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DEVELOPMENT OF ANALYTICAL METHOD FOR SIMULTANEOUS ESTIMATION OF AMOXICILLIN AND CARBOCISTEINE IN SOLID DOSAGE FORM BY RP-HPLC

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Keywords:

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ABSTRACT: Pharmaceutical analysis is the application of principles of analytical chemistry to drug analysis. The analytical chemistry is the science of developing accurate, precise, and sensitive methods for determining the composition of materials in terms of elements or compounds which they contain. Quality means confirming specifications and standards, and it is never an accident; it is a result of intelligent efforts. Quality assurance influences the quality of the product and plays a central role in assuring the quality, safety, purity, and effectiveness of the drug products. Estimation of amoxicillin and carbocisteine in their combined dosage form were done. Official books also do not provide methods for simultaneous analysis. Most of the methods available for the analysis of active ingredients of such formulations are applicable only after prior separation, which makes it tedious and time-consuming and also a costly affair. In experimental work trials of various mobile phases, Analysis of laboratory mixture by proposed method carried out. The calibration curve was found to be linear in the concentration range of 5-25 µg/ml for Carbocisteine and 10-50 µg/ml for Amoxicillin, respectively. The application of the proposed method for the estimation of both drugs in marketed formulation took place. Validation parameters studied in that recovery studies for combinations were found to be within the acceptable limit.

INTRODUCTION: Pharmaceutical analysis plays a very significant role in the quality control of pharmaceuticals. It also plays an important role in building up quality products through in-process quality control [1, 2]. Chromatography involves a sample being dissolved in a mobile phase. The mobile phase is then forced through the stationary phase. These differential rates of migration as the mixture moves over adsorptive materials provide separation [3, 4].

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Analytical technique plays an important role in maintaining and assuring the quality of substance and are critical components of Quality Assurance and Quality Control. Several instrumental methods are used in pharmaceutical analysis, of which important methods are separation techniques, spectrometric techniques, and other analytical techniques.

Modern analytical chemistry is dominated by instrumental analysis. These are extremely sensitive, providing precise and detailed information from a small amount of material [5, 6].

Most Commonly used Methods in HPLC:

Reversed-Phase Chromatography: Retention by the interaction of the stationary phase's non-polar hydrocarbon chain with non-polar parts of sample.

Normal Phase Chromatography: Retention by the interaction of the stationary phase's polar surface with polar parts of the sample molecules.

Components of the HPLC System: [3, 4, 7] HPLC instruments consist of a reservoir of the mobile phase, a pump, an injector, a separation column, and a detector.

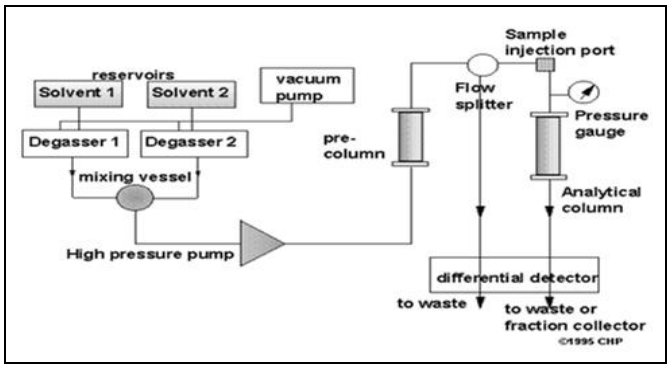


FIG. 1: SCHEMATIC DIAGRAM OF HPLC INSTRUMENT

Pump: Its performance directly affects retention time, reproducibility, and detector sensitivity. The pump should be capable of delivering the mobile phase at flow rates of 0.5-1.5 ml/min and at pressure reaching 5000 psi. There are two types of pumps that are used for HPLC *viz.* constant displacement pumps and the constant volume pumps.

