Research Article

Analytical method development and validation of Ramipril and Candesartan Cilexetil in synthetic mixture

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ABSTRACT

Aim: The aim of the study was to develop a rapid, accurate, and precise ratio spectra derivative spectroscopic high-performance liquid chromatography method and validated for the estimation of Ramipril (RAM) and Candesartan cilexetil (CAN) in synthetic mixture. **Materials and Methods:** The estimation of RAM, CAN (6 μg/ml) was used as a devisor and for the estimation of CAN, RAM (5 μg/ml) used as a devisor. The wavelengths selected for quantitative estimation were 331 nm for RAM and 231 nm and for CAN. **Results:** The result for validation shows that linearity of the developed method was 0.9976 and 0.9994 in the range of 5–10 μg/ml and 6–16 μg/ml for RAM and CAN, respectively. Phenomenex C18 column (250 mm×4.6 mm, 5 μm particle size) column was used. Acetonitrile:water (0.5% TEA, pH 4.5 adjusted with 10% orthophosphoric acid) (85:15 v/v) as a mobile phase, flow rate 1 ml/min and detection carried out at 220 nm. The retention time of RAM and CAN was 3.607 and 5.613 min, respectively. Linearity of the developed method was found to be 0.9956 and 0.9974 in the range of 0.5–0.9 μg/ml and 1.60–2.88 μg/ml for RAM and CAN, respectively. **Conclusion:** From the mentioned results, we can conclude that the developed methods were validated successfully as per the ICH guideline and are accurate, robust, and precise.

Keywords: Candesartan cilexetil, high-performance liquid chromatography, ramipril, simultaneous estimation, validation

Introduction

Ramipril (RAM) is used to treat high blood pressure. It is an angiotensin converting enzyme inhibitor. They act by lowering the production of angiotensin II, thereby relaxing arterial muscles while at the same time enlarging the arteries, allowing the heart to pump blood more easily, and increasing blood flow due to more blood being pumped into and through larger passage ways.[1-3] Candesartan cilexetil (CAN) is used to treat the hypertension. It is in a class of drugs called angiotensin receptor blockers. CAN is a potent, orally active and selective angiotensin II type-I receptor blocker, belongs to benzimidazole class.[4,5] CAN is hydrolyzed to Candesartan in the gastrointestinal tract during absorption. It inhibits the binding of angiotensin II to the AT1 – receptor. Candesartan blocks the angiotensin receptor and there by prevents the action of angiotensin. As a result, blood vessels expand and blood pressure is reduced [Figure 1].[6,7]

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Materials and Methods

The pure drug RAM was kindly provided by Bharat Parenterals and CAN by Alembic pharmaceuticals. Lactose, Starch Magnesium Stearate, and Talc were purchased from SD Fine Chemicals Limited. Micro Crystalline Cellulose was purchased from Qualikems Fine Chem Pvt. Ltd. All the chemicals are used of high-performance liquid chromatography (HPLC) and AR grade.

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HPLC Method Development

Selection of wavelength

Standard solution of RAM (5 µg/ml) and CAN (5 µg/ml) was scanned in the range of 200–400 nm. The wavelength selected for detection of both the drugs was 220 nm.

Preparation of mobile phase

In 100 ml of water, 0.5 ml of TEA was added than adjust the pH 4.5 with 10% ortho phosphoric acid (OPA). Mobile phase was prepared using 85 ml of ACN and 15 ml of water (0.5% TEA, pH 4.5 was adjusted with 10% OPA) as per [Figure 2].

Preparation of stock solution for RAM (10 µg/ml)

A stock solution of 10 µg/ml of RAM was prepared by accurately weighing the 10 mg of RAM and transferred in a 100 ml volumetric flask. 50 ml of acetonitrile was added in volumetric flask and the drug was dissolved properly and then volume was made up to 100 ml with acetonitrile. From above solution pipette, out 5 ml and volume was made up to 50 ml with acetonitrile.

Preparation of stock solution for CAN (10 µg/ml)

A stock solution of 10 µg/ml of CAN was prepared by accurately weighing the 10 mg of CAN and transferred in a 100 ml volumetric flask. 50 ml of acetonitrile was added in volumetric flask and the drug was dissolved properly and then volume was made up to 100 ml with acetonitrile. From above solution pipette, out 5 ml and volume was up to 50 ml with acetonitrile.

