Formulation and Stability of Rifampicin Oral Suspension

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ABSTRACT
Rifampicin is an antitubercular antibioic reported to undergo a variety of chemical changes in aqueous medium, as such, the drug is usually prepared in the dry form for reconstitution by adding a specified quantity of purified water just before dispensing. An affordable and cost-effective paediatric formulation of rifampicin was prepared and investigated. Stability studies were then carried out to establish, predict and to confirm the shelf-life and storage conditions of the drug in solution by preparing both dry and reconstituted samples and subjecting them to different exaggerated temperature storage conditions.

The drug content of the formulation were determined using ultra-violet spectrophotometric method of analysis described in the British Pharmacopoeia 2005. The dry samples were kept at 25°C (room temperature) and 40°C for a period of three months and four weeks respectively and it was observed that those kept at room temperature were most stable while those at 40°C were unstable.

Reconstituted samples were also stored at room temperature and in refrigerator for a period of 30 days. Refrigerated samples remained stable over 14 days period while those kept at room temperature were very unstable.

The dry powder formulation before reconstitution is most stable at room temperature for a period of at least 3 months while the reconstituted oral suspension should be refrigerated immediately after reconstitution for optimal stability and must not be kept longer than 2 weeks after which it should be discarded. Its degradation is pH-dependent and maximum stability is attained at near neutral pH.

KEY WORDS
Formulation, Oral Suspension, Rifampicin, Stability.

INTRODUCTION

Rifampicin, a semi-synthetic antibiotic, is a primary drug in the treatment of tuberculosis. Its introduction marked a major advance in the treatment of tuberculosis and it is at present the most active agent in clinical use for the treatment of tuberculosis. In order to increase its therapeutic effectiveness, rifampicin should be formulated as an oral suspension for paediatric use[1,2].

The paediatric formulation is currently not available in the Nigerian market. The high incidence of tuberculosis in Nigeria makes it imperative that a suitable formulation be made available and affordable to the populace. There is also the need to establish the stability from the viewpoint of immediate reaction and that of progressive reactions which may develop in the product upon ageing. According to the British Pharmacopoeia (B.P.) 2005, the recognized acceptable potency level is 90-110% of labelled content[3].

Suspensions constitute an important class of pharmaceutical preparations in which insoluble particles are dispersed in a liquid medium. They may be defined as preparations containing finely divided drug particles referred to as the suspenoid, distributed somewhat uniformly throughout a vehicle in which drug exhibits a minimum degree of solubility[4,5].

The degradation of a drug in the presence of water may preclude its use as an aqueous solution; it may be possible to reduce the contact time between the solid drug particles and the dispersion medium by preparation of the suspension immediately prior to issue to the patient[6]. Certain drugs are chemically unstable when in solution but stable when suspended. In instances such as this, the oral suspension ensure chemical stability while permitting liquid therapy. For many patients, the liquid form is preferred over the solid dosage forms - tablet or capsule of the same drug because of the ease of swallowing liquids. The flexibility in the administration of doses, the greater convenience in the administration of unusually large doses, and the safety of liquid doses for infant and children are other advantages.

Suspending agents are added to suspension formulations for their thixotropic properties. Tragacanth is one of the commonly used suspending agents in formulations; it is usually added at concentration range of 4 to 6% to give a yield stress value of 10 Pa[7].

In this study, an affordable and cost-effective rifampicin oral suspension was formulated and evaluated for ▶

stability. The effect of tragacanth as suspending agent on the stability of rifampicin formulation was also investigated.

MATERIALS AND METHODS

Rifampicin (Troge Medical GMBH, Hamburg, Germany), sucrose, compound tragacanth (Spectrum Chemical Company, St. Louis, MO, USA).

Spectrophotometric determinations were carried out with the ultraviolet-visible, SP8-400 Pye-Unicam Spectrophotometer (Cambridge, England) and pH readings were taken with the Kent EIL 7020 pH meter.

Standard solutions of rifampicin in phosphate buffer pH 7.4 were prepared in a concentration range of 1.5micrograms to 30micrograms. The absorbances of the standard solution were analysed at 475 nm; a calibration curve of absorbance against concentration was obtained.

Preparation and storage of Rifampicin oral suspensions

Six different formulations, each in triplicate were prepared and designated A, B, C, D, E & F as shown in Table 1 below:

Table 1: Formulations of Rifampicin Oral Suspension

Ingredient	A	B	C	D	E	F
Rifampicin (g)	1.2	1.2	1.2	1.2	1.2	1.2
Compound Tragacanth (g)	1.4	1.4	1.4	1.4	-	-
Sucrose to (g)	30	30	30	30	30	30
Water to (ml)	-	-	60	60	60	60

Appropriate quantities of the ingredients were weighed and triturated together in a glass mortar and uniformly mixed; 30g of the triturated powder was then transferred into amber glass bottles and stored appropriately.

