96	13.53	183.04
	Tt	97.37	81.08	87.56	30min	89.66	101.32	11.66	136.03
15min	Rt	87.29	85.40	86.43	45min	91.09	101.34	10.25	105.00
	Tt	101.67	96.99	99.96	Sum R_t		323.51		
30min	Rt	90.26	88.56	89.66	Sum (R_t-T_t)		40.83		
	Tt	103.31	97.98	101.32	Sum $(R_t-T_t)^2$		443.87		
45min	Rt	97.51	89.28	91.09	**Similarity factor f_2**		**49**		
	Tt	102.41	98.89	101.34	**Difference factor f_1**		**13**		

f_2<50 denotes a lack of similarity, but with f_1<15, there is no significant difference between pyrazinamide dissolution profiles in Formulation C and Formulation F.

Table 11: Dissolution profiles of Ethambutol HCl in Formulation D against Ethambutol HCl in Formulation F

Time		**Max%**	**Min%**	**Mean%**	**Time**	**R_t**	**T_t**	**(R_t-T_t)**	**$(R_t-T_t)^2$**
5min	Rt	16.22	12.93	14.73	5min	14.73	44.70	29.97	898.17
	Tt	48.37	38.36	44.70	10min	34.98	72.06	37.08	1375.13
10min	Rt	37.22	31.96	34.98	15min	43.22	93.00	49.78	2477.87
	Tt	73.47	70.37	72.06	30min	75.21	101.82	26.61	708.31
15min	Rt	47.61	39.65	43.22	45min	89.81	100.60	10.79	116.34
	Tt	83.52	102.73	93.00	Sum R_t			257.95	
30min	Rt	78.89	68.79	75.21	Sum (R_t-T_t)			154.23	
	Tt	107.13	92.91	101.82	Sum $(R_t-T_t)^2$			5575.81	
45min	Rt	103.13	89.28	89.81	**Similarity factor f_2**			**24**	
	Tt	108.67	89.36	100.60	**Difference factor f_1**			**60**	

f_2<50 and f_1>15 show a lack of similarity between the dissolution profiles of ethambutol in Formulation D and Formulation F.

4. DISCUSSION

Quality Control Tests

The samples were evaluated according to the physical and visual inspection criteria, uniformity of mass, assay and dissolution tests. All samples were compliant with the view of visual and physical inspection, as none of them was damaged upon receipt, except Formulation C which featured tablets were easily friable. Evaluation of the physicochemical and pharmaceutical properties of tablets showed that all brands fulfilled the requirements of the USP as shown in Table 3. This justify that the samples of our study are WHO-prequalified drugs. Whether by UV-visible spectrophotometry or HPLC compliance criteria of the system were met with RSDs≤2% according to USP [8, 9]. Table 4 showed that all the samples were consistent with the view of assay specifications met for each molecule and for each dosage form according to USP 36 [8, 9]

and RSDs≤10%. All samples were compliant with dissolution perspective after 45min (specifications of USP [8, 9] and FDA [11] were met for each of the molecules) with RSDs≤10%.

These results are surimposable to those of a study on Linesolid orodispersible tablets [12]. The dissolution profile studies of all six formulations showed that more than 50% of the active drug was released from all the six tablets at the end of 30 minutes whereas about 90% of drug release was seen at the end of 60 minutes.

It was found in another study [13] that 9.1% of samples were non-compliant in terms of disintegration and dosage.

Also another work [4] revealed that two formulations of Rifampicin/Isoniazid (300/150mg tablets and capsules) did not meet the requirement of the dissolution test for rifampicin : 33.70% ± 0.48% and 65.80% ± 1.05%, but the percentages of release of the two others were above 80% (96.20% ± 0.50% and 97.20% ± 1.64%, respectively) and met the requirement of dissolution test for rifampicin.

WHO [14] published a dissolution report that showed 11.30% of non-compliant samples concerning 9 samples of isoniazid tablets (appearance, dissolution testing, content uniformity), 12 samples of rifampicin capsules (dosage related substances), 1 sample of the combination rifampicin / Isoniazid (related substances).
Nevertheless, these results were similar to those reported in another work where all formulations tested were passed the quality control tests with reference to the USP requirements [5].

The ethambutol 400mg simple form and rifampicin 300mg simple form in this study exhibited the lowest percentages of dissolution at 45 min (Table 5) compared to other samples (89.81% and 88.67% respectively). Apart from these, active ingredients in the other samples showed rapid dissolution rates with 85% of active ingredient dissolved in less than 30 min.

