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Extractive spectrophotometric methods for the determination of docetaxel in pure and pharmaceutical formulations

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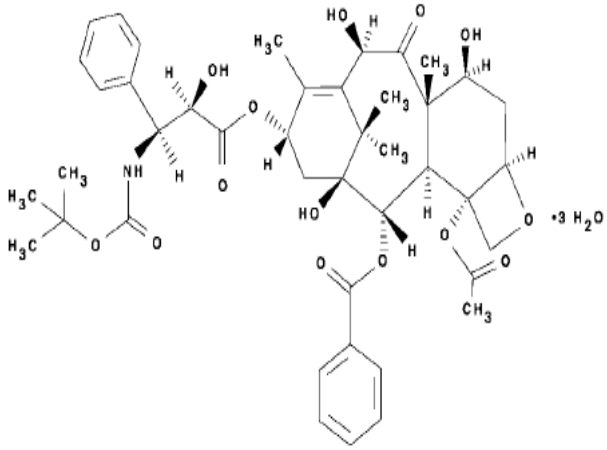
ABSTRACT

Two sensitive extractive spectrophometric methods have been developed for the estimation of Docetaxel in pure and pharmaceutical dosage forms. The developed methods are based on the formation of colored chloroform extractible ion-association complex of the drug with Alizarin red-s [ARS] and Tropaeolin ooo[TPooo]. The extracted complexes showed absorbance maxima at 424 and 430 nm respectively for these methods. Beer's law is obeyed in the concentration ranges between 8-40 µg/ml for the two methods. The effective concentration of dyes, pH and optimum conditions have been established for these methods. The methods are applied for the determination of drugs in commercial tablets and results of analysis were validated statistically through recovery studies.

Keywords: Docetaxel, Spectrophotometric methods, ARS, Tpooo, Chloro form.

INRRODUCTION

Docetaxel, (2R,3S)-N-carboxy-3-phenylisoserine,N-*tert*-butyl ester, 13-ester with 5β-20-epoxy-l,2α,4,7β,10β,13α-hexahydroxytax-ll-en-9-one 4-acetate 2-benzoate, trihydrate is an antineoplastic agent belonging to the taxoid family used in the treatment of breast, ovarian, prostate, and non-small cell lung cancer[1-3].The cytotoxic activity of docetaxel is exerted by promoting and stabilizing microtubule assembly, while preventing physiological microtubule depolymerisation/disassembly in the absence of guanosine triphosphate. This leads to a significant decrease in free tubulin, needed for microtubule formation and results in inhibition of mitotic cell division between metaphase and anaphase, preventing further cancer cell progeny[4,5].



Pharmacokinetic studies [6-8] of Docetaxel has been reported in the literature. Various methods cited in literature for its determinations involve HPLC methods and two HPLC/MS in plasma [9-11]. An ion-pair extractive

spectrophotometric method has been reported in the literature for the determination of a drug using BPB reagent [12]. So far, there has been no ion-pair extractive spectrophotometric method reported for the estimation of docetaxel [DCL] in pure and pharmaceutical dosage forms. This prompted the author to develop two simple and rapid spectrophotometric methods with a one-step extraction procedure for determination of docetaxel [DCL] in pharmaceutical dosage forms.

MATERIALS AND METHODS

Apparatus:

• Spectral and absorbance measurements were carried out by using ELICO UV – Visible Double beam spectrophotometer model SL-159 equipped with 1.0cm thickness matched quartz cells was used for the entire experimental work.

• Systronics digital pH meter was used to adjust and determine the hydrogen ion concentration (pH) of the buffer solution.

Preparation of Standard drug solution: Pharmaceutical grade docetaxel [DCL] was gifted by Hetero drugs India Ltd., (99.8% pure), was used in method development. A stock standard solution containing 1.0mg.mL^{-1} docetaxel [DCL] was prepared by dissolving accurately weighed (100mg) of pure drug with double distilled water in 100mL calibrated flask. This stock solution is further diluted appropriately with double distilled water to get a working standard concentration of 200µg.mL^{-1} for the given below proposed methods respectively.