Preparation of working standard solutions

From the stock solution of RAM (10 µg/ml), 0.5, 0.6, 0.7, 0.8, and 0.9 ml aliquots were transferred in 10 ml volumetric flask. Add 1.6, 1.92, 2.24, 2.56, and 2.88 ml of aliquots of the stock solution of CAN (10µg/ml) and volume was made up to mark with the mobile phase to prepare 0.5, 0.6, 0.7, 0.8, and 0.9 µg/ml of the RAM and 1.6, 1.92, 2.24, 2.56, and 2.88 µg/ml of the CAN.

Analysis of drugs in synthetic mixture

All the ingredients were weighed accurately and mixed properly with the help of spatula. Whole mixture transferred to 100 ml volumetric flask, then 50 ml of acetonitrile was added and sonicated for 15 min, volume was made up to the mark with acetonitrile, from the above solution take 0.2 ml and then volume was made up to 10 ml with mobile phase. The solution was filtered through filter paper. The results for analysis of drugs in synthetic mixture of RAM and CAN are reported in Table 1. Peak shape of both the drugs is good. Theoretical plate is good. Resolution is proper.

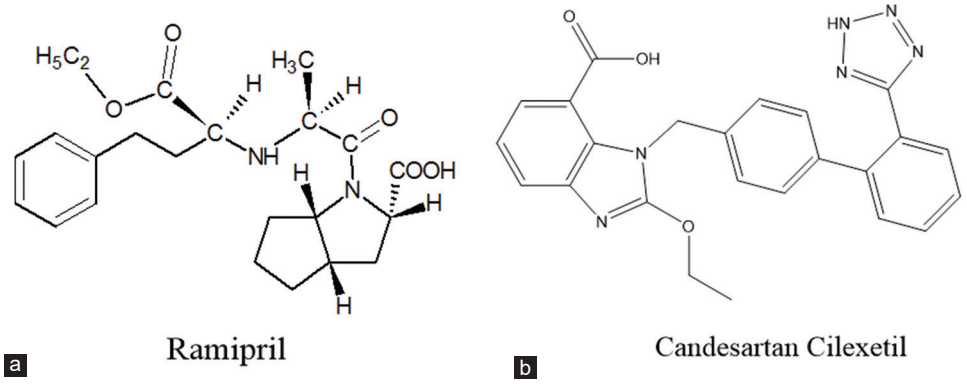

Figure 1: (a and b) Structure of ramipril and candesartan cilexetil

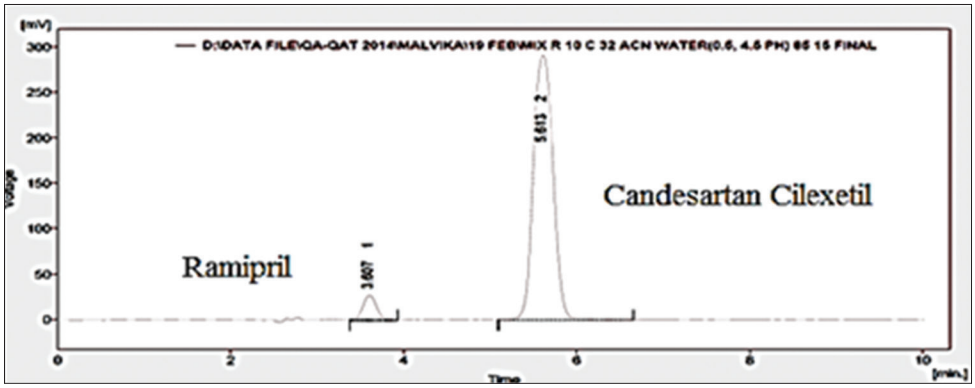

Figure 2: Chromatogram of ramipril and candesartan cilexetil (Acetonitrile:water [0.5% TEA] [pH 4.5 adjusted with 10% OPA] [85:15])

Table 1:The results for analysis of drugs in synthetic mixture

Drug	Retention time (min)	Tailing factor	Theoretical plate	Area (mV.s)	Resolution
RAM	3.607	1.091	2494	293939	-
CAN	5.613	1.136	2650	4600547	5.535

