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SIMULTANEOUS QUANTITATIVE DETERMINATION OF ATENOLOL AND AMLODIPINE BESILATE IN THEIR COMBINED DOSAGE FORM BY USING UV SPECTROPHOTOMETER

Devi Velmurugan*, Ilavarasan K., Lavanya R., Sandhiya P., Sekar V. and Sambathkumar R.

JKK Nattraja College of Pharmacy, Department of Pharmaceutical Analysis, Komarapalayam, Tamilnadu, India.

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***Corresponding Author**
Devi Velmurugan
JKK Nattraja College of Pharmacy, Department of Pharmaceutical Analysis, Komarapalayam, Tamilnadu, India.

ABSTRACT

To develop a simple, sensitive and highly accurate Simultaneous Estimation of Atenolol and Amlodipine Besilate in their bulk and pharmaceutical combined tablet dosage forms. 0.1N NaOH used as a solvent. Then spectra of Atenolol and Amlodipine Besilate exhibit at λ max of 232nm and 236nm respectively. The linearity of calibration curve (Absorbance Vs Concentration) in pure solution was checked over the concentration range of about 4-24µg/ml for Atenolol and 5-30 µg/ml for Amlodipine Besilate respectively. With the correlation coefficient higher than 0.99. The developed method was validated in terms of accuracy, precision, linearity, ruggedness, robustness, limit of detection and limit of quantification which proves suitability of proposed method for routine estimation of Atenolol and Amlodipine Besilate on bulk pharmaceutical formulation. The methods were validated as per ICH guidelines.

KEYWORDS: Amlodipine Besilate; Atenolol; Sodium hydroxide; distilled water, UV Spectrophotometer; validation.

INTRODUCTION

Atenolol is chemically known as 4-(2-hydroxy-3-isopropylaminopropoxy) phenylacetamide[1] (Figure 1). Atenolol is a beta-adrenergic blocking agent that blocks the effects of adrenergic chemicals, released by nerves of the sympathetic nervous system. It is to stimulate the heart muscle to beat more rapidly. By blocking the stimulation by these nerves, Atenolol reduces the heart rate and is useful in treating abnormally rapid heart rhythms. Atenolol also reduce

the force of contraction of heart muscles and lowers blood pressure, against which the heart must pump, Atenolol reduces the work of heart muscles and need of the muscle for oxygen.

About 50% of an oral dose of Atenolol is absorbed. Peak plasma concentrations occur in 2 to 4 hours. Atenolol has low lipid solubility. It crosses the placenta and is distributed into breast milk where concentrations higher than those in maternal plasma have been achieved. Only small amounts are reported to cross the Blood Brain Barrier and plasma protein binding is minimal. The plasma half-life is about 6 to 7 hours. Atenolol undergoes little or no hepatic metabolism and is excreted mainly in the urine. It is removed by hemodialysis.[2-3]

Amlodipine Besilate is chemically known as 3-ethyl 5-methyl (4RS)-2-[(2-amino ethoxy) methyl]-4- (2-chloropheny) -6- methyl-1,4-dihydropyridine-3, 5-dicarboxylate benzene sulphonate[1] (Figure 2). It is considered a peripheral arterial vasodilator that exerts its action directly on vascular smooth muscle to lead to a reduction in peripheral vascular resistance, causing a decrease in blood pressure. Amlodipine is a dihydropyridine calcium antagonist that inhibits the influx of calcium ions into both vascular smooth muscle and cardiac muscle. Experimental studies imply that amlodipine binds to both dihydropyridine and nondihydropyridine binding sites, located on cell membranes. The contraction of cardiac muscle and vascular smooth muscle are dependent on the movement of extracellular calcium ions into these cells by ion channels.

