Research Article **ISSN: 2349 – 7106**

Asian Journal of Research in Chemistry and Pharmaceutical Sciences

Journal home page: www.ajrcps.com

https://doi.org/10.36673/AJRCPS.2020.v08.i03.A36

A PRECISE METHOD DEVELOPMENT AND VALIDATION OF TWO DRUGS IN MIXED TABLET DOSAGE FORM BY RP-UPLC AND RP-HPLC

CH. Vishnu Priya*[1] and G. Subba Rao[1]

[1*]Department of Pharmacy, St. Xavier Institute of Pharmacy, Phirangipuram, Guntur, Andhra Pradesh, India.

ABSTRACT

For RP-UPLC method the mobile phase was consisted of methanol, acetonitrile and potassium phosphate buffer at pH 3.0 (40:20: 40 v/v) was selected as a mobile phase which gives good resolution and good peak shapes. The flow rate was set at 0.2ml/min, and the detection was carried out with PDA detector at 233nm. Thermo fisher C18 column (50mm x mm x 3µm), was used for the separation. At the optimum conditions mentioned above. The total run time required was below 5mins. The linearity and range was established over the range 96.072 to 144.048µg/ml of NAC and 8.073 to 12.01µg/ml for CPC. The correlation coefficient of N-Acetyl Cysteine and Clomiphene Citrate was found to be 0.9998 and 0.9999. This method was validated for accuracy, precision, and system suitability. The percentage of recovery of N-Acetyl Cysteine and Clomiphene Citrate was found to be 100.1%, 99.1% for 100% level. The standard deviation values and good recoveries indicate the reproducibility and accuracy of the developed method. As well the Percentage RSD values for precision study also were within acceptable limit.

KEYWORDS

Clomiphene citrate, N-Acetyl cysteine and RP-UPLC method.

Author for Correspondence:

CH. Vishnu Priya,

Department of Pharmacy,

St. Xavier Institute of Pharmacy,

Phirangipuram, Guntur, Andhra Pradesh, India.

Email: blessyvan21@gmail.com

INTRODUCTON

In this broader sense, it sometimes includes injuries, disabilities, disorders, syndromes, infections, isolated symptoms, deviant behaviors, and typical variations of structure and function, while in other contexts and for other purposes these may be considered distinguishable categories.

A drug is, in the broadest of terms, a chemical substance that has known biological effects on humans or other animals[1]. Foods are generally excluded from this definition, in spite of their physiological effects on animal species.

Pharmacy is involved in the science and technique of preparing as well as dispensing drugs and medicines.

Chromatography

Modern pharmaceutical formulations are complex mixtures containing one or more therapeutically active ingredients, and a number of inert materials like diluents, disintegrates, colors and flavors. For assurance of quality and stability of the final product, the pharmaceutical analyst must be able to separate the mixtures into individual components for quantitative analysis. Among the techniques available to the analyst for the separation of the mixture of compounds, a group of highly efficient methods which are collectively called as chromatography. It is the separation of mixture of compounds into individual components using stationary phase and mobile phase. The fixed phase is called stationary phase, and the other one is the mobile phase.

Ultra-Performance Liquid Chromatography (UPLC)

Now a day's pharmaceutical industries as well as analytical laboratories are in search of new ways to reduce cost and time for analysis of drugs and improve quality of their product. UPLC with better resolution, assay sensitivity and high sample throughput allows a greater number of analyses to be performed in a shorter period of time and it also imparts cost effective advantage over HPLC analysis. So that conventional assay were transferred and optimized for UPLC system.

EXPERIMENTAL WORK

Materials

NAC, and CPC working standards with 99.95 and 99.99% purity, respectively, were provided as a gift sample by Ideal analytical and research laboratories. Tablet dosage form (OVASHEILD; NAC 600mg, and 50mg of CPC per tablet) was purchased from local market.

Methanol and acetonitrile (HPLC grade) were obtained from S.D. Fine Chemicals Ltd. (Mumbai, India).

Buffer solution preparation

Potassium dihydrogen orthophosphate buffer was prepared by dissolving 1.36g of potassium phosphate in 2 liters of water, and pH was adjusted to 3 with orthophosphoric acid.

Mobile phase preparation

Mobile phase was prepared by mixing of buffer, methanol and acetonitrile in the ratio of 40:40:20v/v/v and filtered through 0.45µ of membrane filter and sonicated for 15 minutes.

Standard preparation

Accurately weighed 240mg of NAC and 20mg of CPC was taken individually in 100ml standard flask and dissolved in methanol. 5ml of above solution was diluted to further 100ml with methanol.

