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ARTICLES

Oxidative Coupling Reaction for the Determination of Lurasidone

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Abstract—A simple, sensitive, precise, accurate, highly reproducible and economical, visible spectrophotometric method for the determination lurasidone in bulk form was developed and validated. The method is based on the formation of an oxidative coupling product by the reaction of lurasidone with 3-methylbenzothiazolin-2-one hydrazone as a chromogenic reagent in presence of ferric chloride. The linear regression analysis data for the calibration plot showed good linear relationship within the concentration range of 0–100 μg/mL with a correlation coefficient (*r*) value of 0.9997. The limits of detection and quantitation are 0.6 and 1.7 μg/mL, respectively. The method was tested and validated according to ICH guidelines. The results demonstrated that the procedure is accurate, precise and reproducible (RSD < 2%).

Keywords: lurasidone, validation, oxidative coupling, 3-methylbenzothiazolin-2-one hydrazone (MBTH)

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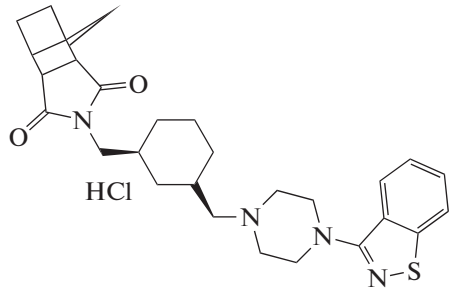
Lurasidone chemically is (3aR,4S,7R,7aS)-2-[[(1R,2R)-2-[[4-(1,2-benzisothiazol-3-yl)-1-piperazinyl]methyl]cyclohexyl]methyl]hexahydro-4,7-methano-1H-isoindole-1,3(2H)-dione hydrochloride [1]. The molecule of lurasidone has six chiral centers (Scheme 1).



Scheme 1. Chemical structure of lurasidone.

Lurasidone was developed by Dainippon Sumitomo Pharma [2] and is widely used as a antipsychotic and antischizophrenia drug. Literature survey revealed that two HPLC methods [3, 4] and three UV spectrophotometric methods [5–7] were developed for the determination of lurasidone in bulk drug, pharmaceutical dosage forms and biological samples. The chromatographic methods utilized for the determination of lurasidone employ delicate and/or expensive instruments that may not be available in quality control laboratories of underdeveloped or developing countries. Visible spectrophotometry may serve as a useful alternative to many of the aforesaid sophisticated techniques, in view of its cost effectiveness, wide applicability, sensitivity, ease of operation, fair accuracy and precision [8–10]. Literature review shows the non-availability of quantitative determination of lurasidone by visible spectrophotometry. In addition, the analytically important functional groups in lurasidone have not been exploited properly so far, and hence, in the present study, an attempt has been made to develop a sensitive and flexible visible spectrophotometric method based on the formation of colored oxidative coupling of lurasidone with 3-methylbenzothiazolinone hydrazone (**MBTH**)–ferric chloride system in bulk drug and dosage formulations.

EXPERIMENTAL

The spectrophotometric measurements were carried out using a Systronics digital spectrophotometer (model 106) provided with 1 cm matched quartz cells. An Elico LI-120 digital pH meter was used for pH measurements. All spectroscopic measurements were carried out at room temperature (25 ± 5°C). Chemicals used were of analytical reagent grade and distilled water was used throughout the investigation. 3% (w/v) $FeCl_3$ solution was prepared by dissolving 3 g of $FeCl_3$ (Merck Pvt. Ltd, India) in 100 mL of 1 M HCl. 0.5% (w/v) MBTH solution (MBTH reagent, Sigma, St. Louis, MO) was prepared in distilled water. The standard stock solution of lurasidone was prepared in methanol and final working standard solution was prepared with distilled water.

