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DEVELOPMENT AND VALIDATION OF FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF LERCANIDIPINE AND VALSARTAN IN SYNTHETIC MIXTURE

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ABSTRACT

A simple, sensitive, accurate, precise first order derivative Spectrophotometric method was developed and validated for the determination of Lercanidipine and Valsartan in Synthetic Mixture. In The first order derivative method absorption at 235 nm (zero crossing point of Lercanidipine) was used for measurement of Valsartan and 244 nm (zero crossing point of Valsartan) was used for measurement of Lercanidipine. The linearity was taken in the concentration range of 1-6 µg/ml for Lercanidipine and 8-48 µg/ml for Valsartan with correlation coefficient (R^2) 0.999 for both the drugs. 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 Lercanidipine and Valsartan in their Synthetic Mixture. The results of analysis have been validated statistically and by recovery studies.

KEYWORDS: Lercanidipine, Valsartan, First order derivative, Synthetic Mixture, Validation method.

INTRODUCTION[1-2]

Lercanidipine: 3-{1-[(3, 3 diphenylpropyl)(methyl)amino]-2-methylpropan-2-yl} 5-methyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate with molecular formula $C_{36}H_{41}N_3O_6$. Chemical structure is shown in figure.

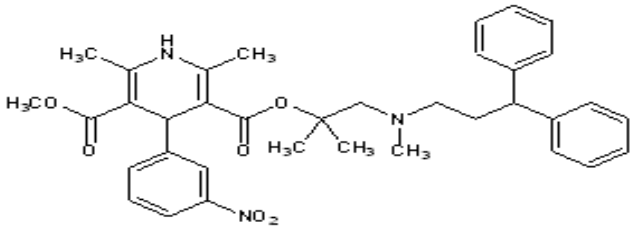

Fig. 1: Structure of Lercanidipine.

Lercanidipine is a calcium channel blocker of the dihydropyridine class. It is commonly used for the treatment of hypertension. Lercanidipine inhibits the influx of extra cellular calcium across the myocardial and vascular smooth muscle cell membranes possibly by deforming the channel, inhibiting ion-control gating mechanisms, and/or interfering with the release of calcium from the sarcoplasmic reticulum. The decrease in intracellular calcium inhibits the contractile processes of the myocardial smooth muscle cells, causing dilation of the coronary and systemic arteries, increased oxygen delivery to the myocardial tissue, decreased total peripheral resistance, decreased systemic blood pressure, and decreased afterload.

Valsartan is a tetrazole derivative; (2S)-3-methyl-2-[pentanoyl-[[4-[2-(2H-tetrazol-5-yl) phenyl] phenyl] methyl] amino] butanoic acid with molecular formula $C_{24}H_{29}N_5O_3$..chemical structure is shown in figure.

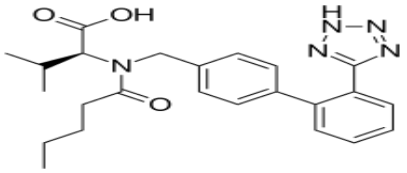

Fig. 2: Structure of Valsartan.

Valsartan is an antihypertensive agent known as angiotensin II receptor antagonist (ARB), which is selective for the type I (AT1) angiotensin receptors. Valsartan is used for the treatment of high blood pressure and congestive heart failure. It blocks the blood pressure by increasing effects of AT2 via the renin-angiotensin- aldosterone system (RAAS). It is an

orally active non-peptide Triazolederived antagonist of angiotensin (AT) II with antihypertensive properties. Valsartan specifically and competitively blocks the binding of AT2 to the AT1 subtype receptor in vascular smooth muscle and the adrenal gland, preventing AT II-mediated vasoconstriction, aldosterone synthesis & secretion, renal reabsorption of sodium, resulting in vasodilation, increased excretion of sodium & water, a reduction in plasma volume, and a reduction in blood pressure.

