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GREEN SPECTROPHOTOMETRY AND SPECTROFLUORIMETRIC METHODS FOR RAPID ECONOMIC DETERMINATION OF TRAVOPROST: STUDY ON INCLUSION COMPLEX WITH BETA CYCLODEXTRIN

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Abstract:

Green spectrophotometry and spectrofluorimetric methods for determination of travoprost(TRAVO) were designed and investigated. Spectrophotometric methods are based on measuring the absorbance of the drug in water and in presence of 1.5 mL of 1.0% (w/v) beta-cyclodextrin (ß-CD) at 277 nm. The absorbance concentration plots were rectilinear over the range of, 10 – 50 µg/mL and 2.5 – 30 µg/mL with limits of detections; 0.018 and 0.009 µg/mL in absence and presence of ß-CD respectively. The fluorescence enhancement of a highly sensitive spectrofluorometric method is based on investigation of the fluorescence spectral behavior of travoprost in aqueous organized system, ß-CD. In aqueous solution travoprost is well incorporated into ß-CD, with enhancement of its native fluorescence. The fluorescence was measured at 305 nm after excitation at 277 nm. Fluorscence spectroscopy of the host – guest interaction between travoprost and ß-CD shows 1:1 inclusion complex with ß-CD, with an association constant of 21.86 M^{-1}. The quantum yield was 0.16 and 0.20 in absence and present of ß-CD. The fluorescence-concentration plots are rectilinear over the range of 10 – 250 and 2.5 – 35 ng/mL, with lower detection limits of, 0.003 and 0.002 ng/mL respectively. The methods were successfully applied to the analysis of pharmaceutical preparation and the results were in good agreement with those obtained with the official HPLC method. The proposed methods are inexpensive, simple, and sensitive for quality control of the drug in QC laboratories.

Keywords: *Travoprost, spectrophotometry, spectrofluorimetry, ßeta-cyclodextrin, inclusion complex, validation*

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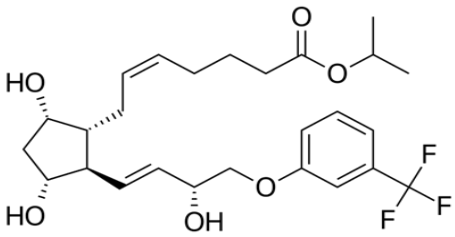
1. INTRODUCTION:

Travoprost is chemically designated as (propan-2-yl 7-[3,5-dihydroxy-2-[3-hydroxy-4-[3-(trifluoromethyl)phenoxy]-but-1-enyl]-cyclopentyl]hept-5-enoate)[1].



Chemical structure of travoprost

Travoprost ophthalmic solution is a topical medication used for controlling the progression of glaucoma or ocular hypertension, by reducing intraocular pressure. It is a synthetic prostaglandin analog (or more specifically, an analog of prostaglandin F2α) that works by increasing the outflow of aqueous fluid from the eyes [2-5].

Literature review revealed that travoprost is official in USP pharmacopeia, and that few high performance liquid chromatographic methods were reported for its determination in biological fluids and pharmaceuticals using HPLC with UV or MS detectors [6-8], however no methods had been reported for the determination of travoprost in pharmaceuticals using spectrophotometry or fluorimetry.

Cyclodextrins (CDs) are neutral and natural oligosaccharides that are prepared by the enzymatic degradation of starch. They have the property of forming an inclusion complex with various guest molecules with suitable polarity and dimension because of their special molecular structure/hydrophobic internal cavity and hydrophilic external surface [9-13].

The most important property of inclusion compounds is that a host component can admit a guest component into its cavity without any covalent bonds being formed. Because inclusion is largely independent of the chemical properties of the guest molecules, the minimum requirement for inclusion complex formation is size compatibility between the host and guest molecules, i.e. the guest must fit, entirely or at least partially, into the cyclodextrin cavity[14].