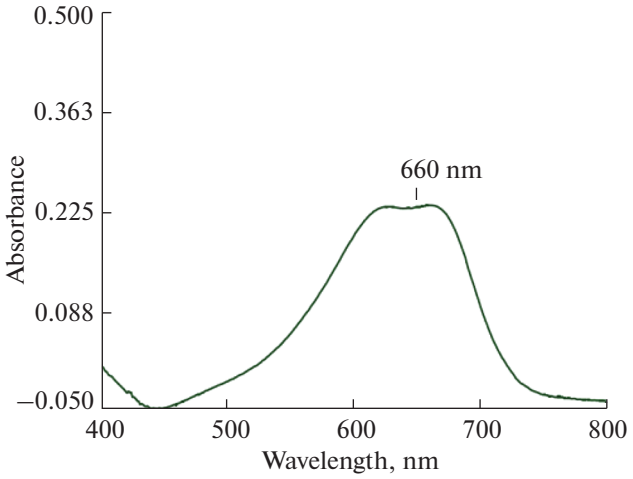


Fig. 1. Visible spectrum of lurasidone.

RESULTS AND DISCUSSION

The chromophore developed for visible spectrophotometric determination of lurasidone shows characteristic absorption maxima at 660 nm for lurasidone (Fig. 1).

Method development. *Optimization of reactions conditions.* The optimum conditions affecting the formation of colored complex (concentrations of MBTH, nature and volume of oxidant, acidity, temperature, order of addition of reagents and diluting solvents) were studied and maintained throughout the experiment to determine the quantity of lurasidone in bulk form. The suitability of MBTH in conjunction with different oxidants (potassium dichromate, ferric chloride, sodium metaperiodate, ceric ammonium sulphate, potassium hexacyanoferrate(III) and chloramine-T) for the determination of lurasidone was examined and ferric ions were found to be the simplest and best with respect to sensitivity, speed and stability of the coloured product formed (Table 1). It was found that the color intensity was dependent on the concentration of both reagents. The highest color intensity was attained when 3 mL of 0.5% MBTH and 3 mL of 3% $FeCl_3$ solutions were used after 15 min of incubation time. These conditions were employed in all the subsequent experiments.

It is noteworthy to mention that the sequence of the addition of reactants had some effect on color development and intensity of the reaction product. In the present case, the addition of the drug followed by the addition of MBTH solution and then ferric ion solution gave the highest color intensity. Though the order of addition varied in diverse reports, the order followed in this case was also adopted by earlier workers with both the oxidants – ferric ion [11–14] and ceric ion [15, 16] for determination of other pharmaceutical drugs. In all these cases, when ferric ion was used as an oxidant in combination with the coupling agent

Table 1. Results of experimental conditions optimization

Variable[a]	Variable parameter	Incubation time, min	λ_{max}, nm	Linear range, μg/mL	Stability of the colored species, min	Sandell's sensitivity, μg/cm ^{2}× 0.001 absorbance uni)	r^b	Conditions[c]
O	$K_2Cr_2O_7$	10	660	0–100	15	0.182	0.999	*1*
O	$FeCl_3$	15	660	0–100	35	0.169	0.999	*1*
O	$NaIO_4$	15	660	0–100	26	0.166	0.999	*1*
O	$Ce(NH_4)_4(SO_4)_4$	8	660	0–100	15	0.192	0.999	*1*
O	$K_3[Fe(CN)_6]$	20	660	0–100	30	0.168	0.999	*1*
O	Chloramine T	12	660	0–100	18	0.198	0.999	*1*
S	Water	15	660	0–100	35	0.169	0.999	*2*
S	Methanol	16	659	0–100	35	0.169	0.998	*2*
S	Ethanol	18	658	0–100	35	0.170	0.995	*2*
S	Acetone	18	658	0–100	35	0.171	0.993	*2*
S	Acetonitrile	16	659	0–100	35	0.172	0.992	*2*
S	Isopropanol	19	657	0–100	35	0.175	0.986	*2*
T	25 ± 5°C	15	660	0–100	35	0.169	0.999	*3*
T	35°C	9	660	0–100	30	0.164	0.991	*3*
T	40°C	7	660	0–100	28	0.161	0.992	*3*
T	45°C	4	660	0–80	21	0.158	0.986	*3*

[a]O – oxidant, S – solvent, T – temperature. [b]Correlation coefficient. [c]Conditions: *1* – solvent – water, 25 ± 5°C; *2* – O – ferric chloride, 25 ± 5°C; *3* – O – ferric chloride, solvent – water.

