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Short communication

Overestimation of rifampicin during colorimetric analysis of anti-tuberculosis products containing isoniazid due to formation of isonicotinyl hydrazone

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Abstract

When present together in fixed-dose combinations (FDC) of anti-tuberculosis drugs, rifampicin (R) and isoniazid (H) interact with each other to form isonicotinyl hydrazone (HYD). In a preliminary study, this product was found to possess similar colorimetric spectrum to that of rifampicin. Therefore, an investigation was undertaken to determine interference of HYD during analysis of rifampicin in FDC products by colorimetry. For the purpose, standard plots were constructed for rifampicin and HYD at 475 nm, the wavelength maximum for both the compounds. The plots were linear in the range of 10–100 μg/ml. Molar absorptivity values for rifampicin and HYD were 15279 and 5034, respectively. It indicated that HYD possessed one-third absorptivity to that of rifampicin. The analysis of combinations of rifampicin and HYD revealed that rifampicin could be overestimated to a maximum extent of 33%, while interference varied at other relative ratios of the two compounds. This was also confirmed by colorimetric and HPLC analysis of a degraded marketed product and samples from a dissolution study. Thus this investigation suggests that any method devoid of interference of HYD should be preferred for analysis of rifampicin, whenever it is present along with isoniazid.

Keywords: Rifampicin; Isoniazid; Isonicotinyl hydrazone; Colorimetry; Overestimation

1. Introduction

Three different techniques, viz. colorimetry [1–4], microbiology [5–8] and HPLC [9–13] are routinely employed for analysis of rifampicin (R) in formulations containing the drug alone or its fixed-dose combinations (FDC) with isoniazid (H), pyrazinamide (Z) and/or ethambutol (E). The colorimetric method is widely used because of its simplicity [1–3]. Even it has been an official method [4] till recently for analysis of dissolution samples of FDCs containing R + H and R + H + Z.

In recent studies, it was shown that R interacts with H, forming isonicotinyl hydrazone (HYD) as the major degradation product. It was established that HYD was formed when R and H were present together under stomach acid conditions [14–15] and during dissolution studies in USP recommended medium. Furthermore, it was found that the product was even generated when suspension of the two drugs and FDC tablets were stored under accelerated stability conditions [16–17].

Preliminary studies showed that HYD possessed similar colorimetric spectrum to that of R. This indicated possibility of overestimation of R in the presence of HYD during colorimetric analysis. Hence the objective of the present study was to explore the extent of interference of HYD during analysis of R in the presence of H by colorimetry at 475 nm.

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2. Experimental

2.1. Materials

R and H were gift samples from Panacea Biotec Ltd., Lalru, India. HYD was prepared by the reported method [18]. The commercial two- and three-drug FDC products (RIMACTAZID®, Novartis India Ltd., Mumbai, India, Batch No. 1X011L, Manufacturing date: October 2001, Expiry date: August 2004 containing 600 mg R and 300 mg H; and CONFEZ®-3, Deepin Pharmaceuticals Pvt. Ltd., Kalaria, India, Batch No. 2001, Manufacturing date: January 2002, Expiry date: December 2003 containing 450 mg R, 300 mg H and 800 mg E) were procured from local market. Buffer materials and all other chemicals were of analytical-reagent grade. HPLC grade acetonitrile and methanol were procured from J.T. Baker (Mexico City, Mexico) and Mallinckrodt Baker Inc. (Paris, KY, USA), respectively. Ultra-pure water was obtained from an ELGA water purification unit (Elga Ltd., Bucks, England).

2.2. Equipment

Samples were analyzed colorimetrically using a UV–vis spectrophotometer (Lambda 20, Perkin–Elmer, Norwalk, USA). pH recordings were made on a research pH meter (MA 235, Mettler Toledo GmbH, Schwerzenbach, Switzerland). Other equipment used were a sonicator (Branson Ultra-sonic Corporation, Danbury, CT, USA), an analytical balance (AG 135, Mettler Toledo, Switzerland) and auto pipettes (Tripette, Merck KGaA, Darmstadt, Germany). The HPLC system consisted of a dual-piston-reciprocating pump (LC-10ATVP), UV–vis dual-wavelength detector (SPD-10AVP), an auto injector (SIL-10ADVP), an on-line degasser (DGU-14AM), and CLASS-VP software (all from Shimadzu, Kyoto, Japan).

2.3. Methods

UV–vis spectra of 50 μg/ml solutions of R and HYD were scanned in the wavelength range of 200–800 nm. Standard curves for R and HYD were established between 10 to 100 μg/ml at wavelength maximum of 475 nm. Absorbance readings were also taken at the same wavelength for combinations of the two compounds in different ratios from 90:10 to 10:90. In addition, the commercial product containing R, H and E (CONFEZ®-3) was exposed to accelerated condition of 40 °C/75% RH in an unpackaged state for one month to induce reaction between R and H and formation of HYD. Separately, a dissolution study was carried out on RIMACTAZID® in 0.1 N HCl, the medium prescribed in USP [4]. In both cases, the samples were analyzed colorimetrically at 475 nm and by HPLC.

HPLC analysis were carried out using a reported gradient stability-indicating method [13]. The separation of HYD, R and H could be successfully achieved on a Zorbax XDB C-18 (250 mm × 4.6 mm, 5 μ) column (Agilent Technologies, Wilmington, USA). The mobile phase composed of acetonitrile and a buffer consisting of 0.01 M sodium dihydrogen orthophosphate (pH adjusted to 6.8 with dilute orthophosphoric acid).