From the literature survey, it was observed that various methods are reported for analysis of Lercanidipine and Valsartan individually as well as in combination with other drugs But no other methods has been reported for analysis of Lercanidipine and Valsartan synthetic mixture. A successful attempt has been made to estimate two drugs simultaneously by First order derivative Spectrophotometric method.

MATERIAL AND METHODS

Instruments

UV Visible Spectrophotometer: A Shimadzu UV–visible double beam spectrophotometer model 1800 (Japan) with spectral width 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell. Spectra were automatically obtained by UV probe system software (UV probe version 2.31).

Digital analytical weighing balance: Wenser DAB-220

Sonicator: Equitron

Chemicals and Materials

Lercanidipine and Valsartan were procured as gift sample from Sun Pharmaceuticals (Baroda) and Torrent Pharmaceuticals (Ahmedabad).

Methanol (Aventor Performance Material, India)

Selection of a Solvent

Both The Drugs were soluble in Methanol. So, Methanol was selected as a solvent for estimation of both the Drugs.

Experimental Work

Preparation of standard stock solution

- Preparation of standard stock solution of Lercanidipine (100µg/ml):

It was prepared by dissolving accurately weighed quantity (10 mg) of Lercanidipine into 100 ml volumetric flask using Methanol as solvent.

- Preparation of standard stock solution of Valsartan (100µg/ml):
  It was prepared by dissolving accurately weighed quantity (10 mg) of Valsartan into 100 ml volumetric flask using Methanol as solvent.

Selection of Wavelength

0.3 ml standard stock solution of Lercanidipine (100 µg/ml) and 2.4 ml standard stock solution of Valsartan (100 µg/ml) was transfer in 10 ml volumetric flask and dilute up to mark with Methanol to get the 3 µg/ml of Lercanidipine and 24 µg/ml of Valsartan. Each solution was scanned in the range 200 – 400 nm.

The Spectra are converted to First Order Derivative. The zero crossing point (ZCP) of Lercanidipine was found to be 235 nm and ZCP of Valsartan was found to be 244 nm. Hence, these 235 nm and 244 nm were selected as wavelengths for measurement.

Calibration curve for Lercanidipine (1-6 µg/ml)

From the Standard stock solution of Lercanidipine (100 µg/ml),aliquots of 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6ml were transferred to 10 ml volumetric flask and diluted up to the mark with Methanol. The resulting solution gives the concentration of 1, 2, 3, 4, 5 and 6µg/ml. The obtained solution was measured at 244 nm in U.V Spectrophotometer.

Graph of Absorbance vs. Concentration (µg/ml) was plotted.

Calibration curve for Valsartan (8-48 µg/ml)

From the Standard stock solution of Valsartan (100 µg/ml), aliquots of 0.8, 1.6, 2.4, 3.2, 4.0 and 4.8 ml were transferred to 10 ml volumetric flask and diluted up to the mark with Methanol. The resulting solution gives the concentration of 8, 16, 24, 32, 40 and 48 µg/ml. The obtained solution was measured at 235 nm in U.V Spectrophotometer.

Graph of Absorbance vs. Concentration (µg/ml) was plotted.

Method Validation[3-4]

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 Lercanidipine and Valsartan was taken to be in the range of 1-6 µg/ml and 8-48 µg/ml respectively. Calibration curve of Absorbance Vs Concentration was plotted and from that slope, intercept, correlation coefficient and regression line equation for Lercanidipine and Valsartan 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 1, 2, 3 µg/ml of Lercanidipine and 8, 16, 24 µg/ml of Valsartan were analyzed three times on the same day and % RSD was calculated.

2) Interday Precision (n=3)

Solutions containing 1, 2, 3 µg/ml of Lercanidipine and 8, 16, 24 µg/ml of Valsartan were analyzed three different successive days and % RSD was calculated.

3) Repeatability (n=6)

Solutions containing 2 µg/ml of Lercanidipine and 16 µg/ml of Valsartan 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.