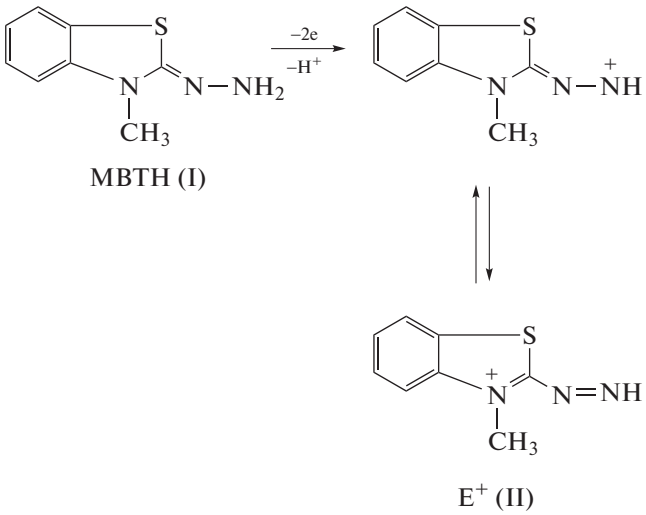
(MBTH), an active coupling species (electrophilic intermediate E^+) is produced. It attacks benzene ring in the drug molecule which has a carbon atom with maximum electron density to give coloured products with absorption maxima at 591 nm with paroxetine hydrochloride [11], at 630 nm with bromhexine hydrochloride [12], 600 nm with cefoperazone [13] and 600 nm with meropenem [14]. Similarly, in the present case the drug molecule produced a green coloured product at $\lambda_{max} = 660$ nm due to coupling of the electrophile with the benzene ring of lurasidone molecule. Although the acidity had no effect on the color development, yet it was found to be important to dissolve and stabilize ferric ions, it was optimized by preparing ferric chloride, and it was 1.0 M.

The reaction was carried out in different solvents (water, methanol, ethanol, acetone, acetonitrile and isopropanol) as diluents and all the solvents gave comparable results. Hence, water was preferred as a diluting solvent on the ground of cost and availability. The reaction was carried out at room (25 ± 5°C) and elevated temperatures (35, 40 and 45°C), using a thermostatically-controlled water bath. It was found that the color intensity decreased significantly when the reaction temperature increased. This was probably attributed to the instability of the colored product and formation of a precipitate at elevated temperatures. Therefore, further experiments were carried out at ambient temperature.

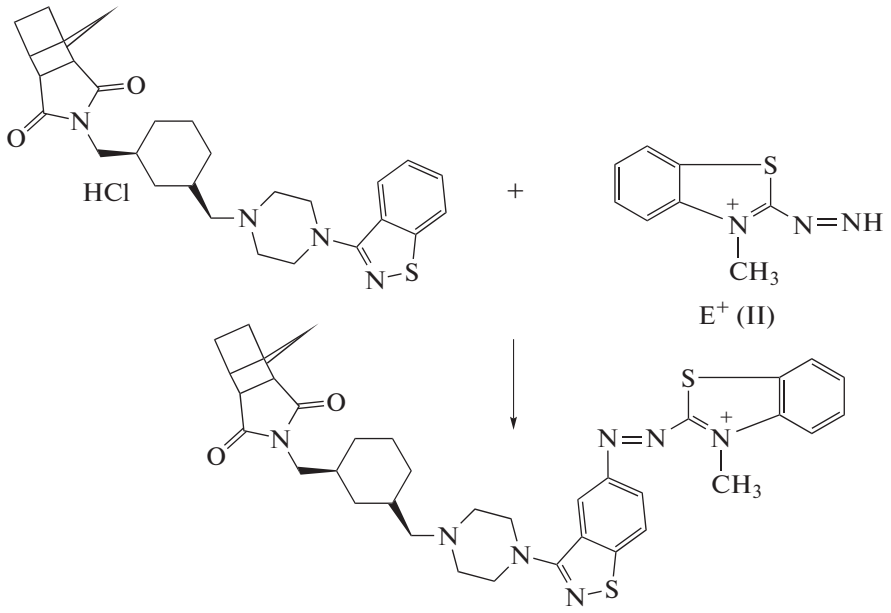
Stoichiometry. The stoichiometry of the reaction of MBTH with lurasidone was investigated by limiting logarithmic method [17]. A plot of log absorbance versus log (lurasidone) at a constant concentration of MBTH and iron(III) gave a straight line with a slope of 1.12. A plot of log absorbance versus log[MBTH] at a constant concentration of lurasidone and iron(III) gave a straight line with a slope of 0.93. Thus, the molar ratio of the reaction (lurasidone : MBTH) is 1.12 : 0.93, which is almost 1 : 1.