Amlodipine is well absorbed after oral doses and peak plasma blood concentrations occur after 6 to 12 hours. The bioavailability varies but is usually about 60 to 65%. Amlodipine is reported to be about 98% bound to plasma proteins. It has a prolonged terminal elimination half-life of 35 to 50 hours and steady state plasma concentrations are not achieved until after 7 to 8 days of use. Amlodipine is extensively metabolized in the liver; metabolites are mostly excreted in urine, with less than 10% of a dose as unchanged drug. Amlodipine is not removed by dialysis.[3-4]

The literature survey was carried out and revealed that very few methods are reported for the simultaneous estimation of these drugs in other combinations. Hence an attempt was made to develop a simple, specific, rapid, precise, accurate, linear, validated and cost efficient UV method for the simultaneous estimation of Atenolol and Amlodipine Besilate in combined dosage forms.[5-10]

To develop a method for the simultaneous estimation of Atenolol and Amlodipine Besilate in bulk and pharmaceutical dosage form and Validate the method proposed in accordance with ICH guidelines for the intended analytical application.[11]

MATERIALS AND METHODS

Chemicals and Reagents

The standard Atenolol was obtained from Wellous Pharma Pvt Ltd, Pondicherry, India. The standard Amlodipine Besilate was obtained from Sai Mirra Innopharm, Pvt Ltd, Chennai, Tamil Nadu, India, Distilled water (AR grade), 0.1N NaOH was obtained from Spectrum Reagents and Chemicals Pvt Ltd, Edayar, Cochin was used throughout the study.

Instruments

The analysis was performed by using the UV Spectrophotometric instrument Lab India with equipped UV detector. Data acquisition was made with UV win software and high precision balance (wensar) was used for the weighing purpose.

Selection of solvent

The solubility of Atenolol and Amlodipine Besilate was determined in a variety of solvents as per Indian Pharmacopoeia standard. Solubility was carried out in polar and non polar solvents. From the solubility data sodium hydroxide was selected as solvent for the analysis of Atenolol and Amlodipine Besilate.

Selection of λ_{max}

Preparation of solution for UV scanning

The quantity containing 100mg of Atenolol and Amlodipine Besilate were taken into two different 100ml standard flask and the volume was made up to the mark with 0.1 N NaOH to obtain 1000µg/ml from which 1ml of solution was taken from above standard flask, and diluted to 10ml and made up to volume to obtain 100µg/ml. From the above 100µg/ml solution, 1ml was taken and transferred into 10ml standard flask and the volume was made up to the mark with 0.1 N NaOH to obtain 10µg/ml concentration of Atenolol and Amlodipine Besilate respectively. Fig No: 3&4.

METHOD DEVELOPMENT BY UV SPECTROSCOPIC METHOD

Simultaneous estimation method

Standard preparation of Atenolol and Amlodipine Besilate

The quantity containing 100mg of Atenolol and Amlodipine Besilate were taken into two different 100ml standard flask and the volume was made up to the mark with 0.1N NaOH to obtain 1000µg/ml from which 0.1ml of solution was taken from above standard flask, and diluted to 10ml and made up to volume to obtain 10µg/ml concentration of Atenolol and Amlodipine Besilate respectively. The absorbance of solution was measured at 232nm and 236 nm.

Analysis of marketed formulation

Ten tablets were weighed and triturated into fine powder. The quantity equivalent to 100mg of Atenolol and Amlodipine Besilate was taken into 100ml clean, dry standard flask and volume was made up to the mark with 0.1 NaOH to obtain 1000µg/ml. From the 1000µg/ml stock solution 0.1ml was taken into 10ml standard flask, and diluted up to the mark with 0.1N NaOH to obtain 10µg/ml concentration of Atenolol and Amlodipine Besilate. The above solution was measured at 232nm and 236nm.

Calculation

At λ_1 A_1= a X_1 b Cx + aY1b Cy ---------- (1)

At λ_2 A2 = a X2 b Cx + aY2 b Cy----------(2)

For measurements in 1 cm cells b=1

Rearrange eq. (2)

$$Cy = \frac{A2 - aX2bCx}{aY2}$$

Substituting for Cy in eq. (1) and rearranging

$$Cx = \frac{A2ay1 - A1ay2}{Ax2ay1 - ax1ay2}$$

$$Cy = \frac{A1ax2 - A2ax1}{ax2ay1 - ax1ay2}$$

aX1& aX2= Mean molar absorbvity of Atenolol

aY1&aY2= Mean molar absorbvity of Amlodipine Besilate

A1&A2 = Absorbance of markedly available sample

Cx = Concentration of Atenolol

Cy= Concentration of Amlodipine Besilate

$$\text{Percentage purity} = \frac{\text{Amount present}}{\text{Label claim}} \times 100$$

VALIDATION OF UV SPECTROSCOPY

Linearity studies

The linearity of calibration curve (Absorbance Vs Concentration) in pure solution was checked over the concentration ranges of about 4-24µg/ml for Atenolol, 5-30µg/ml for Amlodipine Besilate respectively. The Correlation coefficient was found to be 0.995 for Atenolol and 0.997 for Amlodipine Besilate respectively. Table No:1 & 2.Figure No:5 &6.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional or an accepted reference value and the value found. This is sometimes termed as trueness.

