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Visible Spectrophotometric Methods for Quantitative Determination of Doxofylline using Iodine and α, α'-Bipyridyl as Reagents

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ABSTRACT

Doxofylline is a phosphodiesterase inhibitor used in the treatment of bronchial asthma, chronic obstructive pulmonary disease and chronic bronchitis. In this study two simple, sensitive, precise and accurate visible spectrophotometric methods (A and B) have been developed for the determination of doxofylline in bulk and in its dosage forms. Method A is based on the formation of yellow colored charge transfer complex between doxofylline as *n*-donor and iodine as σ-acceptor. Method B is based on the reaction of doxofylline with iron (III) and subsequent reaction with α, α'-bipyridyl in an acid medium to yield a red colored complex. The colored products are quantitated spectrophotometrically at 395 and 535 nm by methods A and B, respectively. The methods determine the cited drug in concentration ranges of 4-32 (method A) and 2-16 (method B) µg mL^{-1}. The optimum experimental conditions have been studied. The proposed methods were successfully applied to the determination of the doxofylline in pure and dosage forms with good accuracy and precision. The results were compared statistically with those given by the reported method.

Key words: Doxofylline, Iodine, α, α' Bipyridyl, Spectrophotometric analysis.

INTRODUCTION

Doxofylline (DFL)[1-5], chemically known as 7-(1, 3-dioxolan-2-ylmethyl)-1, 3-dimethylpurine-2, 6-dione (Figure 1), is a novel bronchodilator xanthine derivative drug used in the treatment of bronchial asthma, chronic obstructive pulmonary disease (COPD) and chronic bronchitis. Animal and human studies has shown similar efficacy to theophylline but with significantly less side effects[6]. DFL differs from theophylline for the presence of a dioxalane group in position 7. DFL acts as a phosphodiesterase inhibitor and have decreased affinities toward adenosine A1 and A2 receptors which may account for the better safety profile of the drug[7, 8]. The safety profile shows a better tolerability on cardiovascular, digestive and the central nervous systems[9]. DFL was found to be

particularly effective in both decreasing the daily asthma attack rate as well as the beta-2- agonist consumption.

The determination of DFL in pharmaceutical preparations is very important for medical and pharmaceutical needs where it is used for the treatment of bronchial asthma, chronic obstructive pulmonary disease and chronic bronchitis. Several types of analytical procedures have been proposed for the analysis of DFL in bulk, pharmaceutical formulations and biological fluids. These procedures include HPLC[10-13], Stability indicating RP-HPLC chromatography[14], LC-MS/MS[15], stability indicating HPTLC[16]. Although the above methods have adequate sensitivity to assay DFL, but require relatively expensive reagents, time-consuming and require expertise. Visible spectrophotometry is considered the most widely used technique, because of its inherent simplicity, low cost and wide availability in most quality control laboratories. Therefore, they are a frequent choice for pharmaceutical analyses. Kamila *et al.,* [17] and Joshi *et al.,* [10] have reported UV spectrophotometric method for the quantification of DFL in pharmaceutical formulations. The literature is still poor in visible spectrophotometric methods for the determination of DFL in dosage forms.

This paper describes two visible spectrophotometric methods for the assay of DFL in pure and dosage forms. Method A is based on charge transfer complexation between the drug as *n*-electron donor and iodine acid as *ó* -acceptor. Method B is based on the oxidation of the drug with Fe^{3+} and the estimation of Fe^{2+} produced after complexation with á, á' bipyridyl. The proposed methods are optimized and validated as per the International conference on Harmonization guidelines[18].

EXPERIMENTAL

Instrumentation

Spectral runs were made on ELICO double beam model SL 159 digital spectrophotometer with 1-cm matched quartz cells.

Materials and reagents

All chemicals used were of analytical reagent grade and all solutions were freshly prepared in doubly distilled water.