CAN: Candesartan cilexetil, RAM: Ramipril

Table 2: System suitability data for RAM

Number of runs	Retention time (min)	Tailing factor	Theoretical plates	Resolution
1	3.490	1.219	3613	-
2	3.483	1.219	3599	-
3	3.483	1.375	3599	-
4	3.480	1.355	3592	-
5	3.493	1.344	3620	-
6	3.480	1.365	3592	-
% RSD	0.155	-	-	-
Limit	<2	<2	>2000	>2

RAM: Ramipril

Table 3: System suitability data for CAN

Number of runs	Retention time (min)	Tailing factor	Theoretical plates	Resolution
1	5.047	1.022	6559	6.466
2	5.040	1.044	6542	6.466
3	5.033	1.022	6525	6.438
4	5.010	1.044	6464	6.355
5	5.040	1.068	6542	6.424
6	5.047	1.020	6548	6.378
% RSD	0.274	-	-	-
Limit	<2	<2	>2000	>2

CAN: Candesartan cilexetil

Validation of HPLC Method

System suitability

System suitability parameters such as theoretical plates, tailing factor, and retention time were studied by injecting six replicates of standard concentration and then % RSD for each parameter was calculated for RAM and CAN as per Tables 2 and 3, respectively.

Linearity and range

The calibration curves were plotted over a concentration range and the linear response was observed over a range of 0.5–0.9 μg/ml for RAM and 1.6–2.88 μg/ml for CAN. The calibration curves of peak area against concentration were plotted [Figure 3]. Correlation coefficient and regression line equations for RAM [Figure 4] and CAN [Figure 5] were calculated. Acceptance criteria for R^2value for linearity are 0.999.[7]

Ramipril [Table 4]

Table 4: Linearity of RAM

Concentration (μg/ml)	Peak area (mV.s)
0.5	16821
0.6	22414
0.7	28026
0.8	31704
0.9	37286

RAM: Ramipril

CAN [Table 5]

Table 5: Linearity of CAN

Concentration(μg/ml)	Peak area (mV.s)
1.60	156112
1.92	214629
2.24	260303
2.56	312414
2.88	355069

CAN: Candesartan cilexetil

Precision

Repeatability

Standard solution was injected 6 times, area of peaks was measured, and % RSD was calculated. % RSD should not be more than 2%. Results are reported in Table 6.

Intraday precision

Three replicates of three concentration of standard solution of RAM (0.6, 0.7, and 0.8 μg/ml) and CAN (1.92, 2.24, and 2.56 μg/ml) total nine determination were analyzed at 3 consecutive times on same day and chromatogram was recorded at 220 nm. % RSD was calculated. Results are reported for RAM and CAN in Tables 7 and 8, respectively.

Intermediate precision

Interday precision

Three replicates of three concentration of standard solution of RAM (0.6, 0.7, and 0.8 μg/ml) and CAN (1.92, 2.24, and 2.56 μg/ml)

Table 6: Repeatability data of RAM and CAN

S. No	Peak area (mV.s)	
	RAM	CAN
1	28026	260303
2	28301	260517
3	28287	260112
4	28118	260210
5	28067	260347
6	28229	260209
Mean peak area	28171.33	260283
SD	116.93	140.92
% RSD	0.4150	0.054
STD % RSD[8]	<2	<2

