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DEVELOPMENT AND VALIDATION OF UV-SPECTROPHOTOMETER METHOD FOR ANALYSIS OF FLUVASTATIN SODIUM IN POLYETHYLENE GLYCOL 6000 AND POLYVINYL PYROLLIDONE K30 SOLID DISPERSIONS

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Abstract

The aim of current research is development and validation of straight forward and cost-effective UV-spectrophotometer analytical method for detection and quantitative analysis of Fluvastatin sodium in polyethylene glycol 6000 and polyvinyl pyrollidone K30 solid dispersion. Polyethylene glycol 6000 and polyvinyl pyrollidone K30 based solid dispersion of Fluvastatin sodium was fabricated by kneading technique. The calibration curve was plotted for FSS in beer's range of 60-100 µg/ml. The linear regression of calibration curve was performed by Graph Pad Prism version 6.01 to find a *p*-value of regression coefficient. The analytical method was validated for linearity, accuracy, specificity, precision and robustness. Limit of detection (LOD), limit of quantification (LOQ) and sandell's sensitivity was determined for validation of sensitivity. Regression equation obtained from calibration curve was y = 0.00164x+0.0084. Developed analytical method for FSS was found linear in concentration range of 60-100 µg/ml with high correlation coefficient of 0.9862 with p-value 0.0007 (*p<0.05). Mean percentage recovery was found in accepted limit of 98%-102% which validated the accuracy of the method. Method exhibited specificity, robustness, intra-day and intermediate precision as demonstrated by relative standard deviation of RSD <2%. Sandell's sensitivity, LOD and LOQ of FSS were found 0.2462, 18.29 and 55.42 µg/ml, respectively. It was concluded that developed UV-spectrophotometer method was accurate, precise, linear, specific, robust and sensitive; therefore, can be employed for routine analysis and quantitative estimation of FSS in polyethylene glycol 6000 and polyvinyl pyrollidone K30 based solid dispersion formulation.

Key words: Fluvastatin sodium, Robustness, Sandell's sensitivity, Limit of Detection, Limit of Quantification.

Introduction

The analytical method validation had been customarily implemented to authenticate that utilized analytical process for definite tests congregate the anticipated requirements and could be deemed to evaluate strength, characteristics, reliability, purity and strength of drug products along with consistency of analytical results (Carr *et al*., 1990). Fluvastatin sodium (FSS) is chemically sodium salt of (3S, 5R, 6E)-7-[3-(4-fluorophenyl)-1-(propan-2-yl)-1H-indol-2-yl]-3,5-dihydroxyhept-6-enoate which is synthetic lipid-lowering agent (Gupta *et al*., 2014; Kamboj *et al*., 2016; Kukati *et al*., 2015; Li *et al*., 2018) (Fig. 1). Its molecular formula is $C_{24}H_{26}FNNaO_4$. It belongs to statin family and competitively restrains hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase to hamper conversion of HMG-CoA to mevalonate required for cholesterol biosynthesis and therefore, lowers plasma lipoprotein and cholesterol levels to prevent cardiovascular disease. It is almost white crystalline powder with molecular weight, melting and flash point values 433.455 g/mol, 191±3°C and 366.1± 31.5°C, respectively (Borgmann *et al*., 2013; El-Helw *et al*., 2015; Tank *et al*., 2013).

The literature discovered that different analytical methods *i.e.* high performance liquid chromatography (HPLC), reverse phase high performance liquid chromatography (RP-HPLC), high performance thin layer chromatography (HPTLC), gas chromatography with flame ionization detection, capillary electrophoresis and UV-spectroscopy in 0.1 N NaOH and 0.1 N HCl have been reported for FSS analysis (Table 1). However, UV-spectrophotometer analytical method for analysis of

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Table 1: Assessment of existing analytical methods.