Accuracy of Atenolol and Amlodipine Besilate was performed with the 80%, 100% & 120% by using 0.1N NaOH used as a solvent. The readings were tabulated in Table no: 3&4.

Precision

Precision was determined by using the method to assay sample for a sufficient number of times to obtain statistically valid results. The precision then expressed in term of relative standard deviation. Acceptance criteria for the precision of the method should not be more than 2%. The results for intraday were shown in the Table no: 5&6.

Ruggedness: (Intermediate precision)

Ruggedness of the method was confirmed by the analysis of formulation was done by using different analysts. The amount and %RSD was calculated. The readings were tabulated in Table No. 7and 8.

Robustness

Robustness of the method was confirmed by deliberate change in the flow rate, wave length and mobile phase composition was made to evaluate the impact on proposed method. The sample were analyzed in three replicates and %RSD was calculated. The readings were tabulated in Table No.9 and 10.

RESULT AND DISCUSSION

The present work, we have developed a newer, simple, accurate and cost effective UV Spectrophotometeric method for the effective quantitative determination of Atenolol and Amlodipine Besilate in bulk and pharmaceutical dosage form. Detection of λ_{max} of Atenolol and Amlodipine Besilate was found to be 232 nm and 236nm respectively. The percentage purity of Atenolol was found to be 95.23% and Amlodipine Besilate was found to be 100.31% respectively. The percentage purity of Atenolol was found to be 95.23% and Amlodipine Besilate was found to be 100.31%. The proposed method was validated as per ICH guidelines. The method validated data showing satisfactory results for method validation tested. The method was found to be linear in the concentration ranges from 4 to 24µg/ml for Atenolol and 5 to 30µg/ml for Amlodipine Besilate with correlation coefficient 0.995 and 0.997 for Atenolol and Amlodipine Besilate respectively. In precision study found that %RSD was less than 2% which indicated that the proposed method has good reproducibility. In accuracy study % recovery of Atenolol and Amlodipine Besilate in bulk drug sample were ranged for Atenolol 100.89%, 99.36% and 100.49% respectively, Amlodipine Besilate 99.31%, 100.86% and 100.50% respectively which indicates that the method was accurate. Ruggedness and Robustness study it was found at %RSD was less than 2%. This indicates that the proposed method was accurate. Limit of detection (LOD) of Atenolol was found to be 2.8555 µg/ml and Amlodipine Besilate was found to be 3.0305 µg/ml respectively. Limit of Quantitation (LOQ) of Atenolol was found to be 8.6530 µg/ml and Amlodipine Besilate was found to be 9.1833 µg/ml respectively.

FIGURES AND TABLES

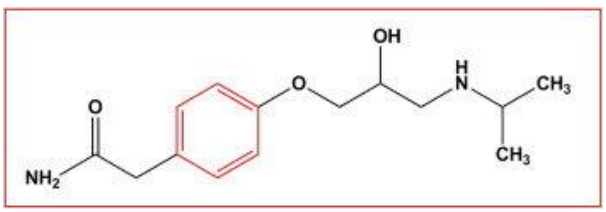

Figure No: 1: Structure of Atenolol.

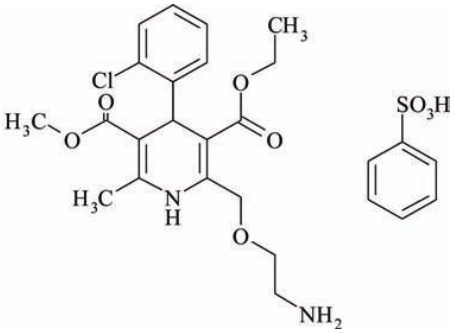

Figure No: 2: Structure of Amlodipine Besilate.