Test preparation

Twenty tablets were crushed to powder a quantity of powder equivalent to 600mg of NAC (50mg of CPC) was taken with an average weight of 1080mg and dissolved in 100ml of methanol and sonicated for 15 minutes filtered through whatmann filter paper, from the filtrate 5ml was diluted to 100ml with methanol.

Development and Optimization of the UPLC Method

Parameters, such as choice of analytical column, pH of buffer, mobile phase composition and proportion, detection wavelength and other factors were exhaustively studied.

Development trial-1

When chromatography was carried out at 25°C on a C18 thermo fisher (50mm x 4.6mm x 3µm) with the isocratic mobile phase of water and methanol (50:50v/v) at a flow rate of 0.5ml/min.

Inference

Resolution was not good but the peaks lost their symmetric. Hence, the second experiment was carried out with acetonitrile as an organic modifier.

Development trial-2

When chromatography was carried out at ambient temperature on a C18 thermo fisher (50mm x 4.6mm x 3µm) with the isocratic mobile phase of methanol, Acetonitrile and Ammonium Acetate at pH 4.8 (40:40: 20 v/v/v) at a flow rate of 0.2ml/min.

Inference

Late elution of analyte with peak tailing and high column pressure were observed. Hence, the third experiments were carried out with changes of mobile phase composition and adjust the pH.

Development trial-3

When chromatography was carried out at ambient temperature on a C18 thermo fisher (50mm x 4.6mm x 3µm) with the isocratic mobile phase of methanol, acetonitrile and Potassium dihydrogen ortho phosphate buffer at pH 3.0(40:20:40 v/v/v) at a flow rate of 0.2ml/min.

Inference

A satisfactory separation of the two drugs was achieved with good resolution and minimal tailing.

Preparation of solutions

Preparation of Buffer solution

Potassium dihydrogen ortho phosphate buffer was prepared by dissolving 1.36g of potassium phosphate in 2 liters of water, and pH was adjusted to 3 with ortho phosphoric acid.

Preparation of mobile phase

Mix buffer, methanol and acetonitrile in the ratio of 40:40:20v/v/v and filtered through 0.45µ of membrane filter and sonicated for 15 minutes.

Preparation of standard solution

Accurately weighed 240mg of NAC and 20mg of CPC was taken individually in 100ml standard flask and dissolved in methanol and diluted to 100ml with same diluent. 5ml of above solution was diluted to further 100ml with methanol.

Preparation of sample solution

Twenty tablets were crushed to powder a quantity of powder equivalent to 600mg of NAC (50mg of CPC) was taken with an average weight of 1080mg and dissolved in 100ml of methanol and sonicated for 15 minutes, filtered through whatmann filter paper, from the filtrate 5ml was diluted to 100ml with methanol.

METHODS DEVELOPMENT

System suitability

The System suitability is defined by ICH as "the checking of a system performance, before or during analysis of unknowns, to ensure system performance."

The System suitability criteria may include such factors as plate count, tailing, retention, and/or resolution, tailing factor. System suitability criteria should also include a determination of reproducibility (%RSD).

System suitability

Area % Report

Sample ID: N-Acetyl Cysteine and Clomiphene Citrate Data File: SYSTEM SUITABILITY-Rep1.dat.

Area % Report

Sample ID: N-Acetyl Cysteine and Clomiphene Citrate Data File: SYSTEM SUITABILITY-Rep2.dat.

Area % Report

Sample ID: N-Acetyl Cysteine and Clomiphene Citrate Data File: SYSTEM SUITABILITY-Rep3.dat.

Area % Report

Sample ID: N-Acetyl Cysteine and Clomiphene Citrate Data File: SYSTEM SUITABILITY-Rep4.dat.

Area % Report

Sample ID: N-Acetyl Cysteine and Clomiphene Citrate Data File: SYSTEM SUITABILITY-Rep5.dat.

Report

From the results the method was said to be system suitable.

Assay

Area % Report

Sample ID: Blank Data File: Blank

Area % Report

Sample ID: N-Acetyl Cysteine and Clomiphene Citrate Data File: Standard-Rep1.dat.

Area % Report

Sample ID: N-Acetyl Cysteine + Clomiphene citrate Data File: ASSAY -Rep1.dat.

Acceptance criteria

Assay value should be in the range of 98% to 102%

Result

Test result is showing that the test method is precise.

RESULTS AND DISCUSSION

An exertion has been made for a simple, accurate and precise method for the estimation of N-Acetyl Cysteine and Clomiphene Citrate in pharmaceutical dosage form by an isocratic RP-UPLC method.

Method development

System suitability

System suitability criteria may includes factors as plate count, tailing, retention, and/or resolution, tailing factor. System suitability criteria should also include a determination of reproducibility (%RSD).

