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Spectrophotometric Estimation of Valsartan and Amlodipine Besylate in Microcrystalline Cellulose and Starch Matrix

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ABSTRACT

Two spectrophotometric methods have been developed and validated for simultaneous estimation of Valsartan and Amlodipine besylate in microcrystalline cellulose and starch Matrix. The first method employed solving of simultaneous equations based on the measurement of absorbance at two wavelengths, 249.6 nm and 237.8 nm, λ_{max} for Valsartan and Amlodipine besylate, respectively. The second method was absorbance ratio method, which involves formation of Q-absorbance equation at 243.8 nm (isoabsorptive point) and also at 237.8 nm (λ_{max} of Amlodipine besylate). The methods were found to be linear between the range of 5 – 25 µg/ml for both Valsartan and Amlodipine besylate using 0.1 N NaOH as solvent. The mean percentage recovery was found to be 97.96 % and 98.56 % for the simultaneous equation method and 97.37 % and 97.53 % for the absorbance ratio method, for Valsartan and Amlodipine besylate, respectively, at three different levels of standard additions. The precision (intra-day, inter-day) of methods were found within limits (RSD < 2%). It could be concluded from the obtained results in the present investigation that the two methods for simultaneous estimation of Valsartan and Amlodipine besylate are simple, rapid, accurate, precise and economical and can be used, successfully in the quality control of pharmaceutical formulations and other routine laboratory analysis.

1. INTRODUCTION

Valsartan (VAL) (Fig. 1), a nonpeptide, is N-(1-oxopentyl)-N-[[2'-(1H-tetrazol-5-yl) [1,1' biphenyl]-4-yl] methyl]-L-valine[1]. It is a potent, highly selective, orally active, specific angiotensin II receptor antagonist used as a hypotensive drug. Amlodipine besylate (AML) (Fig. 2) is (3-Ethyl-5-methyl (±)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1, 4-dihydro- 6-methyl-3, 5-pyridinedicarboxylate, monobenzenesulphonate), is an antianginal, antihypertensive drug[2].

AML is a dihydropyridine calcium channel blocker that inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle. AML is a peripheral arterial vasodilator that acts directly on vascular smooth muscle to cause a reduction in peripheral vascular resistance and reduction in blood pressure.

VAL is a nonpeptide, orally active and specific angiotensin II antagonist acting on the AT1 receptor subtype. VAL does not bind to or block other hormone receptors or ion channels known to be important in cardiovascular regulation. Blockade of the angiotensin II receptor inhibits the negative regulatory feedback of angiotensin II on renin secretion, but the resulting increased plasma renin activity and angiotensin II circulating levels do not overcome the effect of VAL on blood pressure.

Both AML and VAL lower blood pressure by reducing peripheral resistance, but calcium influx blockade, reduction of angiotensin II and vasoconstriction are complementary mechanisms[3].

The goal of antihypertensive therapy is to abolish the risks associated with blood pressure elevation without adversely affecting quality of life. Drug selection is based on efficacy in lowering blood pressure and in reducing cardiovascular end points including stroke, myocardial infarction and heart failure. AML/VAL is indicated as initial therapy in patients who are unlikely to be controlled with a single drug and as second-line therapy in patients not responding adequately to monotherapy[4,5].

Combination therapy is increasingly recommended for selected patients with hypertension to facilitate prompt attainment and maintenance of goal blood pressure (BP). AML/VAL combination therapy simplifies treatment and optimizes long term compliance. AML, a

dihydropyridine calcium antagonist, and VAL, an angiotensin receptor blocker, are well established antihypertensive agents with complementary mechanisms of action. This combination lowers blood pressure (BP) significantly more than either of its components, and VAL reduces the incidence of dose related AML induced edema. Rigorous clinical trial data have proven the BP lowering efficacy and high tolerability of the AML/VAL combination in patients with moderate to severe hypertension as well as other difficult to treat populations[6].

In line with the literature, a lot of work has been carried out on triple combination of VAL, AML and hydrochlorothiazide[7-12]. Literature survey also revealed that a number of methods have been reported for estimation of AML and VAL individually or in combination with other drugs. Chromatographic assessment of AML/VAL combination has been performed by some researchers which comprise of HPLC, RP-HPLC, stability indicating method and HPTLC[13-18].

