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DEVELOPMENT AND VALIDATION OF FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF ROSUVASTATIN CALCIUM AND GEMIGLIPTIN IN SYNTHETIC MIXTURE

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Key words

Rosuvastatin calcium, Gemigliptin, U.V Spectrophotometer, First order derivative, Synthetic Mixture, Validation method.

ABSTRACT

A simple, precise and accurate UV-Spectrophotometric Method for simultaneous estimation of Rosuvastatin calcium and Gemigliptin in Synthetic Mixture. In The first order derivative method absorption at 223 (zero crossing point for Rosuvastatin calcium) was used for Gemigliptin and 253 nm (zero crossing point for Gemigliptin) was used for Rosuvastatin calcium. The linearity was taken in the concentration range of 4-24 µg/ml for Rosuvastatin calcium and 10-60 µg/ml for Gemigliptin with correlation coefficient (R^2) 0.998 and 0.997, respectively. The developed method was validated according to ICH (Q2 R1) guidelines and whereby %RSD values were found to be ≤2% complying the validation requirements. Method can be applied to the simultaneous estimation of Rosuvastatin calcium and Gemigliptin in their Synthetic Mixture. The results of analysis have been validated statistically and by recovery studies.

INTRODUCTION

Gemigliptin is a class of oral antidiabetic which is used to control glucose level in the people with type 2 diabetes mellitus. It is an oral anti-hyperglycemic agent to optimized DPP-4 inhibitor in terms of efficacy, safety, and patient compliance for treatment of type 2 diabetes mellitus. It helps to prevent kidney injury and it is used to increase insulin level and reduce the glucagon level, decrease fasting and postprandial glycemia. Gemigliptin inhibites the glucose lowering effects of Dipeptidyl peptidase-4 the enzyme which is responsible for inactivation of incretin hormones GLP-1 and GIP (gastric inhibitory polypeptide). Rosuvastatin is an indicated class of statin and inhibitor of the enzyme HMG-CoA reductase. It is used for the treatment of dyslipidemia, Hypercholesterolemia, triglycemia. It is reduce the plasma concentration of LDL-C, Apolipoprotein, Triglyceride and increase HDL-C level in patients. Rosuvastatin works by reducing the high cholesterol level in hyperlipidemic patients. Controlling high cholesterol helps in prevention of primary cardiovascular function (event) relative risk of heart attack and stroke in patients. From the literature survey, it was observed that various methods are reported for analysis of Rosuvastatin calcium and Gemigliptin, individually as well as in combination with other drugs. But no analytical method has been

reported for analysis of in Rosuvastatin calcium and Gemigliptin synthetic mixture. A successful attempt has been made to estimate two drugs simultaneously by First order derivative Spectrophotometric method.

MATERIAL AND METHODS: Rosuvastatin calcium and Gemigliptin were procured as generous gift sample by supplied by (West coast Pharma, Ahmedabad, Gujarat). Methanol (Aventor Performance Material, India) (AR Grade)

UV Spectrophotometric method [8]
First order derivative spectrophotometric method for simultaneous estimation of Rosuvastatin calcium and Gemigliptin in synthetic mixture.

Instrument and apparatus: UV Visible spectrophotometer: A shimadzu model 1800 (Japan) with spectral width 2 nm, wavelength accuracy of 0.5 nm and pair of 10 mm matched quartz cell. Spectra was automatically obtained by UV probe system software (UV probe version 2.31) Digital were automatically weighing balance: Wenser DAB-220 Sonicator: Equiton Volumetric Flask: 10, 50, 100 ml (Borosilicate) Pipette: 1, 2,5,10 ml (Borosilicate) Measuring cylinder: 10, 50, 100 ml

Chemical and reagents: Rosuvastatin calcium and Gemigliptin were procured as generous gift sample by supplied by (West coast Pharma, Ahmedabad, and Gujarat). Methanol (Aventor Performance Material, India) (AR Grade)

Preparation of standard stock solution (100µg/ml): Accurately weighed 10 mg of Rosuvastatin calcium and Gemigliptin and transferred into separate 100ml volumetric flask, dissolved to half, sonicated and made up to the mark with Methanol. (100µg/ml)

Procedure of selection of wave length:

0.8 ml standard stock solution of Rosuvastatin calcium (100 µg/ml) and 2 ml standard stock solution of Gemigliptin (100 µg/ml) was transfer in 10 ml separate volumetric flask and dilute up to mark with Methanol to get the 12 µg/ml of Rosuvastatin calcium and 30 µg/ml of Gemigliptin. Each solution was scanned in the range 200 – 400 nm. The Spectra are converted to First Order Derivative. The overlain first derivative spectrums of Rosuvastatin calcium and Gemigliptin at different concentration were recorded. The zero crossing point (ZCP) of rosuvastatin calcium was found to be 223 nm and ZCP of Gemigliptin was found to be 253 nm. Both this wavelength 223 nm and 256 nm were used for the determination of Rosuvastatin calcium and Gemigliptin respectively.

Preparation of calibration curve:

Calibration curve for Rosuvastatin calcium: Aliquots of stock solution of Rosuvastatin calcium (100 µg/ml) 0.4, 0.8, 1.2, 1.6, 2.0, 2.4 ml were pipette out in 10 ml volumetric flask separately and dilute up to the mark with Methanol which will give 4-24µg/ml solution was prepared and absorbance was measured at 256 nm in U.V. Graph of Absorbance vs. Concentration (µg/ml) was plotted ***Calibration curve for Gemigliptin***: Aliquots of stock solution of Gemigliptin (100 µg/ml) 1,2,3,4,5,6 ml were pipette out in 10 ml volumetric flask separately and dilute up to the mark with Methanol which will give 10-60 µg/ml solution was prepared and absorbance was measured at 223 nm in U.V. Graph of Absorbance vs. Concentration (µg/ml) was plotted

Method validation: [9] The developed method was validated with respect to

linearity, accuracy, precision, limit of detection and limit of quantification in accordance with the ICH guideline.

Linearity & range (n=6): The linearity of Rosuvastatin calcium and Gemigliptin was taken to be in the range of 4-24 µg/ml and 10-60 µg/ml respectively. Calibration curve of Absorbance Vs Concentration was plotted and from that slope, intercept, correlation coefficient and regression line equation for Rosuvastatin calcium and Gemigliptin was constructed.

Precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: Intermediate (Intraday) precision, reproducibility (Interday precision), repeatability.

1) Intraday precision (n=3): Solutions containing 4, 8, 12 µg/ml of rosuvastatin calcium and 10, 20, 30 µg/ml of Gemigliptin were analyzed three times on the same day and % RSD was calculated.

2) Interday Precision (n=3): Solutions containing 4, 8, 12 µg/ml of rosuvastatin calcium and 10, 20, 30 µg/ml of Gemigliptin were analyzed three different successive days and % RSD was calculated.

3) Repeatability (n=6): Solutions containing 8 µg/ml of Rosuvastatin calcium and 20 µg/ml of Gemigliptin were analyzed for six times and % R.S.D was calculated. R.S.D was not more than 2%.

limit of detection (LOD): Limit of Detection can be calculated using following equation as per ICH guidelines.

$$\mathbf{LOD = 3.3 \times (\sigma / S)}$$

Where, σ = standard deviation of the Y intercept of calibration curve

S = Mean slope of the corresponding calibration curve.

Limit of quantification (LOQ):

Limit of Quantification can be calculated using following equation as per ICH guidelines.

$$\mathbf{LOQ = 10 \times (\sigma / S)}$$

Where, σ = standard deviation of the Y intercept of calibration curve

S = Mean slope of the corresponding calibration curve.

Accuracy (recovery study) (n=3):

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy of the developed method was confirmed by doing recovery study as per ICH guideline at three different concentration levels 50%, 100%, 150% and the values were measured for Rosuvastatin calcium (8 **µg/ml**) and Gemigliptin (**20µg/ml**). This performance was done in triplicate.

Preparation of sample solution:

Accurately weigh powder equivalent to 8mg of Rosuvastatin calcium and 20mg Gemigliptin from the prepared synthetic mixture and transferred in 100 ml volumetric flask and make up to the mark with Methanol. This solution was sonicated and filtered. The mixture contains Rosuvastatin calcium 80µg/ml and Gemigliptin 200µg/ml. From the above synthetic mixture 1 ml was pipetted out in 3 different 10ml volumetric flask and spiked with standard stock solution of Rosuvastatin calcium and Gemigliptin in 50, 100, 150 level. Made up to the mark with Methanol to obtain final concentration.