Chemistry of the colored chromogens. MBTH was frequently used for the spectrophotometric determination of several pharmaceutical compounds, *viz.* polyhydroxy compounds, aromatic amines, aliphatic and salicylic amines [18–21]. Azodyes, stilbenes and Schiff bases as well as pyrrole derivatives also react with MBTH under oxidative conditions [22]. In the present investigation, the drug lurasidone reacts with MBTH in presence of $FeCl_3$ in acidic medium to give green-colored product.

The reaction of MBTH with lurasidone in the presence of an oxidant (Fe^{+3}), proceeds *via* oxidative coupling. MBTH (I) loses two electrons and one proton on oxidation with oxidizing agent (ferric chloride), forming the electrophilic intermediate (II) (Scheme 2), which is the active coupling species [18]. The literature survey shows that electrophilic intermediate (E^+) formed on oxidation of MBTH with ferric ions attach the drug molecules at one position [23–26], two positions [18, 27, 28] and three positions [11]. In the present study, the stoichiometry of lurasidone: MBTH was found to be 1 : 1 confirming the mono substitution of electrophile on lurasidone. The intermediate would be expected to attack carbon atom with maximum electron density in lurasidone to form the colored complex. The position of substitution on lurasidone can be explained as follows: owing to ortho and para directing nature of thiazole group, the electrophile (E^+) can attach either at 5 (para) or 7 (ortho) positions of benzisothiazole, where the electron density is high. However, due to steric hindrance at ortho position, the elctrophile (E^+) attaches preferably at para position. The tentative reaction scheme was given in Scheme 3.



Scheme 2. Formation of electrophile (E^+).



Scheme 3. Coloured complex formation between lurasidone and electrophile (E^+).

Validation of method. The main validation parameters such as linearity and range, accuracy and precision, recovery, ruggedness, limit of detection (**LOD**) and limit of quantitation (**LOQ**) were evaluated [29].

Linearity and range. Aliquots of working standard drug solution containing 0.2 to 1.0 mL (0.2. 0.4, 0.6, 0.8 and 1.0 mL) were transferred into a series of calibrated 10 mL standard flasks. To each flask 3 mL of 0.5% (w/v) MBTH reagent, 3 mL of 3% (w/v) ferric chloride solution were added and kept for 15 min, with occasional stirring and the volume was made up to mark with distilled water. The absorbance of green-colored chromogen was measured at λ_{max} = 660 nm against the reagent blank (3 mL of 3% $FeCl_3$ and 3 mL of 0.5% MBTH). The amount of drug in the sample was computed from its calibration curve. The linear regression equation obtained was $y = 0.0058x + 0.0046$. The linear regression analysis data for the calibration plot showed good linear relationship within the concentration range of 0–100 μg/mL with a correlation coefficient (*r*) value of 0.9997. The summary of optical and regression parameters was shown in Table 2.

Table 2. Optical characteristics, statistical data of the regression equations and validation parameters for lurasidone

Parameter	Observation
Apparent molar absorptivity, L/mol cm	3.13×10^3
Sandell's sensitivity, μg/cm^2	0.17
Slope	0.0058
Intercept	0.0046
Regression coefficient (*r*)	0.9997
λ_{max}, nm	660
Linear range, μg/mL	0–100
Limit of detection, μg/mL	0.6
Limit of quantitation, μg/mL	1.7

Accuracy. To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80, 100, and 120%) of bulk samples of lurasidone to 40 μg/mL so that overall concentration will be within the linearity range. The accuracy was expressed in terms of percent recovery. The mean of percentage recovery values was 101.28–101.60. The results are given in Table 3. The statistical analysis of data obtained for the estimation of lurasidone indicates a high level of accuracy for the proposed method, as evidenced by the low values of standard deviation and relative standard deviation.