These methods are accurate and precise with good reproducibility, but the cost of analysis is quite high owing to expensive instrumentation, reagent and expertise. Hence it is worthwhile to develop simpler and cost effective method for simultaneous estimation of drugs for routine analysis of formulation. Spectrophotometric analysis fulfils such requirement where the simultaneous estimation of the drug combination can be done with similar effectiveness as that of chromatographic methods[19].

Some spectrofluorimetric and spectrophotometric methods are also available in the literature[20-22]. But the advantage of proposed methods is that 0.1 N sodium hydroxide was used as a solvent which is very economical as compared to methanol utilized in above methods.

The purpose of this research was to establish such a method and after validation in accordance with International Conference on Harmonization (ICH) guidelines[23] and the directives for good laboratory practice, to use the method for analysis of the drug content of tablets. The present work describes two spectrophotometric methods for estimation of VAL with AML in combination that are simultaneous equation method (method 1) and absorbance ratio method (method 2).

2. MATERIALS AND METHODS

2.1 Instrument

A double-beam Shimadzu UV Visible spectrophotometer, with spectral bandwidth of 1 nm, wavelength accuracy ± 0.5 nm, Model- UV 2450 PC (Japan), Software: UV-Probe and a pair of 1 cm matched quartz cells was used to measure absorbance of the resulting solution.

2.2 Materials

Standard gift sample of VAL was provided by Lupin Pharmaceuticals Ltd, Pune and AML by Blue Cross Laboratories Pvt. Ltd (Nashik), India. Sodium hydroxide was procured from S.D. Fine chem. Ltd, India which was used as a solvent for both the methods.

2.3 Selection of an appropriate solvent system

Various solvent systems like distilled water, 0.1 N HCl, 0.1 N NaOH, methanol were tried to select an appropriate solvent with good suitability and stability. A solvent system, 0.1 N NaOH was selected for the determination of VAL and AML, since both drugs were soluble in 0.1 N NaOH.

2.4 Preparation of Stock standard solutions

Stock standard solution of VAL (1000 µg/ml) was prepared by dissolving 10 mg VAL in 10 ml of 0.1 N NaOH in 10 ml volumetric flask with vigorous shaking. From this stock standard solution, 1 ml was withdrawn and diluted to 10 ml using solvent to get working solution of 100 µg/ml. Similarly, Stock standard solution of AML (1000 µg/ml) was prepared by dissolving 10 mg AML in 10 ml of 0.1 N NaOH in 10 ml volumetric flask with vigorous shaking. This solution was further diluted to get working solution of 100 µg/ml.

2.5 Selection of analytical wavelengths

For selection of analytical wavelengths, working solutions of both the drugs were scanned separately between 400 nm to 200 nm. The overlay spectra of both drugs were recorded (Fig 3). From overlay spectra, wavelengths 249.6 nm (λ_{max} of VAL) and 237.8 nm (λ_{max} of AML) were selected for analysis of both drugs using simultaneous equation method. Also, wavelengths 243.8 nm (Isoabsorptivity point) and 237.8 nm (λ_{max} of AML) were selected for analysis of both drugs using Absorbance ratio method.

2.6 Linearity study

The linear absorbances were obtained in the concentration range 5 – 25 µg/ml for VAL and AML. Absorbances of these solutions were measured at 249.6 nm and 237.8 nm, for method 1 and at 243.8 nm and 237.8 nm, for method 2. Calibration curve was constructed by plotting absorbance versus concentration.

2.7 Method 1: Simultaneous Equation Method

Determination of A (1%, 1cm) values of drugs at selected wavelengths:

A (1 %, 1 cm) value of drugs were calculated using following formula;

$$A\ (1\%, 1cm) = \frac{\text{Absorbance}}{\text{Concentration (g/100 ml)}}$$

A set of two simultaneous equations were framed using these A (1 %, 1 cm) values are given below;

$$\mathbf{C_{VAL} = A_2\ ay_1 - A_1\ ay_2 / ax_2\ ay_1 - ax_1 ay_2 \ldots\ldots\ldots\ldots\ldots\ldots\ (1)}$$

$$\mathbf{C_{AML} = A_1\ ax_2 - A_2\ ax_1 / ax_2\ ay_1 - ax_1 ay_2 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (2)}$$

Where, A_1 and A_2 are absorbance of mixture at 249.6 nm and 237.8 nm; ax_1 and ax_2, A (1 %, 1 cm) of VAL at 249.6 nm and 237.8 nm, respectively; ay_1 and ay_2, A (1 %, 1 cm) of AML at 249.6 nm and 237.8 nm , respectively. C_{VAL} and C_{AML} are concentrations of VAL and AML in mixture.

