

## Determination of the dexlansoprazole in bulk and spiked human plasma by extraction spectrophotometry

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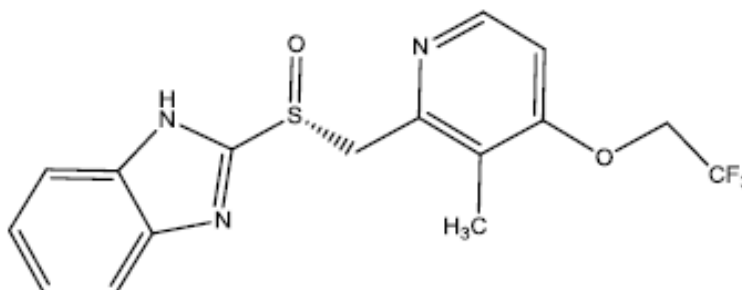
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**ABSTRACT.** This paper describes a simple extraction spectrophotometric method for the quantification of dexlansoprazole in bulk and spiked human plasma. This method involves formation of stable yellow colored chloroform extractable ion-pair complex of the amino derivative of dexlansoprazole with acid dye, namely methyl orange in acidic medium. The ion-pair complexes exhibit absorption maxima at 425 nm. Dexlansoprazole can be determined up to 4-40 µg/mL by the proposed method. The effect of optimum reagent concentration was studied. The relative standard deviations ( $\leq 1.246\%$ ) obtained in the intra-day and inter-day analyses were found to be satisfactory. The accuracy results exhibited the mean recovery and percentage error in the range of 99.137%–100.574% and 0.012%–0.863%. When applied for the assay of the dexlansoprazole in spiked human plasma sample, recovery mean values ranged from 96.495–98.960%. The proposed method is useful for the estimation of the dexlansoprazole in bulk and human plasma samples.

### 1. INTRODUCTION

Dexlansoprazole is an acid- or proton-pump inhibitor/gastric antisecretory agent used in the short-term treatment of all grades of erosive esophagitis and management of symptoms of gastroesophageal reflux in patients without erosive esophagitis [1]. Dexlansoprazole binds to and inactivates hydrogen-potassium ATPase (proton, hydrogen, or acid pump) in gastric parietal cells, blocking the final step in secretion of hydrochloric acid; results in potent, long-lasting inhibition of gastric acid secretion [2]. Dexlansoprazole is the R-enantiomer of lansoprazole. Dexlansoprazole was approved by the U.S. Food and Drug Administration (FDA) on January 30, 2009 [3]. Chemically dexlansoprazole is known as (R)-(+)-2-([3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl]methylsulfinyl)-1H-benzimidazole (Fig. 1).



**Figure 1: Structure of dexlansoprazole**

Few analytical methods have been described in the literature for the determination of enantiomers of lansoprazole in the biological fluids. Katsuki *et al.*, [4] reported a chiral stationary-phase liquid chromatography method for the determination of enantiomers of lansoprazole in the human serum. R(+)- and S(-)-enantiomers from racemic lansoprazole was separated on a Chiralcel OD (cellulose tris(3,5-dimethyl-phenylcarbamate)) and a Chiralpak AS (amylose tris ((S)-1-phenylethylcarbamate)) with ultraviolet detection at 285 nm using acetonitrile and water in the ratio of 35:65 v/v as mobile phase. The developed method was applied to evaluate pharmacokinetic behaviors of the enantiomers in seven subjects

Borner *et al.*, [5] described a simple HPLC method is described for the separation and quantitative determination of the (+)- and (-)- enantiomers of lansoprazole. The enantiomers were separated by chromatography on a Chiral-AGP<sup>R</sup> column which contained covalently bound acid  $\alpha_1$ -glycoprotein as chiral selector. The method was applied to determine the pharmacokinetics of both enantiomers in the human serum. An enantioselective HPLC method developed for determination of simultaneous determination of lansoprazole enantiomers and its major metabolites: 5-hydroxylansoprazole and lansoprazole sulfone in human microsomal liver incubations by Katsuki *et al.*, [6] was carried out using a Chiralcel<sup>®</sup> OD-R column with mobile phase containing methanol–water (75:25 v/v). The method is suitable for the analysis of lansoprazole enantiomers and its metabolites from human microsomal liver incubations.