Drug	Method	λ_{max} (solvent)	LOD and LOQ	Beer's lawRange	Reference
Fluvastatin Sodium	HPLC	230 nm (Methanol)	5.4 and 1.7 µg/ml	2-12 µg/ml	Venkatesh *et al* ., 2016
Fluvastatin Sodium and Valsartan	RP-HPLC	237 nm (Acetonitrile: Buffer)	3.01 and 2.99 µg/ml (LOD) 20.0 and 9.99 µg/ml (LOQ)	20-60 µg/ml and 40-120 µg/ml	Sarigomula *et al*., 2013
Fluvastatin and Fenofibrate	UV	304 nm and 288 nm (Methanol)	-	8-24 µg/ml and 2-16 µg/ml	Prathyusha *et al.*, 2011
Fluvastatin sodium in bulk and tablet dosage form	RP-HPLC	235 nm (methanol: 20 mM Phosphate buffer: acetonitrile)	0.0194 and 0.0588 µg/ml	1-6 µg/ml	Saminathan *et al*., 2009
Fluvastatin sodium	Gas chromatography with flame ionization detection	Methanol	1.0 and 3.0 µg/ml	10-50 µg/ml	Saglik *et al*., 2009
Fluvastatin Sodium	UV	304 nm (0.1 N NaOH) and 229 nm (0.1N HCl)	0.215 and 0.652 µg/ml	10-50 µg /ml	Tuljapure *et al*., 2012
Fluvastatin	Capillary electrophoresis	239 nm (10 mM Borate buffer pH 8)	1×10^{-6} M and 2.89×10^{-6} M	$1.03\text{-}5.15 \times 10^{-5}$ M	Dogrukol-Ak *et al.*, 2001
Fluvastatin sodium	HPTLC (Methanol)	305 nm (in methanol)	65 ng/spot 200 ng/spot	300-800 ng/spot	Tuljapure *et al*., 2012

FSS in polyethylene glycol 6000 and polyvinyl pyrollidone K30 based solid dispersion has not been reported in literature survey. Therefore, a novel and economic UV-spectrophotometer analytical process has been designed for detection and quantitative analysis of FSS in solid dispersion of FSS synthesized using polyvinyl pyrollidone K-30 (PVP K-30) and polyethylene glycol 6000 (PEG 6000). This original analytical method was validated for appropriate parameters as per international conference on harmonization guidelines Q2 (R1) to establish range, linearity, accuracy, specificity, repeatability, intermediate precision, limit of detection (LOD), limit of quantification (LOQ) and sandell's sensitivity of analytical method (ICH Guideline, 2005; Sharma *et al*., 2017; Singh *et al*., 2016).

Materials and Methods

Instruments

Double beam scanning UV-Spectrophotometer (Systronics AU-2701, Ahmedabad, India) and (Systronics 2202, Ahmedabad, India) with 1 cm matched quartz cells coupled to computer with UV-Probe software was utilized for measuring absorbance. Digital pH meters (Deluxe model 101, Ambala, India) and an electronic analytical weighing balance (0.1 mg sensitivity, Denver Instrument SI-234, Ambala, India) were utilized during analytical work.

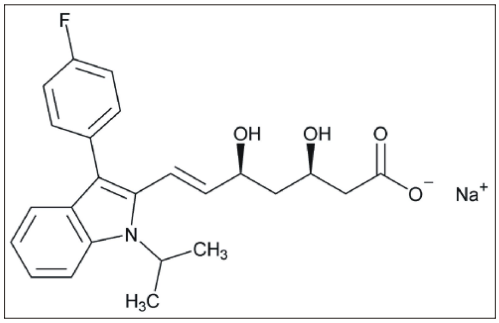

Fig. 1: Chemical structure of fluvastatin sodium.

Reagents and Chemicals

Fluvastatin sodium (CAS NO- 93957-55-2, 99.6% purity) was purchased from All Well Pharmaceuticals Company, Chandigarh. PVP K-30, PEG 6000, potassium dihydrogen phosphate and sodium hydroxide were procured from Loba Chemicals Private Limited, Mumbai, India. All ingredients employed were of analytical grade.

Table 2: Linear regression statistical data of calibration curve for FSS.

Parameter	Value
Best-fit values	
Slope	0.00164 ± 0.0001120
Y-intercept when X=0.0	0.008400 ± 0.009095
X-intercept when Y=0.0	-5.122
1/slope	609.8
95% Confidence intervals	
Slope	0.001284 to 0.001996
Y-intercept when X=0.0	-0.02054 to 0.03734
X-intercept when Y=0.0	-29.00 to 10.32
Goodness of fit	
R square	0.9862
P value	0.0007
Equation	Y = 0.00164*X + 0.008400

Table 3: Linearity determination of UV-spectrophotometer analytical procedure (n=3).