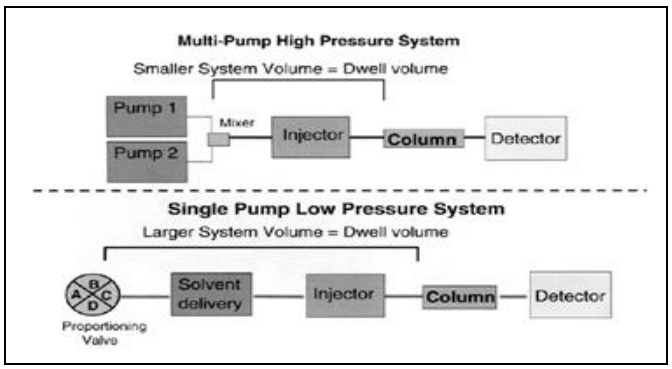


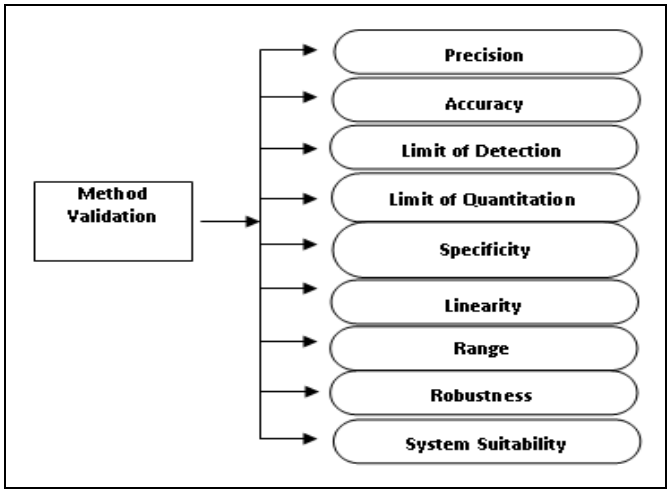
FIG. 2: SCHEMATIC DIAGRAMS OF A LOW-PRESSURE MIXING SYSTEM AND HIGH-PRESSURE MIXING PUMPS

Injector: The highest chromatographic efficiencies are obtained by injection directly on to the top of the column. This can be achieved through the use of septum injectors or by stop-flow injectors. The sample is introduced mainly by using valve and loop injectors. In all valve and loop injectors, the sample is introduced at atmospheric pressure. These type of injectors operates at very high pressure 6000-10000 psi and offers high precision since they depend only on operators skill. The results obtained are very reproducible.

Column: The analytical column is made up of stainless steel tubes with various forms with terminators or end fittings. For optimum efficiency, the inner walls of the column should have a crack-free, smooth, polished surface, but more importantly, the end fittings should contribute minimal dead volume. In most analytical columns, internal diameter ranges from 2-2.5 mm or 4.6-5 mm and is approximately 10-25 cm in length.

Detector: The most popular detectors used for HPLC includes UV and UV-photodiode array (PDA), fluorescence, refractive index, evaporative light scattering (ELSD), charged aerosol (CAD), and the mass spectrometer. The most commonly used detector is the UV detector since a majority of pharmaceutical compounds have some type of chromophores, which makes them amenable to UV-visible detection. There are three types of UV-Visible detectors: Fixed wavelength detector (usually 254 nm), Multiple wavelength detector, which, through use filters, can operate at several wavelengths, Variable wavelength detector which can be set at any desired wavelength ranging from 190 to 600 nm.

Validation: Validation is defined as documented evidence that gives a high degree of confidence that a process, system, facility will consistently produce a product quality attribute. In setting a new analytical method, the following parameters should be considered as per ICH guidelines. Specificity, linearity, range, accuracy, precision, detection limit, Quantization limit, system suitability [9-12].



MATERIALS AND METHODS: All the chemicals and solvents used were of AR and HPLC grades. Double distilled water and Axiva filter paper Grade-I, 0.45-µm filter paper were used throughout the experimental work.

Standard Drug: Pure Amoxicillin and Carbocisteine were generously gifted by Micro Lab. Bangalore and Elder Pharmaceutical Panvel.

Marketed Formulation: The marketed preparations of Amoxicillin and Carbocisteine combination used for the study were purchased from the local market and are given below:

Brand name	Drug in mg		Manufactured by
	Amoxi-cillin	Carbo-cisteine	
MUCOBORN-250	250	150	ELDER Pharmaceuticals

Instruments:

Spectrophotometer: Double beam UV-visible spectrophotometer

- Make: Shimadzu
- Model no.:1800
- Software: win lab
- Detector: UV/VIS Detector

HPLC System:-Agilent Technologies

- Model no.: 1120
- Software: Total Chrom
- Column: C18 (250 × 4.6mm i.d. 10µm)

pH Meter: Elico LI 612 pH analyzer.

Balance: Mettler toledo AG135

Sonicator: Pci Services

Thermostatic Water Bath: Sentwin

Simultaneous Determination of Amoxicillin and Carbocisteine in Solid Dosage Form by RP-HPLC Method:

Preparation of Standard Stock Solution: Standard stock solution of AMO and CAR was prepared by dissolving 100 mg of drug in 100 ml of selected mobile phase to get the concentration of 1000 µg/ml.