Simultaneous assay of lansoprazole enantiomers and their metabolites, 5-hydroxylansoprazole enantiomers and lansoprazole sulfone, in human plasma has been reported by Miura *et al.*, [7]. Chromatographic separation was achieved with a Chiral CD-Ph column using a mobile phase of 0.5 M NaClO<sub>4</sub>–acetonitrile–methanol (6:3:1 v/v/v). The method is applicable for the simultaneous monitoring of the plasma levels of lansoprazole enantiomers and their metabolites in the renal transplant recipients. Gomes *et al.*, [8] developed and validated a two-dimensional HPLC method based on the direct injection of biological samples for the quantification of lansoprazole enantiomers in human plasma. The lansoprazole enantiomers were extracted from the biological matrix using an octyl restricted access media bovine serum albumin column. The enantioseparation was performed on an amylose tris (3,5-dimethoxyphenylcarbamate) chiral column using acetonitrile and water (35:65 v/v). The method was applied to the analysis of the plasma samples obtained from nine volunteers who received a 30 mg oral dose of racemic lansoprazole. The method was able to quantify the enantiomers of lansoprazole in the clinical samples.

All the above described methods are applied for the simultaneous determination of enantiomers of lansoprazole in the biological samples. Only two methods report on the quantification of dexlansoprazole (R-enantiomer of lansoprazole). A bioanalytical method [9] has been developed for the determination of dexlansoprazole using omeprazole as an internal standard in human plasma. The API-4000 liquid chromatography-tandem mass spectrometry (LC-MS/MS) was operated under multiple reaction-monitoring mode using electrospray ionization. This was achieved with a mobile phase consisting of 0.2% ammonia–acetonitrile (20:80 v/v) on an X-terra RP 18 column. The assay method was applied to an oral bioequivalence study in humans.

In a method reported by Geetharam *et al.*, [10], separation and quantification of dexlansoprazole in the presence of their degradants were conducted on Hypersil BDS C18 using 0.01M KH<sub>2</sub>PO<sub>4</sub> buffer (pH adjusted to 7.0 with triethyl amine) and acetonitrile (60: 40 v/v) as a mobile phase. The UV detection was performed at 283 nm. The method can be successfully applied for routine analysis of dexlansoprazole in bulk and capsule dosage form in the presence of their degradants.

The spectrophotometry technique may act as a useful alternative to many of the aforementioned sophisticated techniques for the reason of their low cost, ease of operation, sensitivity, significant accuracy & precision and broad applicability. To the best of our knowledge, there are no reports on the spectrophotometric quantification of dexlansoprazole. In the present study an attempt has been made to develop and validate an extraction spectrophotometric method for the determination of dexlansoprazole in bulk and spiked human plasma sample.

## 2. MATERIALS AND METHODS

### 2.1. Instrumentation

1. Systronics UV/VIS double beam (model SL-2201) digital spectrophotometer was used for all spectrophotometric measurements.
2. One cm matched quartz cells were used for absorbance measurements.
3. Samples were weighed by using electronic weighing balance (K. Roy Balance Instrument Co. Ltd, Varanasi) KEA 210 model.
4. Remi desktop centrifuge R-303 model (Remi elektrotechnik ltd, Thane).

### 2.2. Materials and Reagents

All chemicals were of analytical reagent grade. Double distilled water was used. All the solutions were prepared afresh daily.

#### 2.2.1. Methyl orange (0.02%)

Stock solution (1 mg/mL) was prepared by dissolving 100 mg of methyl orange (Merck Specialities Private Ltd, Mumbai) in 100 mL of water. The working solution was prepared by diluting 20 mL of stock solution to 100 mL with water.

#### 2.2.2. HCl (0.1 N)

This solution was prepared by mixing 0.86 mL of HCl (Sd Fine Chem Limited, Mumbai) in 70 mL of water in a 100 mL volumetric flask and then made up to 100 mL with water.