Tailing factor

Tailing factor of N-Acetyl Cysteine and Clomiphene Citrate was found to be 0.902 and 1.046 respectively with acceptance criteria of not more than 2.0. Where the sample shows tailing factor within the limit as per USP.

Theoretical plates

Theoretical plates of N-Acetyl Cysteine and Clomiphene Citrate was found to be 2312 and 2776 respectively with acceptance criteria of not less than 1500. Where the sample shows tailing factor with in the limit as per USP.

Resolution

Resolution of the sample was found to be 4.99051. Where it was within the acceptance criteria of not less than 2.0 as per USP.

Peak area

Peak area for both the samples was found to be as 217370 for N-Acetyl Cysteine and 199723 for Clomiphene Citrate.

Retention Time

Retention time of both drugs was at the time of 1.722 for N-Acetyl Cysteine and 2.722 for Clomiphene Citrate.

At 233nm N-Acetyl Cysteine and Clomiphene Citrate were eluted at 1.722 min, 2.722 min, respectively using Potassium phosphate buffer, methanol and acetonitrile in the ratio of 40:40:20v/v/vas mobile phase and a flow rate of 0.2mL/min. and values are within the acceptable criteria and found to be system suitable.

Assay

Assay was performed for the sample of ovasheild and observed the values as follows,

Percentage purity of N-Acetyl Cysteine was found to be 99.03% Percentage purity of Clomiphene citrate was found to be 100.14%

Acceptance criteria for percentage purity or Assay value should be in the range of 98% to 102%. So the assay values are within the limit and developed method may be acceptable.

Table No.1: Trail 1 Parameters

Technique	UPLC
Column	C18 thermo fisher (50mmx 4.6 mm x 3µm),
Mobile phase	water and methanol (50:50 v/v)
Column temperature	Ambient
Wavelength	271nm
Flow rate	0.5µl/min
Run time	5min
Detector	PDA
Pressure	18000 psi

Table No.2: Trail 2 Parameters

Technique	UPLC
Column	C18 thermo fisher (50mmx 4.6 mm x 3µm),
Mobile phase	Methanol, Acetonitrile and Ammonium Acetate at pH 4.8 at pH 4.8 (40:40: 20 v/v/v)
Column temperature	Ambient
Wavelength	233nm
Flow rate	0.2µl/min
Run time	4 min
Detector	PDA
Pressure	18000 psi

Table No.3: Chromatographic system conditions

Technique	UPLC
Column	C18 thermo fisher (50mmx 4.6 mm x 3µm),
Mobile phase	methanol, Acetonitrile and potassium di hydrogen phosphate (pH at 3.0) (40:40: 20 v/v/v)
Column temperature	Ambient
Wavelength	233nm
Flow rate	0.2µl/min
Run time	5min
Detector	PDA
Pressure	18000 psi
Mode	Isocratic programmed

Table No.4: System suitability for N-Acetyl cysteine

S.No	Injection No	Drug	Rt time	Area	% Area
1	1	NAC	1.720	217370	52.12
	2		1.722	215788	52.21
	3		1.723	219212	52.20
	4		1.720	216922	52.03
	5		1.722	218230	51.99
2	Mean			217504.4	
3	STD DEV			1298.622	
4	% RSD			0.597055	
5	Theoretical plates			2312	
6	Tailing factor			0.902	
7	Resolution			0.00000	

Table No.5: System suitability for Clomiphene citrate

S.No	Injection No	Drug	Rt time	Area	% Area
1	1	CPC	2.722	199723	47.88
	2		2.723	197485	47.79
	3		2.723	200755	47.80
	4		2.722	199990	47.97
	5		2.722	201540	48.01
2	Mean			199898.6	
3	STD DEV			1524.161	
4	% RSD			0.762467	
5	Theoretical plates			2776	
6	Tailing factor			1.046	
7	Resolution			4.99051	

Table No.6: Assay of n-acetyl cysteine and clomiphene citrate

S.No	Sample ID	Standard Area	Sample Area	Label claim (mg)	Mg / tab	Percentage (%)
1	N-Acetyl Cysteine	217504.4	216819	600	594.161	99.03
2	Clomiphene citrate	199898.6	199478	50	50.068	100.14