Selection of Analytical Wavelength: Using appropriate dilution of standard stock solution, to make AMO (10 µg/ml) and CAR (20 µg/ml), two solutions were prepared and scanned separately. The maximum detection level for Amoxicillin in the range of 210 nm to 240 nm in **Fig. 3** and Carbocisteine in the range of 200 nm to 220 nm in **Fig. 4**. Overlaid spectrum of Amoxicillin and Carbocisteine is given in **Fig. 5**.

Selection of Mobile Phase: The mobile phase was allowed to equilibrate with the stationary phase until a steady baseline was obtained. The standard solution containing AMO and CAR was run, and different individual solvent, as well as combinations of solvents, have been tried to get good separation and stable peak. Each mobile phase was filtered through a membrane filter (0.45 µm) and vacuum degassed.

Selection of Mobile Phase: The standard solution containing AMO and CAR was run and different individual solvent, as well as combinations of solvents, have been tried to get good separation and stable peak. Methanol and water combinations, Methanol and sodium acetate buffer combinations, Methanol and potassium phosphate buffer combinations, and Acetonitrile and water combination.

Preparation of Calibration Curve:

Preparation of Standard Stock Solution: Standard stock solution of AMO and CAR was prepared by dissolving 100 mg of drug in 100 ml of selected mobile phase Acetonitrile: Water at pH 3.0 (10:90 v/v) to get the concentration of 1 mg/ml.

Procedure: The mobile phase was allowed to equilibrate with the stationary phase until a steady baseline was obtained. The various concentrations from 5-25 µg/ml of a standard solution containing CAR in **Fig. 7** and 10-50 µg/ml of a standard solution containing AMO **Fig. 6** were injected separately, and the chromatograms were recorded. The graph was plotted as the concentration of the drug vs. peak area depicted in **Fig. 8**. The CAR and AOX showed good correlation coefficient in concentration range of 5-25 µg/ml (r = 0.998) and 10-50 µg/ml (r = 0.995) respectively.

Chromatographic Conditions: In that established parameter and mobile phase allowed to equilibrate with the stationary phase. The working standard solution was injected in the injector (20 µl), and

chromatograms were recorded for the drug. The mobile phase containing a mixture of Water and Acetonitrile in the ratio of 90:10 v/v, adjusted to pH 3 at a flow rate of 1.0 ml/min was found to yield satisfactory retention time of about 2.43 and 5.20 min with sharp peak for both the drug **Table 1**.

Application of Proposed Method for Estimation of CAR and AMO in Laboratory Mixture: [13, 14]

Preparation of Sample: Five different solutions of CAR and AMO were prepared by appropriately weighing the quantities of drug sample so as to get the mixture of two solutions in the concentration range of 1:2 µg/ml, mean CAR 5µg/ml and AMO 10µg/ml **Fig. 8**.

Application of Proposed Method for Estimation of CAR and AMO in Marketed Formulation:

Procedure: Equal volume (20 µL) of standard and sample solution were injected equilibrium of stationary phase separately. The chromatograms were recorded, and the response, *i.e.*, peak area (AUC) of major peaks were measured. The content of CAR and AMO was calculated by comparing a sample peak with that of standard **Fig. 10**. Results of the analysis of marketed formulation are shown in **Table 5, 6**.

Validation of Proposed Method: [15, 16]

Precision: The chromatographic conditions were set as per the optimized parameters, and the mobile phase was allowed to equilibrate with the stationary phase **Table 8**. After equilibration of the stationary phase, one injection of standard solution and each of five sample solutions was made separately and injected **Fig. 11**.

Accuracy: The chromatographic conditions were set as per the optimized parameters, and mobile phase was allowed to equilibrate with a stationary phase. One replicate injections of standard drug solution and one replicate injection of sample solution at each spiking level were injected separately, and chromatograms were recorded **Fig. 12**. The concentration of each drug was estimated by comparing the sample peak area with that of standard. The results are shown in **Table 9**.