Conc. (µg/ml)	Absorbance 1 ± SD	Absorbance 2± SD	Absorbance 3 ± SD	Mean absorbance ± SD
60	0.106 ± 0.0046	0.104 ± 0.0027	0.109 ± 0.0023	0.106 ± 0.00251
70	0.125 ± 0.0050	0.128 ± 0.0029	0.129 ± 0.0027	0.127 ± 0.00208
80	0.141 ± 0.0051	0.144 ± 0.0036	0.143 ± 0.0033	0.142 ± 0.00152
90	0.151 ± 0.0037	0.156 ± 0.0039	0.153 ± 0.0043	0.153 ± 0.00251
100	0.175 ± 0.0035	0.172 ± 0.0042	0.176 ± 0.0031	0.174 ± 0.00208

Fabrication of PEG 6000 and PVP K30 based solid dispersion of FSS

Solid dispersion of FSS was manufactured by kneading technique (Christian *et al*., 2017; Ghareeb *et al*., 2009; Modi and Tayade, 2006). Physical mixtures of FSS (80 mg), PEG 6000 (295 mg) and PVP K30 (295 mg) were triturated with ethanol-water (1:1) solution to generate thick paste followed by kneading for 30 minutes and oven drying at 45°C to obtain dried mass. Subsequently, it was milled and sifted through sieve #30 and stored in vacuum desiccators for 48 h. solid dispersion formulation was sifted again through sieve #60 and transferred to airtight container.

Preparation of standard solution

100 mg FSS was exactly weighed and dissolved in 100 ml phosphate buffer, pH 7.4 to generate 1000 µg/ml solution. 10 ml solution was diluted to 90 ml with phosphate buffer, pH 7.4 to furnish 100 µg/ml standard stock solutions. Further, 6 ml stock solution was diluted to 10ml using phosphate buffer, pH 7.4 to provide 60 µg/ml working standard solutions. All determinations were conducted in triplicate.

Absorption maxima (λ_{max}) and calibration curve of FSS

60 µg/ml FSS was scanned over an UV-spectroscopic scanning range (200-400 nm) to determine λ_{max} of FSS using phosphate buffer, pH 7.4 as blank. From 100 µg/ml standard stock solution, aliquots (*i.e.* 6, 7, 8, 9 and 10 ml) were diluted with phosphate buffer, pH 7.4 to 10 ml dilutions having 60-100 µg/ml concentration and analyzed for absorbance (n=3).

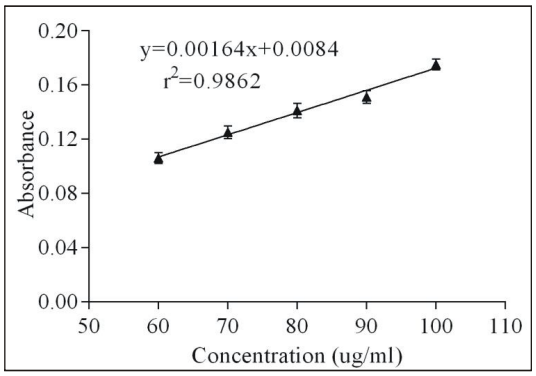

Fig. 2: Standard curve of FSS in phosphate buffer, pH 7.4 using UV-spectroscopy (*n*=3).

Analytical method validation parameters

Linearity

Linearity of analytical procedure was determined using 60-100 µg/ml standard concentration of FSS. Standard solutions of FSS were prepared in triplicate and subjected to determination of absorbance at 300 nm (n=9). Calibration curve was plotted between actual concentration (µg/ml) *vs.* absorbance and correlation coefficient was calculated. Correlation coefficient was used for evaluation of linearity of analytical procedure (Marakkarakath *et al*., 2019; Uyar *et al*., 2007).

Accuracy

Accuracy of analytical method was checked by spiking method. 0.6, 0.7 and 0.8 mg of FSS was dissolved in 10 ml phosphate buffer, pH 7.4 to give concentrations of 60 µg/ml, 70 µg/ml and 80 µg/ml, respectively. Absorbance of prepared dilution was determined at 300 nm. The accuracy was calculated as the mean percentage drug recovery from each dilution. The accepted limits of mean percentage recovery are 98%-102% (Almasri *et al*., 2019; Belal *et al*., 2013).