Fluorescence spectroscopy is a powerful tool to study the host–guest molecular systems with both high sensitivity and high selectivity [15]. The remarkable advantages of fluorescence analysis are that it is capable of measuring much lower concentrations than spectrophotometric analysis, and it is potentially more selective because both the excitation and emission wavelengths can be varied. The sensitivity of the fluorescence detection of a host–guest molecular interaction depends upon the electronic or steric changes of the chromophore responsible for the fluorescence behavior. Here, the analytical application of various CDs to enhance the fluorescence intensity of various analytes and the use of fluorescence signal enhancement to develop cyclodextrin-induced spectrofluorimetric methods for the determination of analyte are reviewed [16].

The aim of this study is to develop simple, sensitive, cheap and eco-friendly spectrophotometric and fluorimetric methods in absent and presence of ß - CD for the quantitative determination of travoprost in drug substance and product. The methods developed were fully validated as per ICH Q2 (R1), [17]. To the best of our Knowledge, no spectrophotometric or spectrofluorimetric methods, were reported for determination of travoprost.

2. EXPERIMENTAL:

2.1 Instruments

For spectrophotometric analysis, Spectrophotometer: Anlytikjena AG bouble beam UV-visible Spectrophotometer (Jena/Germany) using matched 1 cm quartz cells, connected to DELL compatible and HP1102 LaserJet printer. The bundled software is WinASPECT PLUS, version 4.2(Anlytikjena Jena/Germany). The scanning speed is 50 nm/min, with 1 nm interval.

For spectrofluorimetric analysis, Cary Eclipse fluorescence spectrophotometric (USA) connected to IBM-PC computer and HP laser jet 1100 series printer. The emission of all samples was recorded against a solvent blank in 1 cm quartz cuvette and scanning at the following parameters:

Band width = 1.5 nm, Scan speed = 1200 nm/min, Data Interval = normal (1nm), Smoothing = high wavelength calibration was performed by measuring λ_{EX} 277 nm and λ_{Em} 305 nm.

2.2 Chemicals and reagents

All chemicals and reagents used were of analytical grade and solvents were of HPLC. Acetonitrile, methanol, ethanol, isopropanol and acetone (Macron fine chemicals, Poland), 100 mM sodium hydroxide (Merck, Darmstadt, Germany), ß - Cyclodextrin Sigma Aldrich (Germany), and Benzalkolium

chloride Sigma Aldrich(Germany) are used. Double distilled water filtered through a 0.47 µm membrane filter (Alltech Associates, USA) was used throughout all experiments.

2.3. Samples

2.3.1. Pure samples

Travoprost was kindly supplied by Cayman Pharma Co., B.N., 1150S04, Egypt. It was certified to be 98.8%.

2.3.2. Market sample

Travoprost ophthalmic solution (Travonorm) was labeled to contain 0.04 mg travoprost/mL, Orchidia Pharmaceutical Industries, (Industrial Zone- Al – Obour City Egypt, B. NO: 0315158). It was purchased from the market.

2.4. Standard solutions

2.4.1. Standard stock solution

2.4.1.1. Spectrophotometric analysis

A stock standard solution of TRAVO (1 mg/mL) was prepared by dissolving 100 mg of TRAVO in water in 100 mL volumetric flask and the volume was completed to the mark with the same solvent.

2.4.1.2. Spectrofluorimetric analysis

A stock standard solution of TRAVO (0.5 mg/mL) was prepared by dissolving 25.00 mg of travoprost in water in 50 mL volumetric flask and the volume was completed to the mark with the same solvent.

2.4.2. Working standard solution

2.4.2.1. Spectrophotometric analysis

A working standard solution (0.5 mg/mL) was prepared by transferring 25 mL of stock solution into a 50 mL volumetric flask and completed to the mark with water.

2.4.2.2. Spectrofluorimetric analysis

TRAVO working standard solution (0.5 µg/mL) was prepared by transferring 0.1 mL of stock solution into a 100 mL volumetric flask and completed to the mark with water.

2.5. Procedures

2.5.1. Construction of the calibration graph

2.5.1.1. Spectrophotometric analysis

2.5.1.1 .a In absence of 1% ß -CD

Aliquots equivalent to 100.0 – 500.0 µg of the standard solutions were transferred into a series of 10 mL volumetric flasks, and completed to the mark with water to give a final concentration range of 10.00 – 50.00 µg/mL.