Table No.7: System suitability parameters

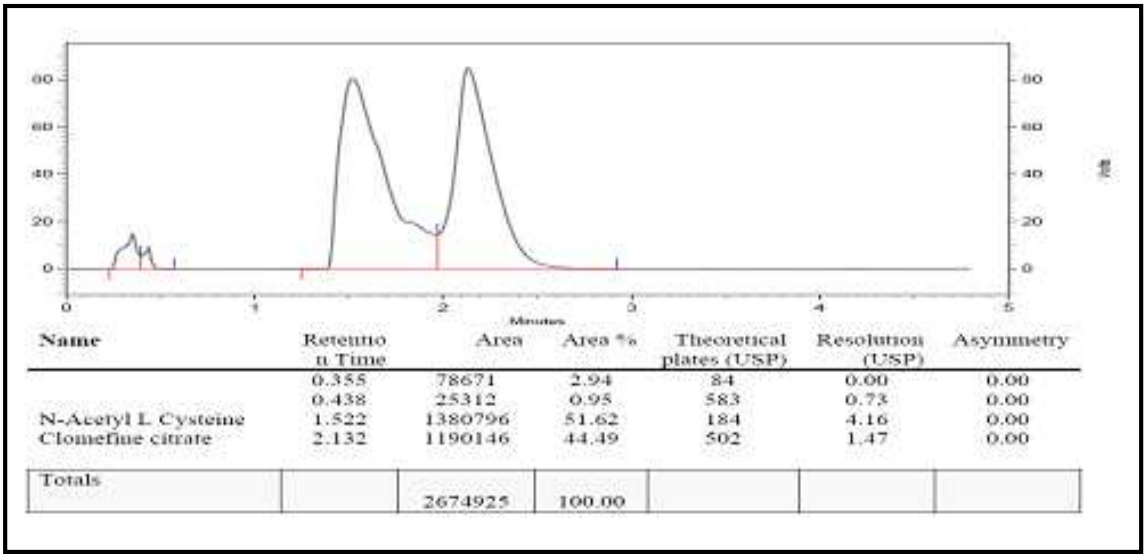
S.No	Parameters	Observed value		Acceptance criteria
		N-Acetyl Cysteine	Clomiphene Citrate	
1	**System suitability**			
2	Tailing factor	0.902	1.046	As per USP NMT 2.0
3	Theoretical plates (N)	2312	2776	NLT 1500
4	Resolution	0.0000	4.99051	
5	Peak area	217370	199723	
6	Retention Time	1.722	2.722	NLT 2.0

Table No.8: Retention time for N-Acetyl Cysteine and Clomiphene Citrate

S.No	Name of the peak	Retention time (min)
1	N-Acetyl Cysteine	1.722
2	Clomiphene Citrate	2.722

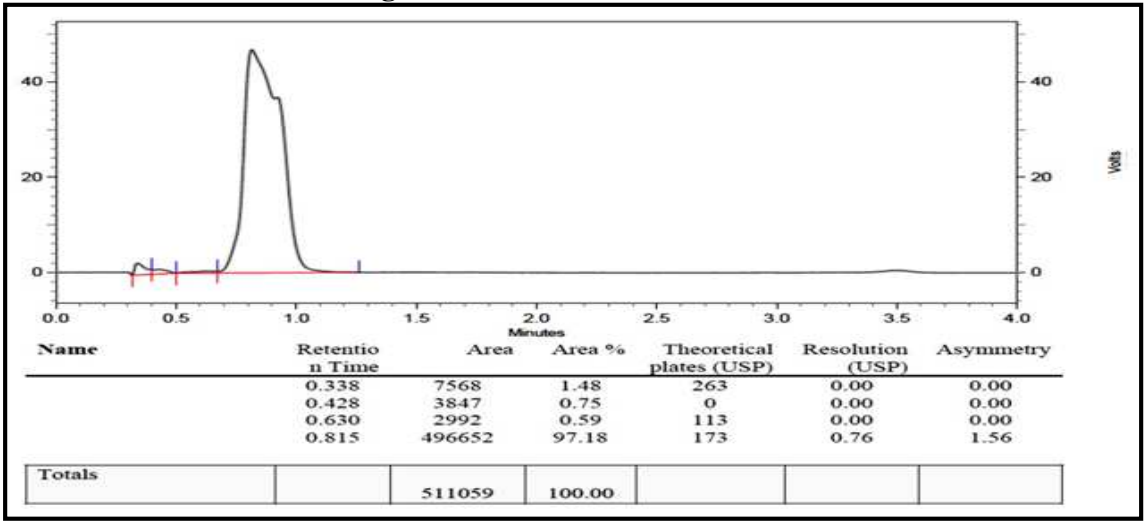
Table No.9: Percentage purity for assay

S.No	Sample ID	Standard Area	Sample Area	Label claim(mg)	mg / tab	Percentage (%)
1	N-Acetyl Cysteine	217504.4	216819	600	594.161	99.03
2	Clomiphene citrate	199898.6	199478	50	50.068	100.14