Specificity

80 mg of FSS was mixed with 100% (80 mg), 200% (160 mg), 300% (240 mg), 400% (320 mg) and 500% (400 mg) of excipients (PVP K-30 and PEG 6000) and analyzed for % recovery of FSS. The accepted limits of % recovery and % relative standard deviation (% RSD) for validating specificity are 98%-102% and < 2%, respectively (Abdelwahab *et al*., 2012; Divya *et al*., 2013; Maleque *et al*., 2012).

Repeatability (intra-day precision)

70, 80 and 90 µg/ml concentrations of FSS were analyzed at three different times within a day. The % RSD should be less than 2% for acceptable repeatability

Table 4: Accuracy determination of UV-spectrophotometer analytical method.

Spiked amount (µg/ml)	Recovered amount (µg/ml)	% Mean recovery	Statistical analysis
60	60.9	101.5	Mean = 101.27 %
70	71.1	101.57	SD = 0.455 %
80	80.6	100.75	RSD = 0.45

Table 5: Specificity determination of UV-spectrophotometer analytical procedure.

PVP K30: PEG 6000 (1:1)	FSS input (mg)	FSS recovered (mg)	FSS recovered (%)	Mean recovered	Statistical analysis
100 %	80	80.7	100.87		Mean = 100.57 % SD = 0.700407% RSD = 0.70
200 %	80	80.2	100.25		
300 %	80	79.7	99.62	100.57 %	
400 %	80	81.2	101.5		
500 %	80	80.5	100.62		

Table 6: Repeatability determined for three different concentrations of FSS.

Concentration (µg/ml)	Absorbance 1	Absorbance 2	Absorbance 3	Mean	S.D	% RSD
70	0.125	0.122	0.123	0.1233	0.00152	1.24%
80	0.140	0.141	0.146	0.1413	0.00155	1.08%
90	0.151	0.154	0.155	0.1533	0.00208	1.36%

(Alamri *et al*., 2016; Divya *et al*., 2013; Prashant *et al*., 2013).

Intermediate precision

It articulates within-laboratories variations: different days (inter-day), different analysts and different equipment. 70, 80 and 90 µg/ml concentrations of FSS were analyzed on three different days (inter-day precision) (% RSD limit: < 2%). 80 µg/ml FSS solutions were analyzed using different equipments (Systronics AU-2701, Ahmedabad, India; Systronics 2202, Ahmedabad, India) (*n*=6) (% RSD limit: < 2%) (Breier *et al*., 2007; Jain *et al*., 2013; Patil *et al*., 2015).

Robustness

The majority of analytical studies involve merely one variant at a time by keeping others as constant. Robustness of UV-spectrophotometer analytical method was determined by analyzing the 80 µg/ml FSS solutions at different wavelengths (λ) *i.e.* 300 ± 15 nm and temperatures *i.e.* 25±20°C. % RSD acceptance limit is < 2% (Christian *et al*., 2017).

Sandell's sensitivity, LOD and LOQ

For sensitivity measurement of UV-spectrophotometer analytical technique for FSS detection, sandell's sensitivity was calculated using following formulas:

$$S = \frac{10^{-3}}{\varepsilon_s} \quad (1)$$

$$\varepsilon_s = \frac{\varepsilon}{\text{Molecular Weight of Determinant}} \times 1000 \quad (2)$$

$$\varepsilon = \frac{A}{C.d} \quad (3)$$

Where, ε_s is specific absorptivity (in ml/g/cm) which is the amount of determinant in cuvette with an optical length of 1 cm, ε is molar absorptivity, d is path length and C is molar concentration of determinant[11]. LOD and LOQ of FSS were assessed from slope (S) of calibration curve and standard deviation of y-intercept of regression equation using subsequent equations:

$$LOD = 3.\frac{3\sigma}{S} \quad (4)$$

$$LOQ = \frac{10\sigma}{S} \quad (5)$$

LOD is least quantity of analyte which can be detected in sample, but not necessarily quantities as an accurate value while LOQ is minimum quantity that can be quantified by the instrument (Divya *et al*., 2013; Divya and Narayana, 2014).

Statistical analysis

Linear regression of calibration curve was executed using GraphPad Prism version 6.01 for windows (GraphPad Software, San Diego California, USA). Statistical difference ($p < 0.05$) was considered significant.