2.5.1.1. b In presence of 1% ß -CD

Aliquots equivalent to 25.0 – 300 µg of the working standard solutions were transferred into a series of 10 mL volumetric flasks, and completed to the mark with water to give a final concentration range of 2.50 - 30.00 µg/mL.

The absorbance was measured at λmax 277 nm for both methods. The calibration graphs were plotted of the absorbance versus concentrations of the drug (µg/mL). The regression equations were computed for the drug in absence and presence of ß -CD respectively.

2.5.1.2. Spectrofluorimetric analysis

2.5.1.2. a In absence of 1% ß -CD

Aliquots equivalent to 100 – 1000 ng/mL of the working standard solutions were transferred into a series of 10 mL volumetric flasks completed to the mark with water to give a final concentration range of 10.00-100.00 ng/mL.

2.5.1.2. b In presence of 1% ß -CD

Aliquots equivalent to 25.0 – 350.0 ng/mL of the working standard solution were transferred into a series of 10 mL volumetric flasks by graduated micropipette and completed to the mark with water to give a final concentration range of 2.50-35.00 ng/mL.

The fluorescence intensity was measured at λ_{Em} 305 nm after excitation at λ_{Ex} 277 nm for both methods. The calibration graph was plotted the emission at 305 nm versus the concentrations of the drug (ng/mL). Then the regression equations were computed for the drug in absence and presence of ß -CD respectively.

2.5.2 Application

The proposed methods were successfully applied for the determination of TRAVO in its pharmaceutical dosage form (Travonorm® eye drops, 0.04 mg TRAVO/mL). A Stock solution was prepared by mixing the content of three bottles (15 mL) in a stopper conical flask. Each milliliter was equivalent to 0.04 mg of TRAVO.

2.5.2.1. Procedure for spectrophotometry

2.5.2.1.a. In absence of 1% ß -CD

Accurately measured volume (2.5 mL) equivalent to 100 µg/mL of TRAVO in Travonorm® solution (0.04 mg TRAVO/ mL) was transferred to 5 mL volumetric flask. The volume was completed to the mark with water to obtain a final concentration of 20 µg/mL. Then the recommended procedure mentioned under; *2.5.1.1 .a In absence of 1% ß -CD* was proceeded.

2.5.2.1.b. In presence of 1% ß -CD
Accurately measured volume (2.5 mL) equivalent to 100 µg/mL of TRAVO in Travonorm® solution (0.04 mg TRAVO/ mL) was transferred to a 10 mL volumetric flask, and completed to the mark with water to obtain a final concentration of 10 µg/mL. Then the recommended procedure mentioned under *2.5.1.1. b In presence of 1% ß -CD* was proceeded.

2.5.2.2. Procedure for spectrofluorimetry
2.5.2.2.a. In absence of 1% ß -CD
Accurately measured volume (2.5 mL) equivalent to 100 µg/mL of TRAVO in Travonorm® solution (0.04 mg TRAVO/ mL) was transferred to a 25 mL volumetric flask, and completed to the mark with water to obtain a final concentration of 4 µg/mL, further dilution step was made to fall in the working range (10 – 200 ng/mL) which is 40 ng/mL. Then the recommended procedure mentioned under *2.5.1.2. a In absence of 1% ß -CD* was proceeded.

2.5.2.2.b. In presence of 1% ß -CD
Accurately measured volume (2.5 mL) equivalent to 100 µg/mL of TRAVO in Travonorm® solution (0.04 mg TRAVO/ mL) was transferred to a 100 mL volumetric flask, and completed to the mark with water to obtain a final concentration of 1 µg/mL, and further dilution was made to fall in the working range (2.5 – 35 ng/mL) which is 10 ng/mL. Then the recommended procedure mentioned under, *2.5.1.2. b In presence of 1% ß -CD* was proceeded. The nominal content of the eye drop was determined using either the calibration graph or the corresponding regression equation.