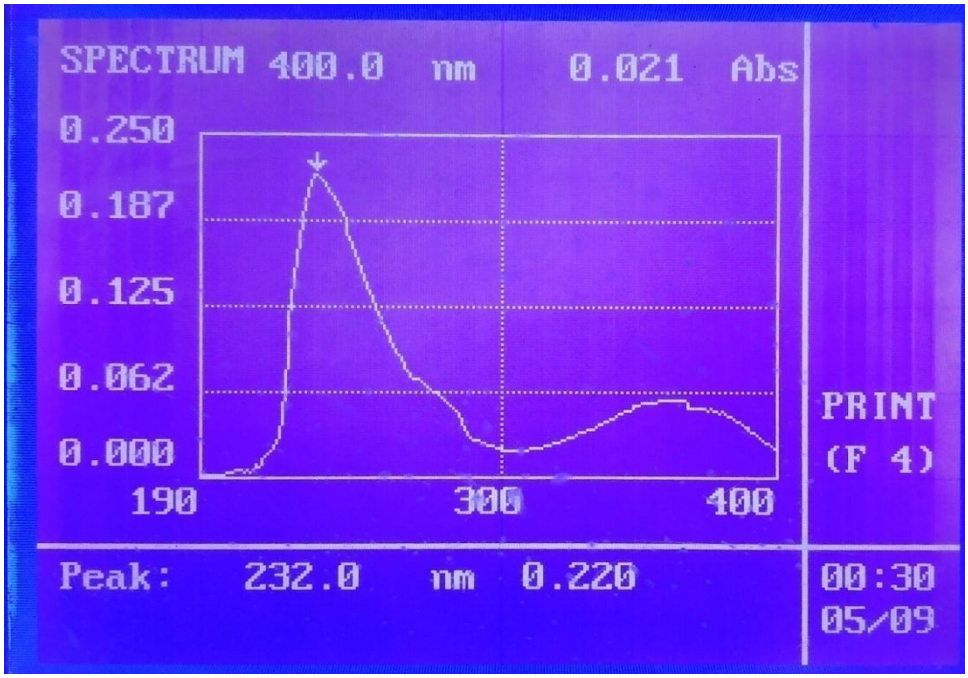

Figure No: 3: UV Spectrum of Atenolol.

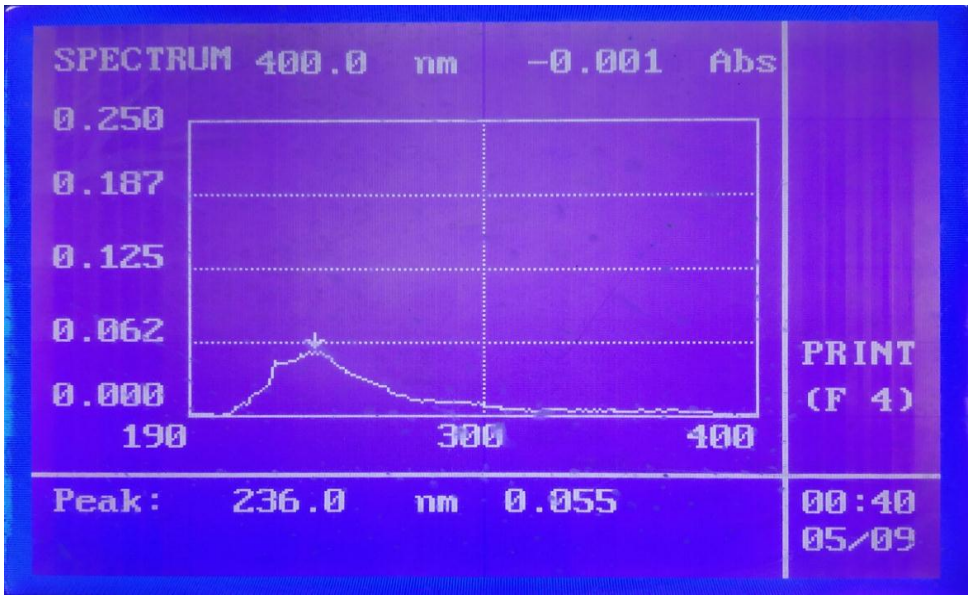

Figure No: 4: UV Spectrum of Amlodipine Besilate.

