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Novel Method for the Spectrophotometric Determination of Isoniazid and Ritodrine Hydrochloride

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A simple, rapid and sensitive spectrophotometric method for the determination of isoniazid (INH) and ritodrine hydrochloride (RTH) in pure form as well as dosage forms is described. The method is based on the diazotisation of 4,4′-sulphonyldianiline (dapsone, DAP) followed by a coupling reaction with either INH or RTH in sodium hydroxide medium. Beer's law is obeyed in the concentration range of 0.5-20 μg ml^{-1} for INH at 440 nm and 0.5-18 μg ml^{-1} for RTH at 460 nm. The method is successfully employed for the determination of INH/RTH in pharmaceutical preparations and the results agree favourably with the official and reported methods. Common excipients used as additives in pharmaceuticals do not interfere in the proposed method. The method offers the advantages of simplicity, rapidity and sensitivity without the need for extraction or heating. Limit of detection (LOD) and limit of quantification (LOQ) are reported.

Key Words: Isoniazid, Ritodrine, Dapsone, Diazotisation, Spectrophotometry

Pyridine-4-carboxylic acid hydrazide, commercially known as isoniazid (INH), is an antitubercular drug and is now widely used together with rifampicin and streptomycin for the chemotherapy of tuberculosis. Ritodrine hydrochloride (RTH), chemically, 1-(4-hydroxyphenyl)-2-[2-(4-hydroxyphenyl)ethylamino]propanol is a potent tocolytic drug and is a selective β2-adrenoceptor agonist and is used to control premature labour and to reverse foetal distress caused by excessive uterine activity.

The importance of these two drugs has prompted many investigators to devise methods for the rapid determination of INH/RTH in its pure form as well as in pharmaceutical preparations. There are various analytical procedures for the assay of INH, may of which were reviewed in one of our earlier works on the spectrophotometric determination of isoniazid[1]. The spectrophotometric reagents for the determination of isoniazid include sodium 1,2-naphthoquinone-4-sulphonate and cetyltrimethyl ammonium

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bromide[1], vanadophosphoric acid[2], 2,5-diphenyl-3-thiazolyltetrazolium chloride[3], o-hydroxyquinolphthalein-Pd(II)-hexadecyl trimethyl ammonium chloride complex[4], 6,7-dichloroquinoline-5,8-dione[5], 1-fluoro-2,4-dinitrobenzene[6], p-benzoquinone[7], chloranil or bromanil[8], Fe(II) and 2,2′-dipyridyl[9], tetrazolium blue chloride[10], ethyl 8-quinolyloxyacetate[11] and N-bromosuccinimide with celestine blue[12]. The reported analytical procedures for the estimation of RTH in bulk samples and in unit dosage forms are reviewed in a recent spectrophotometric work for the determination of RTH[13]. The other spectrophotometric reagents for RTH include 3-methylbenzothiazolin-2-one hydrazone (MBTH)-Ce(IV)[14] and 4-aminoantipyrine with phenols in the presence of an alkaline oxidising agent[15]. The recent three methods[13−15]for the determination of RTH makes use of an oxidizing agent like Ce(IV) or chloramine-T(CAT) along with reagents like MBTH, N,N-dimethylamino- paraphenylenediamine (DMPD), 4-aminophenazone and 4-aminoantipyrine. There are several oxidising agents which give colour with these reagents and MBTH is becoming a universal reagent for the determination of various drugs. The reported methods[13,14] make use of MBTH-Ce(IV), as MBTH is not economical and the results are inconsistent. Bakry et al.[16] have determined RTH with 2,6-dihaloquinone chlorimides. Both isoniazid and ritodrine hydrochloride are listed in the United States Pharmacopoeia[17,18].

In continuation of our work[19−26] on the spectrophotometric determination of organic compounds of pharmaceutical importance, the present work reports an elegant method for the determination of INH/RTH in pure form or in pharmaceutical preparations. The method is based on the diazotisation of DAP followed by a coupling reaction with either INH or RTH in alkaline medium. The method does not make use of any oxidising agent and avoids heating or extraction. This colour reaction is being reported for the first time.

Experimental

A JASCO MODEL UVIDEC-610 UV-VIS spectrophotometer with 1.0 cm matched cells was used for absorbance measurements. INH (BDH, Poole, England), RTH (Duphar-Interfran Ltd., Mumbai, India), and DAP (Sigma, USA) were used without further purification. Sodium nitrite (BDH), sodium hydroxide (GR), hydrochloric acid (AR) and all other reagents and solvents were of analytical grade. Commercial dosage forms were purchased from local sources.