CAN: Candesartan cilexetil, RAM: Ramipril

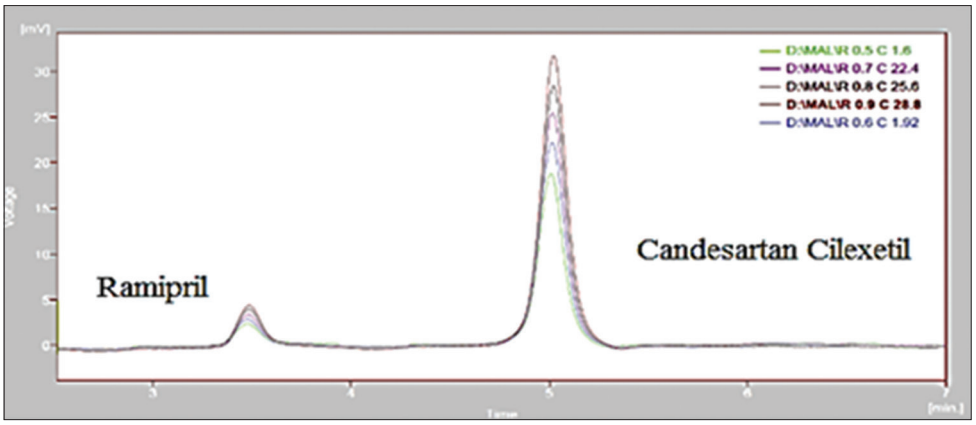

Figure 3: Overlay chromatogram of ramipril and candesartan cilexetil

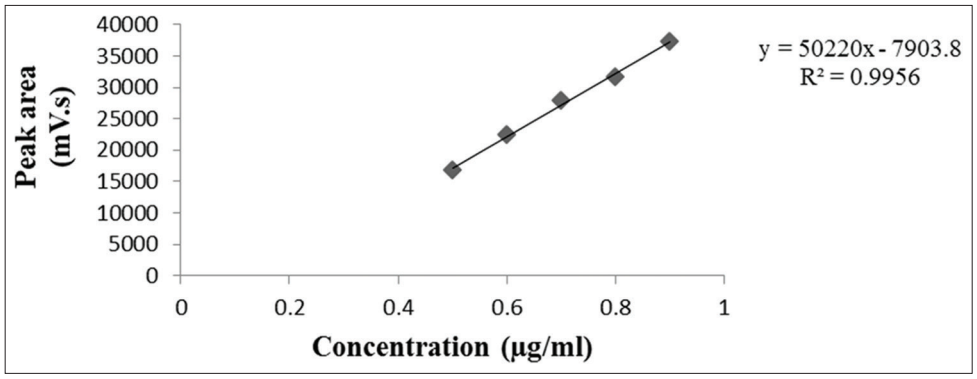

Figure 4: Calibration curve for ramipril

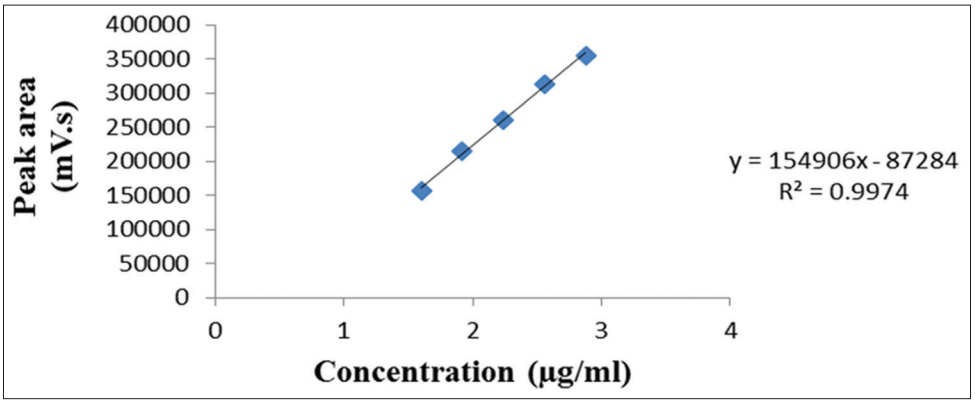

Figure 5: Calibration curve for candesartan cilexetil

Table 7: Intraday precision data of RAM

Concentration (µg/ml)	Peak area (mV.s)			Mean peak area (mV.s)	SD	% RSD
	10 pm	1 pm	4 pm			
0.6	22414	22280	22110	22268	152.35	0.68
0.7	28026	28107	28117	28083.33	49.90	**0.17**
0.8	31704	31213	31181	31366	293.15	**0.93**
					Limit[8]=	<2

RAM: Ramipril

Table 8: Intraday precision data of CAN

Concentration (µg/ml)	Peak area (mV.s)			Mean Peak area (mV.s)	S.D	% RSD
	10 pm	1 pm	4 pm			
1.92	214629	214485	214295	214469.7	167.52	0.07
2.24	260303	260299	260647	260416.3	199.77	0.07
2.56	312414	312327	312110	312283.7	156.56	0.05
					Limit[8]=	<2

CAN: Candesartan cilexetil

total nine determination were analyzed at 3 consecutive days and chromatogram was recorded at 220 nm. % RSD was calculated. Results are reported for RAM and CAN in Tables 9 and 10, respectively.

Different analyst

Three concentrations of standard solution of RAM (0.6, 0.7, and 0.8 µg/ml) and CAN (1.92, 2.24, and 2.56 µg/ml) were analyzed by three different analysts and chromatogram was recorded at 220 nm. % RSD was calculated. Results are reported for RAM and CAN in Tables 11 and 12, respectively.