Application of method to synthetic mixture: Preparation of synthetic mixture: Preparation of Synthetic Mixture of Rosuvastatin calcium and Gemigliptin: [8] The synthetic mixture of Rosuvastatin and Gemigliptin was prepared in ratio of 1:2.5. Powder weight to 20 mg of Rosuvastatin calcium and 50 mg Gemigliptin was taken and transferred into 100 ml volumetric flask than add polyvinyl Pyrolidine, microcrystalline cellulose, lactose, talc, mg. stearate into the flask and mark up to the half mark with Methanol. This solution was sonicated till the drug and excipients dissolves and was made upto the mark with methanol. Powder Weighted equivalent to 8 mg of Rosuvastatin calcium and 20mg of Gemigliptin was taken and transferred into 100 ml volumetric flask. Added 50 ml of methanol and sonicated it. Diluted up to the mark with methanol. This solution filtered through Whatmann filter paper. This mixture contains rosuvastatin calcium 80 µg/ml Gemigliptin 200 µg/ml.

Preparation of Sample Solution:

From the above synthetic mixture 1 ml was pipetted out in volumetric flask and made up to the mark with Methanol to make final concentration of rosuvastatin calcium 8 µg/ml and Gemigliptin 20 µg/ml.

RESULTS AND DISCUSSION:

Selection of wavelength for Rosuvastatin calcium and Gemigliptin

To determine the wavelength for measurement, Rosuvastatin calcium (8µg/ml) and Gemigliptin (20µg/ml) solution were scanned between 400-200 nm. Absorbance maximum were obtained at their λmax 223 nm and 256 nm for Rosuvastatin calcium and Gemigliptin, respectively.

A Simple, Precise and Accurate First Order Derivative Spectrophotometric Method have been developed for simultaneous estimation of Rosuvastatin calcium and Gemigliptin in Synthetic Mixture. Rosuvastatin calcium shows ZCP (Zero Crossing Point) at 223 nm and Gemigliptin show ZCP at 256 nm. At 256 (ZCP of Gemigliptin) Rosuvastatin shows considerable absorbance while at 223 nm (ZCP of Rosuvastatin) Gemigliptin shows considerable absorbance. Linearity Range of 4-24 µg/ml for Rosuvastatin calcium and 10-60 µg/ml for Gemigliptin with Correlation Coefficient of 0.998 and 0.997 Rosuvastatin calcium and Gemigliptin for respectively was obtained and the Precision data obtained with less than 2% RSD. Accuracy was carried out by Recovery Studies and was obtained in the range of 98.14-99.06 for Rosuvastatin calcium and 98.06-98.85 for Gemigliptin. LOD and LOQ values were found to be µg/ml respectively for Rosuvastatin calcium and Gemigliptin value were found to be µg/ml respectively.

CONCLUSION

The results of present study indicate that the proposed UV spectroscopic method is simple, precise and accurate. Statistical analysis proves that the method is repeatable and selective for the analysis of Rosuvastatin calcium and Gemigliptin in combination. It can therefore be concluded that the developed analytical method was precise & accurate and can be use for routine Analysis of both the drug in combination.

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Table 1: Melting Point of Rosuvastatin calcium and Gemigliptin [7]

Sr no.	Drug	Reported melting point	Observed Melting point
1	Rosuvastatin calcium	155-160℃	152-157℃
2	Gemigliptin	174-177℃	179-180℃

Table 2: Wavelength of Rosuvastatin calcium and Gemigliptin

Drug name	Observed λmax (Methanol)	Reported λmax (Methanol)
Rosuvastatin Calcium	244	248
Gemigliptin	256	260