RESULTS AND DISCUSSION:

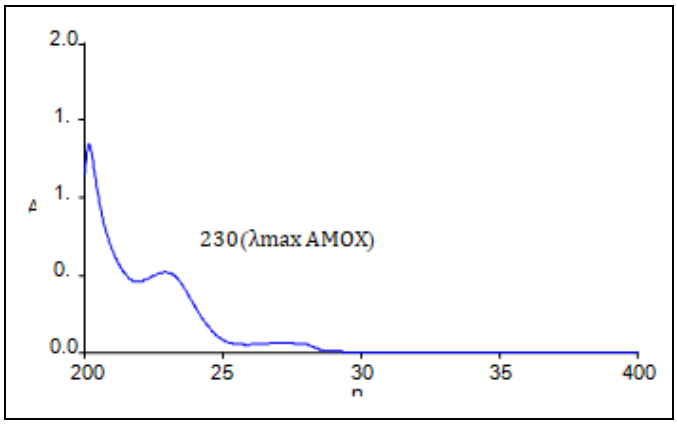


FIG. 3: SPECTRUM OF AMO λ_{max} = 230

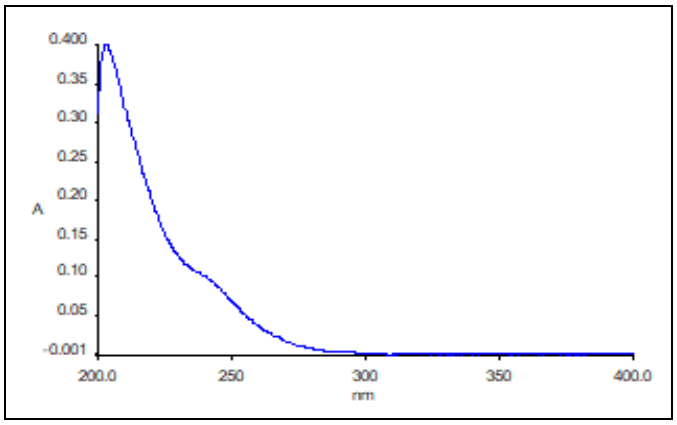


FIG. 4: SPECTRUM OF CAR λ_{max} = 210

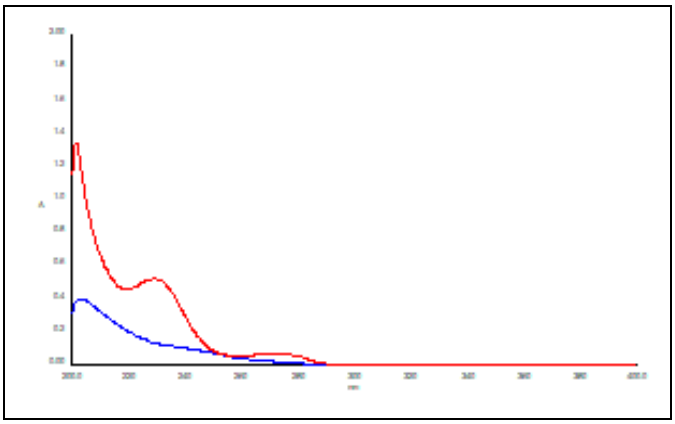


FIG. 5: SIMULTANEOUS DETERMINATION OF AMOXICILLIN AND CARBOCISTEINE IN SOLID DOSAGE FORM

Selection of Mobile Phase: From various mobile phases tried, mobile phase containing Acetonitrile: Water (10:90) at pH 3.0 was selected, since it gives sharp peak, well-resolved with symmetry and significant reproducible retention time for both AMO and CAR. Sample info: Amoxicillin 10 µg/ml + Carbocisteine 5 µg/ml STD

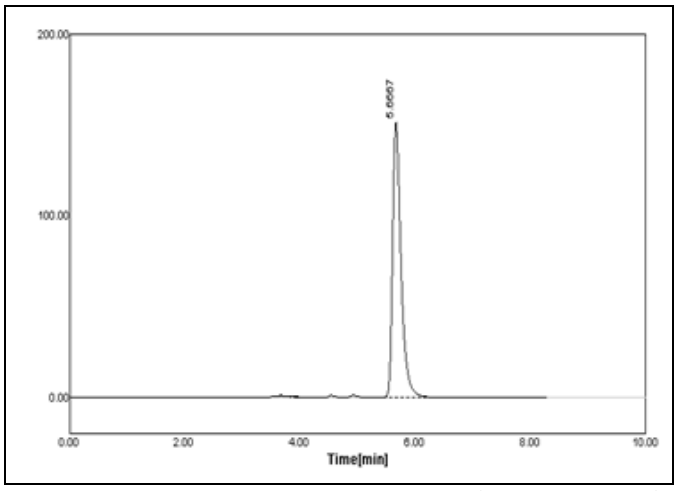


FIG. 6: CHROMATOGRAM OF AMO (RT = 5.66 MIN)

