TABLE 2: ANALYSIS DATA OF TABLET FORMULATION.

Formulation (Tablet)	Label Claim (mg/tab)	Amount found (mg/tab)	% of Label claim* ± S.D.	% COV	S.E. of Mean	% Recovery
Brand 1	5	4.97	98.72 ± 0.99	1.0028	0.6877	101.5395
Brand 2	5	4.96	98.66 ± 1.02	1.0338	0.6897	100.3246

*Mean of five determinations.

diazonium salt with NED in presence of ammonium sulphamate producing purple chromogen[4,5]. Stability of the chromogen was determined by measuring the absorbance values of chromogen at 540 nm at time interval of 30 min and was found to be stable for 5 h.

The optical characteristics such as absorption maxima, Beer's law limits, correlation coefficient (r), slope (m), y-intercept (c), molar absorptivity, Sandell's sensitivity and percent range of error have been calculated from measurements containing 3/4th of upper Beer's law limit and are summarized in Table 1. The molar absorptivity and Sandell's sensitivity show that the method is sensitive. Percent COV (coefficient of variance) and percent range of error reveal the precision of the method.

To test the accuracy and reproducibility of the proposed method, recovery experiments were performed by adding known amount of drug to the preanalyzed formulations and reanalyzing the mixture by proposed method. The results of analysis of marketed formulation are shown in Table 2. The reproducibility and accuracy of the method was found to be good which is evidenced by low standard deviation. The percent recovery values indicate non-interference from the excipients used in formulation. In conclusion, the method developed in the present investigation is simple, sensitive, precise and accurate. Hence it can be successfully applied in estimation of mosapride citrate in pharmaceutical solid dosage forms such as tablets.

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REFERENCES

1. Parfitt, K., Eds., In; Martindale: The complete drug reference, 32nd Edn., The Pharmaceutical press, London, 1999, 1203.
2. Mishra, L., Eds., In; Drugs Today, Vol. 9, Lorina Publications Inc., New Delhi, 2002, 19.
3. Morie,T. and Kato, S., **Chem. Pharm. Bull.**, 1994, 42, 877.
4. Choudhary, K.P.R., **Indian Drugs**, 1997, 34, 396.
5. Sethi, P.D., In; Quantitative Analysis of Drug in Pharmaceutical Formulations, 2nd Edn., CBS Publisher and Distributors, New Delhi, 1993, 48.

Spectrophotometric Determination of Isoniazid in Pure and Pharmaceutical Formulations

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A simple, sensitive and accurate spectrophotometric method has been proposed for the determination of isoniazid in pharmaceutical formulations. The method is based on the oxidation of tiron

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by potassium periodate followed by coupling with isoniazid in alkaline medium leading to the formation of a red coloured product having maximum absorbance at 505 nm. The reaction conditions were optimized to obtain the maximum colour intensity. The absorbance was found to increase linearly with increase in concentration of isoniazid, which was corroborated by the calculated correlation coefficient (0.9991). The system obeyed Beer's law in the concentration range of 1.5-18 μg/ml. The common excipients and additives used in pharmaceutical formulations do not interfere with the proposed method. The method was successfully applied for the assay of INH in various pharmaceutical formulations and the results compare favorably with those of the official method.

Pyridine-4-carboxilic acid hydrazide commercially known as isoniazid (INH) is an antitubercular drug and is widely used together with rifampicin and streptomycin for the chemotherapy of tuberculosis[1,2]. This has prompted many researchers to develop accurate and rapid methods for the determination of INH in pure and pharmaceutical formulations. Of the various techniques used for the determination of this drug, FIA-spectrofluorimetry[3-4], polarography[5] and adsorptive stripping voltammetry[6] are of high cost and are not available at most of the quality control laboratories. Other methods include high performance liquid chromatography[7] and indirect potentiometnc titration[8]. Many spectrophotometric methods have been described for the assay of INH in pharmaceutical preparations; some of these are either time bound[9-11], involve extraction[12], require heating[13-15], need cooling to lower temperature for long time[16] or less sensitive[10,13,15]. The USP method[17] involves titration procedure, which is laborious and time consuming. Hence, it was planned to develop reasonably sensitive and economically viable technique like spectrophotometric procedure.