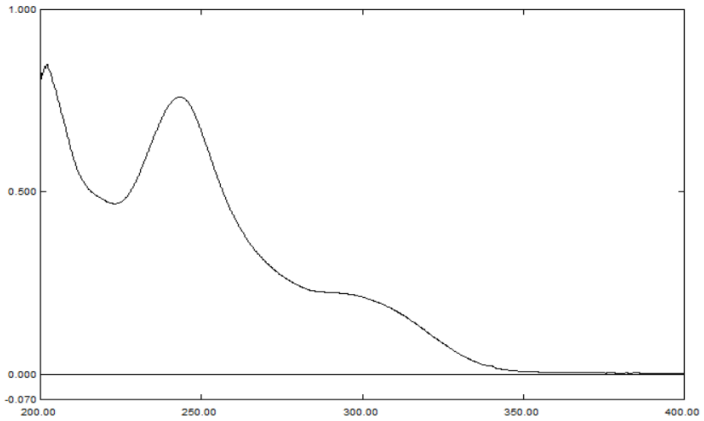

Fig 1: Rosuvastatin calcium UV Spectra 244 nm (12 µg/ml)

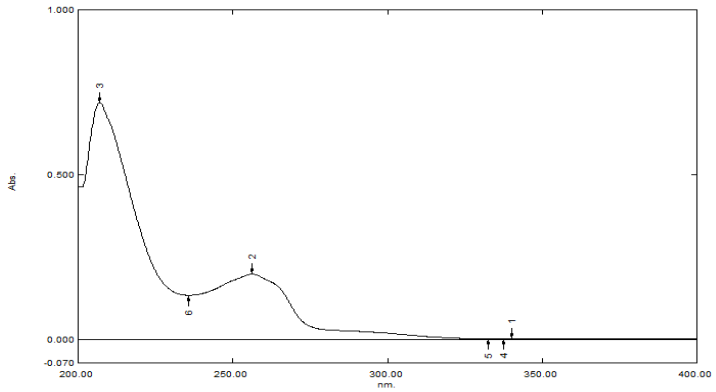

Fig 2: Gemigliptin UV Spectra 256 nm (30 µg/ml)

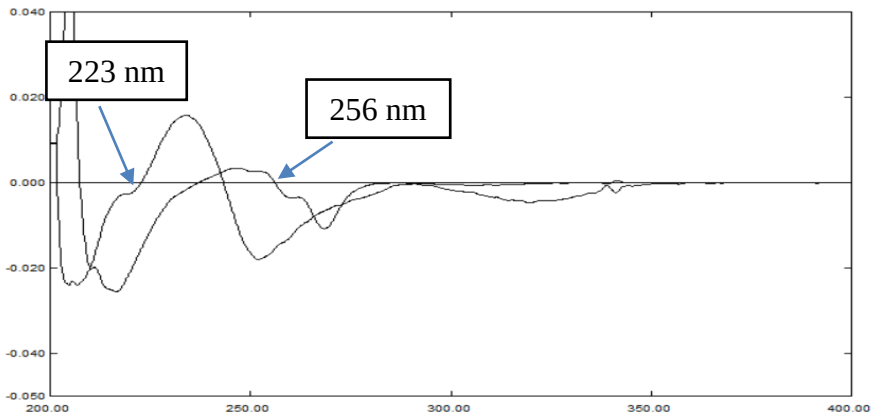

Fig 3: Zero crossing point of Rosuvastatin calcium at 223 nm (8µg/ml) and Gemigliptin at 256 nm (20 µg/ml)

LINEARITY AND RANGE:

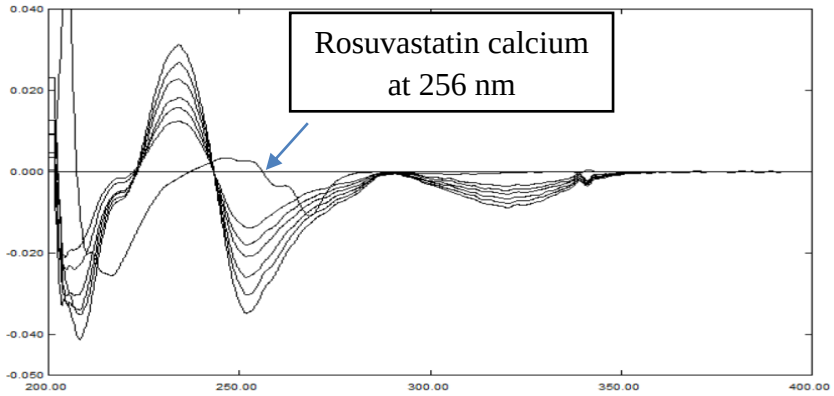

Fig 4: Linearity of 1st Derivative spectra of Rosuvastatin calcium (256 nm)