1. 0.2% iodine in chloroform: Prepared by dissolving 200 mg of iodine (Sdfine-Chem limited, Mumbai) in 100 mL chloroform (Merck, Mumbai).
2. 0.15% a, a' Bipyridyl: Prepared by dissolving 150 mg of á, á' Bipyridyl (Merck, Mumbai) in 100 mL of distilled water
3. 0.27% Ferric chloride: Prepared by dissolving 270 mg of Ferric chloride (Sdfine-Chem limited, Mumbai) in 100 mL of distilled water.
4. 0.2M ortho phosphoric acid: Prepared by diluting 8.5 mL of ortho phosphoric acid (Merck, Mumbai) to 100 mL with doubly distilled water.
5. Pharmaceutical grade DFL was kindly gifted by local pharmaceutical industry.
6. Tablet dosage forms of DFL such as Doxobid (400 mg, Reddy's Lab, Hyderabad), Synasma (400 mg, Ranbaxy, Mumbai), Doxfree (400 mg, Maceleods pharmaceuticals, Mumbai) were purchased from local market.

Preparation of stock and working standard drug solutions

A stock standard solution containing 1 mg mL^{-1} of DFL was prepared in chloroform for method A and in water for method B. Working standard solution equivalent to 200 ìg mL^{-1} and 100 ìg mL^{-1} of DFL was obtained by appropriate dilution of stock solution by chloroform and water for methods A and B, respectively.

Recommended procedure

Method A

Into a series of 10 mL flasks, different volumes (0.2-1.6 mL) of DFL (200 ìg mL^{-1}) were pipetted. 2 mL of 0.2 % iodine was added in each flask at room temperature (25±?1 °C) and diluted up to the mark with chloroform. The absorbance was measured after 15 min at 390 nm against the reagent blank prepared similarly omitting the drug. The concentration of DFL was calculated either from calibration curve or from regression equation.

Method B

Into a series of boiling test tubes, different volumes (0.2-1.6 mL) of DFL (100 ìg mL^{-1}) were

pipetted. To each test tube, 1.5 mL of 0.27 % $FeCl_3$, 1 mL of 0.15 % á, á' bipyridyl and 1 mL of 0.2 M orthophosphoric acid were added, mixed well, and heated on a water bath at 65°C for 15 minutes. The tubes were cooled at room temperature, and then the contents of the tubes were transferred to 10 mL volumetric flasks and diluted to volume with doubly distilled water. The absorbance was measured at 535 nm against a reagent blank treated similarly except without drug. The concentration of DFL was calculated either from calibration curve or from regression equation.

Procedure for the analysis of DFL in tablet dosage forms

Ten tablets were weighed accurately and ground into a fine powder. An amount of powder equivalent to 100 mg of DFL was weighed into a 100 mL volumetric flask, 50 mL of the chloroform (method A) or water (method B) was added and shaken thoroughly for about 10 min, then the volume was diluted up to the mark with the same solvents, mixed well and filtered using a quantitative filter paper. The filtered solution was further diluted with the respective solvents according to the need and then analyzed following the proposed procedures.

RESULTS AND DISCUSSION

Mechanism of the reaction

The results obtained in method A were due to the charge transfer reaction between the DFL and iodine to yield a yellow colored Tri-iodide ion pair having maximum absorption at a wavelength of 390 nm against the corresponding reagent blank. Iodine is an *ó*-electron acceptor. The DFL has tertiary amino group, which act as *n*-electron donor. Therefore, the DFL react with electron acceptor to form charge transfer complex. Formation of the tri-iodide ion in solution is most probably due to a transformation of the initially formed outer complex into an inner electron donor accepter complex followed by a reaction of the resulting inner complex with iodine to form a triiodide ion. The colored complex was stable for about 1.5 hrs. A general reaction mechanism is proposed in figure 2.

