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Spectrophotometric Estimation of Melatonin and Pyridoxine Hydrochloride in Combined Dosage Forms

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Simple colorimetric methods for the estimation of melatonin and pyridoxine hydrochloride in combined dosage form are described. Estimation of melatonin in presence ot pyridoxine hydrochloride is based on oxidative coupling reaction using 3-methylbenzothiazolin-2-one hydrazone (MBTH) and cerric ammonium sulphate. The reddish colored melatonin complex is measured at 530 nm against reagent blank it obeyed linearity over 1.2 to 15.6 μg/ml. Pyridoxine hydrochloride is estimated using ferric nitrate and 1,10-phenanthroline producing reddish brown colored chromogen, measured at wavelength of maximum absorption 510 nm against reagent blank. The chromogen obeyed linearity over 12.0 to 32.0 μg/ml for pyridoxine hydrochloride in presence of melatonin.

Chemically melatonin is N-[2-(5-methoxy-I-H-indol-3-yl)ethyl] acetamide[1]. It is recommended in psychiatric neurological and cardiovascular disorders[1]. It is not official in any pharmacopoeia and available in tablet form (Meloset tablets, Aristo Pharma). Literature survey reveals that the drug has been analyzed by voltametry[2], immunoassay[3], amperometry[4], electrophoresis[5], HPLC[6,7] and spectrophotometric methods[8]. Chemically, pyridoxine hydrochloride is 5-hydroxy-6-methyl-3,4-pyridinedimethanol hydrochloride[9]. It is used as a vitamin or a enzyme cofactor. It is available as pyridoxine hydrochloride powder[10-13], tablets[10,12] and injection[12-13]. Literature survey revealed that pyridoxine has been analyzed by non-aqueous[10,11,13], HPLC[12] and spectrophotometric methods.[10,12,13] Spectrophotometric methods were also reported for estimation of these drugs in combined dosage forms[14,15].

The present work describes simple colorimetric methods for estimation of melatonin and pyridoxine hydrochloride in combined dosage forms (Eternex, Dabur India Ltd.). A Systronic spectrophotometer 106 with 1 cm-matched cuvettes was used for spectrophotometric estimation. Cerric

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ammonium sulphate (0.01% in 1 M sulphuric acid), MBTH (0.3% solution in distilled water), 10% ferric nitrate solution in 5% nitric acid, 1,10-phenanthroline solution (0.02 M in distilled water) were freshly prepared. Standard solutions of melatonin (60 µg/ml) and pyridoxine hydrochloride (200 µg/ml) were prepared in distilled water.

Twenty tablets (each containing melatonin 3 mg and pyridoxine hydrochloride 10 mg) were weighed and the average weight was determined. The tablets were powdered in a glass mortar and amount equivalent to 6 mg of melatonin and 20 mg of pyridoxine hydrochloride was transferred to a 100 ml volumetric flask, dissolved in distilled water and the final volume was made up to the mark with the same solvent.

Aliquots of 0.2 ml to 3.0 ml portion of standard solution of melatonin were transferred to a series of 10 ml corning test tubes. To each test tube, 3.5 ml of cerric ammonium sulphate reagent and 1.5 ml of MBTH were added. The solution was heated on a boiling water-bath for 5 min, cooled and diluted to 10 ml with distilled water. The absorbance was measured at 530 nm against reagent blank. The calibration curve was constructed and found to be linear over 1.2 to 15.6 µg/ml of melatonin. Similarly the absorbance of sample solution was measured and the amount of melatonin was determined by referring to the calibration curve.

Aliquots of 0.2 to 2.0 ml portions of standard solution of pyridoxine hydrochloride were transferred to a series of 10 ml corning test tubes. To each test tube, 0.5 ml of ferric nitrate reagent and 3.0 ml of 1,10-phenanthroline were added. After thorough shaking the test tubes was set-aside for 10 min and diluted to 10 ml with distilled water. The absorbance of the solution in each test tube was measured at 510 nm against reagent blank prepared in similar manner without addition of drug. The calibration curve was plotted and found to be linear over the concentration range of 12.0 to 32.0 µg/ml. Similarly the absorbance of sample solution was measured and the amount of pyridoxine hydrochloride in sample solution was determined by referring to the calibration curve. To test the accuracy and reproducibility of the proposed method, recovery experiments were performed by adding known amount of drug to the preanalyzed formulation, reanalyzing the mixture by proposed method and the results were shown in Table 1.