#### 2.2.3. Chloroform

This was obtained from Sd Fine Chem limited, Mumbai and used for the extraction of ion-pair complex formed in methods 1 and 2.

### 2.3. Dexlansoprazole standard solutions

Stock solution of dexlansoprazole (1 mg/mL) was prepared by dissolving 100 mg of dexlansoprazole in 20 mL of 0.1 N HCl and then diluted to 100 mL with the same solvent in a 100 mL volumetric flask. Working standard solution containing 200 µg/mL of dexlansoprazole was prepared by diluting 20 mL of stock solution to 100 mL with 0.1 N HCl in a 100 mL volumetric flask.

### 2.4. Preparation of placebo blank

The placebo consisting of hydroxypropyl cellulose (10 mg), magnesium stearate (10 mg), sucrose (10 mg), glucose (10 mg), talc (10 mg), starch (10 mg), lactose (10 mg) and fructose (10 mg), sodium alginate (10 mg) and acacia (10 mg) was accurately weighed into a 100 mL beaker, 30 mL of methanol (Merck, Mumbai, India) was added and the mixture was shaken for 20 minutes. The mixture was filtered using Whatman No. 1 filter paper and the filtrate was evaporated to dryness on a water bath. The residue was then transferred into a 100 mL volumetric flask containing 20 mL of 0.1N HCl. The solution was mixed well and made up to the mark with 0.1N HCl.

### 2.5. General analytical procedure

Into a series of 100 mL separating funnels, volumes (0.2-2.0 mL) of dexlansoprazole standard solution (200 µg/mL) equivalent to 4–40 µg of the drug were transferred. The volume in each separating funnel was adjusted to 2.0 mL with 0.1 N HCl. Then, to each separating funnel 1.0 mL of 0.02% methyl orange was transferred and mixed well. The funnels were shaken vigorously with 5 mL of chloroform for 2 min and then allowed to stand for clear separation of the two phases. The

chloroform phase thus separated was transferred into a 10 mL volumetric flask. Then the extract was made up to the mark with chloroform and mixed well. The absorbance of the chloroform phase was measured at 425 nm against a reagent blank prepared similarly omitting the drug.

The calibration graph was constructed by plotting the concentration of the dexlansoprazole in  $\mu\text{g/mL}$  versus the absorbance values. The amount of the dexlansoprazole was computed either from the calibration graph or from the regression equation.

### 2.6. Determination of dexlansoprazole in spiked human plasma

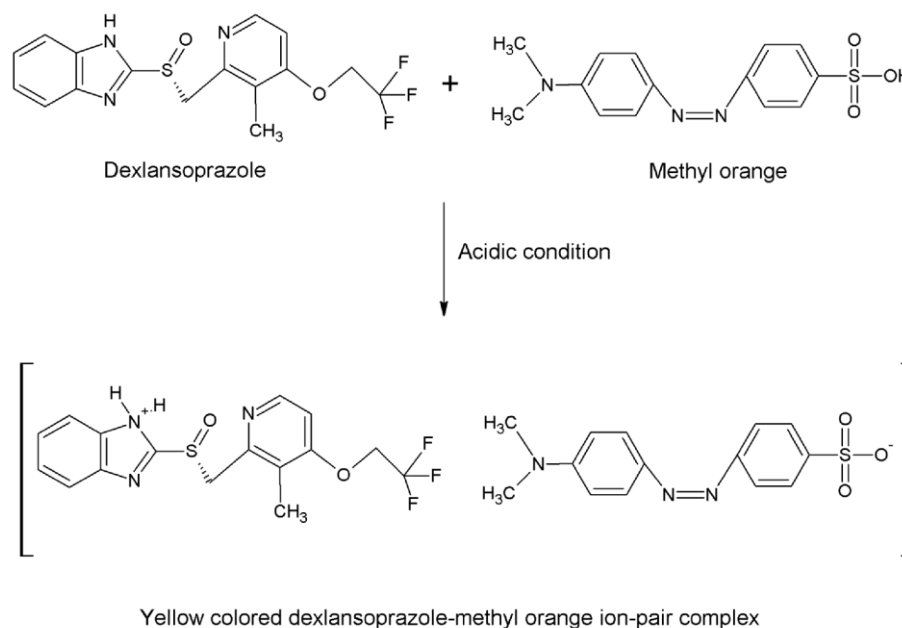
A liquid-liquid extraction method was followed for extraction of dexlansoprazole from human plasma. To an aliquot of 1 mL plasma, 1 mL of dexlansoprazole standard solution (600  $\mu\text{g/mL}$ ) was added and mixed for 5 minutes. The spiked plasma sample was added with 2 mL of ethyl acetate, the mixture was vortexed for 2 min, followed by centrifugation for 5 min at 3000 rpm. The organic layer was separated and evaporated to dryness at on a water bath. The dry residue was dissolved in 1 mL 0.1 N HCl. The solution with a concentration of 400  $\mu\text{g/mL}$  dexlansoprazole concentration was analyzed by proposed method. The dexlansoprazole concentration in the plasma was calculated from the calibration graph prepared under identical conditions or from the regression equation derived.