Sample storage and sampling

Sample storage and sampling were carried out as shown in Table 2 below:

Table 2: Storage conditions and Sampling Time

Formulation	Storage condition	Sampling Time
A	Dry sample, room temp. (25°C)	0, 3, 6, 9 & 12 weeks
B	Dry sample, 40° C	0, 1, 2 , 3 & 4 weeks
C	Reconstituted, room temp. (25°C)	0, 1, 2 , 5, 10,14 & 30 days
D	Reconstituted, 4°C	0, 1, 2 , 5, 10, 14 & 30 days
E	Reconstituted, room temp. (25°C)	0, 1, 2 , 5, 10, 14 & 30 days
F	Reconstituted, 4°C	0, 1, 2 , 5, 10, 14 & 30 days

Spectrophotometric Analysis

Specptrophotmetric analysis of samples was carried out by weighing a quantity of powder containing 0.4g of rifampicin, this was diluted to 500ml with methanol and mixed thoroughly; 2ml of this was then diluted to 100 ml with phosphate buffer pH 7.4 and the absorbance was measured at the maximum of 475nm to determine the amount of rifampicin remaining at each sampling time.

Organoleptic tests and pH determinations

Organoleptic assessments of colour, odour, taste and pH determinations of samples were carried out.

RESULTS

The assay method employed in the study is robust, accurate and reproducible; correlation coefficient of calibration curve is 0.995. From the assay of the samples, it was observed that rifampicin is liable to pH-dependent degradation in aqueous medium and also sensitive to changes in temperature. ►

Table 3: Effect of temperature on dry powder samples for reconstitution

Formulation A Stored at room temp (25°C)			
Time (weeks)	Assay (%) Mean ± s.d. N=3	Time (weeks)	Assay (%) Mean ± s.d. N=3
0	100.00±1.21	0	100.00±0.57
3	99.39±2.23	1	93.85±1.39
6	99.08±2.12	2	86.15±3.42
9	98.46±4.27	3	84.62±2.71
12	96.92±3.14	4	69.23±4.11

Table 4: Effect of temperature on stability of reconstituted samples

Time (days)	Assay (%) Mean ± s.d. n=3 FORMULATION			
	C	D	E	F
0	100.00±2.11	100.00±1.74	100.00±3.24	100.00±4.01
1	85.31±4.31	99.69±3.14	83.60±2.71	100.00±3.17
2	84.05±3.74	99.38±4.42	82.61±1.87	99.35±2.86
5	82.81±2.16	99.06±1.62	79.19±0.93	99.06±3.21
10	81.88±2.91	98.94±2.19	72.98±2.47	97.81±1.07
14	75.00±4.12	96.88±2.41	68.32±1.38	95.33±2.14
30	54.68±3.21	86.56±2.34	55.91±2.44	88.13±3.27

Table 5: pH values of reconstituted samples

Time (weeks)	pH Mean ±s.d. n=3 FORMULATION			
	C	D	E	F
0	6.8±0.2	6.9±0.2	6.7±0.3	6.8±0.1
1	4.3±0.1	6.8±0.3	4.2±0.2	6.7±0.3
2	4.1±0.3	6.8±0.1	4.1±0.1	6.7±0.2
4	4.0±0.2	6.7±0.4	4.0±+0.2	6.6±0.2

Results of organoleptic assessment shown in Table 6 remained constant throughout the sampling time with products being brick red in colour, odourless with a sweet taste.

Table 6: Results of Organoleptic Tests

Time (weeks)	FORMULATION			
	C	D	E	F
0	O/BR/S	O/BR/S	O/BR/S	O/BR/S
1	O/BR/S	O/BR/S	O/BR/S	O/BR/S
2	O/BR/S	O/BR/S	O/BR/S	O/BR/S
4	O/BR/S	O/BR/S	O/BR/S	O/BR/S

Key: O-odourless; BR-brickred; S-sweet ▶

DISCUSSION

Rifampicin being a chemically unstable drug in aqueous medium necessitated its formulation as powder for reconstitution into an oral suspension prior to use. The scarcity of paediatric oral suspension of rifampicin in the Nigerian market has limited its use in the tuberculosis treatment programme in Nigerian children. Current work on formulation of paediatric oral suspension of rifampicin has confirmed the feasibility of an affordable and cost-effective product.

From the results obtained, it was observed that rifampicin is liable to pH-dependent degradation in aqueous medium and also sensitive to changes in temperature.

Formulation A samples which are dry powders kept at room temperature (25°C) remained very stable throughout the sampling period of 12 weeks. Fig. 1 shows that the concentration of the drug remained relatively constant and well within the stipulated B.P. range of 90-110% of stated quantity at room temperature for at least 12 weeks before reconstitution.

Formulation B samples were kept in an oven at 40°C; Fig.1 showed some chemical instability as the concentration of the drug dropped below the stipulated minimum of 90% of the stated amount after about 2 weeks. It can thus be stated that though rifampicin in the dry state is more stable, it is grossly affected by high temperature. It is recommended that the dry powder formulation for reconstitution should be kept at room temperature for maximum stability.