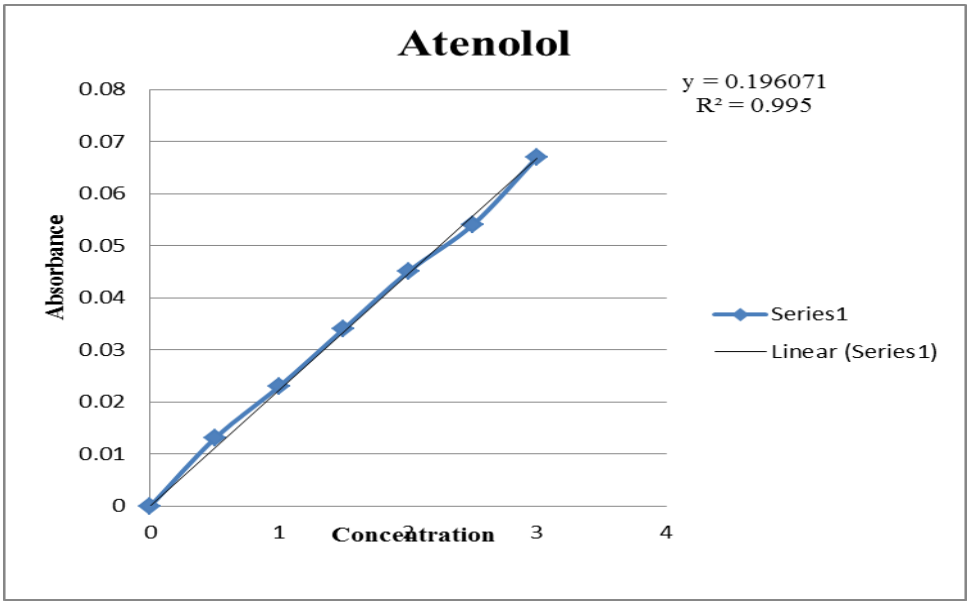

Figure No. 5: Calibration curve of Atenolol.

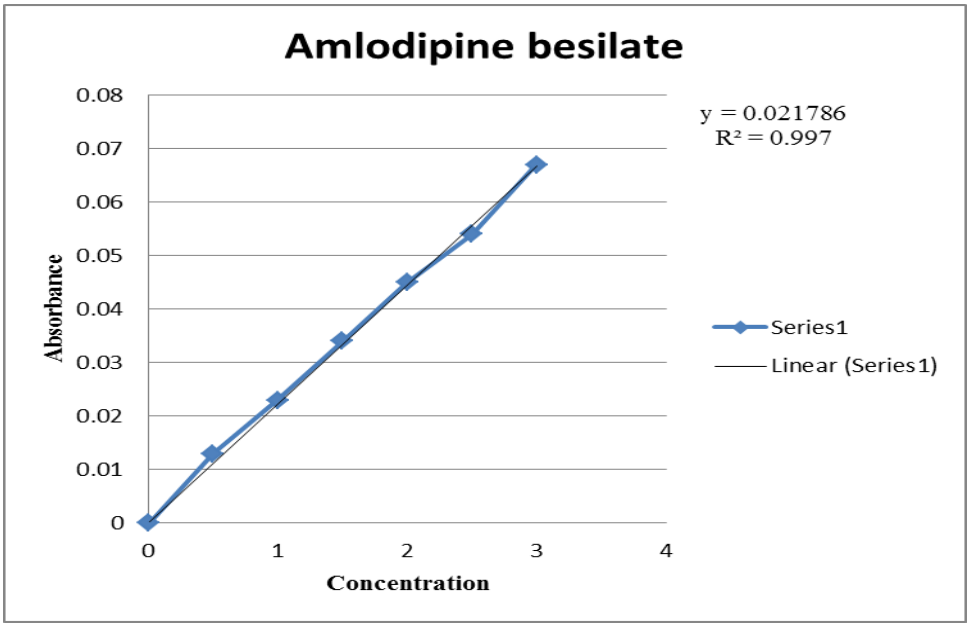

Figure No. 6: Calibration curve of Amlodipine Besilate.

Table No. 1: Linearity data for Atenolol.