Deionised water was used to prepare all solutions. Standard solutions of INH or RTH (1000 μg ml^{-1}) were prepared by dissolving 100 mg of drug in water and then diluting to the mark in a 100 ml volumetric flask. A working standard solution of the drug containing either 25 or 50 μg ml^{-1} was prepared by further dilution. Isoniazid and ritodrine hydrochloride solutions were standardised by the official method[17] and 2,6-dihaloquinone chlorimide method[16] respectively. A 0.2% solution of dapsone in water (add 1 ml of dilute hydrochloric acid), 1% solution of sodium nitrite in water, 2% sulphamic acid solution, 5 N hydrochloric acid solution and a solution of 5 N sodium hydroxide were used.

Recommended Procedure

For Pure INH or RTH

Three millilitres of 0.2% solution of dapsone was taken in a series of 25 ml standard flasks. A 5 N solution of hydrochloric acid (0.5 ml) was added to the dapsone solutions and cooled for 5 min in an ice bath. Then 1 ml of a 1% solution of sodium nitrite was added to each flask with swirling. It was cooled for 5 min and then 2 ml of 2% sulphamic acid was added. The solutions were swirled and allowed to stand for 5 min.

Then, INH solution (12.5-500 μg) or RTH solution (12.5-450 μg) was added followed by the addition of 2 ml of 5 N sodium hydroxide solution, made up to the mark with water, mixed thoroughly and after 5 min the absorbance was measured at 440 and 460 nm respectively for INH and RTH against the corresponding reagent blank and the calibration graph was constructed.

For dosage forms

Twenty tablets were weighed and finely powdered. A powdered amount equivalent to 50 mg was dissolved in water (dilute hydrochloric acid for RTH) and filtered (for the syrup or injection, an appropriate volume was taken). The filtrate was made up to 100 ml and appropriate aliquots of the tablet/syrup/injection solutions were treated as described in the recommended procedure for pure samples.

Results and Discussion

The method involves the diazotisation of dapsone followed by coupling with INH or RTH in an alkaline medium to produce a yellow (for INH) or orange yellow (for RTH) product.

Spectral characteristics

The absorption spectra of the yellow product (1) with maximum absorption at 440 nm and of the orange yellow product (2) with maximum absorption at 460 nm are shown in the Figure. The colourless reagent blank has practically negligible absorption at these wavelengths in both cases. The optical characteristics and precision data for both methods are given in Table 1.

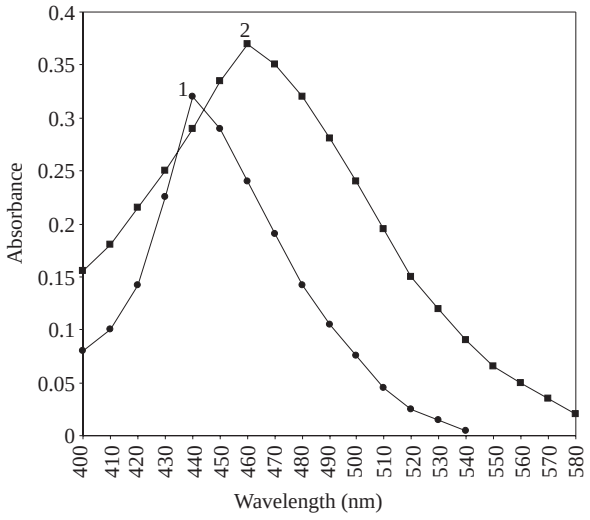


Figure. Absorption spectra of the reaction product (1) of INH with DAP and of reaction product (2) of RTH with DAP. Final drug concentration = 10 μg ml^{-1}.

Optimum reagent concentrations

It was found that a 0.2% solution of dapsone in the range of 2-4 ml, a 5 N solution of hydrochloric acid in the range of 0.25-1.0 ml, a 1% solution of sodium nitrite in the range of 0.5-2.0 ml, a 2% solution of sulphamic

acid in the range of 1-3 ml and 1-3 ml of 5 N NaOH solution were necessary to achieve maximum colour intensity with both INH and RTH. Hence, the required volumes of all the reagents and solutions were used (as mentioned in the recommended procedure) to produce the coloured product. The excess of nitrite during diazotisation could be removed by the addition of sulphamic acid solution and an excess of sulphamic acid solution has no effect on the colour intensity.