$LOD = 3.3 \times (\sigma / S)$

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.

$LOQ = 10 \times (\sigma / S)$

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 Lercanidipine (**2 µg/ml**) and Valsartan (**16 µg/ml**). This performance was done in triplicate.

Assay

Preparation of Synthetic Mixture of Lercanidipine and Valsartan[8]

The synthetic mixture of Lercanidipine and Valsartan was prepared in ratio of 1:8

Accurately weighed equivalent weight of Lercanidipine (10mg) and Valsartan (80mg) which transferred in 100 ml volumetric flask dissolved with methanol and sonicated. Then this concentration of Lercanidipine 100 µg/ml and Valsartan 800 µg/ml.

Preparation of Sample Solution

From the above synthetic mixture 0.2 ml was pipetted out in volumetric flask and made up to the mark with Methanol to make final concentration of Lercanidipine was 2µg/ml and Valsartan was 16µg/ml.

RESULT AND DISCUSSION

Selection of wavelength for Lercanidipine and Valsartan

To determine the wavelength for measurement Lercanidipine (3 µg/ml) and Valsartan (24 µg/ml) solutions were scanned between 400-200 nm. Lercanidipine shows ZCP at 235nm and Valsartan shows ZCP at 244nm. At ZCP of Lercanidipine 235nm, Valsartan shows measurable absorbance, whereas at ZCP of Valsartan 244nm Lercanidipine shows a measurable absorbance. Hence, these wavelengths 235.74 and 244.35nm were selected as analytical wavelengths.

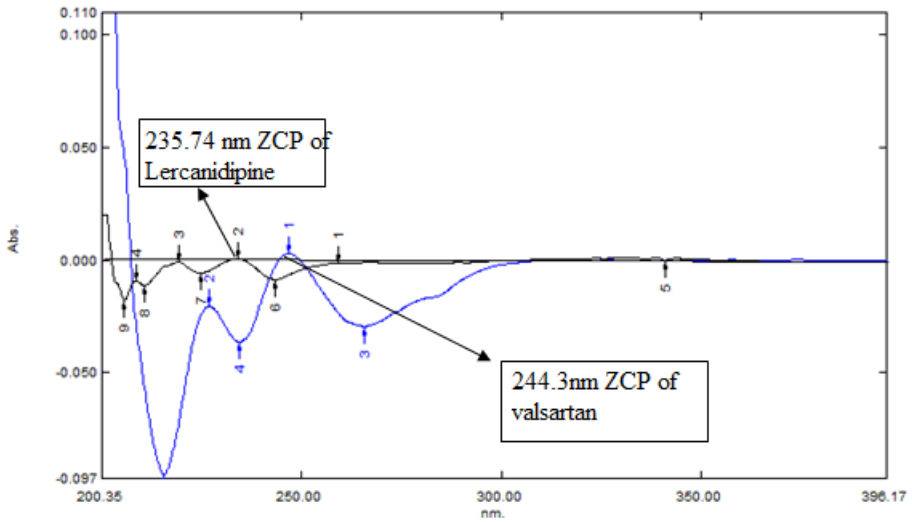

Fig. 3: Zero crossing point of Lercanidipine at 235 nm (3µg/ml) and Valsartan at 244 nm (24µg/ml).