Reagents and Solutions:

All chemicals and reagents used were of analytical grade or pharmaceutical grade. All solutions were prepared in doubly distilled water.

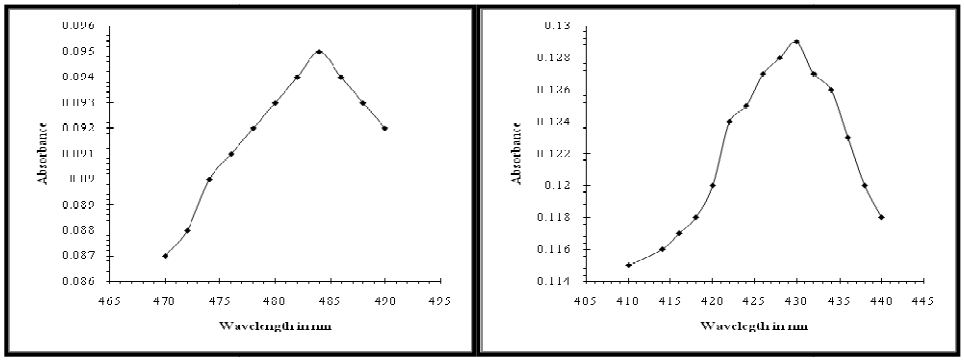
Hydrochloric acid (0.1 M, Sd Fine chemicals, India): Prepared by diluting 8.5 mL of concentrated acid to 1 litre of double distilled water.

Buffer solution, pH 3.0: Prepared by mixing 50mL of 1.0 M sodium acetate solution with 39.5mL of 1.0 M HCl solution and diluted to 250mL with doubly distilled water. The pH of the solution was adjusted to an appropriate value with the aid of a pH meter.

Assay procedure for tablets: Twenty tablets were weighed and finely powdered. A quantity of tablet powder equivalent to 100 mg of docetaxel [DCL] taken in volumetric flask (100mL) was shaken with methanol (10.0mL) for 10 min and the volume was made upto the mark with distilled water. The solution was then filtered through whatman filter paper and the aliquot portion of the filtrate was diluted to 100.0mL with distilled water to get sample solution and analyzed as given in the above proposed assay procedures.

RESULTS AND DISCUSSION

Fig. 1: Absorption spectrum of DCL with TPooo **Fig. 2: Absorption spectrum of DCL with ARS**



1. Method Development:

In order to ascertain the optimum wavelength of maximum absorption (λ_{max}) of the colored species formed in the above methods, specified amounts of docetaxel [DCL] were taken and colors were developed separately by following the above proposed procedures. The absorption spectras were scanned on a spectrophotometer in the wave length region of 340 to 900nm against similar reagent blank or distilled water and are shown in **(Figs. 1 & 2)**. These

spectrums show a single well-defined peak with characteristics absorption maxima where as the blank in each method has low or no absorption in this region.

The wavelengths (absorption maxima) for each proposed methods were used for the visible spectrophotometric analysis of docetaxel [DCL] in bulk samples respectively.

2. Optimization Studies of Experimental Variables for the Proposed Procedures:
The Optimization studies for the color development for the proposed methods for the assay of docetaxel [DCL] were established by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the colored species and were found to be same as described in **Table.1 & 2**.

For ARS: The Optimization studies for the color development for the proposed method with ARS was established by varying the one parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the colored species and the conditions so obtained **(Table.2)** were incorporated in recommended procedures**.**

3. Method Validation:
A) Optical Characteristics: In order to test whether the colored species formed in the above methods, adhere to Beer's law the absorbance's at appropriate wavelengths of a set of solutions containing varying amounts of docetaxel [DCL] and specified amounts of reagents (as given in the recommended procedures for each method) were recorded against the corresponding reagent blanks. The Beer's law plots of these systems are recorded against the corresponding reagent blanks. The Beer's law plots of these systems are recorded graphically **(Figs.3 & 4)**.