## 3. RESULTS AND DISCUSSION

### 3.1. Basis of the reaction

The ion-pair complex is a special form of molecular complex resulting from two oppositely charged ions extractable into organic solvents from aqueous phase at suitable pH [11,12]. In the recent years ion-pair extraction spectrophotometry has received substantial significance for the quantification of many pharmaceutical compounds [13-16].

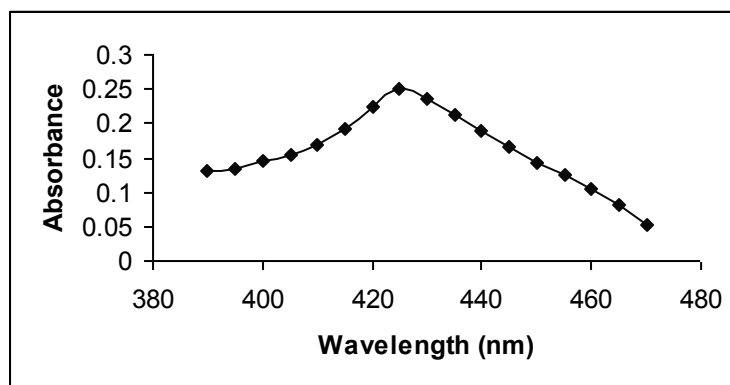
Considering basic drugs capacity to generate ion-pairs with anionic dyes, one new extraction spectrophotometric method for dexlansoprazole assay was developed. Dexlansoprazole exhibits basic character owing to the presence of secondary amino group. In acidic media, the secondary amino group of dexlansoprazole is protonated, while sulphonic group present in methyl orange undergoes dissociation to form an anionic derivative. The results obtained in the proposed method are based on ion-pair formation of dexlansoprazole with methyl orange under acidic condition. The resulted yellow colored dexlansoprazole-methyl orange ion-pair complex was extracted with chloroform and the absorbance was measured at 425 nm. The proposed reaction mechanism of dexlansoprazole with methyl orange has been given in the Fig. 2.



**Figure 2. Dextansoprazole-methyl orange ion-pair complex formation**

### 3.2. Optimum wavelength

The absorbance of the ion-pair complex, formed between dextansoprazole & methyl orange, was measured against the reagent blank in the range of 380-500 nm (Fig. 3). The yellow colored dextansoprazole-methyl orange ion-pair complex exhibited maximum absorbance at 425 nm.



**Figure 3. Absorption spectra of dextansoprazole-methyl orange ion-pair complex**

### 3.3. Optimization of dye concentration

The effect of concentration of dye solution on the formation of the ion-pair complex was extensively studied to determine the optimal dye concentration for the determination of dextansoprazole. The optimum value of the dye concentration was maintained throughout the experiment.