The proposed method B was based on oxidation of DFL by Fe^{3+} in $FeCl_3$. The resulting Fe^{2+} complexes with unshared pair of electrons on each of the two nitrogen atoms of α, α' bipyridyl to produce colored chromogen having maximum absorption at 535 nm against the corresponding reagent blank. Fe^{3+} interferes to a little extent (especially in the lower range of Beer's law limits) in the determination of Fe^{2+} by method B. The reactivity of the interfering entity (Fe^{3+}) has made insignificant by complexing it with orthophosphoric acid. The colored complex was stable for about 2 hrs. The probable reaction mechanism is proposed in figure 3.

Table 1: Optimization of experimental variables for method A

Parameter	Investigation conditions	Conditions in procedure	Remarks
λ_{max} (nm)	350 - 600	390	The chloroform was selected as the best for the iodine charge-transfer complex formation as it produces maximum sensitivity and product stability.
Effect of solvent	Acetonitrile, Chloroform Dichloromethane, Chloroform and Dioxane		
Volume of 0.2% Iodine (mL)	0.2 – 3.6	2	2 mL of 0.2% iodine gave the maximum absorbance and remained constant by further addition of iodine.
Effect of reaction time (min)	0-45	15	15 min of reaction time was required for maximum color intensity. Further increase in the reaction time does not cause any change in intensity of color.

Table 2: Optimization of experimental variables for method B

Parameter	Investigation conditions	Conditions in procedure	Remarks
λ_{max} (nm)	400 - 700	535	
Volume of 0.27% $FeCl_3$ (mL)	0.5 – 3.1	1.5	1.5 mL of $FeCl_3$ was sufficient to produce maximum color. Beyond this volume the intensity of the color remains constant.
Volume of 0.15% á, á' Bipyridyl (mL)	0.5 – 2.5	1	For optimum color development 1mL of á, á' Bipyridyl is required. Beyond 1mL there is no change in the intensity of color.
Volume of 0.2 M orthophosphoric acid (mL)	0.5 – 5	1.0	1 mL of OPA was sufficient to complex with the interfering Fe^{3+} remaining in the solution after reaction was completed.
Effect of Temperature (^{0} C)	30 - 80	65	From 30^{0}C, the color intensity was increased upto 65^{0}C. Raising the temperature above 65^{0}C, color intensity and the absorbance started to decrease. Hence 65^{0}C was selected.
Effect of heating time (min)	5 – 30	15	15 min of heating time was required for maximum color intensity. Further increase in the heating time does not cause any change in intensity of color.

Table 3: Spectral and Statistical Data for the Determination of DFL by the proposed methods

Parameters	Method A	Method B
λ_{max} (nm)	390	535
Beer's Limit (µg mL^{-1})	4-32	2-16
Molar Absorbtivity (L/ mole/ cm)	8.012 x10^3	1 . 8 1 2 x10^4
Sandell's sensitivity (µg cm^{-2}/0.001 Absorbance unit)	0.0333	0.0327
Stability of colored products (hrs)	1.5	2.0
Regression equation (Y= mx + c)$^{\$\$}$		
Slope (m)	0.0301	0.0300
Intercept (c)	-0.0008	0.0017
Correlation coefficient (r)	0.9999	0.9993
LOD (µg mL^{-1})	0.138	0.101
LOQ (µg mL^{-1})	0.418	0.306
Standard deviation$^{\$}$	0.00126	0.00184
Relative standard deviation (%)	1.041	1.520
% Range of error (Confidence Limits)		
0.05 level	0.870	1.270
0.01 level	1.287	1.880

$^{\$\$}$Y = mx + c, where Y is the absorbance and x is the concentration of drug in ìg mL^{-1}.

$^{\$}$Average of six determinations.

Optimization of experimental variables

The optimization of proposed methods is commonly accomplished by sequentially optimizing one variable at a time while keeping all other variables constant. In this work, the influence of experimental variables on the absorbance of colored product was studied to obtain the optimum conditions for assay procedures. The conditions so obtained were incorporated in Table 1 and 2. The optimum values of the variables were maintained throughout the determination process.