3. Results and discussion

3.1. UV–vis spectra and linearity plots of R and HYD

Fig. 1 shows the UV–vis spectra of R and HYD in the wavelength range of 200–800 nm. It clearly indicates that both compounds have similar profiles, with wavelength of maximum absorbance in the colorimetric region as 475 nm. Evidently, HYD absorbed less strongly as compared to R at the same concentration.

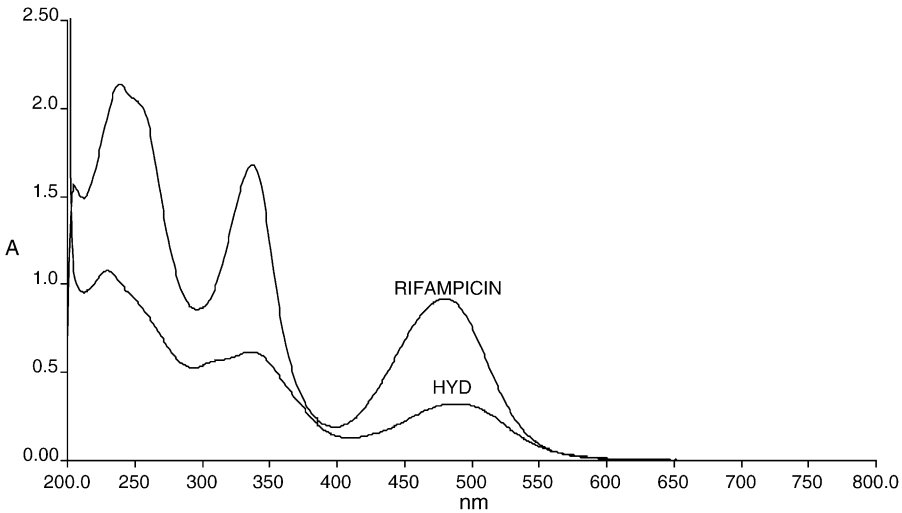

Fig. 1. UV–vis spectra of R and HYD at equal concentration of 50 μg/ml.

Table 1
Regression parameters of the linearity plots and specific and molar absorptivity values of R and HYD

Parameters	R	HYD
Slope	0.0174 (0.0006)[a]	0.0061 (0.0006)
Intercept	0.0251 (0.0046)	−0.0012 (0.0005)
Regression coefficient	0.999	0.9982
Specific absorptivity	182.6 (5.4)	59.6 (2.9)
Molar absorptivity	15278.9 (180.3)	5034.4 (151.4)

[a] The values in the parenthesis gives the S.D. of $n = 3$.

The regression parameters of the linearity curves of R and HYD are included in Table 1, along with specific and molar absorptivity values. The value of molar absorptivity of R is almost equal to that reported by Gallo and Radaelli [19]. The corresponding value for HYD is apparently one-third as compared to R.

3.2. Colorimetric analysis of R in the presence of different ratios of R:HYD

Fig. 2 shows the plots of absorbance values at varying concentrations (0–50 μg/ml) of R and HYD, both when present alone and in different ratios in combination. It is evident that the plot for R:HYD lies above the plots for R or HYD alone, indicating possibility of overestimation of R in the presence of HYD during colorimetric analysis. The plots show that extent of interference due to HYD increases, as its proportion gets higher in the mixture.

3.3. Testing of degraded and dissolution samples

Table 2 gives comparison of the results of colorimetric and HPLC analysis of R from a degraded marketed product and the samples from dissolution study. Clearly, R gets overestimated in the degraded sample by as much as 30% when analyzed colorimetrically against HPLC. This confirms that there can be wrongful estimation of R in the presence of H, if the samples on shelf or stability are analyzed colorimetrically. Further support is also provided by the result of the dissolution study where the determined overestimation is ∼6.2%, which is reasonable as only ∼20% of R gets converted to HYD in the presence of H in 45 min [14].

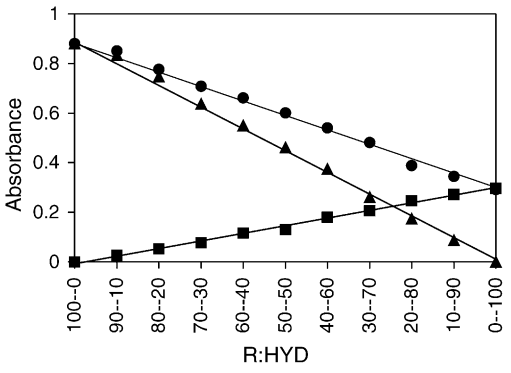

Fig. 2. Overestimation of R in the presence of HYD by colorimetric method. Key: (▲) R alone; (■) HYD alone; (●) R in the presence of HYD.

Table 2
Comparison of colorimetric and HPLC analysis of marketed formulation stored at 40 °C/75% RH for 1 month and dissolution samples

Sample	Assay value (%)		Percentage overestimation by colorimetry with respect to HPLC
	Colorimetry	HPLC	
Degraded product	43.5	30.4	30.11
Dissolution study	103.9	97.4	6.25

4. Conclusion

A strong possibility exists for overestimation of R if there is formation of HYD in the sample and direct analysis is done colorimetrically at 475 nm. Thus a method, which allows specific determination of R in the presence of HYD, should only be used for assay of R and its analysis in dissolution samples. This is important keeping into view the serious concern on the quality of anti-tuberculosis FDC products in worldwide circulation [20].

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