Table.1 Optimum conditions established in method TPooo for Docetaxel

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm) Tpooo	470 - 500	484	
Effect of acid or buffer on color development.	0.08 - 0.12 HCl for TPooo	0.1M HCl for TPooo	Variation of concentration or pH of acid beyond the upper and lower limits resulted in low absorbance values.
Choice of organic solvent for extraction of the colored complex.	Chloroform for TPooo	Chloroform for TPooo	The water immiscible solvents tested for the extraction of the colored complex into organic phase, which include (chlorobenzene, carbontetrachloride, benzene, n-butanol and chloroform). Chloroform was preferred for its selective extraction of the colored drug-dye complex from the aqueous phase.
Effect of shaking time on extraction.	1- 5 min	2 min	Constant absorbance values were obtained for shaking periods between 1-5 min.
Effect of temperature on the colored species.	Laboratory temperature (28+30C)	Laboratory temperature	At low temperature (< 20^0C) the extraction of colored species was found to be improper. At high temperature (> 35^0C) the stability of the colored species was found to be less.
Stability of the colored species in organic solvent.	1 - 60 min	5 min	

Table.2 Optimum conditions established in method ARS for Docetaxel

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm) ARS	415 - 440	430	
Effect of acid or buffer on color development.	0.08 - 0.12 HCl for ARS	0.1M HCl for ARS	Variation of concentration or pH of acid beyond the upper and lower limits resulted in low absorbance values.
Choice of organic solvent for extraction of the colored complex.	Chloroform for ARS	Chloroform for ARS	The water immiscible solvents tested for the extraction of the colored complex into organic phase, which include (chlorobenzene, carbontetrachloride, benzene, n-butanol and chloroform). Chloroform was preferred for its selective extraction of the colored drug-dye complex from the aqueous phase.
Effect of shaking time on extraction.	1- 5 min	2 min	Constant absorbance values were obtained for shaking periods between 1-5 min.
Effect of temperature on the colored species.	Laboratory temperature (28+30C)	Laboratory temperature	At low temperature (< 200C) the extraction of colored species was found to be improper. At high temperature (> 350C) the stability of the colored species was found to be less.
Stability of the colored species in organic solvent.	1 - 60 min	5 min	

B) Linearity Range and Analytical data: Linearity ranges for each proposed spectrophotometric method for quantitative analysis of docetaxel [DCL], were made by plotting calibration curves over the concentration ranges cited. The statistical parameters (optical characteristics) such as Beer's law limits, Correlation coefficient, Sandell's sensitivity, molar absorptivity, percent relative standard deviation (calculated from six replicate samples containing

3/4th of the amount of the upper beer's law limits) were calculated for all the proposed methods and the results are summarized in **Table.3**.

Fig. 3: Beer's Law plot of DCL with TPooo

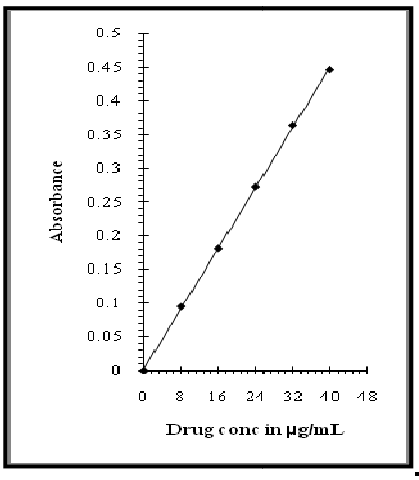
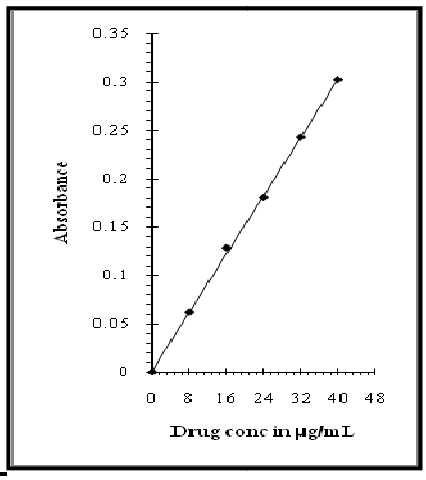


Fig. 4: Beer's Law plot of DCL with ARS



C) Precision: The precision of each proposal methods was ascertained from the absorbance values obtained by actual determination of six replicates of a fixed amount of docetaxel [DCL] (10.0 µg/ml) for the proposed methods. The percent relative standard deviation and percent range of error (at 0.05 and 0.01 confidence limits) were calculated for the proposed methods **(Table.4)**.