Linearity and Range

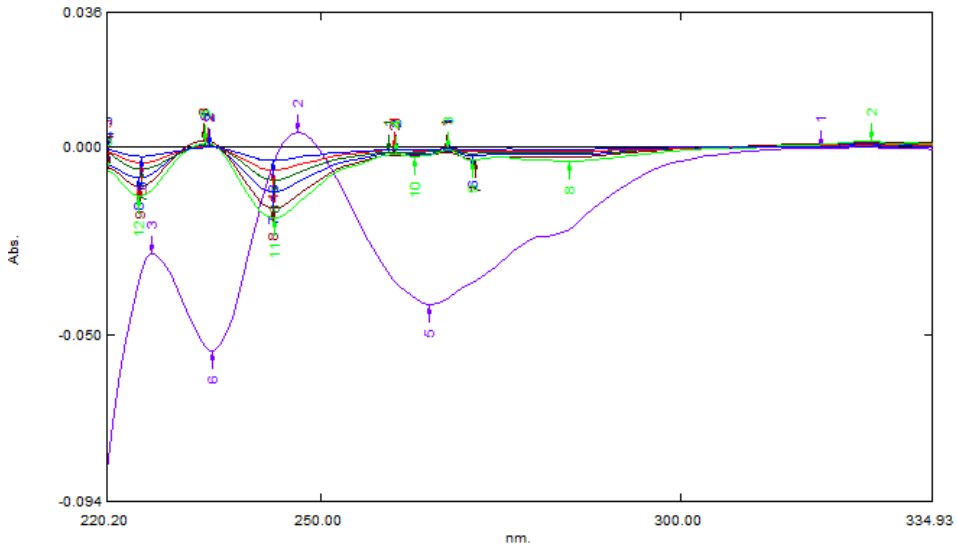

Fig. 4: Linearity of 1st Derivative spectra of Lercanidipine (244 nm).

Table 1: Linearity data of Lercanidipine.

Concentration (µg/ml)	Mean Absorbance ± SD (n=6)	% RSD
1	|-0.0063|± 0.00010	1.638
2	|-0.0094|± 0.00015	1.630
3	|-0.0131|± 0.00016	1.227
4	|-0.0163|± 0.00018	1.162
5	|-0.0196|± 0.00017	0.917
6	|-0.0226|± 0.00018	0.811

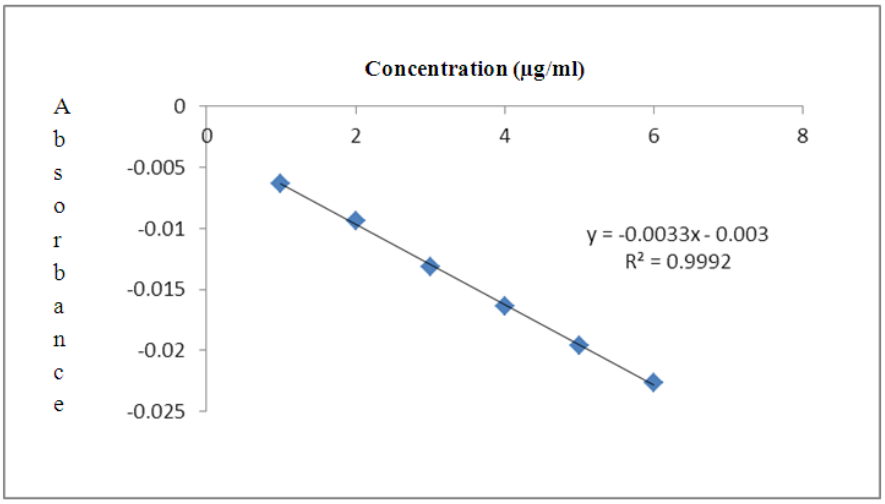

Fig. 5: Calibration Curve of Lercanidipine (1-6 µg/ml).