The effect of the concentration of methyl orange was studied by treating 20 µg/mL dextansoprazole with varying volumes (0.5–2.5 mL) of 0.02% methyl orange. The absorbance of the dextansoprazole-methyl orange ion-pair complex at 425 nm was increased with increasing volume of 0.02% methyl orange upto 1.0 mL. Above this volume, the absorbance of dextansoprazole-methyl orange ion-pair complex slightly decreased (Fig. 4). Therefore, a volume of 1.0 mL of 0.02% methyl orange was chosen for the quantification process.

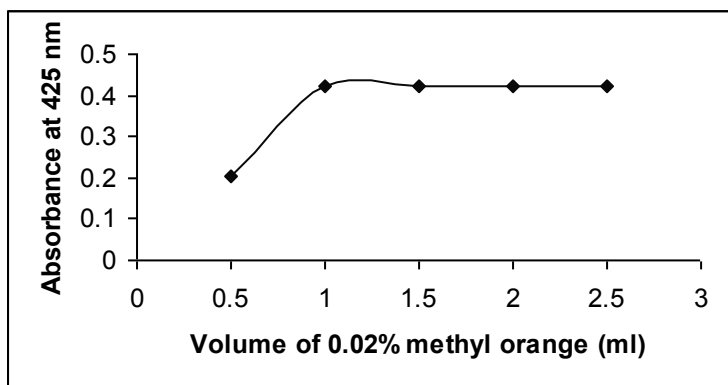


Figure 4. Effect of concentration of methyl orange

### 3.4. Stability of ion-pair complex

The stability of the dexlansoprazole-methyl orange ion-pair complex was monitored by keeping the solution at room temperature ( $25\pm 1^\circ\text{C}$ ) and then measuring the absorbance of the solution at 425 nm at regular intervals of time. There was no change in the absorbance for at least 4 hours. This indicates that dexlansoprazole-methyl orange ion-pair complex was stable for at least 4 hours at  $25\pm 1^\circ\text{C}$ . The stability of these ion-pair complex helped in proceeding with large batches of samples and their comfortable measurements easily.

### 3.5. Method validation

#### 3.5.1. Linearity

At described experimental conditions for dexlansoprazole determination, standard calibration curves for dexlansoprazole with methyl orange was constructed by plotting an increase in absorbencies vs concentrations (Fig. 5). A linear correlation was found between absorbance and concentration of dexlansoprazole in the range given in Table 1. The statistical parameters given in the regression equation were calculated from the calibration graph. The high values of the regression coefficient ( $R^2$ ) and low values y-intercepts of the regression equation, proved the linearity of the calibration curve (Table 1).

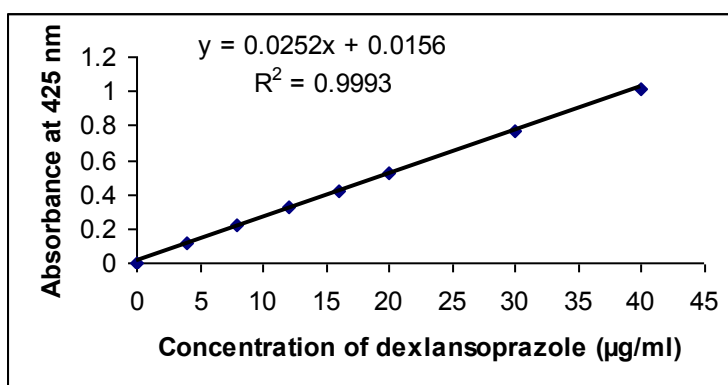


Figure 5. Linearity curve for the proposed method

#### 3.5.2. Sensitivity

The sensitivity of the proposed method was assessed by calculating molar absorptivity, Sandell's sensitivity, limit of detection (LOD) and limit of quantification (LOQ) according to ICH guidelines [17]. The LOD and LOQ was determined by taking the ratio of standard deviation of the reagent blank ( $n=5$ ) with respect to water and slope of calibration curve multiplied by a factor of 3.3

and 10, respectively. The results presented in Table 1 reveal the high sensitivity of the proposed method.