By rearranging equations 1 and 2,

Concentration $\mathbf{C_{VAL}}$ and $\mathbf{C_{AML}}$ can be obtained as;

$$\mathbf{C_{VAL} = \frac{A_2 \times 370.7 - A_1 \times 342.2}{-25875.58} \quad \ldots\ldots\ldots\ldots (3)}$$

$$\mathbf{C_{AML} = \frac{A_1 \times 258.9 - A_2 \times 356.1}{-25875.58} \quad \ldots\ldots\ldots\ldots (4)}$$

2.8 Method 2: Absorbance ratio Method

A set of equations for Absorbance ratio method were framed using these A (1 %, 1 cm) values are given below;

$$Qx = \frac{ax_2}{ax_1}, QY = \frac{ay_2}{ay_1} \text{ and } QM = \frac{A_2}{A_1} \quad \ldots\ldots\ldots\ldots\ldots\ldots (5)$$

Where, A_1 and A_2 are absorbance of mixture at 243.8 nm and 237.8 nm; ax_1 and ax_2, A (1 %, 1 cm) of VAL at 243.8 nm and 237.8 nm, respectively; ay_1 and ay_2, A (1 %, 1 cm) of AML at 243.8 nm and 237.8 nm, respectively. C_{VAL} and C_{AML} are concentrations of VAL and AML in mixture.

Concentration C_{VAL} and C_{AML} can be obtained as;

$$C_{VAL} = \frac{Q_M - QY}{Q_X - QY} \cdot \frac{A_1}{ax_1} \quad \ldots\ldots\ldots\ldots\ldots\ldots (6)$$

$$C_{AML} = \frac{A_1}{ax_1} - C_{VAL} \quad \ldots\ldots\ldots\ldots\ldots\ldots (7)$$

2.9 Application of proposed methods for standard mixture

Standard mixture of VAL and AML was prepared by weighing 80 mg of VAL and 5 mg of AML in 10 ml of volumetric flask. 5 ml of 0.1 N NaOH was added to the volumetric flask and both drugs were dissolved completely. Finally, volume was adjusted up to the mark with 0.1 N NaOH to get the concentration of 8000 µg/ml of VAL and 500 µg/ml of AML. Appropriate volume 0.1 ml was transferred to 10 ml of volumetric flask and diluted up to mark with the same solvent to obtain strength 80 and 5 µg/ml of AML and VAL, respectively; the solutions were scanned in the range 400 – 200 nm, absorbances of the sample solutions were recorded at 249.6 nm and 237.8 nm i.e. A_1 and A_2 respectively. The concentrations of the two drugs in sample solution (C_{VAL} and C_{AML}) were determined, by using equation 3 and 4. For method 2, absorbances of the sample solutions were recorded at 243.8 nm and 237.8 nm i.e. A_1 and A_2, respectively. The concentrations of the two drugs in sample solution (C_{VAL} and C_{AML}) were determined, by using equation 6 and 7. Results are shown in Table 1.

2.10 Application of proposed method for analysis of tablets

A quantity of matrix sample equivalent to 80 mg of VAL and 5 mg of AML was transferred into 10 ml volumetric flask containing 0.1 N NaOH, sonicated for 30 min, volume was adjusted to mark with same solvent and filtered through Whatmann filter paper no. 41. The resulting solution was further diluted to get concentration 80 and 5 µg/ml of VAL and AML, respectively. Prepared solution was scanned within 400 to 200 nm and absorbance of sample solution at selected wavelengths was recorded against blank. The concentrations of the two drugs in sample solutions (C_{VAL} and C_{AML}) were determined, using equation 3 and 4. For method 2, the concentrations of the two drugs in sample solutions (C_{VAL} and C_{AML}) were determined, using equation 6 and 7. The results of the same are reported in the Table 2.

VALIDATION OF PROPOSED METHODS

The method was validated in terms of linearity, accuracy, precision, specificity LOD, LOQ, ruggedness and robustness.

2.11 Accuracy

Accuracy of the method was assessed by percentage recovery experiments performed at three different levels i.e. 80, 100 and 120 %. Known amount of standard VAL and AML solutions were added to the pre-analyzed sample solutions, absorbances were recorded and re-analyzed by proposed method. The % recovery was calculated by using formula;

$$\% \text{ Recovery} = \frac{A - B}{C} \text{ X } 100$$

Where,

A = Total amount of drug estimated

B = Amount of drug found on pre-analyzed basis

C = Amount of bulk drug added

Results of recovery studies are shown in Table 3.