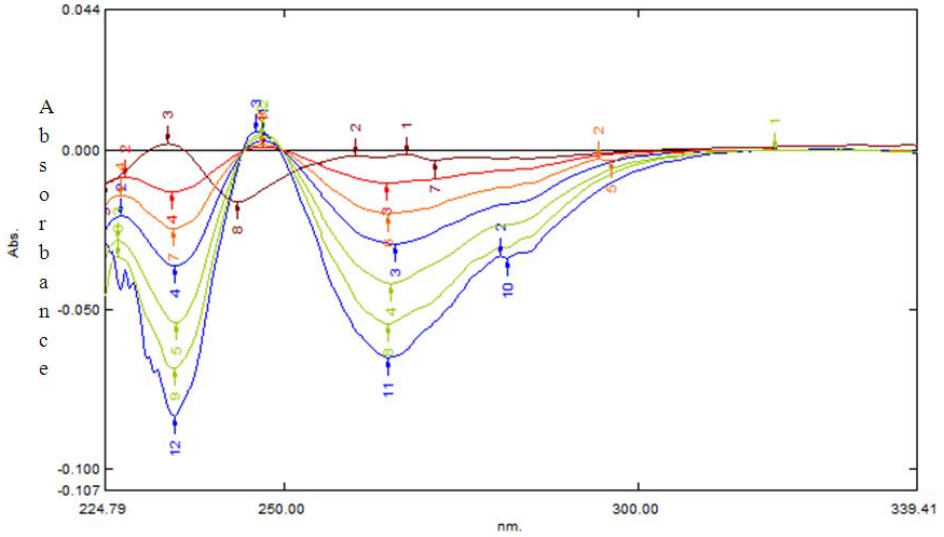

Fig. 6: Linearity of 1st Derivative spectra of Valsartan (235 nm)

Table 2: Linearity data of Valsartan.

Concentration (µg/ml)	Mean Absorbance ± SD (n=6)	% RSD
8	|-0.0124|± 0.00019	1.581
16	|-0.0244|± 0.00028	1.146
24	|-0.0384|± 0.00036	0.941
32	|-0.0523|± 0.00048	0.922
40	|-0.0669|± 0.00054	0.811
48	|-0.0813|± 0.00057	0.706

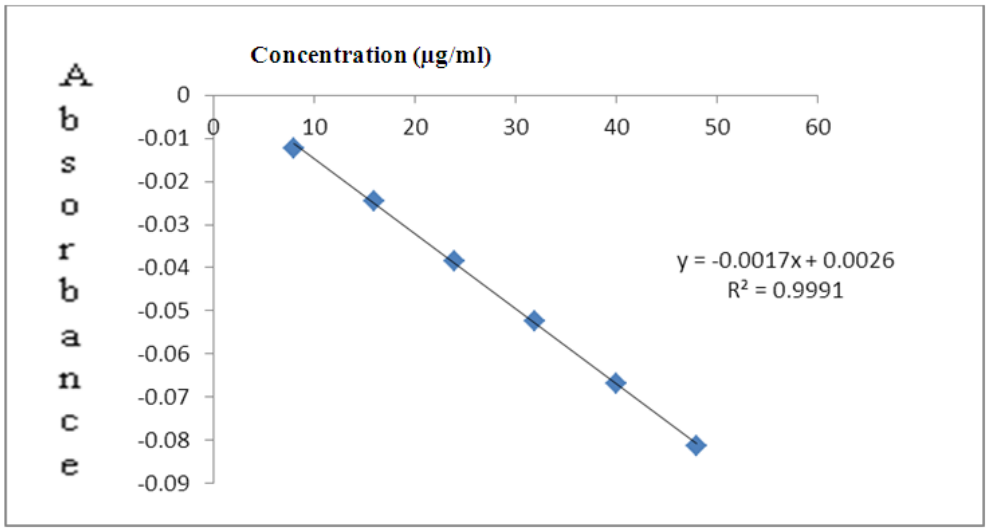

Fig. 7: Calibration Curve of Valsartan (8-48 µg/ml).

Precision

Table 3: Precision study of Lercanidipine.