Table 9: Interday precision data of RAM

Concentration (µg/ml)	Peak area (mV.s)			Mean peak area (mV.s)	SD	% RSD
	Day 1	Day 2	Day 3			
0.6	22414	22861	22337	22537.33	282.93	1.25
0.7	28026	28373	28112	28170.33	180.70	0.64
0.8	31704	31168	31295	31389	280.09	0.89
					Limit[8]=	<2

RAM: Ramipril

Table 10: Interday precision data of CAN

Concentration (µg/ml)	Peak area (mV.s)			Mean peak area (mV.s)	SD	% RSD
	Day 1	Day 2	Day 3			
1.92	214629	214717	214429	214591.7	147.58	0.06
2.24	260303	260726	260422	260483.7	218.13	0.08
2.56	312414	313548	312744	312902	583.27	0.18

CAN: Candesartan cilexetil

Table 11: Different analyst precision data of RAM

Concentration (µg/ml)	Peak area (mV.s)			Mean peak area (mV.s)	SD	% RSD
	Analyst 1	Analyst 2	Analyst 3			
0.6	22414	22565	22670	22549.67	128.68	0.57
0.7	28026	28210	28115	28117	92.01	0.32
0.8	31704	31220	31130	31351.33	308.71	0.98
					Limit[8]=	<2

RAM: Ramipril

Table 12: Different analyst precision data of CAN

Concentration (µg/ml)	Peak area (mV.s)			Mean peak area (mV.s)	SD	% RSD
	Analyst 1	Analyst 2	Analyst 3			
1.92	214629	214585	214220	214478	224.51	0.10
2.24	260303	261020	260455	260592.7	377.80	0.14
2.56	312414	312315	312768	312399	238.16	0.07
					Limit[8]=	<2

CAN: Candesartan cilexetil

Accuracy

Accuracy of the method was determined by calculating the % recovery of RAM and CAN by Standard addition method, in which known amounts of standard samples of RAM and CAN at 80%, 100%, and 120% level were added to the pre analyzed synthetic mixture (0.5µg/ml for RAM and 1.6µg/ml for CAN). The recovered amounts of RAM and CAN were calculated at each level. The results for accuracy of RAM and CAN are reported in Tables 13 and 14, respectively.

Limit of detection (LOD) and limit of quantification (LOQ)

Calibration curve repeated for 5 times and the standard deviation of intercepts was calculated. The acceptance criteria for quantization limit for mean recovery are 50–150% with % RSD <25%. The LOD was found to be 0.05 and 0.24 (µg/ml) of RAM and CAN, respectively.

Robustness

Different wavelength

Three concentrations of standard solution of RAM (0.6, 0.7, and 0.8 µg/ml) and CAN (1.92, 2.24, and 2.56 µg/ml) were analyzed at three different wavelength (219, 220, and 221 nm) and chromatogram was recorded at 220nm. % RSD was calculated. Results for different wavelength of RAM and CAN are reported in Tables 15 and 16, respectively.

Different flow rate

Three concentrations of standard solution of RAM (0.6, 0.7, and 0.8 µg/ml) and CAN (1.92, 2.24, and 2.56 µg/ml) were analyzed at three different flow rates (0.9, 1, and 1.1 ml/min) and chromatogram was recorded at 220 nm. % RSD was calculated. Results for different flow rate of RAM and CAN are reported in Tables 17 and 18, respectively.

Conclusion

HPLC method was developed by using Phenomenex Luna C_{18} column (250 mm×4.6 mm id, 5 µm particle size), Acetonitrile:water (0.5%

Table 13: Accuracy data for RAM

Level	S. No	Amount of sample taken(µg/ml)	Amount of standard spike(µg/ml)	Total amount (µg/ml)	Total amount recovered	% Recovery
	1	0.5	0.24	0.74	0.741	100.18
80	2	0.5	0.24	0.74	0.743	100.40
	3	0.5	0.24	0.74	0.738	99.72
	1	0.5	0.3	0.8	0.785	98.12
100	2	0.5	0.3	0.8	0.798	99.75
	3	0.5	0.3	0.8	0.792	99.00
	1	0.5	0.36	0.86	0.852	99.06
120	2	0.5	0.36	0.86	0.864	100.46
	3	0.5	0.36	0.86	0.876	101.86
					Limit[9]=	98–102%