TABLE: 3 Linearity data of Rosuvastatin calcium

Rosuvastatin calcium		
Concentration (µg/ml)	Mean Absorbance ± SD (n=6)	%RSD
4	|-0.012|± 0.00021	1.76
8	|-0.016|± 0.00026	1.61
12	|-0.019|± 0.00029	1.54
16	|-0.023|± 0.00030	1.30
20	|-0.026|± 0.00031	1.18
24	|-0.029|± 0.00032	1.08

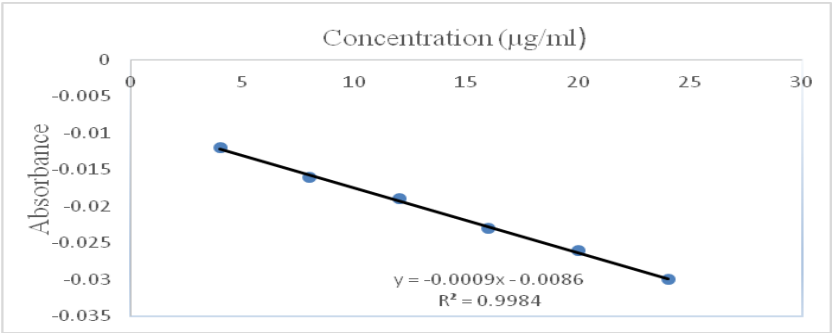

Fig 4.1 Calibration Curve of Rosuvastatin calcium (4-24 µg/ml)

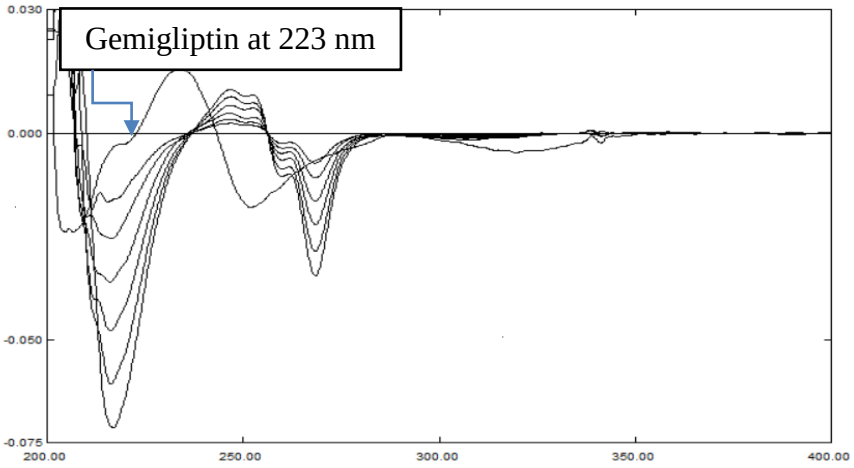

Fig 5: Linearity of 1st Derivative spectra of gemigliptin (223nm)

TABLE: 4 Linearity data of Gemigliptin

Gemigliptin		
Concentration (µg/ml)	Mean Absorbance ± SD (n=6)	%RSD
10	|-0.010|± 0.00016	1.57
20	|-0.016|± 0.00024	1.47
30	|-0.024|± 0.00033	1.37
40	|-0.031|± 0.00037	1.18
50	|-0.037|± 0.00040	1.08
60	|-0.046|± 0.00045	0.98

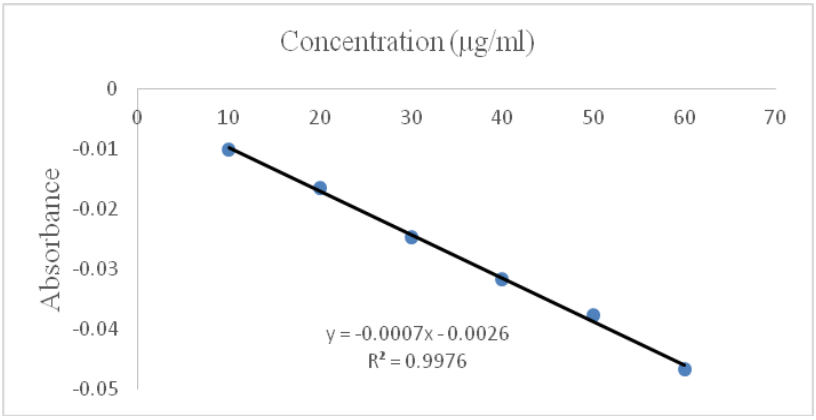

Fig 5.1: Calibration Curve of Gemigliptin (10-60 µg/ml)

