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Dissolution test method for rifampicin–isoniazid fixed dose formulations

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Abstract: A dissolution procedure for a rifampicin–isoniazid combination formulation was evaluated using 0.1 N hydrochloric acid solution and 0.4% (w/v) sodium lauryl sulphate solution as dissolution media. Rifampicin and isoniazid along with degradation components were chromatographed using reversed-phase liquid chromatography on a 10 μm octadecylsilica column using methanol–0.01 M disodium hydrogen phosphate (70:30, v/v; pH 4.6 ± 0.1) as mobile phase. The detection was carried out at 254 nm. The data obtained indicate that the dissolution medium consisting of 0.4% (w/v) sodium lauryl sulphate solution is suitable for such a combination. The degradation observed in dissolution medium consisting of 0.1 N hydrochloric acid was 10–23%.

Keywords: *Rifampicin and isoniazid; rifampicin; rifampin; isoniazid; dissolution medium effect; sodium lauryl sulphate.*

Introduction

Dissolution analysis when correlated with bioavailability is the single most important factor to ensure the consistency and quality of a pharmaceutical dosage form. To do so the test should be so designed as to mimic closely the biological environmental conditions [1]. Dissolution of a dosage form *in vivo* is often the rate-limiting factor determining the physiological availability of a drug. Dissolution is the most important test employed in the development and quality control of solid dosage forms.

The disintegration rates of tablets or capsules occasionally do not coincide with the dissolution rates [2]. Most pharmaceutical dosage forms are polydisperse systems. Drug release from such systems involves various aspects, such as simultaneous dissolution of more than one phase and differing dissolution rates within phases. These factors may affect the overall dissolution rate of the system [3].

Many carrier materials or active drugs readily form soluble complexes with drugs, thereby enhancing or reducing the drug's apparent solubility [4, 5]. Solid drugs administered orally are not immediately available to the biological system since they are normally absorbed only from solution [6, 7]. For simulating *in vivo* conditions, parameters like pH, surface tension, viscosity and sink conditions need to be controlled in dissolution medium. The system generally employed is 0.1 N hydrochloric acid or buffered solutions with pH close to 1.2, similar to gastric fluid [8–10].

For any method of analysis to be acceptable, the sample should be unaffected during the analysis process. The dissolution medium specified in the USP XXII for rifampin capsules has been reported to be unsuitable, as decomposition up to 17% was observed within 45 min in this medium [11]. Systems containing more than one component can interact in different ways, thereby influencing the dissolution behaviour [1]. Rifampicin interacts with isoniazid in solutions to form a soluble complex [14]. Because of the known incompatibility of rifampicin and isoniazid in solution, it was decided to develop a suitable methodology for *in vitro* evaluation of rifampicin and isoniazid fixed dose combination formulations.

Experimental

Reagents

Hydrochloric acid, methanol, disodium hydrogen phosphate, all of reagent grade, were obtained from Glaxo (Bombay, India); sodium lauryl sulphate, of reagent grade was obtained from Suyesh Chemicals (Bombay, India); rifampicin and isoniazid reference standards were obtained from the US Pharmacopeial Convention (Rockville, MD, USA).

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Instrumentation

An electrolab TDT06 (Bombay, India) dissolution test apparatus equipped with a six-basket assembly and a built in system to regulate temperature and rotation of the baskets was employed. A Waters (Milford, USA) HPLC system consisting of a 510 dual pump solvent delivery system, a 680 automated gradient controller, a 490E multiwavelength UV–vis detector, a fixed loop of 100 μl with 7125 rheodyne (Cotati, USA) injector and a Waters (Milford, USA) microbondapak C18 (30 × 3.9 cm i.d.) column was used for analysis.

Dissolution conditions

Two dissolution media solutions were prepared for the study: solution A, 0.1 N hydrochloric acid (pH 1.2); and solution B, 0.4% (w/v) sodium lauryl sulphate (pH 7.0). The *in vitro* evaluation was done using 900 ml of each dissolution fluid. The temperature of the dissolution fluid was maintained at 37° ± 0.1°C and the baskets were rotated at 50 rpm.

