SHORT COMMUNICATIONS

Spectrophotometric Estimation of Melatonin and Meloxicam Using Folin-Ciocalteu Reagent

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A simple, accurate, rapid and sensitive method has been developed for the estimation of melatonin and meloxicam using Folin-Ciocalteu reagent in the presence of 20% sodium carbonate solution. The blue coloured chromogen formed is measured at wavelength of maximum absorption 700 nm against reagent blank. The chromogen obeyed linearity over 1.5 to 15 µg/ml for melatonin and 1.5 to 22.5 µg/ml for meloxicam.

Chemically, melatonin is N-[2-(5 methoxy-1H-indole-3yl) ethyl]acetamide[1]. It is used in psychiatric, neurological and cardiovascular disorders[1]. It is available in the tablet form and not official in any pharmacopoeia. Literature survey reveals that this drug could be analyzed by voltametry[2], immunoassy[3], amperometry[4], electrophoresis[5] and spectrophotometric[6] methods. Chemically, meloxicam is 4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl) 2-H,-1,2-benzothiazine-3-carboxamide-1,1-dioxide. It is used as an antiinflammatory drug[7]. It is official in BP. Literature survey reveals that this drug could be analyzed by UV spectrophotometric methods[8-9].

The present work describes a new, simple colourimetric estimation of melatonin and meloxicam based on the formation of blue colour when they are reacted with Folin-Ciocalteu reagent in presence of 20% sodium carbonate solution. Folin-Ciocalteu reagent has been successfully used in colourimetric estimation of drugs and their formulations such as mefanimic acid[10].

A Spectronic 20 Bausch and Lomb Spectrophotometer with 1 cm matched cuvettes was used for spectrophotometric estimation. Folin-Ciocalteu Reagent was prepared as per reported method[11] and was diluted to (1:2) with distilled water, 20% sodium carbonate solution. A standard solution of melatonin containing 3 mg/100 ml was prepared in distilled water. A standard solution of meloxicam 15 mg/100 ml was prepared in 0.1 N NaOH.

Twenty tablets of melatonin or meloxicam were weighed and powdered in a glass morter and amount equivalent to 3 mg of melatonin or 15 mg of meloxicam was transferred to a 100 ml volumetric flask, dissolved in distilled water or 0.1 N NaOH and was made up to the mark with respective solvent.

Aliquots of 0.5 to 5.0 ml or 0.1 to 1.5 ml portion of standard solution of melatonin or meloxicam were transferred to series of 10 ml corning test tubes. To each test tube, 2 ml of Folin-Ciocalteu reagent for melatonin or 1 ml for meloxicam and 2 ml of 20% sodium carbonate solution were added. Then the volume of each test tube was adjusted to 10 ml with distilled water for melatonin and with 0.1 N NaOH for meloxicam. After thorough shaking the test tubes were set aside for 15 min for reaction to complete. The absorbance of solution in each test tube was measured at 700 nm against reagent blank prepared in a similar manner without addition of drug. The calibration curve was plotted and the amount of melatonin or meloxicam was determined by referring to the respective calibration curve. Results of tablet analysis

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TABLE 1: OPTICAL CHARACTERISTICS AND PRECISION

Observations	MLT	MLX
Absorption maxima	700nm	700nm
Beers Law Limit (mcg/ml)	1.5-15	1.5-22.5
Correlation coefficient	0.9982	0.9977
Molar Absorptivity (lit mole^{-1} cm^{-1})	1.1253×10^4	1.4784×10^4
Sandell's Sensitivity (mcg/cm^2/0.001)	0.0206	0.0237
Regression equation (y=mx+c)		
Slope (m)	0.05046	0.0420
Intercept (c)	0.06853	-0.0218

MLT stands for Melatonin while MLX stands for Meloxicam

TABLE 2: RECOVERY STUDY DATA

Sample (TAB)	Labelled amount (mg)	Proposed Method		Reported Method	
		Amount found* ±S.D. (mg)	% Recovery ±S.D.	Amount found* ±S.D. (mg)	% Recovery ±S.D.
MXL1	7.5	7.46±0.07	99.4±0.83	7.44±0.02	99.4±0.44
MXL2	15	14.80±0.06	99.9±0.66	14.89±0.04	99.3±0.38
MLT	3	3.01±0.01	99.7±0.70	2.96±0.01	99.8±0.38

Each value is a mean of five determinations. MXL1 and MXL2 represent meloxicam tablets 1 and 2, respectively, while MLT represents melatonin tablets.

as well as recovery study by the proposed method and by reference method[6,9] are given in Table 2.