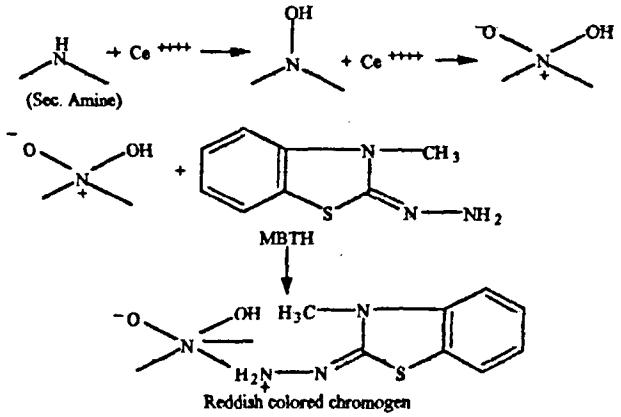
Melatonin is estimated on the basis of oxidative coupling reaction of the drug with 3-methylbenzothiazolin-2-one hydrazone (MBTH) in presence of cerric ammonium sulphate in 1M sulphuric acid producing the reddish colored chromogen (Scheme 1). The phenolic hydroxyl groups in pyridoxine hydrochloride are oxidized with ferric ions to produce ferrous ions that subsequently forms reddish brown colored complex with 1,10-phenanthroline (Scheme 2). The color intensity of chromogen in estimation of melatonin in presence of pyridoxine hydrochloride was intensified with 3.5 ml of cerric ammonium sulphate reagent and 1.5 ml of MBTH. The chromogen for pyridoxine hydrochloride in presence of melatonin was intensified with 0.5 ml of ferric nitrate reagent and 3.0 ml of 1,10-phenanthroline. Stability of the colored complex was determined by measuring absorbance value of chromogen at time intervals of 15 min and was found to be stable for 2.0 h for melatonin and 1.5 h for pyridoxine hydrochloride.

The calibration curve yielded coefficient of correlation (*r*) of 0.9996 for melatonin and 0.9982 for pyridoxine hydrochloride over the Beer's range of 1.2 to 15.6 µg/ml and 12 to 32 µg/ml, respectively. The regression equation for melatonin was found to be y=0.04133 x + 0.01532 and y=0.02729 x + 0.1623 for pyridoxine hydrochloride The molar absorp-

TABLE 1: ANALYSIS DATA OF TABLET FORMULATION.

Formulation		Label claim (mg/tab)	Amount found (mg/tab)	% of label claim* ± Standard Deviation	Standard Error	% Recovery*
Tablet	Melatonin	3	2.99	99.74±0.97	0.7356	99.3615
	Pyridoxine Hydrochloride	10	9.97	99.67±1.02	0.4198	99.6541

* Mean of five determinations.



SCHEME 1:

Oxidative coupling reaction of melatonin with cerric ammonium sulphate and MBTH.

tivity and Sandell sensitivity are 9.6022 x 10^3 l/mole/cm and 0.02419 µg/sq.cm/0.001 for melatonin and 5.6125x10^3 l/mole/cm and 0.03664 µg/sq.cm/0.001, respectively for pyridoxine hydrochloride, which indicate that the method is highly sensitive. The results of analysis of marketed formulation are shown in Table 1. The low values of 95% confidence limit of 0.3708 for melatonin and 0.9082 for pyridoxine hydrochloride obtained indicating high precision of the method.

The reproducibility and accuracy of the methods was found to be good, which is evidenced by low standard deviation. The percent recovery values obtained, indicate non-interference from drug in combination and the excipients used in formulation. In conclusion, the methods developed in the present investigation are simple, sensitive, precise and accurate. Hence it is successfully applied in estimation of melatonin and pyridoxine hydrochloride in combined dosage forms in presence of each other.

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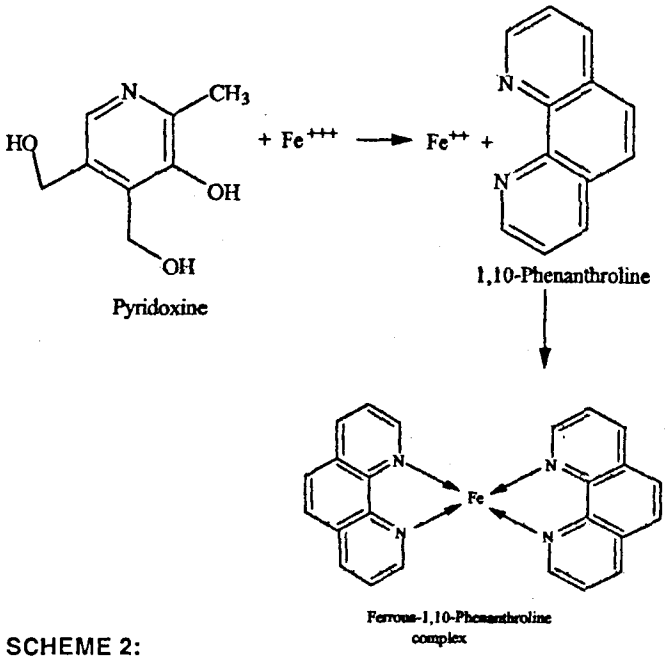

SCHEME 2:

Reaction of pyridoxine hydrochloride with ferric nitrate and 1,10-phenanthroline.

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