Method Validation

Method validation includes all of the procedures required to demonstrate that a method to quantify the concentration of MSZ is reliable for the intended application.

In order to test whether the colored species formed in the above methods adhere to Beer's law, the absorbance's of a set of solutions containing varying amounts of DFL and specified amounts of reagents (as given in the recommended procedures) were recorded at appropriate wavelengths against the corresponding reagent blank. Beer's law limits, molar absorptivity and Sandell's sensitivity for DFL in each method developed with mentioned reagents were calculated. Least square regression analysis was carried out for getting the slope, intercept and the correlation coefficient values. The results were summarized in the Table 3.

The limit of detection (concentration of drug corresponding to a signal equal to the blank mean plus three times the standard deviation of the blank) and limit of quantification (concentration of drug corresponding to the blank mean plus ten times the standard deviation of the blank) were calculated according to the current ICH guidelines[18] and the results are presented in Table 3.

Table 4: Standard addition method for the determination of DFL in tablet dosage forms

Method	Brand name of tablet	Labeled claim (mg)	Pure drug added (mg)	Found ± S.D (n=5)	Recovery (%)	RSD
A	Doxobid	400	10	410.05±0.348	100.01	0.848
	Synasma	400	10	408.95±0.856	99.74	0.209
	Doxfree	400	10	409.75±0.728	99.93	0.177
B	Doxobid	400	10	410.39±0.536	100.09	0.130
	Synasma	400	10	411.54±0.485	100.37	0.117
	Doxfree	400	10	411.09±0.658	100.26	0.160

Table 5: Comparison of the proposed methods with the reference method for the determination of DFL

Method	Brand name of tablet	Labeled claim (mg)	Found ± S.D (n=5)	Recovery (%)	F* value	t* value
Reference	Doxobid	400	399.80±0.569	99.95	-	-
	Synasma	400	405.80±0.324	101.45	-	-
	Doxfree	400	400.24±0.445	100.06	-	-
A	Doxobid	400	406.38±0.726	101.59	2.30	1.59
	Synasma	400	397.98±0.406	99.49	2.59	0.98
	Doxfree	400	400.15±0.518	100.03	1.86	0.49
B	Doxobid	400	399.86±0.598	99.96	3.49	1.99
	Synasma	400	404.19±0.452	101.04	2.67	1.79
	Doxfree	400	397.97±0.603	99.49	1.66	0.83

*Tabulated t value at 95 % confidence level = 2.77 and Tabulated F value at 95% confidence level = 6.39.

The intraday precision and accuracy of the proposed method was examined by carrying out six replicate determinations of fixed concentration of DFL (within Beer's law range) by the proposed methods. The standard deviation, relative standard deviation and percentage of error were calculated for the proposed methods and were found to be acceptable (Table 3). The results shown that the proposed methods are effective for the determination of DFL.

The accuracy of the proposed methods was further evaluated by performing recovery studies by standard addition technique. The recovery studies were carried out by adding 10 mg of bulk sample of DFL to the pre-analyzed formulation and the mixture were analyzed by the proposed methods. The results of this study (Table 4) indicate