Table 1. Optical characteristics and precision data

Parameters / Characteristics	INH - DAP	RTH – DAP
Colour	Yellow	Orange yellow
λ_{max} (nm)	440	460
Stability (Hrs)	01	72
Beer's law range (μg ml^{-1})	0.5-20	0.5-18
Limit of detection (μg ml^{-1})	0.3243	0.2182
Limit of quantification (μg ml^{-1})	1.0811	0.7274
Molar absorptivity (l mol^{-1} cm^{-1})	5.729 x 10^3	1.163 x 10^4
Sandell's sensitivity (μg cm^{-2})	0.0239	0.02471
Optimum photometric range (μg ml^{-1})	0.7-18	0.8-16
Regression equation (Y = bx + a, where x is the concentration in μg ml^{-1})		
Slope (b)	0.0259	0.03593
Intercept (a)	0.0473	0.00588
Correlation coefficient (r)	0.9961	0.9998
Relative standard deviation (%) for n = 10	0.35	0.45
Range of error (%)	±0.4908	±0.6196

Reaction sequence

For the diazotisation process, dapsone could be readily diazotised in acidic medium and each diazonium cation would then react with a molecule of INH or RTH by electrophilic substitution at the position ortho to the hydrazide group (INH) or ortho to the phenolic hydroxyl group (RTH), and this results in the formation of the coloured products. An investigation of the continuous molar variation of the INH/RTH and DAP showed that the drug interacts with DAP at a ratio of 2:1. Similar results have been observed with the mole ratio method. A reaction sequence based on the above results is shown in the Scheme.

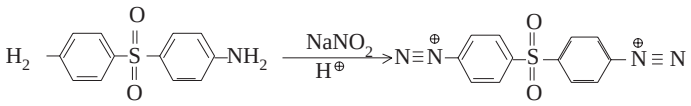
Stability of the products

The yellow product (INH-DAP) was stable for about 1 h. The orange yellow product (RTH-DAP) was stable for more than 3 days. Attempts to increase the stability of the yellow product beyond 1 h were unsuccessful. This was done by varying the concentrations and volumes of all the reagents involved in the experiment. A temperature range of 20-30°C is preferred for both coupling reactions.

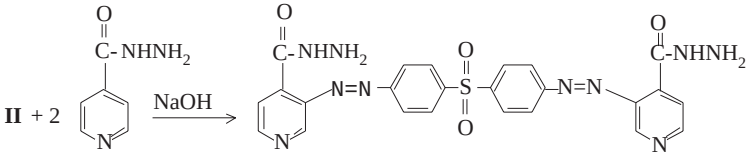
Quantification, accuracy and precision

The validity of the proposed procedure for the determination of the studied drugs in their pure state and in their pharmaceutical formulations was tested by analysing these drugs using the proposed procedure and the official methods[17,18]. The results obtained for pure drugs (Table 1) were reproducible with low relative

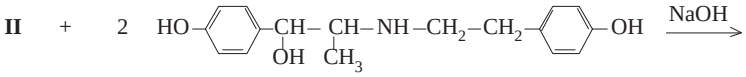
standard deviations (0.35-0.45%) and the mean recoveries were comparable to those obtained using the official methods for each of the studied drugs.



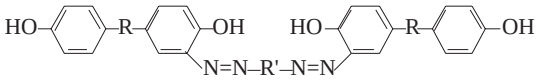
DAP (I) **(II)**



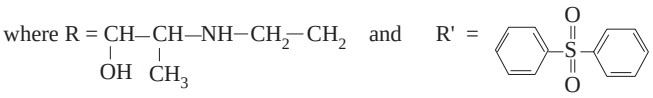
INH (III) **Coupled Product (IV)**



RTH (V)



Coupled product (VI)



Scheme

LOQ is determined by taking the ratio of standard deviation (σ) of the blank with respect to water and the slope of calibration curve (s) multiplied by a factor of 10. This means that LOQ is approximately 3.3 times LOD. Naturally, the LOQ slightly crosses the lower limit of Beer's law range. However, LOD is well below the lower limit of Beer's law range. The upper limit of the Beer-Lambert range is determined by a plot of absorbance against concentration at the value of λ_{max}. Beyond this limit, the correlation results were strongly affected. Hence, the measurements were excluded above these limits to keep the relationship linear.