2.5.3. Interference
Accurately transfer 10 mg of benzalkolium chloride (excipient)into 10 mL volumetric flask and completed to the mark with water to obtain a final concentration of 1mg/mL. Further dilution step was made to obtain a concentration in the working range of each of the developed methods. Then the recommended procedure mentioned under, *2.5.1* was followed.

3. RESULTS AND DISCUSSION:

3.1. Spectral characterization
The characteristic absorption spectra of TRAVO were observed at 277 nm. Significant increase of absorbance intensity was observed at 277 nm after addition of ß-CD, which depends on the concentration of ß-CD (Fig. 1). The formation of complex of TRAVO with ß-CD in aqueous solution was characterized by UV spectroscopy. The results suggest the formation of inclusion complex between TRAVO and ß-CD. The specific absorbance [A1%, 1cm] was found to be, 100.1 and 448.8, and molar absorbtivity (Ɛ) of 80.60 × 10^{2} L. $mol^{-1}.cm^{-1}$ and 36.175 × 10^{3} L. $mol^{-1}.cm^{-1}$ in absence and presence of 1.5 mL of 1.0% (*w/v*) ß-CD.

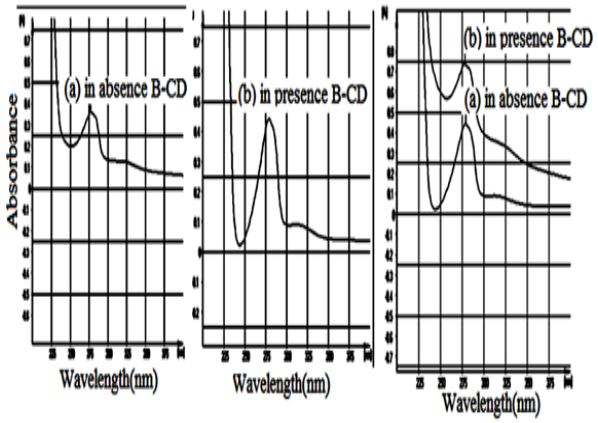


Fig. 1. Absorption spectra of travoprost (20 µg/mL) at ƛ 277 nm in absence and presence of ß – CD.

As a consequence fair sensitivity was achieved by direct spectrophotometric measurements, and this sensitivity was greatly increased when the native fluorescence of TRAVO was measured at λem 305 nm after excitation at λ_{Ex} 277 nm in water. Travoprost is characterized by having a native fluorescence due to its fused aromatic rings and extended conjugated structure. Emission and excitation spectra of TRAVO were given in Fig.2. Fluorescence spectra of TRAVO in absence and presence of ß-CD were investigated (Fig. 2). Maximum emission wavelength of TRAVO and TRAVO/ ß -CD complex was observed at 305 nm. The results suggest that a stable complex was formed between ß-CD and TRAVO. The quantum yield [QY] (18,19) was calculated in absence and presence of ß-CD and it was increased from 0.16 to 0.2. The enhancement of native fluorescence intensity in aqueous organized media is due to change in viscosity, polarity and binding capacity [20]. Quantum yield was calculated according the equation (21):

QY = Ys . Fu / Fs . As /Au
QY = Quantum yield
As = Absorbance of standard
Au = Absorbance of unknown
Ys = Quantum yield of standard
FU = Integrated emission of unknown
Fs = Integrated emission of standard

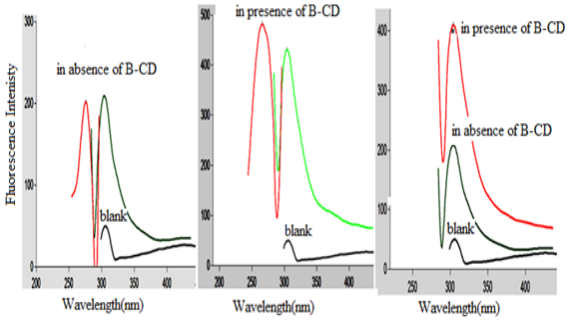


Fig. 2. Emission and exitation spectra of travoprost (30 ng/mL) in absence and presence of ß –CD at λ_{Em} 305 nm and λ_{Ex} 277 nm.