2.12 Precision

Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions.

Intra-day and inter-day precision

Intra-day and inter-day variations were determined by analyzing three different solutions of VAL and AML within the same day and three different days over a period of week.

Intra-day precision was determined by analyzing 10 µg/ml, 15 µg/ml and 20 µg/ml for VAL and AML for three times within the same day.

Inter-day precision was determined by analyzing above mentioned concentrations of both the drugs for three different days over period of week. The results are shown in Table 4.

2.13 Specificity

The interference of other excipients was evaluated. 10 µg/ml Dextrose and Lactose were added separately to standard solutions of 25 µg/ml VAL and 25 µg/ml AML using the same experimental and environmental conditions.

2.14 Ruggedness

Ruggedness of the method was proved by analyzing the standard solutions 25 µg/ml of VAL and 25 µg/ml of AML by two different analysts using the same experimental and environmental conditions.

2.15 Robustness

Robustness of the method was proved by analyzing the standard solutions 25 µg/ml of VAL and 25 µg/ml of AML by two different solvents using the same experimental and environmental conditions.

3. RESULTS AND DISCUSSIONS

3.1 Method 1

Analytical method has been developed for simultaneous estimation of VAL and AML in combined pharmaceutical dosage form using simultaneous equation. In 0.1 N NaOH, VAL

showed maximum absorbance at 249.6 nm and AML at 237.8 nm. Linearity was observed in the range 5 – 25 µg/ml (R^2 = 0.999) of VAL and 5 – 25 µg/ml (R^2 = 0.998) of AML. The proposed method was applied for matrix and percentage label claim of VAL and AML was found to be 97.83 and 98.12, respectively. The amount of drug estimated by proposed method was in good agreement with the label claim. Accuracy of the method was checked by the recovery studies at three different levels i.e. 80 %, 100 % and 120 %. The mean percentage recovery for VAL and AML was found to be 97.96 and 98.56 respectively. The method was found to be precise as indicated by the inter-day, intra-day analysis, showing % R.S.D. less than 2. The results did not show any statistical difference between operators suggesting that method developed was rugged. Also, there was no any statistical difference between various strengths of solvents suggesting that method was robust. The sensitivity of method was assessed by determining LOD and LOQ. For VAL, LOD and LOQ was found to be 0.24 and 0.73 µg/ml respectively. For AML, the LOD and LOQ was found to be 0.54 and 1.64 µg/ml respectively.

3.2 Method 2

Analytical method has been developed for simultaneous estimation of VAL and AML in combined pharmaceutical dosage form using Absorbance Ratio Method. In 0.1 N NaOH, wavelengths 243.8 nm (Isoabsorptivity point) and 237.8 nm (λ_{max} of AML) were selected for analysis of both drugs using Absorbance ratio method. Linearity was observed in the range 5 – 25 µg/ml (R^2 = 0.999) of VAL and 5 – 25 µg/ml (R^2 = 0.997) of AML. The proposed method was applied for matrix and percentage label claim of VAL and AML was found to be 97.26 and 98.12 respectively. The amount of drug estimated by proposed method was in good agreement with the label claim. Accuracy of the method was checked by the recovery studies at three different levels i.e. 80 %, 100 % and 120 %. The mean percentage recovery for VAL and AML was found to be 97.37 and 97.53 respectively. The method was found to be precise as indicated by the inter-day, intra-day analysis, showing % R.S.D. less than 2. The results did not show any statistical difference between operators suggesting that method developed was rugged. Also, there was no any statistical difference between various strengths of solvents suggesting that method was robust. The sensitivity of method was assessed by determining LOD and LOQ. For VAL, LOD and LOQ was found to be 0.52 and 1.16 µg/ml respectively. For AML, the LOD and LOQ was found to be 0.31 and 0.93 µg/ml respectively.

The summary of validation parameters are presented in Table 5.

4. CONCLUSION

The proposed spectrophotometric methods were found to be simple, sensitive, accurate, precise, reproducible, specific, robust, and economical. They can be used for the routine simultaneous estimation of VAL and AML in pharmaceutical formulations.

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CONFLICT OF INTEREST STATEMENT

There are no competing interests amongst authors. The authors declare that there are no conflicts of interest.