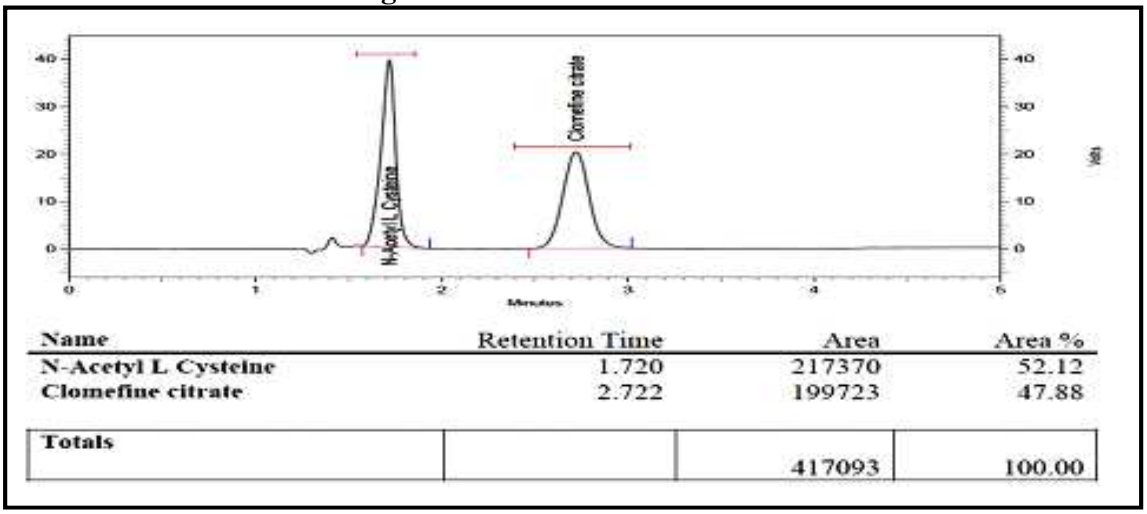
Name	Retention Time	Area	Area %	Theoretical plates (USP)	Resolution (USP)	Asymmetry
	0.355	78671	2.94	84	0.00	0.00
	0.438	25312	0.95	583	0.73	0.00
N-Acetyl L Cysteine	1.522	1380796	51.62	184	4.16	0.00
Clomefine citrate	2.132	1190146	44.49	502	1.47	0.00
Totals		2674925	100.00			

Figure No.1: Trail 1 Parameters



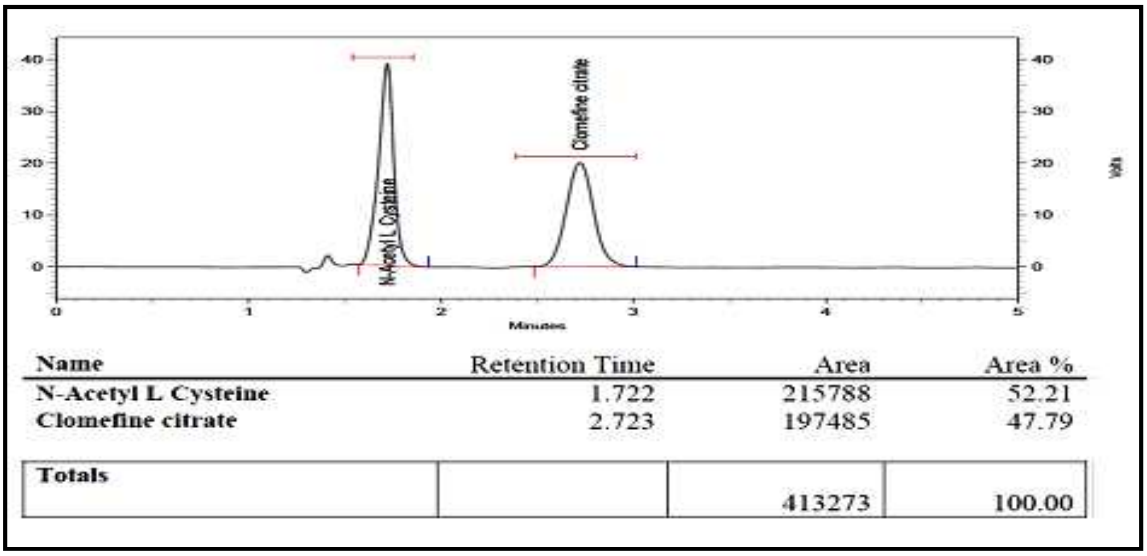
Name	Retention Time	Area	Area %	Theoretical plates (USP)	Resolution (USP)	Asymmetry
	0.338	7568	1.48	263	0.00	0.00
	0.428	3847	0.75	0	0.00	0.00
	0.630	2992	0.59	113	0.00	0.00
	0.815	496652	97.18	173	0.76	1.56
Totals		511059	100.00			