Chromatographic conditions

The mobile phase consisted of methanol–0.01 M disodium hydrogen phosphate (70:30, v/v; pH 4.6 ± 0.1). The mobile phase was operated at a flow rate of 1.2 ml min^{-1} at ambient temperture. The UV detector was operated at 254 nm. The volume of solution injected onto the column was 100 μl.

Preparation of reference samples

The rifampicin reference sample, 0.04 mg ml^{-1} and isoniazid reference sample 0.04 mg ml^{-1} were prepared in both dissolution fluids. These samples were chromatogrammed immediately to avoid degradation on storage.

Procedure

Two marketed capsule dosage forms containing rifampicin 450 mg and isoniazid 300 mg, were evaluated using the two dissolution fluids. Six baskets containing one capsule each were immersed in the dissolution fluid, maintained at 37° ± 0.1°C and rotated at 50 rpm. At predetermined intervals, 10 ml samples were withdrawn and replaced with dissolution fluid. The samples were withdrawn at 10, 20, 30, 45, 60 and 120 min and the content of rifampicin and isoniazid determined by high-performance liquid chromatography (HPLC). The concentrations of rifampicin and isoniazid were computed by comparing peak areas of reference standard and test sample solutions.

Results and Discussion

Rifampicin degrades to 3-formylrifamycin SV in acidic solution and to rifampin quinone in alkaline solution. The degradation of rifampicin is further catalysed by the presence of isoniazid in solution forming a soluble complex which interferes in spectrophotometric analysis. Rifampicin contains an azomethine bond (—N=CH—) which degrades in acidic aqueous solution by reversible azomethine bond cleavage. Reversible addition of water across the azomethine bond forms a transient carbinolamine, which then rearranges to give 3-formylrifamycin SV and 1-amino-4-methylpiperazine [12, 13]. The 3-formylrifamycin SV and isoniazid then probably undergo a Schiff's reaction to form a soluble complex. Further investigation is in progress to identify the soluble complex so formed. The analytical procedure based on the method proposed by Shah *et al.* [14], was used for the determination of rifampicin and isoniazid simultaneously along with separation of the degradation complex. The current trend for the assay of multiple dosage forms requires more complicated assay techniques, like HPLC and UV photodiode array spectrophotometry.

The dissolution procedure given for rifampin capsules in the US Pharmacopeia XXII is unsuitable for evaluating rifampicin and isoniazid fixed dose combination dosage forms [15]. The analytical procedure followed is spectrophotometric which does not allow for the presence of degradation products. The dissolution medium is not suitable as rifampicin is unstable in acidic medium. To overcome this problem, a search for a dissolution medium which would not cause degradation was made. Unfortunately water was not suitable as the rifampicin is not sufficiently soluble. Supramicellar surfactant concentrations are used for drugs of low solubility [16] and sodium lauryl sulphate in a dissolution medium is considered to have solubilizing activity similar to that of the naturally occurring bile salts, sodium cholate, and sodium taurocholate [17]; hence use of this medium was investigated.

The dissolution rate data of the two dissolution fluids are tabulated in Tables 1 and 2. The results indicate that in the case of 0.1 N

Table 1
Dissolution of rifampicin and isoniazid fixed dose combinations in 0.1 N hydrochloric acid solution

	Cumulative per cent dissolved			
	Product A		Product B	
Time (min)	Rifampicin	Isoniazid	Rifampicin	Isoniazid
0	0	0	0	0
10	10.11 ± 1.80	88.16 ± 1.56	16.78 ± 1.56	27.61 ± 5.31
20	43.63 ± 3.42	99.43 ± 1.34	44.35 ± 4.38	54.61 ± 4.43
30	77.19 ± 3.28	102.95 ± 1.85	62.51 ± 4.40	76.18 ± 5.44
45	87.27 ± 2.71	102.13 ± 1.34	68.34 ± 3.61	90.58 ± 2.65
60	85.21 ± 2.08	105.18 ± 1.34	69.32 ± 1.32	98.02 ± 2.23
120	64.59 ± 4.87	101.64 ± 1.42	57.10 ± 2.93	94.67 ± 2.91

All values are the mean of 12 determinations (mean ± SE).