Precision. The precision of a method is defined as the closeness of agreement between independent test results obtained under optimum conditions. Two different concentrations of lurasidone in the linear range (40 and 60 μg/mL) were analyzed in six independent series on the same day (intra-day precision) and on six consecutive days (inter-day precision) (Table 4). The RSD values of intra-day studies varied from 0.416 to 0.364 and inter-day studies varied from 0.490 to 0.566, which showed that the precision of the method was satisfactory.

Ruggedness. The ruggedness of the proposed method was evaluated by applying the developed pro-

Table 3. Recovery of lurasidone using the proposed method

Nominal concentration used, μg/mL (a)	Amount of drug added, μg/mL (b)	Total amount of drug, a + b, μg/mL (theoretical)	Amount of drug recovered, μg/mL (practical), mean[a] ± SD	Recovery = practical/theoretical × 100%
40	32	72	73.1 ± 0.5	101.2
40	40	80	81.0 ± 0.8	101.3
40	48	88	89.5 ± 0.4	101.6

[a]Average of six determinations.

Table 4. Intra- and inter-day precision

Concentration of lurasidone added, μg/mL	Found intra-day, mean[a] ± SD, μg/mL	RSD, %	Found inter-day, mean[a] ± SD, μg/mL	RSD, %
40	40.7 ± 0.2	0.41	41.2 ± 0.2	0.49
60	59.9 ± 0.2	0.36	60.2 ± 0.3	0.56

[a]Average of six determinations.

cedure for assay of 40 and 60 μg/mL of lurasidone using the same instrument by two different analysts under the same optimized conditions on different days. There was no significant difference between the analysts as the obtained results were found to be reproducible. Thus, the proposed methods could be considered rugged (Table 5).

Limits of detection and determination. For characterization of sensitivity of the proposed method, LOD and LOQ were calculated according to the ICH guidelines (2005) [30] by using S (slope of the calibration curve) and σ (relative standard deviation of the response) [29]. The values calculated for lurasidone are: LOD = $3.3\sigma/S$ = 0.6 μg/mL, LOQ = $10\sigma/S$ = 1.7 μg/mL

Analysis of pharmaceutical formulations. The drug content per tablet (on an average weight basis) was calculated from the absorbance values of the extracts of lurasidone tablets (Latuda) (Table 6). Good recovery values show that the proposed method can be successfully applied to the determination of lurasidone in pharmaceutical formulations without any interference from common excipients.

CONCLUSIONS

The proposed method involves measurements in visible region and is more selective than the previously reported spectrophotometric methods that involved measurements in the ultraviolet region. Further, the proposed method is straightforward as it does not require elaborate treatment of the samples, careful adjustment of the critical optimum pH of the reaction medium and/or tedious liquid-liquid extraction for the chromophores. The proposed method does not require any expensive or sophisticated instrumentation and/or critical analytical reagents. These advantages encourage the application of the proposed method in routine analysis of bulk drugs and pharmaceutical dosage forms of lurasidone in quality control

Table 5. Ruggedness of lurasidone determination by two analysts on different days

Concentration oflurasidone, μg/mL	Found by analyst 1, μg/mL		Found by analyst 2, μg/mL	
	mean[a] ± SD	RSD, %	mean[a] ± SD	RSD, %
40	40.9 ± 0.3	0.62	40.8 ± 0.2	0.56
60	60.1 ± 0.2	0.38	60.0 ± 0.2	0.30

[a]Average of six determinations.

Table 6. Estimation of lurasidone in its formulation Latuda® tablets by visible spectrophotometric method

Labeled amount, mg	Amount found[a], mg	Recovery, %
40	39.2	98.0
80	79.1	98.8

[a]Average of six determinations.

laboratories as alternatives to the HPLC and UV methods.

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