3.2. Optimization of reaction conditions

Different experimental parameters affecting the absorbance and native fluorescence intensity of the drug and its stability were carefully studied and optimized.

3.2.1 Influence of diluting solvents

The effect of different diluting solvents on FI of TRAVO was investigated using water, methanol, ethanol, diluted aqueous acid, diluted aqueous alkali, and acetonitrile. It was found that water was the best solvent for dilution as it gave the highest FI as shown in Fig. 3.

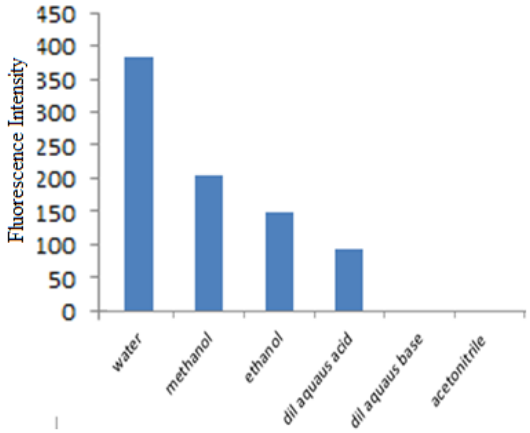


Fig. 3. Effect of different solvents on fluorescence intensity of travoprost (35 ng/mL) at λ_{Em} 305 nm and λ_{Ex} 277 nm.

3.2.2. Influonce of different concentrations of ß-CD

The absorbance and fluorescence intensity of TRAVO in different concentrations of ß-CD from 0.5 to 3.0 % (*w/v*) were investigated. The results revealed that the highest intensity was observed at concentration of 1 % (*w/v*) ß-CD. The results are shown in Fig. 4.

3.2.3 Influence of different volumes of 1.0% (w/v) ß-CD

The effect of different volumes of, 0.5 – 3.0 mL (ß-CD, 1% *w/v*) was investigated. It was found that 1.5 mL, is the best volume, as it gave the highest absorbance and FI.

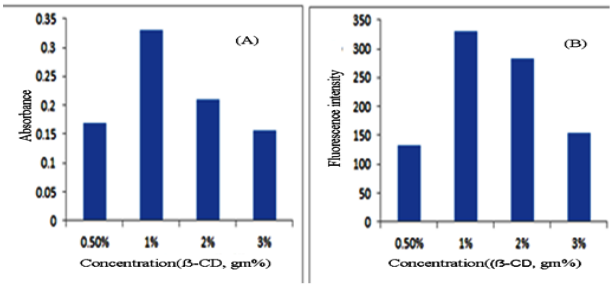
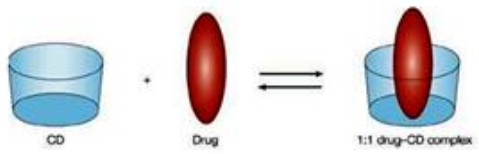


Fig. 4: Effect of different concentrations of ß-CD (gm%) on the (A)absorbance intensity, 25 µg/mL and (B) fluorescence intensity, 35 ng/mL of travoprost at λ_{Em} 305 nm and λ_{Ex} 277 nm.

3.3. Determination of complex-ratio and formation constant

Standard fluorescence spectroscopy analyzes the variation of a spectroscopic property (quantum yield, spectral shift, lifetime, or anisotropy) of a fluorescent guest or host due to the complexation. A significant variation of any of these parameters requires an intimate participation of the fluorophore in the complexation process. The formation of a host-guest inclusion complex of Travo with (ß -CD) in aqueous organized solution has been characterized by fluorimetry and UV-Vis absorption spectroscopy. The nature of the host-guest inclusion complex between TRAVO and ß -CD has been elucidated. The experimental results confirmed the existence of 1:1 inclusion complex. The binding constants describing the extent of formation of the complex have been determined, using modified Benesi-Hildebrand plots. The schematic presentation of the inclusion is represented in Scheme 1.