Results and Discussions

Absorption maxima (λ_{max}) and calibration curve of FSS

Absorption Maxima (λ_{max}) of FSS acquired through UV scan of 60 µg/ml FSS in phosphate buffer was found 300 nm. Calibration curve of FSS was acquired using UV-spectrophotometer technique by plotting a graph between concentrations of FSS *vs.* absorbance value obtained at 300 nm (Fig. 2). Statistical analysis of calibration curve was performed by curve linear regression. Regression coefficient and *p*-value was found 0.9862 and 0.0007 ($p<0.05$), respectively, which illustrated goodness of fit as well as statistical significance of proposed method (Table 2).

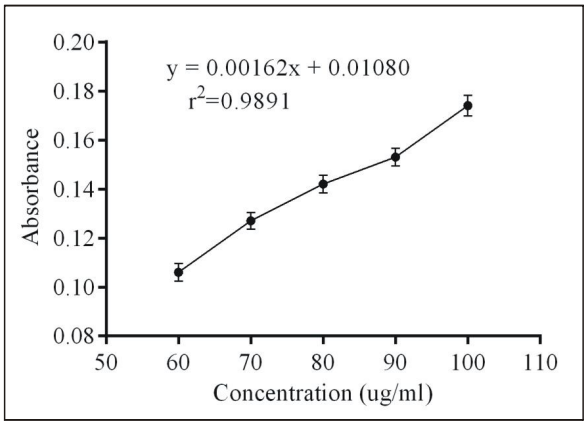

Fig. 3: Graphical representation of linearity.

Table 7: Inter-day precision determined for FSS.

Concentration (μg/ml)	Day 1	Day 2	Day 3	Mean	S.D	% RSD
70	0.125	0.122	0.123	0.1233	0.00152	1.24%
80	0.140	0.141	0.146	0.1413	0.00155	1.08%
90	0.151	0.154	0.155	0.1533	0.00208	1.36%

Linearity

The linearity range for FSS at 300 nm was found 60-100 μg/ml which has been confirmed by correlation coefficient value of 0.9891 (*n*=3) (Table 3 and Fig. 3) (Marakkarakath *et al*., 2019; Uyar *et al*., 2007).

Accuracy

Accuracy validation of UV-spectrophotometer analytical method was performed by spiking method. Accuracy of an analytical process articulates the proximity of agreement among spiked and recovered amount using UV-spectrophotometer analytical procedure (Fig. 4). The accuracy was determined as mean percentage drug recovery from 60, 70 and 80 μg/ml FSS concentrations. The % mean recovery of FSS was found to be 101.5%, 101.57%, 100.75% respectively for 60, 70 and 80 μg/ml solutions (Table 4). Average % recovery of FSS was 101.49% which lies in acceptable limits of mean percentage recovery are 98%-102% with % RSD value 0.45 which indicated good accuracy (Almasri *et al*., 2019; Belal *et al*., 2013).

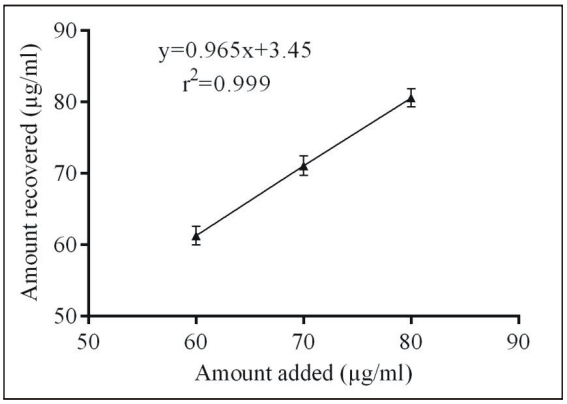

Fig. 4: Graphical illustration of accuracy.

Specificity

Specificity of UV-spectrophotometer analytical method was determined by analyzing FSS in presence and absence of excipients (PVP K-30 and PEG 6000). Mean recovery of FSS was found 99.93 % which was within accepted limit (98%-102%). The % RSD was found 0.8644 % (< 2%) which validated specificity of analytical method (Table 5) (Abdelwahab *et al*., 2012; Divya *et al*., 2014; Maleque *et al*., 2012).

Repeatability (intra-day precision)

The % RSD for absorbance values of 70, 80 and 90 μg/ml FSS at three different time periods within a day was found to be 1.24, 1.08 and 1.36% (< 2%), which validated repeatability of analytical method (Table 6) (Alamri *et al*., 2016; Divya *et al*., 2013; Prashant *et al*., 2013).

Table 8: Intermediate precision determined for FSS (*n*=6).