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FIGURES

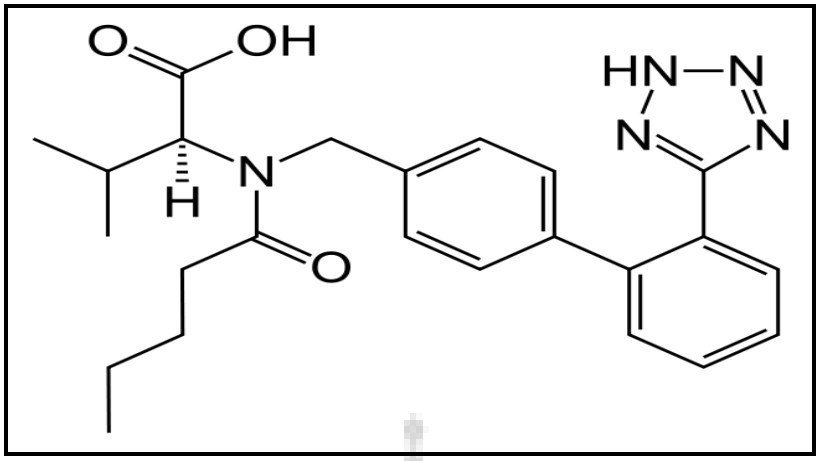

Fig. 1 Structure of VAL

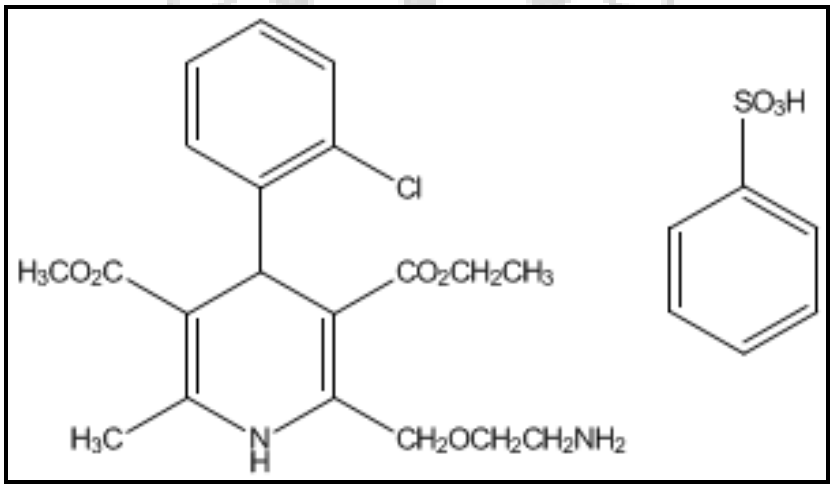

Fig. 2 Structure of AML

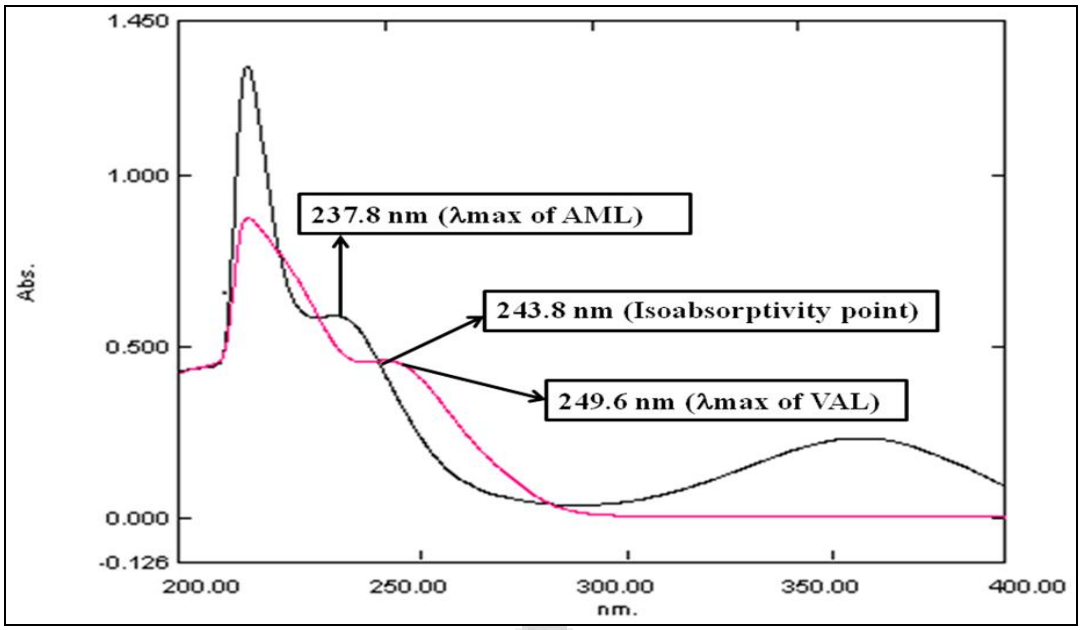

Fig. 3 Overlay spectra of VAL and AML

Table 1: Application of proposed method for standard mixture

Name of drug	Amount taken (mg)	% Amount found (n = 6)		% R.S.D.	
		Method 1	Method 2	Method 1	Method 2
VAL	80	101.5	100.4	0.7	0.9
AML	5	101.6	100.5	0.6	1.2

mg – milligram, n – Number of observations, RSD – Relative Standard Deviation

Table 2: Application of proposed method for analysis of tablets

Tablet sample	Label claim (mg)	% Label claim (n = 6)		% R.S.D.	
		Method 1	Method 2	Method 1	Method 2
VAL	80	97.83	97.26	1.6	1.3
AML	5	98.12	98.12	1.8	1.4

mg – milligram n – Number of observations, RSD – Relative Standard Deviation

Table 3: Results of recovery studies

Recovery level	Initial amount (µg/ml)		Concentration of std drug added (µg/ml)		% Recovery (n = 3)			
					Method 1		Method 2	
	VAL	AML	VAL	AML	VAL	AML	VAL	AML
80 %	20	1.25	16	1.0	98.48	98.22	97.32	98.14
100 %	20	1.25	20	1.25	97.53	98.64	97.81	96.87
120 %	20	1.25	24	1.5	97.89	98.81	96.98	97.58
Mean					**97.96**	**98.56**	**97.37**	**97.53**

µg/ml – microgram per milliliter, n – Number of observations

Table 4: Results of intra-day and inter-day precision

Drug	Amount taken (µg/ml)	Intra-day (n =3) % R.S.D.		Inter-day (n = 3) % R.S.D.	