**Table 1. Linearity, regression and sensitivity characteristics**

Parameters	Value
Beer's Limit ( $\mu\text{g/mL}$ )	4-40
Molar Absorptivity (L/mole/cm)	$9.788 \times 10^4$
Sandell's sensitivity ( $\mu\text{g cm}^{-2}/0.001$ Absorbance unit)	0.0032
Regression equation ( $A = mC + I$ ) <sup>§§</sup>	-----
Slope (m)	0.0252
Intercept (I)	0.0156
Regression coefficient ( $R^2$ )	0.9993
LOD ( $\mu\text{g/mL}$ )	0.053
LOQ ( $\mu\text{g/mL}$ )	0.161

<sup>§§</sup> $A = mC + I$ , where A is the absorbance and C is the concentration of drug in  $\mu\text{g/mL}$

### 3.5.3. Selectivity

The selectivity of the proposed method was evaluated by analysis of placebo blank. The placebo blank solution was analyzed by the general analytical procedure. The absorbance value of the placebo blank solution was almost equal to the absorbance of the reagent blank. This revealed no significant interference from the excipients. The results also confirm the selectivity of the proposed method.

### 3.5.4. Precision

The precision of the proposed method was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was carried out by performing five repeated analysis of dexlansoprazole at three different concentrations levels on the same day, under the same experimental conditions. The intermediate precision of the method was assessed by carrying out the analysis for three consecutive days (inter-day). The results of this study are given in Tables 2 & 3. The RSD values of repeatability (intra-day) and intermediate precision (inter-day) showed that the precision of the proposed methods was acceptable.

### 3.5.5. Accuracy

The intra-day and inter-day accuracy of the proposed method was determined by analyzing a known amount of dexlansoprazole at three different concentrations levels by the proposed methods. Accuracy was evaluated as percentage relative error between the measured mean concentrations and taken concentrations. The results are shown in Tables 2 & 3, from which it is clear that the accuracy of the proposed method is excellent.

Table 2. Intra-day precision and accuracy of the proposed method

Concentration of Dexlansoprazole ( $\mu\text{g/mL}$ )		% Recovery
Taken	Found	
4	3.931	98.275
4	3.965	99.137
4	3.965	99.137
4	4.000	100.000
4	3.965	99.137
	<b>Mean - 3.9652</b> <b>SD - 0.0243</b> <b>%RSD - 0.612</b>	<b>Mean - 99.137</b> <b>%Error - 0.863</b>
20	20.153	100.766
20	20.191	100.957
20	20.114	100.574
20	19.961	99.808
20	20.153	100.766
	<b>Mean - 20.114</b> <b>SD - 0.899</b> <b>%RSD - 0.446</b>	<b>Mean - 100.574</b> <b>%Error - 0.574</b>
40	40.000	100.000
40	39.960	99.901
40	40.118	100.295
40	40.039	100.098
40	39.960	99.901
	<b>Mean - 40.015</b> <b>SD - 0.0660</b> <b>%RSD - 0.164</b>	<b>Mean - 100.039</b> <b>%Error - 0.039</b>

Table 3. Inter-day precision and accuracy of the proposed method

Day	Concentration of Dexlansoprazole ( $\mu\text{g/mL}$ )		% Recovery
	Taken	Found	
1	4	4.0288	100.72
2	4	3.9652	99.137
3	4	4.0643	101.621
		<b>Mean - 4.019</b> <b>SD - 0.0501</b> <b>%RSD - 1.246</b>	<b>Mean - 100.494</b> <b>%Error - 0.494</b>
1	20	20.114	100.574
2	20	19.954	99.772
3	20	19.945	99.732
		<b>Mean - 20.048</b> <b>SD - 0.0950</b> <b>%RSD - 0.474</b>	<b>Mean - 100.026</b> <b>%Error - 0.026</b>
1	40	40.0154	100.039
2	40	40.0232	100.058
3	40	39.9758	99.940
		<b>Mean - 40.048</b> <b>SD - 0.0254</b> <b>%RSD - 0.634</b>	<b>Mean - 100.012</b> <b>%Error - 0.012</b>



### 3.5.6. Robustness

Robustness of the proposed method was determined by deliberately varying experimental parameter i.e., volume of 0.02% methyl orange solution (1.9, 2.0 and 2.1 mL). The assay was carried out in triplicate at two different concentration levels. The absorbance values were measured at 425 nm. The relative standard deviation of the absorbance values was calculated. The results are shown in Table 4. The minor changes in the volume of dye that may take place during the experimental operation did not affect the assay of dexlansoprazole. The results indicating the excellent robustness of the proposed method.