Figure No.2: Trail 2 Parameters



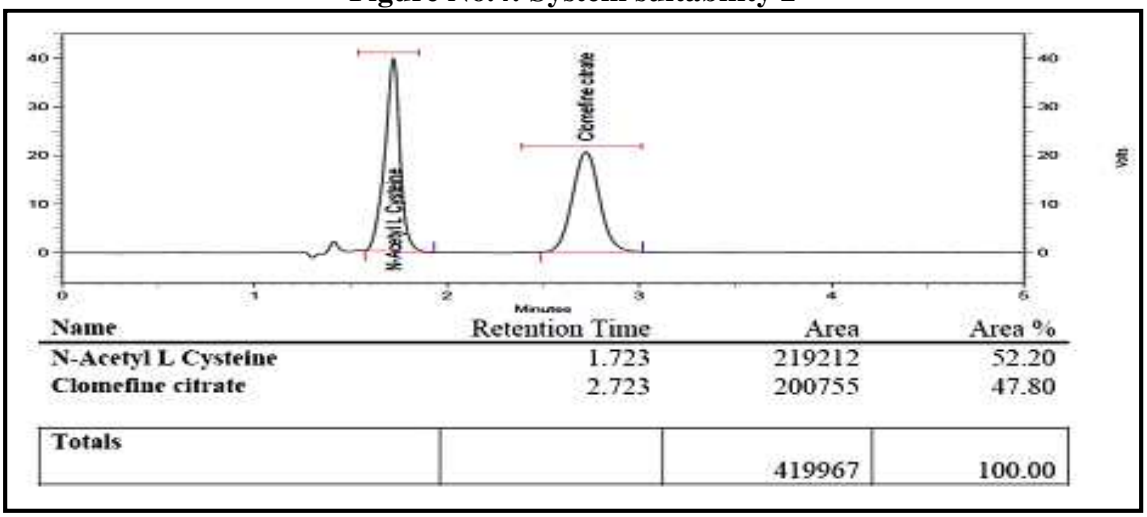
Name	Retention Time	Area	Area %
N-Acetyl L Cysteine	1.720	217370	52.12
Clomefine citrate	2.722	199723	47.88
Totals		417093	100.00

Figure No.3: System suitability 1



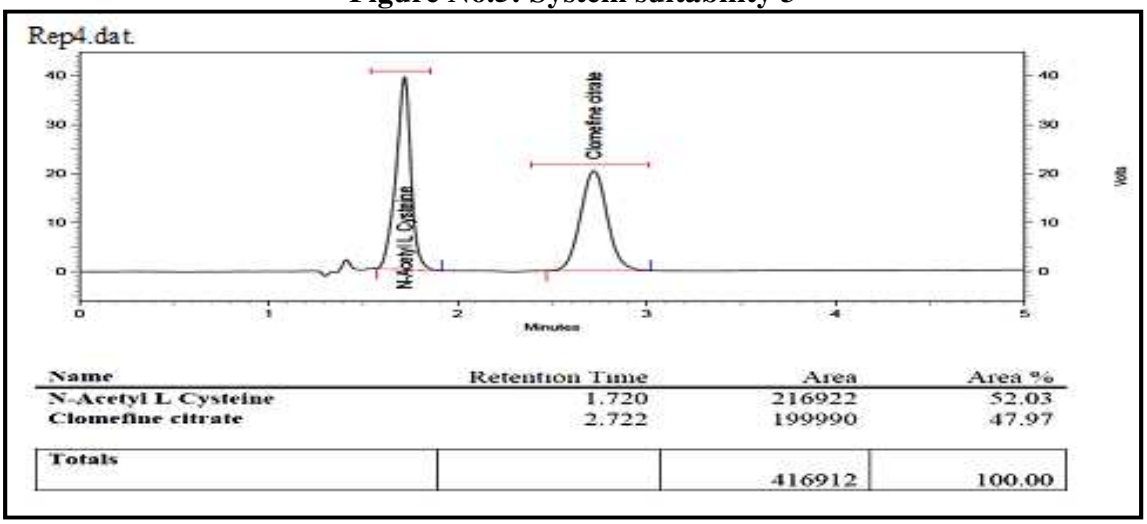
Name	Retention Time	Area	Area %
N-Acetyl L Cysteine	1.722	215788	52.21
Clomefine citrate	2.723	197485	47.79
Totals		413273	100.00