Table 2
Dissolution of rifampicin and isoniazid fixed dose combinations in 0.4% (w/v) sodium lauryl sulphate solution

	Cumulative per cent dissolved			
	Product A		Product B	
Time (min)	Rifampicin	Isoniazid	Rifampicin	Isoniazid
0	0	0	0	0
10	17.85 ± 1.86	86.11 ± 2.16	12.50 ± 1.11	30.02 ± 6.78
20	45.05 ± 3.18	87.39 ± 5.59	28.54 ± 3.87	47.98 ± 5.76
30	60.31 ± 2.43	96.63 ± 0.63	41.66 ± 4.18	63.39 ± 5.62
45	85.05 ± 1.94	97.07 ± 0.75	62.14 ± 3.39	90.57 ± 3.61
60	90.07 ± 3.03	97.42 ± 0.54	73.52 ± 2.47	99.48 ± 1.99
120	97.16 ± 2.51	99.72 ± 2.09	81.36 ± 1.15	104.18 ± 1.00

All values are the mean of 12 determinations (mean ± SE).

Table 3
In vitro–in vivo correlation coefficients for rifampicin and isoniazid fixed dose combinations

	In vivo–in vitro correlation for rifampicin	
Dissolution medium	Product A	Product B
0.4% (w/v) Sodium lauryl sulphate solution	0.9465	0.9478
0.1 N Hydrochloric acid solution	0.8021	0.7894

hydrochloric acid dissolution fluid, rifampicin degradation starts after 30 min. Two additional peaks were observed in the chromatograms at the retention time of 6–9 min, which increase with respect to area and height in subsequent analyses. This increase in additional peaks is reflected by the decrease in the peak area of rifampicin (Fig. 1). In the second dissolution medium such a phenomenon was not observed even in the 120 min sample (Fig. 2), indicative of no degradation during the total analysis time.

The mean serum levels obtained from three groups comprising of six volunteers each after oral ingestion of 450 mg rifampicin and two test formulations containing a mixture of 450 mg rifampicin and 300 mg isoniazid (products A and B), were correlated (Fig. 3). The *in vivo* correlation coefficients of 0.959 and 0.957 were obtained for products A and B, respectively. The *in vitro–in vivo* correlation coefficient obtained for both products in the two given dissolution media are shown in Table 3. The data indicate a good correlation with results obtained from the dissolution medium containing 0.4% (w/v) sodium lauryl sulphate. Furthermore, the *in vitro–in vivo* correlation values obtained from 0.4% (w/v) sodium lauryl sulphate solution medium are in conformity with the *in vivo* correlation coefficient.

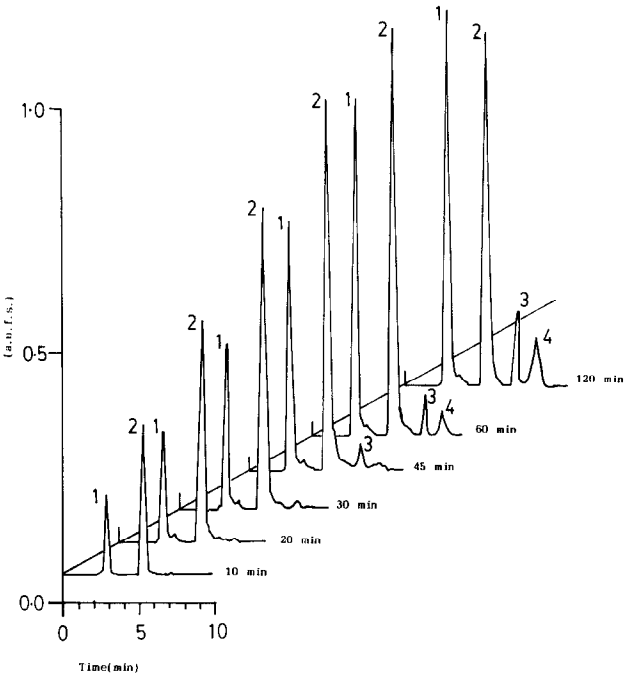

Figure 1
Separation and detection of isoniazid (1), rifampicin (2), and its two degradation components (3) and (4) in 0.1 N hydrochloric acid dissolution medium. The samples were collected after 10, 20, 30, 45, 60 and 120 min and analysed immediately. a.u.f.s. = absorbance unit full scale.