Scheme 1. Schematic presentation of incluion complex of travoprost with, 1% (*w/v*) ß-CD (1:1).

The ratio of complex, and formation constant were calculated from the modified Benesi-Hildebrand equation, 1/(F-Fo)= 1/(Kk[P]o[CD]o) + 1/(Kq[P]o)
Where F, Fo represent the fluorescence intensity of TRAVO in absence and presence of ß-CD, respectively, K is the formation constant, and [p] is constant. The reciprocal plots of 1/(F-Fo) versus 1/[CD] showed good linearity (Fig 5), indicating that the inclusion complex has a stoichiometry of 1:1. The value of k was found to be 21.86 M^{-1}.

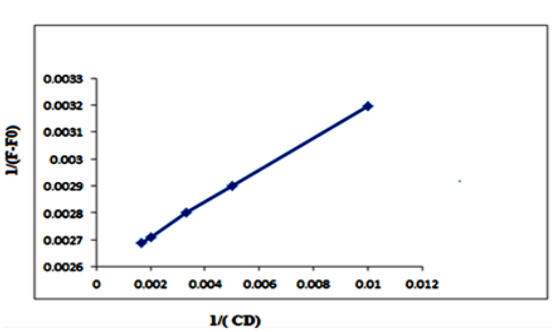


Fig. 5: Incluion complex of TRAVO – ß-CD (1:1).

3.4. Method validation

The validity of the proposed methods was assessed by studying the following parameters: linearity, range, LOD, LOQ, accuracy, precision, robustness and specificity, according to ICH guidelines [17] and the results are presented in Table 1.

3.4.1. Linearity and range

There was linear relationship between TRAVO concentration and both absorption and native fluorescence obtained over the concentration range of (10.00 – 50.00 µg/mL), (2.50-30.00 µg/mL), and (10.00 – 200.00 ng/mL), (2.50-35.00 ng/mL) for spectrophotometric and spectrofluorimetric methods in absence and presence of 1.5 mL of 1% ß - CD, respectively. The results showed good linearity with regression parameters calculated according to ICH guidelines as presented in Table 1.

The regression equations were computed and found to be:
A = 0.0125C - 0.0222 R2 = 0.9996 for spectrophotometry in absence of ß -CD
A = 0.0182 C - 0.0606 R2 = 0.9993 for spectrophotometry in presence of ß -CD
FI = 3.9328 C + 91.85 R2 = 0.9992 for spectrofluorimetry in absence of ß -CD
FI = 7.9342 C + 70.291 R2 = 0.9996 for spectrofluorimetry in presence of ß -CD

Where: A: is the Absorbance. C: is the concentration in µg/mL and ng/mL for spectrophotometric and spectrofluorimetric methods, respectively. FI: is the fluorescence intensity.

The high values of correlation coefficient (R2) and low values of standard deviation (SD), standard error (SE), and relative standard deviation (RSD) showed the assemblage of the points around the calibration graph and proved the linearity of the method over the specified concentration range as shown in Table 1.

3.4.2. Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were calculated according to the following equations as specified by ICH guidelines and the results are summarized in Table 1.

$$LOD = 3.3\ \sigma / S, \qquad LOQ = 10\ \sigma / S$$

Where σ is the standard deviation of the response and S is the slope of linearity.

Table 1: Regression parameters and validation results of the proposed spectrophotometric and spectroflourimetric methods for determination of travoprost drug substance