		Method 1	Method 2	Method 1	Method 2
VAL	10	0.67	1.8	0.53	0.8
	15	0.88	0.8	0.54	0.5
	20	0.74	0.9	0.50	0.6
	Mean	**0.76**	**1.2**	**0.52**	**0.7**
AML	10	0.025	2.4	0.15	0.7
	15	0.014	1.2	0.13	0.8
	20	0.035	1.1	0.55	0.4
	Mean	**0.025**	**1.6**	**0.28**	**0.6**

n – Number of observations, RSD – Relative Standard Deviation

Table 5: Summary of validation parameters

Methods	Method 1		Method 2	
	VAL	AML	VAL	AML
λmax	249.6 nm	237.8 nm	258.4 nm	237.8 nm
Linearity range (µg/ml)	5 - 25	5 – 25	5 – 25	5 - 25
Regression equation	Y = 0.190 x + 0.051	Y = 0.0374 X + 0.0171	Y = 0.0378 X + 0.025	Y = 0.0351 X + 0.032
Slope (m)	0.190	0.0374	0.0378	0.0351
Y – intercept (c)	0.051	0.0171	0.025	0.032
Correlation coefficient (r^2)	0.999	0.998	0.999	0.997
% Recovery (n = 3)	97.96	98.56	97.37	97.53
LOD (µg/ml)	0.24	0.54	0.52	0.31
LOQ (µg/ml)	0.73	1.61	1.16	0.93
Molar absorptivity (lit/mole/cm)	15591.61	19336.40	14894.78	21264.38
Sandell's sensitivity (µg/sqcm/0.001)	0.02793	0.02932	0.02923	0.02666
Standard error	0.369×10^{-3}	0.0881×10^{-3}	2.8034×10^{-3}	3.3236×10^{-3}
Precision (% R.S.D.)				
Intra- day (n = 3)	0.76	0.025	1.2	1.6
Inter-day (n = 3)	0.52	0.28	0.7	0.6
Specificity (%R.S.D.)				
Addition of Dextrose	1.6	1.8	1.0	1.2
Addition of Lactose	0.9	0.8	1.2	1.6
Ruggedness (%R.S.D.)				
Analyst I (n = 3)	0.6	0.5	1.2	0.8
Analyst II (n = 3)	0.5	0.3	1.5	1.8
Robustness (%R.S.D.)				
0.2 N NaOH (n = 3)	1.9	1.2	1.1	1.4
0.5 N NaOH (n = 3)	0.7	0.5	0.8	0.6