This result is similar to that obtained by WHO [14] for whom the dissolution percentage means were between 78 and 89% in 45min.

Comparison of dissolution profiles

Rifampicin is the most important and most effective component in FDCs, its bioavailability in the past is of paramount importance. Other TB drugs belong to the highly soluble class of biopharmaceutical classification system (BCS) 1 or class 3, while rifampicin appears in the low solubility (class 2).

Rifampicin

The comparison of dissolution profiles of Formulation A and rifampicin through Formulation E by independent model gave $f_2<50$ ($f_2=14$) and $f_1>15$ ($f_1=94$) (Table 6) and that of Formulation A with rifampicin in Formulation F gave $f_2<50$ ($f_2=24$) and $f_1>15$ ($f_1=59$), (Table 7).

There would be a difference between the dissolution profiles of rifampicin in Formulation A and that in Formulation E and F (150mg). For them to be similar, f_2 must be more than 50 and $f_1>15$ according to the FDA specifications [11].

At this level we can issue such a case the difference between the dosage forms (tablets vs capsules), the difference in dosage, the type of device used for the dissolution test and finally a potentiation of the release (increased solubility) rifampicin through its association with other molecules when one appreciates the look of the dissolution curves (Figure 1). It is important to notice that rifampicin is twice less important combinations than in simple formulations.

Indeed, it could be a synergistic solubility favored by the presence of other molecules and would increase the rate of dissolution of rifampicin (BCS Class 2) in combined forms or high concentration of active ingredient in the simple form that would reduce its rate of release.

Isoniazid

The comparison of dissolution profiles through the independent model of Formulation B and isoniazid in Formulation E gave $f_2>50$ ($f_2=52$) and $f_1<15$ ($f_1=7$) (Table 8) and that of Formulation B and isoniazid in Formulation F gave $f_2<50$ ($f_2=34$) and $f_1>12$ ($f_1=19$) (Table 9).

The percentage of isoniazid released is higher than 85% for the first 15 min in both cases, which justifies the classification BCS class 3/1, high solubility [15]. The only circumstance where f_2 is not required according to the Guidance [11], is when 85% or more of the labeled amount of the drug dissolves in fifteen min.

According to the WHO [16], when the molecule test and the reference have a dissolution rate ≥85% within 15 min, the profiles can be considered similar; the comparison therefore requires no calculations [11].

The dissolution profiles of Formulation B and isoniazid in Formulations E and F can be considered similar.

Pyrazinamide

Comparison of Formulation C dissolution profiles and pyrazinamide in Formulation F through independent model provides a close to 50 f_2 (f_2=49) and f_1<15 (f_1=13) (Table 10). Only the first 4 sample times are considered for the calculation of f_1 and f_2 as recommend by the FDA, one sample time must be considered after the dissolution percentage reaches 85%. The pyrazinamide dissolution percentages in single and combined form are ≥85% for the first 15 min, which justifies the BCS class 3/1 [15].

Indeed, for isoniazid, WHO [16] stated that when the molecule test and reference exhibit a dissolution percentage ≥85% within 15 min, the profiles can be considered similar. The comparison therefore requires no calculations. The hypothesis that isoniazid and pyrazinamide in single and combined forms have similar dissolution profiles could be accepted.

Ethambutol HCl

The comparison of Formulation D's dissolution profile and that of Formulation F through self-model presents a f_2<50 (f_2=24) and f_1>15 (f_1=60) (Table 11).

Formulation D's dissolution profile differs from ethambutol in Formulation F.

Referring to the shapes of the dissolution curves (Figure 4), ethambutol release rate observed in Formulation D (400mg) was lower than ethambutol in Formulation F (275mg) both film-coated tablets. Being ethambutol BCS class 3 [15], it should have a high solubility. Indeed, during the dissolution test, if the concentration of the active ingredient in the medium reaches its solubility limit, the dissolution rate decreases. This could be explained by the difference in formulation of the two samples (as different manufacturers), the potentiating effect of the release rate of ethambutol in the combined form due to the presence of other molecules, and also the close

expiry date of Formulation D. It should be noted at this level as for rifampicin the decreased dose of ethambutol in the combined form of 30% compared to the simple form.

These results are similar to those revealed by a comparative study of *in-vitro* dissolution profiles of paracetamol and caffeine combination in different formulations using similarity and difference factors [17].