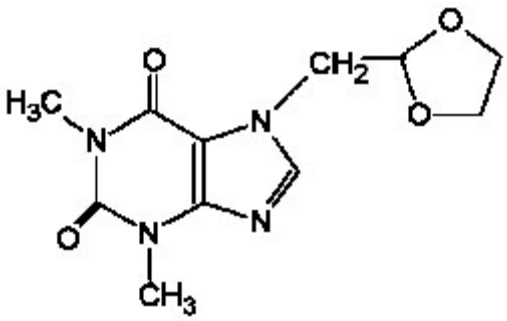

Fig. 1: Structure of Doxofylline

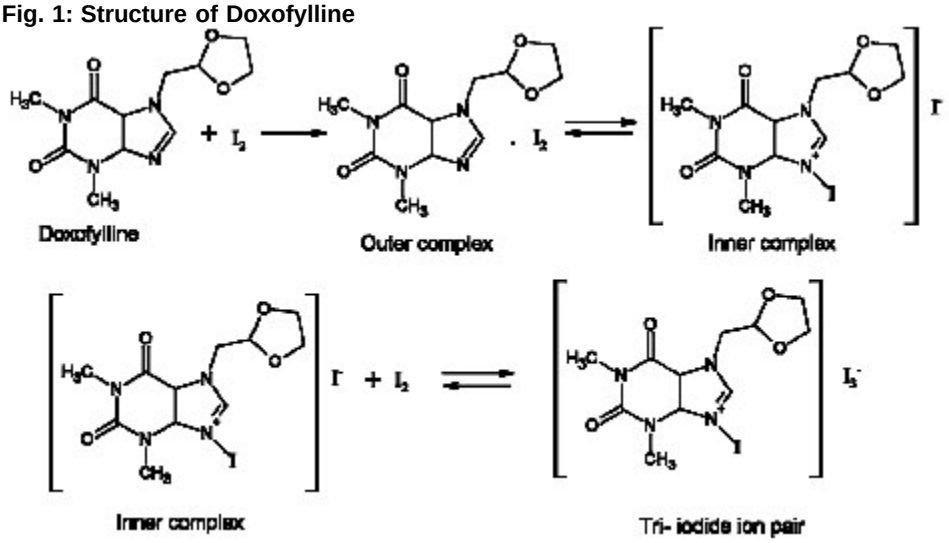

Fig. 2: Probable reaction sequence of the proposed method A

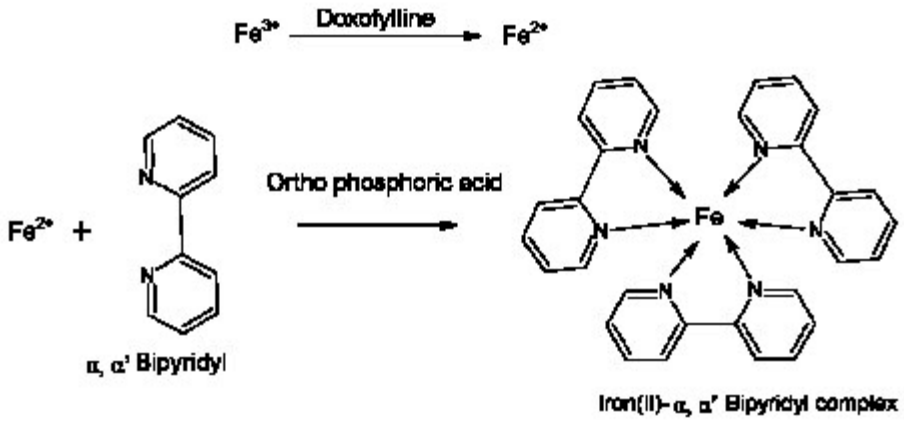

Fig. 3: Probable reaction sequence of the proposed method B

that neither the accuracy nor the precision of the methods is not affected by the coformulated substances.

Application of the proposed methods for tablet dosage forms

The proposed methods were successfully applied to the analysis of different dosage forms containing DFL. The results obtained by the proposed methods are compared statistically with the reference method[10]. The *t*-test and *F*-test were carried out, which showed that the proposed methods and official method are of comparable accuracy and precision. The results are summarized in Table 5.

CONCLUSION

Doxofylline was quantified successfully in bulk and tablet formulations by the two inexpensive, simple, sensitive, accurate and precise visible spectrophotometric methods that were developed. The proposed methods do not require any sophisticated equipment, pretreatment of the drug and tedious extraction procedure prior to its analysis. The sample recoveries in tablet dosage forms were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence, these methods can be easily and conveniently adopted for routine analysis of doxofyline in pharmaceutical industries, hospitals and research laboratories.

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