**Table 4. Robustness of the proposed method**

Volume of 0.02% Dye (mL)	Amount of drug ( $\mu\text{g/mL}$ )	OD at 425 nm
0.9	4	0.114
1.0	4	0.114
1.1	4	0.113
		Mean – 0.1136 SD - 0.0057 %RSD - 0.440
0.9	40	1.013
1.0	40	1.013
1.1	40	10.13
		Mean – 1.0136 SD -0.0011 %RSD - 0.108

### 3.5.7. Ruggedness

The ruggedness of the proposed method was assessed by comparison of the results for the assay of dexlansoprazole performed by four different analysts in the same laboratory with two different spectrophotometers. The RSD for inter analyst analysis and inter instrumentation analysis was calculated. The results are reported in the Tables 5 & 6. The low RSD values indicated the ruggedness of the proposed method.

**Table 5. Ruggedness of the proposed method (inter-analyst variation)**

Analyst	Amount of drug ( $\mu\text{g/mL}$ )	OD at 425 nm
1	4	0.114
2	4	0.114
3	4	0.113
4	4	0.114
		Mean – 0.1137 SD - 0.00050 %RSD - 0.439
1	40	1.016
2	40	1.015
3	40	1.015
4	40	1.015
		Mean – 1.0152 SD - 0.0005 %RSD - 0.049

Table 6. Ruggedness of the proposed method (inter-instrument variation)

Amount of drug ( $\mu\text{g/mL}$ )	OD with instrument 1	OD with instrument 2
4	0.114	0.113
4	0.114	0.114
4	0.113	0.113
	<b>Mean</b> – 0.1134 <b>SD</b> - 0.0005 <b>%RSD</b> - 0.441	
40	1.016	1.015
40	1.016	1.016
40	1.015	1.015
	<b>Mean</b> – 1.0155 <b>SD</b> - 0.0005 <b>%RSD</b> - 0.481	

### 3.6. Application to the spiked human plasma

The developed and validated extraction spectrophotometric method was applied to the analysis of dexlansoprazole in spiked plasma samples. The results are shown in Table 7. The assay results were very close to the 100 % and relative standard deviation values were low, thus confirming that the developed method is suitable for determination of dexlansoprazole in plasma sample.

Table 7. Assay of dexlansoprazole in spiked plasma sample

Concentration of Dexlansoprazole ( $\mu\text{g/mL}$ )		% Recovery
Spiked	Spiked	
40	38.598	96.495
40	39.584	98.960
40	39.548	98.870
40	38.955	97.387
40	38.886	97.215
	<b>Mean</b> – 39.114 <b>SD</b> - 0.433 <b>%RSD</b> – 1.109	<b>Mean</b> – 97.785 <b>%Error</b> – 2.214

## 4. CONCLUSION

A simple, accurate and sensitive extraction spectrophotometric method is proposed for the rapid and accurate determination of dexlansoprazole using methyl orange. The calculated LOD and LOQ, Sandell's sensitivity and molar absorptivity for dexlansoprazole indicate the sensitivity of the method. The relative standard deviation, mean recovery and percentage error obtained in the intra-day and inter-day analyses were found to be acceptable. The selectivity test indicated the non interference from the common excipients. The proposed method has been successfully applied for the assay of the dexlansoprazole in spiked human plasma sample. Good recovery value indicating the absence of significant matrix effects on the measurements. The proposed method therefore is generally applicable to the determination of the dexlansoprazole in bulk and human plasma sample.

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