In the present paper we report a rapid and sensitive spectrophotometric method for the determination of INH in pure and pharmaceutical formulations based on the oxidation of tiron by potassium periodate (PPI) followed by coupling with INH in alkaline medium.

A Shimadzu 160 UV/vis spectrophotometer with 1 cm matched quartz cells was used for absorbance measurements. All chemicals used were of analytical reagent or pharmaceutical grade and quartz-processed high purity distilled water was used throughout.

Standard solution of INH (Cadila Pharmaceuticals Ltd., Ahmedabad) was prepared by dissolving 100 mg of INH in distilled water in a 100 ml calibrated flask to obtain a final concentration of 1000 μg/ml. Solution of lower concentration was prepared by suitable dilution whenever necessary. Stock solutions of 0.05% PPI, 0.5% tiron and 0.1 M sodium hydroxide were prepared separately in distilled water.

Aliquots of standard drug solution containing 15-180 μg of INH were transferred in to a series of 10 ml calibrated flasks. Then, 1.0 ml of 0.05% PPI, 0.5 ml of 0.5% tiron and 1.0 ml of 0.1 M sodium hydroxide were added to each of the flask and diluted to the mark with distilled water. The absorbances of the red coloured product were determined at 505 nm against the reagent blank. Calibration graph was constructed or regression equation calculated.

Twenty tablets of INH were weighed, finely powdered and an amount equivalent to 50 mg of INH was treated with water and filtered. The filtrate was made up to 100 ml and a suitable amount of an aliquot was treated as described for pure sample. For the syrup, an appropriate volume of the sample was taken and analysed for INH using the procedure described earlier.

Tiron readily undergoes oxidation by PPI which further couples with INH in presence of sodium hydroxide to form an intense red colored product having absorption maximum at 505 nm as shown in fig. 1. The reagent blank does not absorb around this wavelength. The tentative reaction mechanism of the formation of oxidative coupled product[2] may be represented as shown in fig. 2. Other oxidising agents such as, quinolinium dichromate, chloramine T, sodium orthovanadate, pyridinium chlorochromate, iodic acid and potassium bromate did not give the red colored product when tried in place of PPI. Less intense coloured products were obtained with N-bromosuccinimide, potassium ferricyanide, ceric ammonium sulphate, bromine chloride, iodine trichloride and potassium dichromate. The oxidative coupled product was stable in the temperature range of 10-40°. The absorbance values remained constant for 45 min at room temperature (27°). At higher temperatures, absorbance values decreased indicating the dissociation of the product. The effects of the reagents such as, PPI, tiron and sodium hydroxide were studied by measuring the absor-

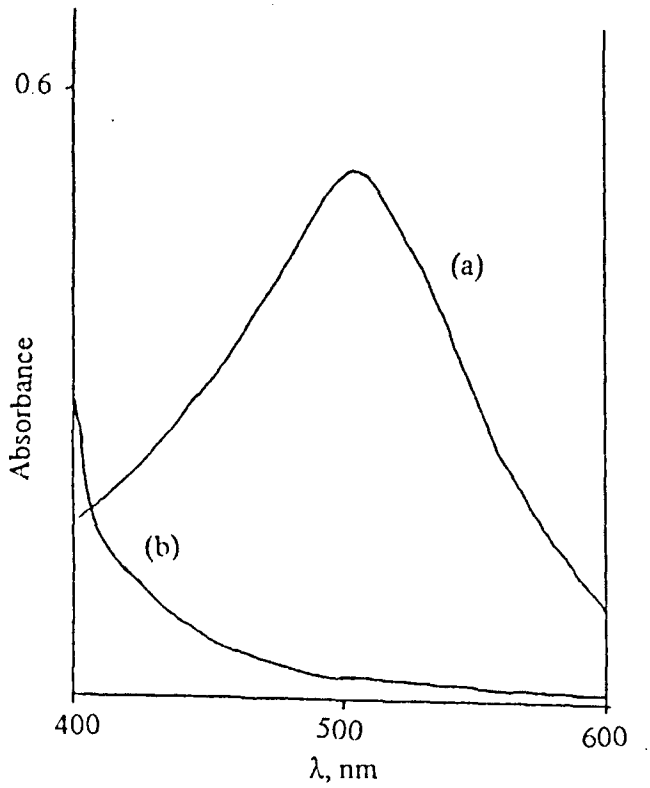

Fig. 1: Absorption spectra of (a) INH-Tiron-PPI system and (b) the reagent blank.