Parameters	Spectrophotometric method		Spectrofluorimetric method	
	Absence ß-CD	Presence ß-CD	Absence ß-CD	Presence ß-CD
Linearity range	10 – 50 µg/mL	2.5 – 30 µg/mL	10 – 200 ng/mL	2.5 – 35 ng/mL
LOD	2.4 µg/mL	0.65 µg/mL	2.12 ng/mL	0.14 ng/mL
LOQ	8.01 µg/mL	2.19 µg/mL	7.08 ng/mL	0.53 ng/mL
Accuracy* Mean ± RSD%	99.86 ± 1.61	99.19 ± 0.93	99.45 ± 0.90.01	100.118 ± 0.38
Regression				
Slope (s)	0.01	0.018	3.39	7.93
SE of slope	0.00014	0.00028	0.06	0.08
Confidence limit**	0.012 - 0.013	0.02 - 0.019	3.72- 4.14	7.7- 8.16
Intercept	-0.022	0.06	91.85	70.29
SE of intercept	0.0047	0.0047	8.01	1.745
Confidence limit **	- 0.034 to -0.0069	0.045-0.075	66.35-117.34	65.44-75.13
Correlation coefficient	0.9996	0.9998	0.9991	0.9995
SE of estimation	0.0045	0.00664	9.93	2.49

*mean of five different determinations. **Confidence at p=0.05%. RSD, is the relative standard deviation and SE, is the standard error.

3.4.3. Accuracy

To prove the accuracy of the proposed methods, the results of the assay of drug substance assessed by the proposed spectrophotometric and spectrofluorimetric methods were compared with those obtained using official HPLC methods [22].

Statistical comparison of the results obtained by the proposed methods and those obtained by the official method using mean recoveries, Student's t-test and variance ratio F-test revealed no significant difference between the two methods regarding accuracy and precision as shown in Table 2, indicating high accuracy and precision of the proposed methods [23].

Table 2: Statistical comparison of the results obtained by the proposed and official methods for the determination of travoprost in drug substance

Parameters	Travoprost								
	Spectrophotometry		Spectrophotometry+		Fluorimetry		Fluorimetry+		Official Method**
	Conce.	%Found	Conce.	%Found	Conce.	%Found	Conce.	%Found	%Found
	15 25 35	99.16 100.17 100.26	7.5 15 25	99.41 98.37 99.77	40 80 160	98.65 98.83 99.42	7.5 15 25	99.83 100.30 100.22	98.02 98.34 99.53
Mean*± SD	99.86± 1.61		99.19±0.92		99.45±0.90		100.12±0.39		98.63 ±0.79
Variance S^2	2.59		0.85		0.81		0.15		0.63
Student *t*-test (2.91)***	1.96		1.95		0.72		1.94		
F- test (19)***	1.69		1.19		2.49		1.29		

+ in presence of 1.5 mL of 1% ß-CD.
*Average of three separate determinations.
**Official HPLC method.
***Value between parentheses is the theoretical values of *t* and F.

3.4.4. Precision (repeatability and intermediate precision)

The intra- and inter-day precision were assessed by assaying freshly prepared solutions in triplicate on the same day and on three different days, respectively using the proposed methods. The low RSD of the repeatability (intra-day) and intermediate precision (inter-day) of the results obtained by means of the proposed methods indicate a high precision of these methods and proved to be suitable for quality control of TRAVO as shown in Table 3.

3.4.5. Specificity

The specificity of the proposed spectrophotometric and spectrofluorimetric methods were proven by its ability to determine TRAVO in pharmaceutical preparation without interference from benzalkolium chloride that commonly present in the matrix(excipient). The results indicate that the methods are specific as the RSD% less than 1.89 as stated in Table 4.

Table 3: Repeatability and intermediate precision data of the proposed methods for the determination of travoprost in drug substance

Drug substance	Amount added				Precision* RSD%							
	Spectrophotometric methods(µg/mL)		Spectroflourimetric methods(ng/mL)									
	absence ß- CD	presence ß- CD	Absence ß -CD	Presence ß -CD	Intraday (Repeatability)				Interday (intermediate precision)			
					UV (µg)	UV+ ß CD (µg)	Fl(ng)	Fl+ ß CD (ng)	UV (µg)	UV+ ß CD (µg)	Fl (ng)	Fl+ ß CD (ng)
Travoprost	20	10	50	10	1.43	1.94	1.304	0.16	0.39	1.83	0.304	1.8
	30	20	100	20	1.87	0.72	0.944	0.31	1.66	0.4	1.77	0.54
	40	30	150	30	0.64	1.08	0.46	0.28	0.699	1.09	0.6	0.21

*Mean of three different determinations.