Condition	Trials	Absorbance	Mean	SD	%RSD
Analyst-1	1	0.143	0.144	0.0018973	1.32%
	2	0.146			
	3	0.142			
	4	0.145			
	5	0.142			
	6	0.146			
Analyst-2	1	0.145	0.1441666	0.0014719	1.02%
	2	0.143			
	3	0.142			
	4	0.146			
	5	0.145			
	6	0.144			
Equipment-1	1	0.147	0.1446666	0.0018618	1.29%
	2	0.145			
	3	0.146			
	4	0.143			
	5	0.142			
	6	0.145			
Equipment-2	1	0.143	0.1443333	0.0021602	1.50%
	2	0.145			
	3	0.144			
	4	0.147			
	5	0.146			
	6	0.141			

Intermediate Precision

The % RSD for absorbance values of 70, 80 and 90 μg/ml FSS on three different days (inter-day) was found 1.25, 1.07 and 0.99 % (< 2%), which validated inter-day precision of analytical method (Table 7) (Breier *et al*., 2007; Jain *et al*., 2013; Patil *et al*., 2015).

% RSD of absorbance values of 80 μg/ml FSS analyzed by two different analysts and using two different equipments was found < 2% which indicated intermediate precision of developed analytical method (Table 8).

Robustness

% RSD of absorbance values of sample solutions analyzed at different wavelengths and temperatures was found 1.48% and 1.73%, respectively. The % RSD values were < 2% which

Table 9: Robustness studies of UV-spectrophotometer analytical method.

Condition	Parameter	Absorbance	Mean	SD	% RSD
Change in Wavelength	285 nm	0.141	0.140	0.002081	1.48
	300 nm	0.138			
	315 nm	0.142			
Change in temperature	5°C	0.145	0.145	0.0025166	1.73
	25°C	0.143			
	45°C	0.148			

indicated that proposed analytical method remained unaffected by small but deliberate variations in method parameters and provided an indication of its reliability during normal usage (Table 9) (Christian *et al*., 2017).

Sandell's sensitivity, LOD and LOQ

The sensitivity of measurement of FSS by proposed method was estimated sandell's sensitivity value. Sandell's sensitivity (μg/cm^2/0.001 absorbance unit) was found 0.2462 which illustrated that method is highly sensitive[11]. LOD and LOQ of FSS in phosphate buffer, pH 7.4 were found 18.29 and 55.42 μg/ml, respectively which illustrated high sensitivity of developed analytical method (Divya *et al*., 2013; Divya *et al*., 2014). Results of several validation parameters of UV-spectrophotometer analytical method for FSS has been summarized in table 10.

Conclusion

The proposed spectrophotometer analytical method for determination of Fluvastatin Sodium was found straightforward, specific, accurate, precise and cost-effective. It was concluded that developed method was robust and negligibly affected by smaller variations in temperature and wavelength. Furthermore, analytical method was highly sensitive and therefore, it was concluded that UV-spectrophotometer method could be employed for routine analysis of Fluvastatin sodium in polyethylene glycol 6000 and polyvinyl pyrollidone K30 solid dispersions.

Table 10: Validation parameters of UV-spectrophotometer analytical method.

Parameter	Result
λ_{max} (nm)	300
Regression equation (y= mx + c)	y = 0.0016x + 0.0084
Regression coefficient (r^2)	R^2 = 0.9862
Linearity (r^2)	0.9891
Accuracy (% drug recovery)	% RSD = 0.45
Specificity	% RSD = 0.70
Repeatability indicated by % RSD for FSS (70, 80 and 90 ìg/ml)	1.24%, 1.08% and 1.36%
Intermediate precision indicated by % RSD (day-1, day-2, day-3)	1.25%, 1.07%, 0.99%
Intermediate precision indicated by % RSD (analyst-I, analyst-2)	1.32%, 1.02%
Intermediate precision indicated by % RSD (equipment-1, equipment-2)	1.29%, 1.50%
Robustness indicated by % RSD (λ_{max}, 300 ± 15 nm)	1.48%
Robustness indicated by % RSD (Temp. 25± 20°C)	1.73%
Limit of detection (LOD)	18.29 μg/ml
Limit of quantitation (LOQ)	55.42 μg/ml
Sandell's sensitivity	0.2462 μg/cm^2/0.001 absorbance unit

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Conflict of interests

The authors report no conflicts of interest in this work.

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