Lercanidipine		
Intraday Precision of Lercanidipine		
Conc. (µg/ml)	**Mean Absorbance ± SD (n = 3)**	**% RSD**
1	|-0.0068|± 0.00011	1.689
2	|-0.0098|± 0.00010	1.020
3	|-0.0139|± 0.00015	1.093
Interday Precision of Lercanidipine		
1	|-0.0063|± 0.00011	1.813
2	|-0.0092|± 0.00015	1.654
3	|-0.0134|± 0.00020	1.492
Repeatability of Lercanidipine		
Conc. (µg/ml)	**Mean Absorbance ± SD (n = 6)**	**% RSD**
2	|-0.0098|± 0.00011	1.174

Table 4: Precision study of Valsartan

Valsartan		
Intraday Precision of Valsartan		
Conc. (µg/ml)	**Mean Absorbance ± SD (n = 3)**	**% RSD**
8	|-0.0123|± 0.00015	1.218
16	|-0.0242|± 0.00020	0.857
24	|-0.0375|± 0.00025	0.670
Interday Precision of Valsartan		
8	|-0.0126|± 0.00025	1.986
16	|-0.0245|± 0.00035	1.429
24	|-0.0375|± 0.00040	1.076
Repeatability of Valsartan		
Conc. (µg/ml)	**Mean Absorbance ± SD (n = 6)**	**% RSD**
16	|-0.0243|± 0.00015	0.627

LOD AND LOQ

Table 5: LOD and LOQ for Lercanidipine and Valsartan.

Parameter	Lercanidipine	Valsartan
LOD (µg/ml)	0.5680	0.2323
LOQ (µg/ml)	1.7213	0.7041

Accuracy

Table 6: Recovery study.

Name of Drug	% Level of recovery	Amount Taken (µg/ml)	Amount Added (µg/ml)	Total Amount (µg/ml)	Amount Recovered (µg/ml)	% recovery (n=3)
Lercanidipine	50	2	1	3	2.96	98.6
	100	2	2	4	3.97	99.2
	150	2	3	5	5.06	101.2
Valsartan	50	16	8	24	23.81	99.2
	100	16	16	32	31.92	99.7
	150	16	24	40	40.46	101.1%

Table 7: Analysis of synthetic mixture

Name of Drug	Amount in Synthetic Mixture Taken (µg/ml)	Amount Found (µg/ml)	% Assay
Lercanidipine	2	1.97	98.5%
Valsartan	16	15.72	98.2%

Table 8: Summary of validation parameter.

Parameter	Lercanidipine	Valsartan
Wavelength (nm)	244 nm	235 nm
Beer's Law Limit	1-6 µg/ml	8-48 µg/ml
Regression equation (y = mx +c)	y = -0.003x - 0.003	y = -0.001x + 0.002
Correlation Coefficient (r^2)	0.999	0.999
Intraday Precision (% RSD, n=3)	1.09-1.68	0.67-1.21
Interday Precision (% RSD, n=3)	1.49-1.81	1.07-1.98
Repeatability (% RSD, n=6)	1.174	0.627
Accuracy (% Recovery, n=3)	98.6-101.2%	99.2-101.1%
LOD (µg/ml)	0.5680	0.2323
LOQ (µg/ml)	1.7213	0.7041
Assay	98.5%	98.2%

RESULT AND DISCUSSION

A Simple, Precise and Accurate First Order Derivative Spectrophotometric Method have been developed for simultaneous estimation of Lercanidipine and Valsartan in Synthetic Mixture. Lercanidipine shows ZCP (Zero Crossing Point) at 235 nm and Valsartan show ZCP at 244 nm. At 244 nm (ZCP of Valsartan) Lercanidipine shows considerable absorbance while at 235 nm (ZCP of Lercanidipine) Valsartan shows considerable absorbance. Linearity

Range of 1-6 µg/ml for Lercanidipine and 8-48 µg/ml for Valsartan with Correlation Coefficient of 0.999 for both the drugs and the Precision data obtained with less than 2% RSD. Accuracy was carried out by Recovery Studies and was obtained in the range of 98.4-99.3% for Lercanidipine and 98.7-101.1% for Valsartan. LOD and LOQ values were found to be 0.5680µg/ml and 1.7213µg/ml respectively for Lercanidipine and Valsartan value were found to be 0.2323µg/ml and 0.7041µg/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 Lercanidipine and Valsartan in combination. It can therefore be concluded that the developed analytical method was precise & accurate and can be used for routine Analysis of both the drug in combination.

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