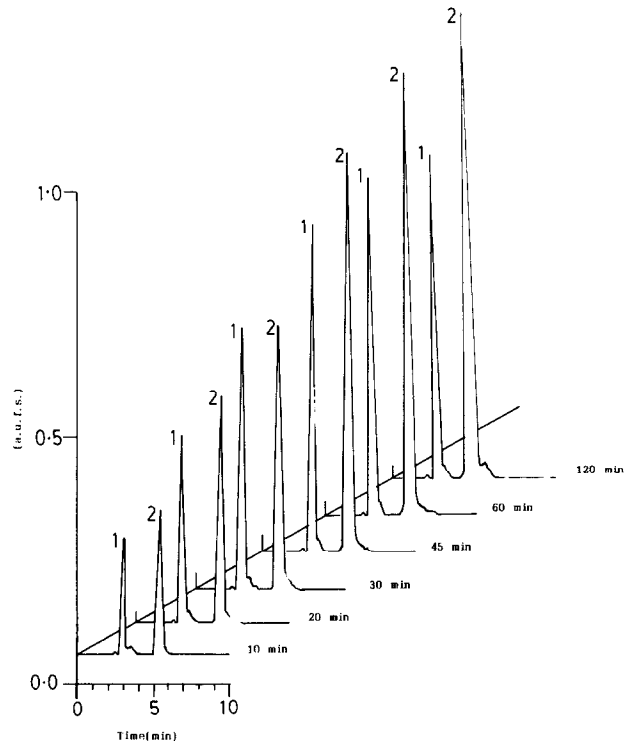

Figure 2
Separation and detection of isoniazid (1), rifampicin (2), in 0.4% (w/v) sodium lauryl sulphate dissolution medium samples, collected after, 10, 20, 30, 45, 60 and 120 min and analysed immediately. The chromatogram indicates absence of two degradation components (3) and (4). a.u.f.s. = absorbance unit full scale.

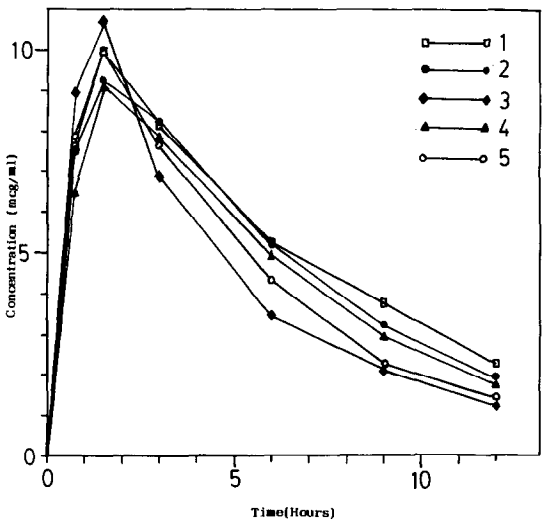

Figure 3
Mean serum concentration of rifampicin from 450 mg rifampicin (1), product A (2); product B (4); and of isoniazid from product A (3) and product B (5).

However after 45 min the concentration of rifampicin started to decrease in the dissolution fluid containing 0.1 N hydrochloric acid. The decrease in concentration of rifampicin ranged between 10 and 23% for six dosage units under test on keeping the solution at room temperature for 2 h. There was no significant effect on the dissolution behaviour of the second component as the isoniazid concentrations determined in both fluids were similar.

In summary, a 0.4% (w/v) solution of sodium lauryl sulphate has been found to be an appropriate dissolution medium for rifampicin–isoniazid fixed dose combinations.

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