bances of solutions containing a fixed concentration of INH and varying amounts of reagents at 505 nm. The constant and maximum absorbance readings were obtained with 1.0 ml of 0.05% PPI, 0.5 ml of 0.5% tiron and 1.0 ml of 0.1 M NaOH. The order of addition of reagents was critical for colour development and NaOH should be added at the end as stated in the procedure.

The Beer's law limits, molar absorptivity and Sandell's sensitivity values, regression equation and correlation coefficient are given in Table 1. The precision value of the proposed method was good as indicated from the low relative standard deviation (less than 1.0%) calculated from six replicate analyses of INH.

In order to assess the possible analytical applications of the proposed method, the effects of some substances that often accompany INH in various pharmaceutical products were studied by adding different amounts of the substances to 10 μg/ml of INH. An attractive feature of the method is its relative freedom from interference by the usual tablet excipients and additives in amounts far in excess of their normal occurrence in the pharmaceutical preparations.

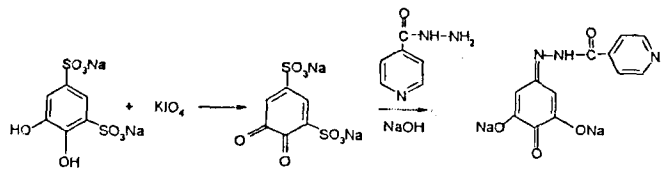

Fig. 2: Reaction scheme.

The results are given in Table 2.

The recovery technique was applied to judge the suitability of the proposed method by adding definite amounts of INH to preanalysed mixtures. The total amount of the drug was then determined using the proposed method and the amount of the added drug was calculated by difference. The results were found to be satisfactory.

The applicability of the proposed method was examined to the assay of pharmaceutical preparations containing INH. The results of the assay of tablets and syrups (Table 3) compare favorably with the quoted values and those obtained by official[17] method.

The performance of the proposed method was judged by calculating the Student t-test and variance ratio F-test. At the 95% confidence level, the calculated t-and F-values do not exceed the theoretical values (Table 3), indicating that there is no significant difference between the proposed

TABLE 1: OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY DATA.

Parameter	Value
λmax (nm)	505
Beer's law limits (μg/ml)	1.5-18
Molar absorptivity (l/mol.cm)	1.77×10^4
Sandell's sensitivity (ng/cm²)	7.74
Regression equation*, Y	
Slope, a	0.0824
Intercept, b	0.0573
Correlation coefficient	0.9991
Relative standard deviation **, %	0.84
% Range of error **	
(95 % confidence limit)	0.75

* Y=ax+b, where x is the concentration in μg/ml, ** Average of six determinations.

TABLE 2: DETERMINATION OF 10 MG OF INH IN THE PRESENCE OF EXCIPIENTS AND OTHER SUBSTANCES.

Interfering substance	Amount taken (mg)	Amount of INH found* (mg)	RSD
Magnesium stearate	40	9.96	0.71
Glucose	40	9.91	0.89
Lactose	30	9.93	0.81
Dextrose	30	9.92	0.88
Starch	40	9.90	0.75
Talc	40	9.92	0.91
Gelatin	30	9.97	0.93
Gum acacia	40	9.93	0.86
Streptomycin sulphate	20	9.89	0.88
Vitamin B_6	20	9.96	0.92
Nicotinamide	40	9.91	0.85

* Average of six determinations.

TABLE 3: ANALYSIS OF INH IN PHARMACEUTICAL FORMULATIONS.