Figure No.4: System suitability 2



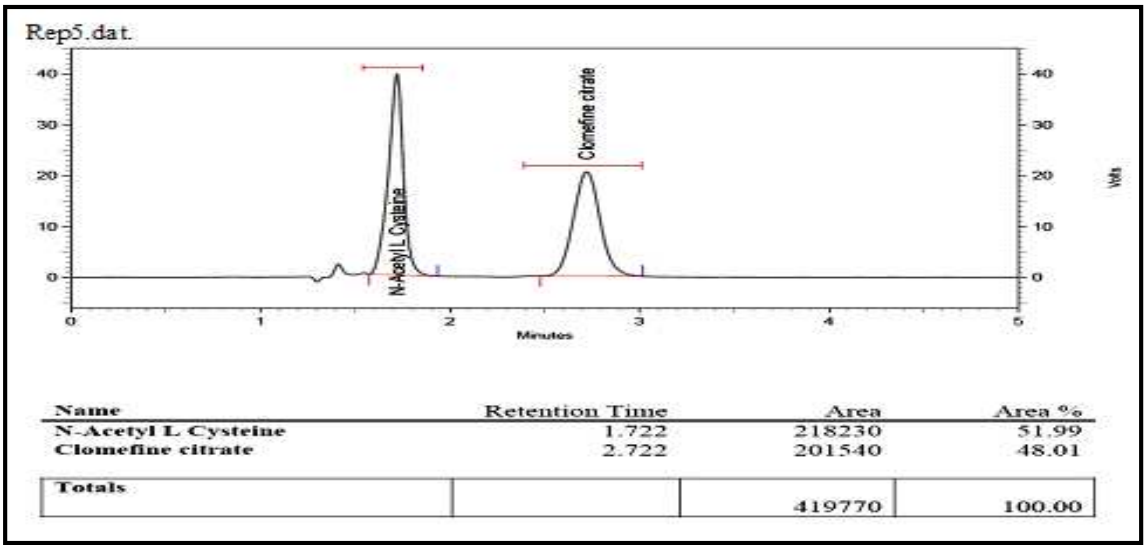
Name	Retention Time	Area	Area %
N-Acetyl L Cysteine	1.723	219212	52.20
Clomefine citrate	2.723	200755	47.80
Totals		419967	100.00

Figure No.5: System suitability 3



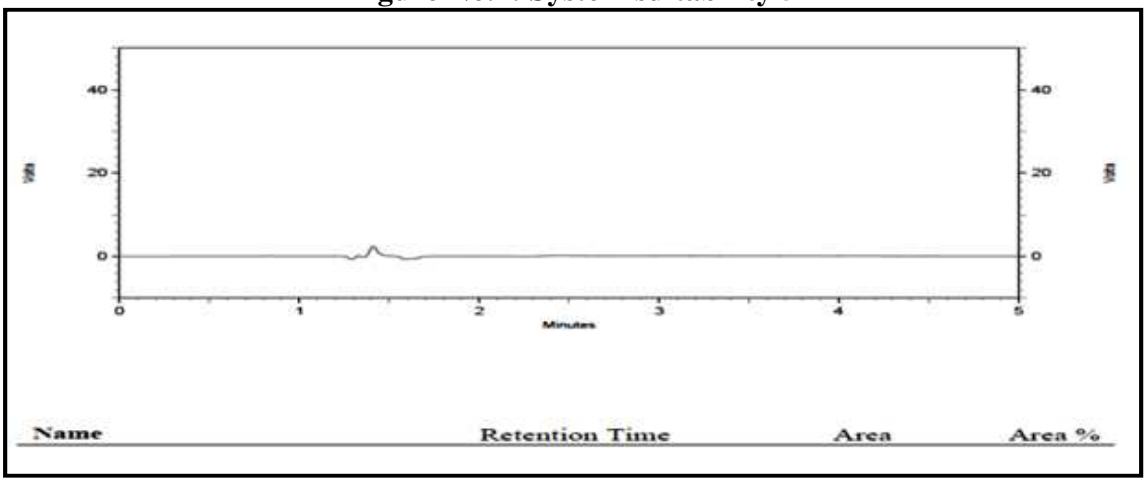
Name	Retention Time	Area	Area %
N-Acetyl L Cysteine	1.720	216922	52.03
Clomefine citrate	2.722	199990	47.97
Totals		416912	100.00

Figure No.6: System suitability 4



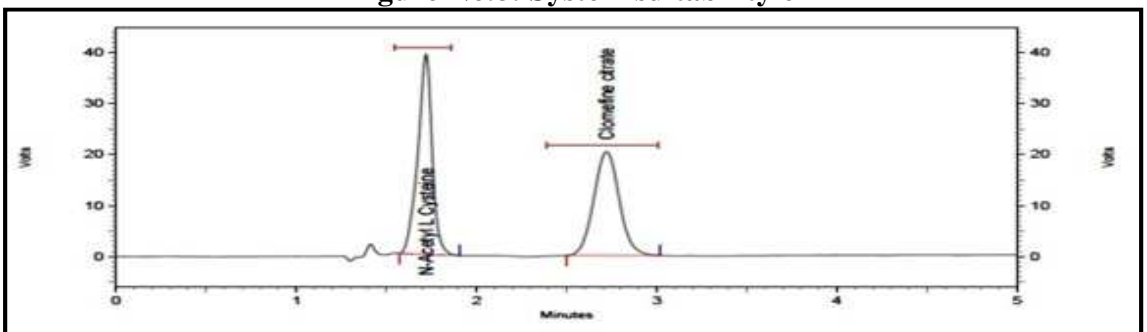
Name	Retention Time	Area	Area %
N-Acetyl L Cysteine	1.722	218230	51.99
Clomefine citrate	2.722	201540	48.01
Totals		419770	100.00