Table 4: Specificity of the proposed methods for the determination of travoprost in presence of benzalkolium chloride

Benzalkolium chloride				Amount added				Found recovery*% ± RSD			
Stock solution (1 mg benzal /mL)				Spectrophotometric method(µg)		Spectroflourimetric method(ng)		Spectrophotometric methods (µg)		Spectrofluorimetric methods (ng)	
				UV	UV+ ß- CD	FL	FL+ ß- CD	UV	UV+ ß- CD	FL	FL+ ß- CD
UV (µg)	UV+ ß -CD (µg)	Fl (ng)	Fl+ ß-CD (ng)	40	20	200	30	98.02±1.89	98.7±1.76	101.11± 1.06	98.82±1.23
150	75	750	112.5								

3.4.6. Stability of the drug solutions

Solutions of the drug were stable for one month when kept in the refrigerator. No change in absorbance or florescence intensity appeared throughout the whole validation procedures.

3.5. Application

The proposed methods were successfully applied for the determination of TRAVO in its pharmaceutical dosage form (Travonorml® eye drops, 0.04 mg TRAVO/mL) and the results are shown in Table 5.

Standard addition technique was used to assess the matrix effect of the solution additives and its contribution in the deviation of the results obtained by the proposed methods. The obtained results revealed no significant matrix effect as shown in Table 6.

Table 5: Results obtained by applying the proposed methods for the determination of travoprost in Travonorm® eye drops

Drug product	Spectrophotometric method		Spectrofluorimetric method		Official Method
Travonorm® (0.04mg TRAVO/mL)	Absence ß-CD Recovery*	Presence ß-CD Recovery*	Absence ß-CD Recovery*	Presence ß-CD Recovery*	Recovery* of claimed amount
Mean±RSD%	93.77 ± 1.57	94.02 ± 1.87	94.31 ± 2.0	96.83 ± 2.0	92.25 ± 1.98

* Average of five determinations.

Table 6: Application of standard addition technique for determination of travoprost in eye drop by the proposed spectrophotometric and spectrofluorimetric methods

Preparation				Amount added				Found recovery*% ± RSD			
Travonorm® (0.04 mg TRAVO /mL)				Spectrophotometric method(µg)		Spectroflourimetric method(ng)		Spectrophotometric methods (µg)		Spectrofluorimetric methods (ng)	
				Absence ß -CD	Presence ß- CD	Absence ß -CD	Presence ß- CD	Absence ß -CD	Presence ß- CD	Absence ß -CD	Presence ß- CD
UV (µg)	UV+ ß -CD (µg)	Fl (ng)	Fl+ ß-CD (ng)	10 20 30	5 10 20	20 40 80	5 10 20	98.8±1.8 100.7±1.4 101.1±1.9	101.7±1.34 98.5±1.07 99.13±1.18	100.25±0.86 98.65±0.73 98.83±1.07	99.52±0.03 100.14±0.2 100.2±0.06
20	10	40	10								

* Recovery of three different determinations.

CONCLUSION:

In the present work, spectral characteristic and emission spectra of travoprost in absence and presence of ß-CD were investigated. The fluorscence spectroscopy of the host – guest interaction between travoprost and ß-CD shows 1:1 inclusion complex, with an association constant of 21.86 M^{-1}. The quantum yield was 0.16 and 0.20 in absence and present of ß-CD. To the best of our knowledge, no report of spectrophotometry or fluorimetric methods were studied before. The proposed methods are challenging to green chemistry as the solvent used is water. The advantages of the methods are cheapness, eco-friendly, rapid and specific and can be used for the routine quality control of drug in drug substance and pharmaceutical eye drop with no potential interferences from excipients. The results showed that the quantity of drug substance in drug product was in a good agreement with given labeled quantity. The proposed spectrophotometry and spectrofluorimetric methods are characterized by being, simple, available, and having shorter time of analysis when compared to tedious chromatographic methods [4-6].

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