Concentration (µg/ml)	Absorbance of Atenolol	Statistical analysis of Atenolol
0	0	Slope= 0.196071 Intercept= 0.015714 R^2= 0.995
0.4	0.103	
0.8	0.179	
1.2	0.256	
1.6	0.326	
2.0	0.404	
2.4	0.477	

Table No: 2: Linearity data for Amlodipine Besilate.

Concentration (µg/ml)	Absorbance of Amlodipine Besilate	Statistical analysis of Amlodipine Besilate
0	0	Slope= 0.021786 Intercept= 0.001036 R^2= 0.997
0.5	0.013	
1.0	0.023	
1.5	0.034	
2.0	0.045	
2.5	0.054	
3.0	0.067	

Table no: 3: Accuracy for Atenolol by UV method.

Level	Amount Present (µg/ml)	Amount Added (µg/ml)	Amount found (µg/ml)	Amount recovered	% recovery	SD	%RSD
80%	10.05	8.90	18.95	8.98	100.89	0.7934	0.7915
100%	10.01	10.401	20.411	10.335	99.36		
120%	1.03	12.120	22.150	12.185	100.49		

***n=3**

Table No: 4: Accuracy for Amlodipine Besilate by UV method.

Level	Amount Present (µg/ml)	Amount Added (µg/ml)	Amount found (µg/ml)	Amount recovered	% recovery	SD	%RSD
80%	10.03	8.70	18.73	8.64	99.31	0.8111	0.8093
100%	10.04	10.469	20.509	10.56	100.86		
120%	10.02	12.179	22.199	12.24	100.50		

***n=3**

Table No: 5: Intraday analysis of Atenolol by UV method.

S.No	Atenolol	
	12(µg/ml)	16(µg/ml)
	0.273	0.375
	0.276	0.381
	0.271	0.385
Average	0.2733	0.3803
S.D	0.002517	0.005033
%RSD	0.9207	1.3233

Table No: 6: Intraday analysis of Amlodipine Besilate by UV method.

S.No	Amlodipine Besilate	
	12(µg/ml)	16(µg/ml)
	0.035	0.039
	0.034	0.038
	0.034	0.039
Average	0.0343	0.0386
S.D	0.000577	0.000577
%RSD	1.6816	1.4931

Table No. 7: Ruggedness of Atenolol (different analysts).

S. No.	Atenolol			
	Analysts 1		Analysts 2	
	12(µg/ml)	16(µg/ml)	12(µg/ml)	16(µg/ml)
	0.221	0.29	0.213	0.309
	0.219	0.292	0.214	0.308
	0.221	0.29	0.211	0.306
Average	0.220333	0.290667	0.212667	0.307667
SD	0.001155	0.001155	0.001528	0.001528
% RSD	0.5240	0.3972	0.7182	0.4964

Table No. 8: Ruggedness of Amlodipine Besilate (different analysts).

S. No.	Amlodipine Besilate			
	Analysts 1		Analysts 2	
	15(µg/ml)	20(µg/ml)	15(µg/ml)	20(µg/ml)
	0.041	0.035	0.033	0.038
	0.04	0.034	0.034	0.037
	0.041	0.035	0.033	0.038
Average	0.040667	0.034667	0.033333	0.037667
SD	0.000577	0.000577	0.000577	0.000577
% RSD	1.4197	1.6654	1.7320	1.5327

Table No. 9: Robustness of Atenolol.

S. No.	Atenolol	
	230nm(12µg/ml)	**234(12µg/ml)**
1.	0.175	0.186
2.	0.172	0.189
3.	0.173	0.188
Average	0.173333	0.187667
SD	0.001528	0.001528
%RSD	0.8812	0.8139

Table No. 10: Robustness of Amlodipine Besilate.

S. No.	Amlodipine Besilate	
	234(15µg/ml)	238(15µg/ml)
1.	0.037	0.031
2.	0.037	0.030
3.	0.036	0.030
Average	0.036667	0.030333
SD	0.000577	0.000577
%RSD	1.5745	1.9033

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CONCLUSION

A simple, precise and accurate method was developed UV Spectrophotometeric method has been developed for quantitative determination of Atenolol and Amlodipine Besilate in bulk and pharmaceutical formulation. The method was validated for linearity, precision accuracy, ruggedness, robustness and LOD & LOQ. The methods were found to be simple and accurate when compared to other existing methods found in literature and journal.

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