RAM: Ramipril

Table 14: Accuracy data for CAN

Level	S. No	Amount of sample taken(µg/ml)	Amount of standard spike(µg/ml)	Total amount (µg/ml)	Total amount recovered	% Recovery
	1	1.6	0.76	2.36	2.333	98.85
80	2	1.6	0.76	2.36	2.340	99.15
	3	1.6	0.76	2.36	2.351	99.61
	1	1.6	0.96	2.56	2.583	100.89
100	2	1.6	0.96	2.56	2.571	100.42
	3	1.6	0.96	2.56	2.558	99.92
	1	1.6	1.15	2.75	2.700	98.18
120	2	1.6	1.15	2.75	2.732	99.34
	3	1.6	1.15	2.75	2.742	99.70
					Limit[9]=	98–102%

CAN: Candesartan cilexetil

Table 15: Robustness (different wavelength) data for RAM

Concentration (µg/ml)	Peak area (mV.s)			Mean Peak area (mV.s)	SD	% RSD
	219 nm	220 nm	221 nm			
0.6	22529	22414	22216	22386.33	158.32	0.70
0.7	28526	28026	28579	28377	305.12	1.07
0.8	31654	31704	31320	31559.33	208.77	0.66

RAM: Ramipril

Table 16: Robustness (different wavelength) data for CAN

Concentration (µg/ml)	Peak area (mV.s)			Mean Peak area (mV.s)	SD	% RSD
	219 nm	220 nm	221 nm			
1.92	214579	214629	214818	214675.3	126.05	0.05
2.24	260117	260303	260225	260215	93.40	0.03
2.56	312213	312414	312377	312334.7	106.97	0.03

CAN: Candesartan cilexetil

Table 17: Robustness (different flow rate) data for RAM

Concentration (µg/ml)	Peak area (mV.s)			Mean peak area (mV.s)	S.D	% RSD
	0.9 ml/min	1.0 ml/min	1.1 ml/min			
0.6	22369	22414	22215	22332.67	104.35	0.46
0.7	28110	28026	28333	28156.33	158.65	0.56
0.8	31617	31704	31531	31617.33	86.50	0.27

RAM: Ramipril

Table 18: Robustness (different flow rate) data for CAN

Concentration (µg/ml)	Peak area (mV.s)			Mean peak area (mV.s)	SD	% RSD
	0.9 ml/min	1.0 ml/min	1.1 ml/min			
1.92	214717	214629	214924	214756.7	151.44	0.07
2.24	260492	260303	260620	260471.7	159.47	0.06
2.56	312597	312414	312772	312594.3	179.01	0.05

CAN: Candesartan cilexetil

TEA, 4.5 pH adjusted with 10% OPA) (85:15 v/v) as a mobile phase, flow rate of 1 ml/min and detection carried out at a 220 nm. The retention time of RAM and CAN was 3.607 and 5.613 min, respectively. Linearity of the developed method was found to be 0.9956 and 0.9974 in the range of 0.5–0.9 µg/ml and 1.60–2.88 µg/ml for RAM and CAN, respectively. % RSD was found to be <2 for intraday precision and intermediate precision. % recovery was found to be 98.12–101.86% and 98.18–100.89% for RAM and CAN, respectively.

Discussion

Based on the literature review, there is no UV and HPLC method was reported for estimation of RAM and CAN in synthetic mixture. The result of system suitability and applicability of the method in the estimation of drugs confirms that the proposed method is suitable and applicable for routine laboratory analysis. The method validation results of the present study are within the specified acceptance criteria as per the ICH guidelines. The % RSD of precision and robustness is <2% which indicates the reproducibility and strength of the method to withstand the variation in method conditions. The % recovery is within the limit, correlation coefficient calculated from linearity is >0.99 and low LOD and LOQ prove that the intended method is accurate, linear, and extremely sensitive.[8,9]

Conclusion

A rapid, accurate, and precise ratio spectra derivative spectroscopic HPLC method has been developed and validated. The method provides a good resolution between the drugs with less retention time. The result of validation parameters has proved that the proposed method is precise, accurate, robust, and sensitive. From the validation results, it was concluded that the developed methods is suitable technique for simultaneous estimation of RAM and CAN synthetic mixture.

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