D) Accuracy: To determine the accuracy of each proposed method, different amounts of bulk samples of docetaxel [DCL] within the Beer's law limits were taken any analyzed by the proposed method. The results (percent error) are recorded in **Table.4**.

Table.3 Optical and regression characteristics, precision and accuracy of the proposed methods for Docetaxel

Parameter	TPooo	ARS
λ_{max} (nm)	484	430
Beer's law limits (µg/ml)	8.0 - 40.0	8.0 - 40.0
Molar absorptivity (1 $mol^{-1}.cm^{-1}$)	7.213 x 10^3	2.366 x 10^4
Sandell's sensitivity (µg.cm^{-2}/0.001 absorbance unit)	0.05329	0.02578
Optimum photometric range (µg/ml)	9.5 - 35.0	8.0 - 38.5
Regression equation (Y=a+bc) ; Slope (b)	0.0110	0.0075
Standard deviation on slope (S_b)	0.000117	0.000139
Intercept (a)	0.0063	0.0048
Standard deviation on intercept (S_a)	0.0000675	0.0000807
Standard error on estimation (S_e)	0.002960	0.003540
Correlation coefficient (r)	0.9998	0.9994
Relative standard deviation (%)*	1.232	1.478
% Range of error (confidence limits)		
0.05 level	1.030	1.236
0.01 level	1.524	1.829

** Average of six determinations considered*

Table.4 Determination of Docetaxel in dosage forms by the proposed methods and official method

Formulations*	Amount taken (mg)	Amount found by proposed Methods**		Reference method[8]	Percentage recovery by proposed methods***	
		TPooo	ARS		TPooo	ARS
Tablet I	20	19.94±0.13 F=1.5 t=0.23	19.89±0.09 F=3.16 t=0.96	19.96±0.16	98.97±0.81	96.42±0.95