Statistical analysis of the results in comparison with the official method

The performance of the proposed method was compared statistically in terms of Student's t-test and the variance ratio F-test. At 95% confidence level, the calculated t-values and F-values do not exceed the theoretical values for either INH or RTH. The theoretical t-value was 2.776 (for n = 5) and F-value was

6.39 (for n = 5). Therefore, there is no significant difference between the proposed method and the official method, indicating that the proposed method is as accurate and precise as the official method.

Interference

The extent of interference by some excipients which often accompany pharmaceutical preparations were determined by measuring the absorbance of solutions containing 10 μg ml^{-1} of INH or RTH and various amounts of diverse species. It was found that the excipients studied do not interfere in the present method, even when present in excess. An error of 2.0% in the absorbance readings was considered tolerable. Typical results are given in Table 2.

Table 2. Determination of INH/RTH (10 μg/ml of drug taken) in presence of excipients

Excipient	Excipient taken (μg/ml)	% Recovery of drug ± % RSD (n = 5)	
		INH	RTH
Talc	50000	99.7 ± 0.3	99.6 ± 0.35
Gum acacia	50000	99.5 ± 0.25	99.4 ± 0.3
Sodium chloride	50000	99.6 ± 0.35	99.5 ± 0.4
Glucose	35000	100.4 ± 0.25	100.2 ± 0.3
Lactose	35000	100.5 ± 0.28	99.8 ± 0.4
Sodium alginate	40000	99.2 ± 0.28	99.3 ± 0.3
Carboxy methylcellulose	40000	99.4 ± 0.35	99.5 ± 0.4
Magnesium stearate	40000	99.5 ± 0.3	99.7 ± 0.25
Starch	50000	99.6 ± 0.35	99.9 ± 0.35
Dextrose	40000	100.3 ± 0.25	100.4 ± 0.4
Vitamin-B_6	20000	99.3 ± 0.35	99.4 ± 0.45

Application

The reproducibility of the method was checked by 10 replicate determinations at 10 μg ml^{-1} level of INH or RTH and the maximum relative standard deviation (%) was found to be 0.45. The present method has been successfully employed for the analysis of INH or RTH in commercial dosage forms. The results of the analysis of pharmaceutical preparations are given in Table 3 and compare favourably with those of the official methods[17,18].

Conclusion

The proposed method is found to be simple, rapid and economical and will compete with most of the spectrophotometric methods available in the literature. The proposed method is advantageous over many spectrophotometric methods, as heating or extraction is avoided. The method has a higher sensitivity and stability than some of the spectrophotometric methods available and is sufficiently sensitive to permit determination even up to 0.5 μg ml^{-1}. The method does not make use of any oxidant, organic dye or catalyst, thereby avoiding possible errors in the determination of INH and RTH. The statistical parameters and recovery study data clearly indicate the reproducibility and accuracy of the method. The recommended procedure is well-suited for the assay and evaluation of drugs in pharmaceutical preparations to assure high standards of quality control.

Table 3. Determination of INH/RTH in pharmaceutical preparations

Drug	Label claim per tablet or per ml (mg)	Amount of drug found in mg ± SD (n = 5)			
		Proposed method	Official method[17,18]	t value	F value
Isoniazid					
Isokin tablet (Warner)	100	99.6 ± 0.4	99.4 ± 0.5	1.396	1.56
Isokin liquid (Warner)	20	19.5 ± 0.3	19.4 ± 0.5	0.767	2.78
Isonex tablet (Pfizer)	100	99.7 ± 0.3	99.5 ± 0.4	1.789	1.78
	300	299.2 ± 0.4	299.0 ± 0.5	1.396	1.56
Solonex tablet (Macleods)	100	99.7 ± 0.3	99.4 ± 0.5	2.301	2.78
	300	298.5 ± 0.4	298.3 ± 0.5	1.396	1.56
Ipcazide tablet (IPCA)	100	99.2 ± 0.3	99.0 ± 0.4	1.789	1.78
Ipcazide liquid (IPCA)	20	20.2 ± 0.3	20.4 ± 0.5	1.534	2.78
Ritodrine					
Ritodine tablet (Triokaa)	10	9.95 ± 0.4	9.92 ± 0.5	2.095	1.56
Ritodine injection (Triokaa)	10	9.95 ± 0.5	9.92 ± 0.3	2.301	2.78
Yutopar tablet (Duphar-Interfran)	10	9.90 ± 0.4	9.92 ± 0.3	1.789	1.78
Yutopar injection (Duphar-Interfran)	10	9.92 ± 0.3	9.95 ± 0.4	2.684	1.78

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