The proposed method is based on the reduction of phopsomolybdotungustic acid, the Folin-Ciocalteu reagent by melatonin as well as by meloxicam in presence of an alkali, sodium carbonate (20%), thereby producing reduced species, the blue coloured chromogen. In the method developed, the colour intensity of chromogen was intensified with 2 ml of Folin-Ciocalteu reagent for melatonin or in case of meloxicam with 1 ml. Stability of the coloured complex was determined by measuring absorbance of the chromogen at specified time intervals and was found to be stable for 1.5 h for melatonin and 2.5 h for meloxicam. The optical characteristic such as absorption maxima, beers law limit, correlation co-efficient [r], slope [m], y intercept [c], molar absorptivity and Sandell's sensitivity were determined and are shown in Table 1. Linearity was observed over 1.5 to 15 µg/ml for melatonin and 1.5 to 22.5 µg/ml for meloxicam.

The reproducibility and accuracy of this method was found to be very good, which is evidenced by low standard deviation. The % recovery obtained indicates non-interference from the excipients used in the formulation. In conclusion, the method developed in the present investigation is a new, simple, sensitive, accurate and precise one that could perhaps be used to analyse these drugs from tablet formulations.

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REFERENCES

1. Budavari, S., Eds., In;The Merck Index, 12th Edn., Merck and Co. inc., Whitehouse station, NJ, 1996, 5860.

2. Philips, P.E.M. and Stanford, J.A., **Electroanalysis,** 1999, 11, 301.
3. Snavali, S., Somejima, M., Uchida, K., Morita, Y. and Fukuda, A., **Clin. Chem.,** 1999, 45, 690.
4. You, T.Y., Liv, Z.M., Yang, X.R. and Wang, E.K., **Talanta,** 1999, 49, 517.
5. Kim, Y.O., Chung, H.J., Chung, S.T., Kim, J.V., Park, J.H., Han, S.Y. and Kil, K.S., **J. Chromatogr.,** 1999, 850, 369.
6. Gazy, A.A., Abdine, H.H., Abdel-Hay and Mohammed, H., **Spectrsc. Lett.,** 1998, 31, 177.
7. Budavari, S., Eds., In; The Merck Index, 12th Edn., Merck and Co. Inc., Whitehouse, NJ 1996, 5872.
8. You, W.W., Liv, Y. and Wang, Z.B., **Fenxi Huaxue,** 1999, 27, 841.
9. Joseph, C. and Bertuatt, M., **Anal. Lett.,** 1999, 32, 2051.
10. Rao, G.R., Avadhanula, A.B. and Vatsa D.K., **Eastern Pharmacist,** 1990, 33, 115.
11. Raghuveer S., Avadhanula A.B. and Pantulu, A.R.R., **Eastern Pharmacist,** 1992, 35, 129.

Comparative Evaluation of Chromogenic Limulus Assay and Rabbit Pyrogen Test for Water For Injection

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A comparative study of application of CLAL (Chromogenic Limulus Amebocyte Lysate) and rabbit pyrogen test on 35 samples of Water for Injection was performed. With the pyrogen test, the accumulated temperature rise ranged from + 0.2° to + 1.3° passing all the samples by the rabbit pyrogen test. The endotoxin content varied from as low as 0 EU/ml (Endotoxin Unit per milliliter) to 0.15 EU/ml as tested by CLAL test suggesting that all samples had endotoxin levels below the prescribed levels of 0.25 EU/ml. No correlation could be established between the endotoxin content and temperature rise.

The preparations for which the LAL (Limulus Amebocyte Lysate) test has proven itself as a final product release test for pyrogen include large volume parenteral[1], radiopharmaceuticals[2], intravenous fat emulsions[3], and iron dextran[4]. However there are no published studies/reports available on application of LAL to parenterals from India. Thus in the present study, comparison of the application of CLAL test and rabbit pyrogen test with respect to a simple parenteral preparation like Water for Injection was performed.

All glassware used in this study was rendered free of endotoxin by heating in an oven at 250° for 2 h. An ELISA reader (FLOW Laboratories, USA) was used for measuring the absorbance at wavelength 405 nm and temperature sensing probe (M/S Electrolab) was used.

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As many as 35 samples of Water for Injection were randomly collected from different commercial sources all over Mumbai. Rabbits having detailed specifications as described later were obtained from M.K. Rangnekar Laboratories, Bombay College of Pharmacy, Kalina, Mumbai. The CLAL assay kit used for this study, was obtained from M/S Bio Whittaker, Inc. 8830, Brggs Ford Road, Walkesville, MD 21793, U.S.A.

All reagents in the kit were reconstituted as per the instructions supplied along with the kit. In each series of the determinations, four standard endotoxin (0111B4; 19 EU/ml) solutions viz., 0.1, 0.25, 0.5, 1.0 EU/ml were prepared as per manufacturer's instructions. Each dilution was vigorously vortexed for at least 1 min before using it in the experiment. Since the chromogenic assay is time-dependent, the reagents were pipetted in the same order