TABLE 3.1: Precision study of Rosuvastatin calcium

Intraday Precision of Rosuvastatin calcium (n=3)		
Conc. (µg/ml)	Mean Absorbance ±SD (n=3)	% RSD
4	|-0.0117|±0.00017	1.50
8	|-0.0151|±0.00020	1.33
12	|-0.0191|±0.00023	1.23
Interday Precision of Rosuvastatin calcium (n=3)		
Conc. (µg/ml)	Mean Absorbance ±SD (n=3)	% RSD
4	|-0.0117|±0.00019	1.63

8	|-0.0141|±0.00021	1.53
12	|-0.0181|±0.00025	1.38
Repeatability of Rosuvastatin calcium (n=6)		
Conc. (µg/ml)	Mean Absorbance ±SD (n=6)	% RSD
8	|-0.0149|±0.00022	1.48

TABLE 4.2: Precision study of Gemigliptin

Intraday Precision of Gemigliptin (n=3)		
Conc. (µg/ml)	Mean Absorbance ±SD (n=3)	% RSD
10	|-0.0090|±0.00015	1.51
20	|-0.0151|±0.00023	1.42
30	|-0.0244|±0.00032	1.31
Interday Precision of Gemigliptin (n=3)		
Conc. (µg/ml)	Mean Absorbance ±SD (n=3)	% RSD
10	|-0.0091|±0.00016	1.77
20	|-0.0150|±0.00025	1.67
30	|-0.0241|±0.00037	1.55
Repeatability of Gemigliptin (n=6)		
Conc. (µg/ml)	Mean Absorbance ±SD (n=6)	% RSD
20	|-0.0146|±0.00020	1.36

TABLE: 5 LOD and LOQ for Rosuvastatin calcium and Gemigliptin

Parameter	Rosuvastatin calcium	Gemigliptin
LOD (µg/ml)	0.63	1.09
LOQ (µg/ml)	1.91	3.31

TABLE 6: Recovery study

Name of Drug	% Level of recovery	Test Amount (µg/ml)	Amount of drug taken (µg/ml)	Total Amt (µg/ml)	Total amount Recovered	Recovery ±SD (n=3)
Rosuvastatin calcium	50	8	4	12	11.77	98.14%±0.0152
	100	8	8	16	15.79	98.68%±0.0251
	150	8	12	20	19.81	99.06%±0.0305
Gemigliptin	50	20	10	30	29.31	98.06%±0.0208
	100	20	20	40	39.16	98.21%±0.0264
	150	20	30	50	49.14	98.85%±0.0360

TABLE: 7 Analysis of synthetic mixture

Name of drug	Amount taken (µg/ml)	Mean Amount found (µg/ml)	%Assay
Rosuvastatin Calcium	8	7.87	98.3%±0.0288
Gemigliptin	20	19.71	98.5%±0.0346

TABLE: 8 Summary of validation parameter

S. no	Parameters	Rosuvastatin calcium	Gemigliptin
1	Wavelength (nm)	256 nm	223 nm
2	Beer's Law Limit	4-24 (µg/ml)	10-60 (µg/ml)
3	Regression equation (y = mx +c)	y = -0.0009x - 0.0086	y = -0.0007x - 0.0026
4	Correlation Coefficient (r^2)	0.998	0.997
5	Intraday Precision (%RSD, n=3)	1.50-1.23	1.68-1.31
6	Interday Precision (% RSD, n=3)	1.63-1.38	1.77-1.55
7	Repeatability(% RSD, n=6)	1.48	1.36
8	Accuracy(% Recovery,	98.14-99.06%	98.06-98.85%
9	LOD (µg/ml)	0.63 (µg/ml)	1.09 (µg/ml)
10	LOQ (µg/ml)	1.9 (µg/ml)	3.3 (µg/ml)
11	Assay	98.3%	98.5%

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