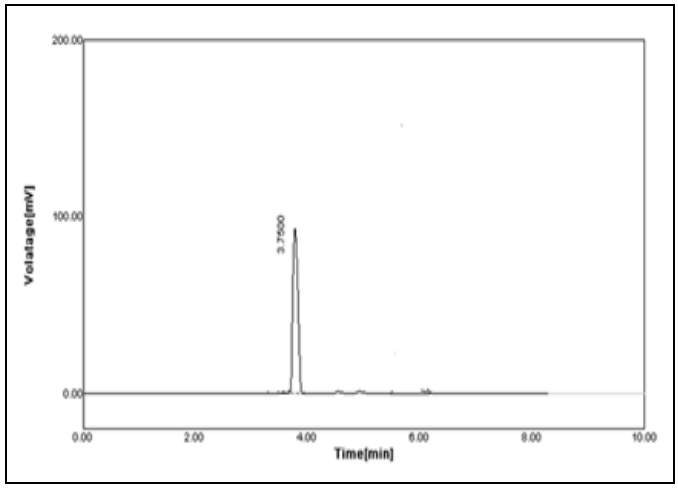


FIG. 7: CHROMATOGRAM OF CAR (RT = 3.75 MIN)

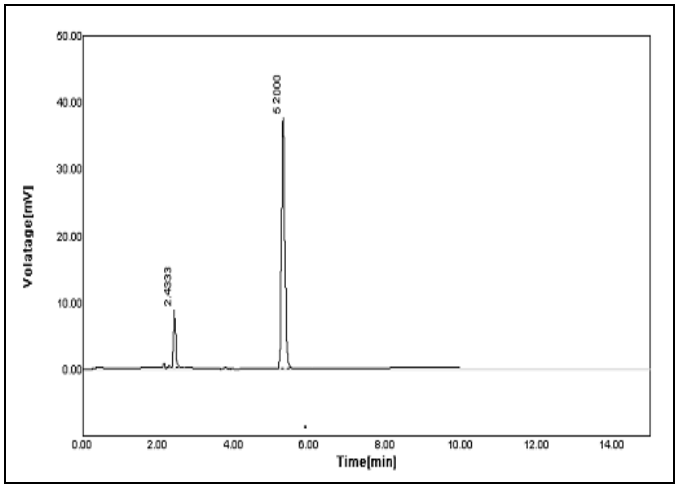


FIG. 8: MIX.OF AMO 10 µg/ml AND CAR 5 µg/ml

TABLE 1: CHROMATOGRAM OF LABORATORY MIXTURE CONTAINING AMO 10 µg/ml AND CAR 5µg/ml

S. no.	RT (min)	Area (mV*s)	Area%	TP	TF	Resolution
1	2.4333	37.0080	13.82	7387.6	1.1250	-
2	5.2000	230.6871	86.18	11443.9	1.0833	15.6364

Preparation of Calibration Curve:

Preparation of Standard Stock Solution: Standard stock solution of AMO and CAR was prepared by dissolving 100 mg of drug in 100 ml of selected mobile phase Acetonitrile: Water at pH 3.0 (10:90 v/v) to get the concentration of 1 mg/ml.

TABLE 2: OBSERVATION FOR STANDARD CALIBRATION CURVE

S. no.	Conc. µg/ml of Amoxicillin	Peak area of Amoxicillin	S. no.	Conc. µg/ml of Carbocisteine	Peak area of Carbocisteine
1	10	230.68	1	5	33.0080
2	20	463.73	2	10	76.6691
3	30	861.70	3	15	128.4408
4	40	1164.80	4	20	171.1429
5	50	1452.71	5	25	216.4441