Reconstituted samples of the oral suspension were also subjected to effects of temperature and suspending agent on their stability. Formulations C and D were reconstituted samples which contained compound tragacanth as suspending agent, and were kept at 25°C and 4°C respectively. Assay results of formulation C stored at 25°C showed a drastic reduction in concentration below the stipulated B.P. minimum of 90% after about 24 hours and dropped even further to about 60% by the 30th day as shown in Fig. 2.

According to Seydel, rifampicin suspension is not stable at extremely low pHs and is subject to increased degradation at alkaline pH. The pH of the samples as shown in Table 4 confirms the fact that degradation of rifampicin is pH-dependent and maximum stability is attained at near neutral pH[8].

From Table 3, reconstituted Formulation D samples at 4°C in the refrigerator showed considerable stability for at least 14 days; the concentration remained within the B.P. stipulated range of 90-110% of labelled content for this period after which the concentration dropped below the stipulated minimum (Fig. 2).

Due to the presence of a suspending agent in formulations C and D, samples were also very well dispersed in the liquid medium and also easily re-suspended without any caking.
It is clearly observed from all reconstituted samples that the effect of temperature on such samples is very important. It is recommended that reconstituted samples MUST be stored in refrigerator immediately after reconstitution as the drug potency is lost within 24 hours after reconstitution if not refrigerated immediately.

Formulations E and F samples were reconstituted samples without suspending agent and kept at 25°C and 4°C respectively. It was also observed from Fig. 3, that the Group E samples kept at room temperature degraded below the stipulated B.P. minimum concentration of 90% after 24 hours, Group F samples remained very stable in the refrigerator for 14 days as shown in Fig. 3 and the pH of the samples were maintained at near neutral pH. This further confirms the effect of temperature and pH on the aqueous reconstituted formulation of the drug.

The effect of the absence of a suspending agent in formulations E and F was not exactly seen as the suspensions were very well dispersed and easily resuspended without any caking. The suspensions were homogeneous with a viscosity high enough to minimize settling of the particles.

The oral suspension exhibited high viscosity due to the use of simple syrup (50%w/v sucrose) employed; oral suspension of rifampicin can be prepared without a suspending agent using simple syrup thereby making the formulation affordable and cost-effective.
The characteristics of colour, odour and taste are important in orally administered suspensions. Changes in these organoleptic properties can be indicative of chemical instability[10]. All samples remained stable with respect to organoleptic tests of colour, odour and taste.

CONCLUSION

Rifampicin can be formulated into an oral suspension by preparing it as a dry powder for reconstitution prior to use. The dry powder formulation before reconstitution is most stable at room temperature for a period of at least 3 months while the reconstituted oral suspension should be refrigerated immediately after reconstitution for optimal stability and should not be kept longer than two (2) weeks after which it should be discarded. Its degradation is pH-dependent and maximum stability is attained at near neutral pH.

REFERENCES

1. Petri, Jr. W A : Antimicrobial Agents. In: Hardman J G, Limbird L E and Gilman A G, eds., Goodman & Gilman's Pharmacological Basis of Therapeutics, 10th ed. N.Y.; McGraw-Hill Co., 2001:1273

2. Physician's Desk Reference 59th ed., Thomson PDR, Montvale, New Jersey, 2005:736

3. British Pharmacopoeia (2005): Her Majesty Stationery Office, London:164, 378

4. Martin, A.N. (1961) Physico-chemical approach to the formulation of pharmaceutical suspensions. J. Pharm. Sci., 50, 513.

5. Nash, R A: Suspensions. In.: Swarbrick, J and Boylan, J C, eds, Encyclopaedia of Pharmaceutical Technology Vol. 3, 2nd ed. N.Y. ▶

Marcel Dekker Inc., 2002:

6. Ball, D., Lee, T., Ryder, J., Seager, H. and Sharland, D. (1978): Improving the stability of a product containing interactive ingredients. A comparison of different approaches. J. Pharm. Pharmac., 30, 43.

7. Howard C. Ansel, Introduction to Pharmaceutical Dosage forms: Henry Kimpton Publishers, London, 1981: 140, 145, 152-153.

8. Seydel, J K (1970): Stability of Rifampicin. Antibiot. et. Chemother 16:380

9. Connors K A, Amidon GL and Stella V J: Chemical Stability of Pharmaceuticals 2nd ed. N.Y.; John Wiley & Sons, Inc., 1986:728.

10. Tingstad J E (1964): Physical Stability testing of Pharmaceuticals. J. Pharm. Sci., 53, 955.

1. World Health Organisation, Counterfeit Medicines; WHO Drug Information, 1997; 11(2):57

2. Fake Drugs in Nigeria: Topical Issues and facts You Need to Know,: Atueyi, I, ed., Pharmanews Ltd., 2004:7

8. WHO Model Formulary, Couper, M R and Mehta, D K, eds., World Health Organisation, Geneva, 2002:254

10. The Japanese Pharmacopoeia 14th ed. (2001): Society of Japanese Pharmacopoeia, Tokyo:49-51