Dosage form	Amount taken (mg)	Found values* ± SD, % and its comparison with the official method	
		USP[17] method	Proposed method
Tablets			
Isokin (Warner)	100	98±1.30	99.12±1.01 F=1.65; t=1.34
Isonex(Dumex)	100	102±1.10	101.1±0.91 F=1.46; t=1.64
Isonex Forte (Dumex)	300	296±0.85	298±0.92 F=1.17; t=1.45
Syrup Isokin (Warner)	20	20.5±1.00	19.92±0.86 F=1.35; t=1.62
Isocaldin Retort (Warner)	30	29.8±0.96	30.4±0.73 F=1.72; t=1.68

* Average of five determinations.

method and the official method.

The reagents used in the proposed method are cheaper and readily available. Moreover, the procedure does not involve any critical reaction conditions such as heating, extraction or tedious sample preparation and is free from interferences by common excipients and additives. Hence it

could be adopted for routine quality control.

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REFERENCES

1. Reynolds, J.E.F., Eds., In; Martindale, The Extra Pharmacopoeia, 30th Edn., The Pharmaceutical Press, London, 1993, 21.
2. Nagaraja, P., Srinivasa Murthy, K.C. and Yathirajan, H. S., **Talanta,** 1996, 43, 1075.
3. Xingwang, Z. and Zhujun, Z., **Analyst,** 1999, 124, 763.
4. Garcia, B.J.A., Garcia, M.J.U. and Martinez, C.J., **Anal. Lett.,** 1998, 31, 1209.
5. Darwish, A., Fathy, A. and Mohammed, A.A.G., **Iraqi J. Sci.,** 1988, 29, 149.
6. Liquiang, L., Zhen Hui, W. and Shuping, Z., **Fenxi Shiyanshi,** 1998, 17, 9.
7. Pawar, S.P., Pal, S.C., Kasture, S.B., Kasture, V.S., Chatterjee, B.P. and Nandy, A., **Eastern Pharmacist,** 1998, 41, 123.
8. Riyazuddin, P. and Nazer, M.M.A., **Indian J. Pharm. Sci.,** 1998, 60, 158.
9. Manna, A., Ghosh, I., Datta, S., Ghosh, P.K., Ghosh, L.K. and Gupta, B.K. **Indian J. Pharm. Sci.,** 2000, 62, 185.
10. Mahfouz, N.M.A. and Emara, K.M., **Talanta,** 1993, 40, 1023.
11. Siraj, P., Rama Krishna, R., Murty, S.S.N., Reddy, B.S. and Sastry, C.S.P., **Talanta,** 1981, 28, 477.
12. Sawsan, A.M., El-Sherif, Z. and Aamer, M.M., **Egypt J. Pharm. Sci.,** 1994, 35, 627.
13. Issopoulos, P.B., **Indian Drugs,** 1991, 29, 171.
14. Abou-Ouf, A.A., Taha, A.M. and Saidhom, M.B., **J. Pharm. Sci.,** 1973, 62, 1700.
15. Issopoulos, P.B., **Pharm. Acta Helv.,** 1989, 64, 280.
16. Devani, M.B., Shishoo, C.J., Doshi, K. and Bhalara, D.D., **Indian J. Pharm. Sci.,** 1980, 42, 179.
17. United State Pharmacopoeia, XXII, National Formulary XXI, Rockville, MD, 1990, 728.

Simultaneous Spectrophotometric Estimation of Cefuroxime Axetil and Probenecid from Combined Dosage Form

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Three simple, accurate and economical procedures for simultaneous estimation of cefuroxime axetil and probenecid in two component capsule formulations have been developed. The methods employ program in the multicomponent mode of analysis of the instrument used, the area under curve method and the graphical absorption ratio method. All these methods utilize 0.05 M NaOH as a solvent. In this solvent system cefuroxime axetil shows maximum absorbance at a wavelength of 278 nm and probenecid shows maximum absorbance at a wavelength of 244.2 nm. The results of analysis have been validated statistically and by recovery studies.

An extensive literature survey revealed HPLC[1-2], spectroflurimetric[3], and first derivative spectrophotometric and liquid chromatographic determination methods[4] for the analysis of cefuroxime axetil, whereas HPLC[5-7] and spectrophotometric[9] methods for the analysis of probenecid. Not a single method has been reported for the simultaneous estimation of both these components from a combined dosage form. The objective of this investigation was to devise

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