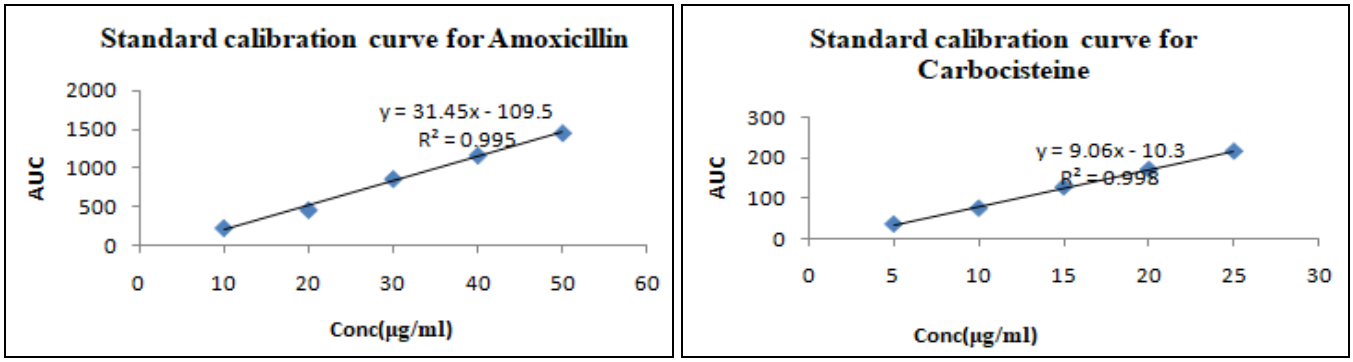


FIG. 9: LINEARITY GRAPH OF AMO AND CAR

The linearity of the calibration curve and adherence of the system to Beer's law was validated by the high value of the correlation coefficient, and S.D. for intercept value was less than 1.5.

Study of System Suitability Parameters:

TABLE 3: STUDY OF SYSTEM SUITABILITY PARAMETERS

S. no.	Peak area		Theoretical plate		Retention time		Tailing factor	
	CAR	AMO	CAR	AMO	CAR	AMO	CAR	AMO
1	37.0080	230.6871	7387.6	11443.9	2.433	5.200	1.1250	1.0833
2	37.0084	230.6859	7385.9	11445.2	2.434	5.201	1.1350	1.1019
3	37.0072	230.6881	7389.0	11448.0	2.450	5.209	1.1249	1.0912
4	37.0083	230.6877	7388.0	11446.2	2.429	5.206	1.1300	1.0732
5	37.0085	230.6875	7387.5	11447.5	2.435	5.201	1.1260	1.0855
				Statistical data				
Mean	37.0080	230.6872	7387.6	11446.16	2.436	5.203	1.1281	1.0870
±S.D.	0.000471	0.000752596	1.001998	1.497465	0.007194	0.003499	0.003885	0.009445
%R.S.D.	0.001273	0.0003261	0.01356	0.0130	0.29532	0.06724	0.34443	0.8689

TABLE 4: RESULTS OF ANALYSIS OF STANDARD LABORATORY MIXTURE

Standard mixture conc. (µg/ml)		AUC of standard solution		AUC of Mixture		% Drug estimation	
CAR	AMO	CAR (5µg/ml)	AMO (10µg/ml)	CAR	AMO	CAR	AMO
5	10			37.010	230.6579	100.005	99.98
5	10			37.109	230.6789	100.27	99.99
5	10	37.0080	230.6871	37.067	230.6865	100.15	99.99
10	20			76.669	463.7321	103.58	100.51
10	20			76.685	463.7401	103.60	100.51
					Mean	101.521	100.196
					±S.D.	1.691427	0.25640
					%R.S.D.	1.666	0.2558

Application of Proposed Method for Estimation of CAR and AMO in Marketed Formulation:

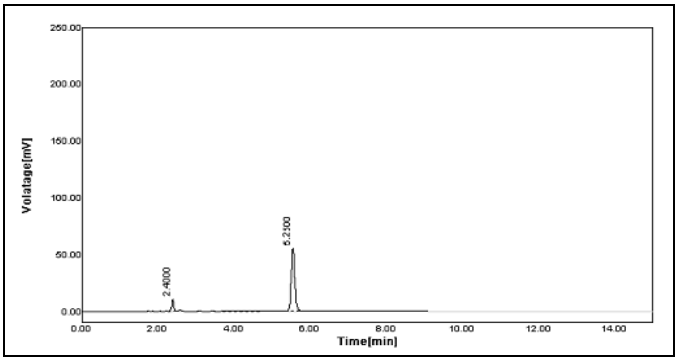


FIG. 10: CHROMATOGRAM OF TABLET MIXTURE CONTAINING AMO 10 µg/ml AND CAR 5µg/ml

TABLE 5: TABLET MIXTURE CONTAINING AMO 10 µg/ml AND CAR 5µg/ml

S. no.	RT (min)	Area (mV*s)	Area%	TP	TF	Resolution
1	2.4000	39.3326	9.83	4599.4	1.0000	-
2	5.2500	239.309	90.17	12549.0	1.0714	15.7500