Figure No.7: System suitability 5



Name	Retention Time	Area	Area %

Figure No.8: System suitability 6



Name	Retention Time	Area	Area %
N-Acetyl L Cysteine	1.720	216819	52.08
Clomefine citrate	2.722	199478	47.92
Totals		416297	100.00

Figure No.9: System suitability 7

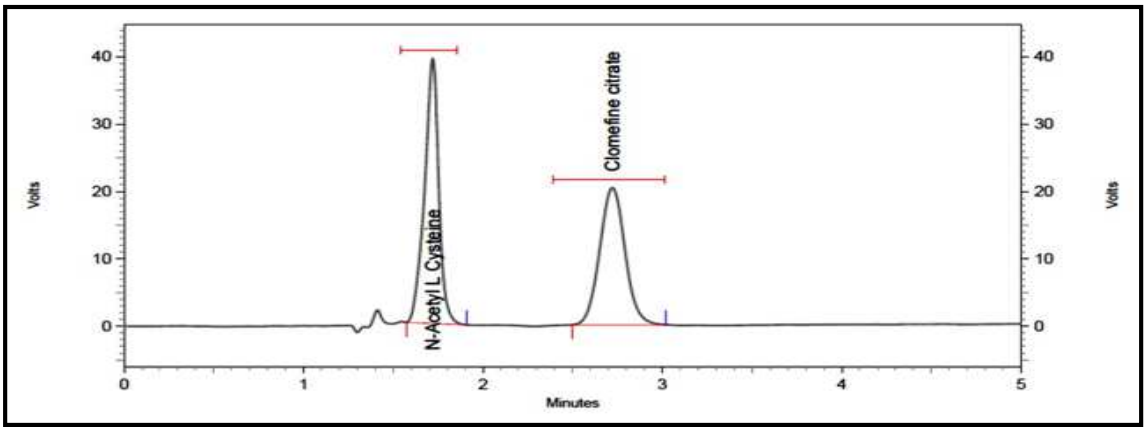


Figure No.10: System suitability 8

SUMMARY AND CONCLUSION

The scope and objective of the present work is to optimize the chromatographic condition to develop RP-UPLC method for the estimation of drugs in selected multicomponent dosage forms and the same is validated. Literature survey revealed several analytical methods such as spectrometry, stability indicating HPLC, LC-MS-MS have been reported for the determination of NAC and CPC in pharmaceutical dosage forms and biological samples. To our present knowledge, there is no UPLC method reported for the estimation of NAC, CPC in combination formulation. The scope and objective of the present work is to optimize condition to develop simultaneous estimation of N-Acetyl Cysteine and Clomiphene Citrate by RP-UPLC method. For RP-UPLC method the mobile phase was consisted of methanol, acetonitrile and potassium phosphate buffer at pH 3.0 (40:20: 40v/v) was selected as a mobile phase which gives good resolution and good peak shapes. The flow rate was set at 0.2 ml/min, and the detection was carried out with PDA detector at 233nm. Thermo fisher C18 column (50mm x mm x 3µm), was used for the separation. At the optimum conditions mentioned above. The total run time required was below 5mins. The linearity and range was established over the range 96.072 to 144.048µg/ml of NAC and 8.073 to 12.01µg/ml for CPC. The correlation coefficient of N-Acetyl Cysteine and Clomiphene Citrate was found to be 0.9998 and 0.9999. The percentage of recovery of N-Acetyl Cysteine and Clomiphene Citrate was found to be 100.1%, 99.1% for 100% level. The Percentage RSD values for precision study also were within acceptable limit.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to Department of Pharmacy, St. Xavier Institute of Pharmacy, Phirangipuram, Guntur, Andhra Pradesh, India for providing necessary facilities to carry out this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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Please cite this article in press as: CH. Vishnu Priya and Subba Rao G. A precise method development and validation of two drugs in mixed tablet dosage form by RP-UPLC and RP-HPLC, *Asian Journal of Research in Chemistry and Pharmaceutical Sciences,* 8(3), 2020, 287-297.