With reference to the dissolution profiles it can be noted that except isoniazid, the other molecules: rifampicin, pyrazinamide and ethambutol in the combined formulations gave higher percentages of release compared to those in simple formulations. This finding would support the hypothesis of synergistic solubilization favored by the association with other molecules. For isoniazid, release percentages in the simple form were higher than in the combined forms.

Media dissolution apparatus, rotation frequency

Formulation A was dissolved in 0.1N HCl while combinations had as pH 6.8 buffer dissolution medium. Another study [5] reported *in vitro* dissolution media for rifampicin in FDCs using 0.1N HCl, pH 6.8 buffer and 0.001N HCl. In this study the dissolution profiles obtained were comparable to rifampicin in 0.1N HCl circles and 0.001N HCl and a similar profile in phosphate buffer medium (pH 6.8). It was also noted an excellent dissolution of rifampicin in this buffer medium. Further, Formulation A was dissolved with apparatus 1 (baskets), for combinations (Formulations E and F), apparatus 2 (paddles) was used. The frequencies of rotation were the same (100 rpm).

For Formulation B, the medium used was 0.01 N HCl and the apparatus used was apparatus 1 (baskets), while for isoniazid in combined forms, the medium used was the pH 6.8 buffer and the device used was the apparatus 2 (paddles). The rotation frequencies were the same (100 rpm).
For Formulation C, water was used as dissolution medium, while for the combination, it was performed in pH 6.8 buffer. According to the FDA [11], the use of water as dissolution medium is not recommended because the test conditions such as pH and the surface tension that can vary depending on the water source and may change during the dissolution test, due to the influence of the active and inactive ingredients. The apparatus used was the same: paddles. The rotation frequencies were different: 50 rpm for Formulation C and 100 rpm for Formulation F. Based on another study [5], the frequency 50 rpm can be considered as an appropriate rotation frequency

since more than 75% of the active ingredient was released within 45 min in all mediums. Then some authors [4] conducted their dissolution study at 75 rpm and 37°C using phosphate buffer saline for two drugs and four drug-FDCs.

Regarding ethambutol, the dissolution media (pH 6.8 buffer) and rotation frequencies (100 rpm) were similar for the two formulations (D and F); Apparatus 1 (baskets) was used for Formulation D and apparatus 2 (paddles) for Formulation F.

It is important to note that for Formulation A and Formulation D which had somewhat slow dissolution rates, dissolution assay was conducted using the apparatus 1 (baskets); All other samples had faster dissolution rates (more than 85% dissolved in 30 min) and dissolution was carried out using the apparatus 2 (paddles) except Formulation B. Apparatus 1 could be the cause of the low percentage of dissolution. This finding is similar to that reported in another study [18]. According to them, many experts agree that the method of the rotating basket (apparatus 1) is not always effective, because the lack of reproducibility and reliability of this method, mainly due to the heterogeneity of the distribution of the solute in the liquid. A clogging of the grid basket with hydrophilic nature excipients can also explain of these variations.

Overall, the media used temperatures and the rotational frequencies for *in vitro* dissolution tests were the same as those recommended by the FDA [11] and European Medicines Evaluation Agency (EMEA) [19], to establish the dissolution profiles except single pyrazinamide for whom, water was used as dissolution medium. Even so, all methods used, whether for assay and dissolution tests were those of the USP 36 [8, 9].

Indeed, other *in vivo* studies conducted around the world [20, 21, 22] on the comparison of bioavailability between combined forms of TB-drugs: rifampicin, isoniazid, pyrazinamide, ethambutol and simple formulation, showed a bioequivalence thereof at similar dosages. Nevertheless, a study [4] about 4 formulations marketed in China tested revealed that the concentrations of rifampicin for the three two-drug FDCs were within the reported acceptable therapeutic range. But, they displayed lower rifampicin bioavailability compared with the reference; only formulation F (four-drug FDCs) was bioequivalent to the reference product.

5. CONCLUSION

One solution against tuberculosis is the necessity to ensure the safety, efficacy and quality of FDCs and other TB drugs, ensuring that they are available and affordable. All formulations tested in this study met the quality control requirements for the mass uniformity, dosage and dissolution tests. This work revealed that the samples tested are fast-release drugs with generally a dissolution percentage higher than 85% in 30 min. Concerning the comparison of dissolution profiles between different active ingredients in simple and combined formulations tested, it appeared that excepted pyrazinamide and isoniazid which gave a similar dissolution percentage higher than 85% in 15 min, rifampicin and ethambutol HCl showed a lack of similarity. This lack of similarity could be due to the difference of formulations, the content of each active ingredient in these different formulations and the type of media and apparatus used.

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