TABLE 6: RESULTS OF ANALYSIS OF MARKETED FORMULATION

S. no.	Weight of std. (g)		Weight of tablet powder (g)	AUC of standard mixture		AUC of sample mixture		% Drug estimation	
	CAR	AMO		CAR (5µg/ml)	AMO (10µg/ml)	CAR	AMO	CAR	AMO
1	0.010	0.010	0.544	37.0080	230.6871	37.3326	235.3094	100.877	102.00
2						110.8031	701.8367	101.31	101.88
3						188.4529	1166.0663	101.84	101.08
4						187.4876	1159.5623	101.32	100.48
5						185.4568	1150.8745	100.22	99.70
							Mean	100.7894	101.028
							±S.D.	0.764966	0.864555
							%R.S.D.	0.7589	0.85575

Validation of Proposed Method: Linearity of Response

TABLE 7: LINEARITY PERFORMANCE PARAMETER

S. no.	Parameters	AMO values (at 220 nm)	CAR values (at 220 nm)
1	Linearity range (µg/ml)	10-50	5-25
2	Slope	31.45	9.06
3	Y-intercept	109.5	10.3
4	Correlation coefficient (R)	0.995	0.998

Precision:

TABLE 8: RESULT OF PRECISION

S. no.	Sample	Weight taken in (mg)		Peak area		%Assay	
1	Std.	CAR	AMO	CAR	AMO	**CAR**	**AMO**
		(5µg/ml)	(10µg/ml)	37.0080	230.6871	-	-
2	Solution	0.150	0.250	110.8031	701.8367	101.31	101.88
		0.152	0.251	109.8055	700.9899	98.61	101.88
		0.154	0.250	110.1237	701.8456	98.98	101.31
		0.150	0.256	111.9823	702.0001	100.566	101.33
		0.153	0.254	112.8967	701.8521	101.387	101.31
					Mean	100.1706	101.542
					±S.D.	1.165093	0.276072
					%R.S.D.	1.1631	0.271877

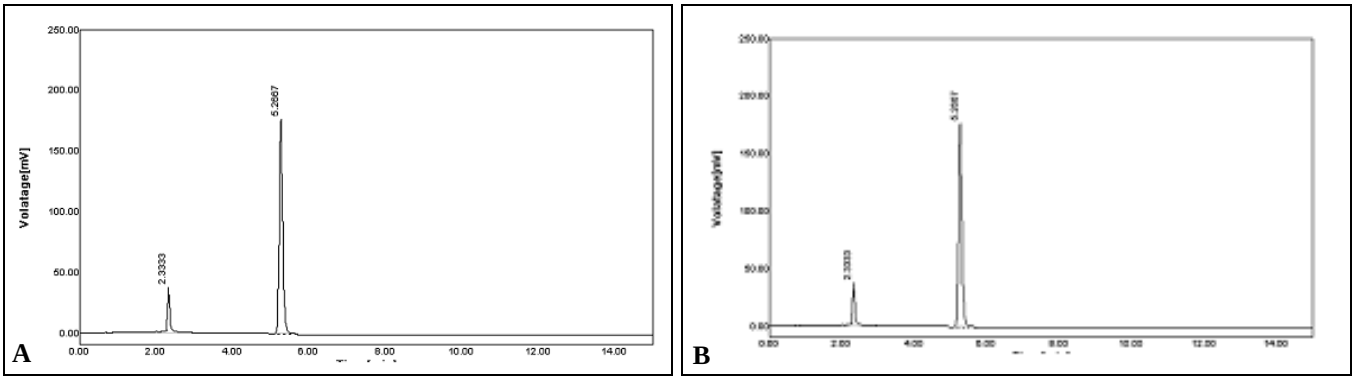


FIG. 11: CHROMATOGRAM OF METHOD PRECISION A) SAMPLE 1 B) SAMPLE 2

Accuracy:

TABLE 9: RESULTS OF RECOVERY STUDY

S. no.	% Spiking Level	Weight of tablet powder (g)	Peak area of std (AUC)		Amount of pure drug added (µg/ml)		Peak area of sample (AUC)		% Recovery	
			CAR	AMO	CAR	AMO	CAR	AMO	CAR	AMO
1	80	0.544	37.0080	230.6871	12	24	222.9165	1152.1611	100.33	101.01
2	100				15	30	248.1429	1694.8075	99.89	100.00
3	120				18	36	276.1490	1886.0836	100.00	100.00
								Mean	100.0733	100.3367
								±S.D.	0.186964	0.476119
								%R.S.D.	0.18682	0.47452