**Tablets from four different pharmaceutical companies.*

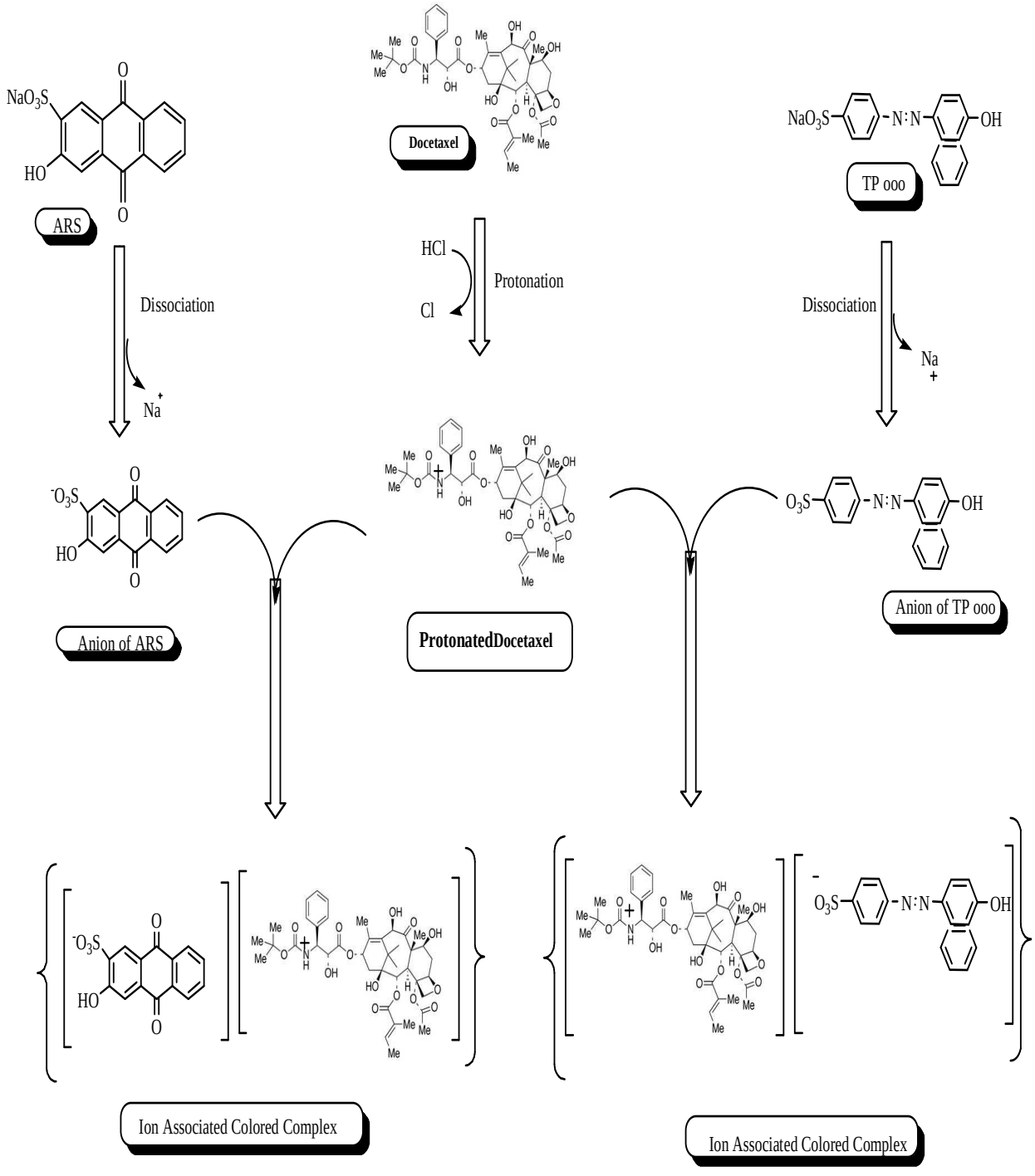
*** Average ± standard deviation of six determinations, the t-and F-test values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limit, F = 5.05, t = 2.26*

E) Application to formulations: In order to evaluate the analytical applicability of the proposed methods the results obtained by the proposed methods in label claim compared statistically with those obtained by a literature UV-spectrophotometric method by applying student's t-test for accuracy and F-test for precision. **Table.4** gives the

results of the assay and reveals that there is close agreement between the results obtained by the proposed methods (label claim) the reference method with respect to accuracy and precision for docetaxel [DCL].

At the 95% confidence level, the calculated *t*- and *F*-values did not exceed the tabulated values (t = 2.77 and F = 6.39), suggesting that the proposed methods are as accurate and precise as the literature method.

F) Nature of colored species:
For Method – TPooo & ARS: Docetaxel [DCL] possesses a secondary amino group. It forms an ion association complex with two acid dyes [Tpooo & ARS] which is extractable into chloroform from aqueous phase. The protonated nitrogen (positive charge) of docetaxel [DCL] is expected to attract the appositely charged part (negative charge) of the dye and behave as a single unit being held together by electrostatic attraction [Scheme].



Scheme: Reaction mechanism of Docetaxel with TPooo & ARS

CONCLUSION

Two sensitive spectrophotometric methods has been developed, optimized and validated for the determination of docetaxel [DCL] in pure drug and in tablets. The simplicity, sensitivity and selectivity make the method a suitable alternative to the HPLC methods. Other characteristics such as short performance time, ease of handling of organic solvents and does not requiring either expensive equipments or specialized technicians, also suggest that the procedures developed by the author can be adopted as routine laboratory methods in quality control laboratories where modern instruments are not available.

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