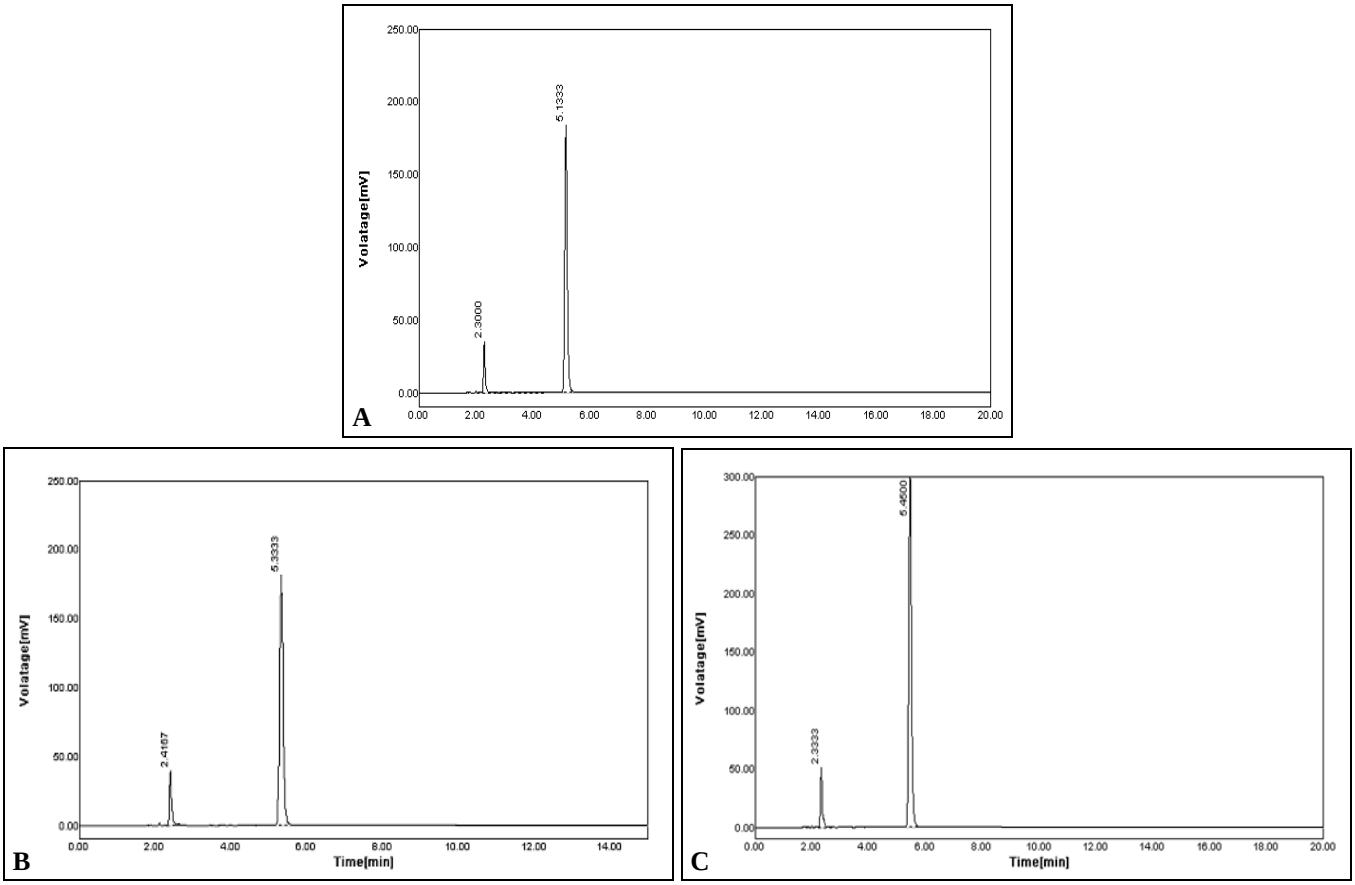


FIG. 12: CHROMATOGRAM OF RECOVERY STUDIES A) 80% B) 100% C) 120%

CONCLUSION: The RP-HPLC technique can be successfully used for the estimation of the Amoxicillin and Carbocisteine in their combined solid dosage formulations. The method shows good reproducibility; the RP-HPLC method is accurate, precise, specific, reproducible, and sensitive.

The analysis of the combined dose formulation of Amoxicillin and Carbocisteine can also be successfully performed by the RP-HPLC method. No interference of additives, matrix, etc. is encountered in these methods.

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CONFLICTS OF INTEREST: The authors declare that there is no conflict of interest regarding the publication of this paper.

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