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Simultaneous estimation of Cetirizine hydrochloride, Phenylpropanolamine hydrochloride and Paracetamol by RP-HPLC method

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Abstract

The combination of Cetirizine hydrochloride, Phenylpropanolamine Hydrochloride and Paracetamol is used in treatment of anti-allergic, over-the count (OTC) drug products as a nasal decongestant to relieve stuffy nose or nasal congestion & antipyretic. Cetirizine hydrochloride, Phenylpropanolamine hydrochloride and Paracetamol are a potential combination for us. The analytical research is carried out on cetirizine hydrochloride i.e. high-performance Liquid chromatographic determination of cetirizine in human plasma, urine, serum and phenylpropanolamine hydrochloride high-performance liquid chromatographic determination of phenylpropanolamine hydrochloride in tablet, OTC preparations multicomponent formulations and analysis Paracetamol in tablets by HPLC. We study RP-High Performance Liquid Chromatographic method for the analysis of Cetirizine hydrochloride, Phenylpropanolamine hydrochloride and Paracetamol drugs that is a ternary combination which is simple sensitive and new method and not yet reported in the market.

Key-Words: Cetirizine Hydrochloride, Ternary combination, RP-HPLC method

Introduction

The multicomponent formulations have gained a lot of importance now days due to greater patient acceptability, increased potency and decreased side effects. The quantitative analysis of such multicomponent formulations is very important. Many methods are available for the analysis of such multicomponent formulations. Spectrophotometric, HPLC and HPTLC methods are mainly used for such analyses. Chromatography is probably the most powerful and versatile analytical technique available to the modern chemist, its power arises from its capacity to determine quantitatively many individual components present in mixture in one, single analytical procedure. The sample can range in complexity from a single substance to multicomponent mixture containing widely differing chemical species**.** Validation is defined as "documented evidence which gives a high degree of confidence that a process, system, facility will consistently produce a product meeting its predetermined specifications and quality attributes."

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Method validation is the process of proving that an analytical method is acceptable for its intended purpose. For pharmaceutical methods, guidelines from the United States Pharmacopoeia (USP), International Conference on Harmonization (ICH), World Health Organization (WHO) and the Food and Drug Administration (FDA) provide a framework for performing such validations. Linearity, range, specificity, accuracy, precision, limit of detection, robustness are some of the parameters studies in the Validation of any analytical method

Material and Methods

Cetirizine Hydrochloride, Phenylpropanolamine Hydrochloride , Paracetamol was procured as a gift sample from Cipla LTD. HPLC grade Methanol, HPLC grade Water, Hexane sulphonic acid ,HPLC grade acetonitrile was procured from Merck Chemicals. Jasco HPLC system 2000 comprising of Jasco Model PU2080 Plus pump, Rheodyne sample injection port, Hemsil C18 column, Jasco Model UV-2075 plus UV/VIS detector, Borwin software version 1.5.

Selection of analytical wavelength

From the standard stock solution dilutions were done using methanol and scanned over the range of 200-400 nm and the spectra were overlain. The wavelength

selected for the analysis was 215 nm for Cetirizine hydrochloride, Phenylpropanolamine hydrochloride and Paracetamol showed considerable absorbance

Selection of Mobile Phase

The solutions of Cetirizine hydrochloride, Phenylpropanolamine hydrochloride, Paracetamol working standards were injected into the HPLC system and run in different solvent systems. Different mobile phases containing methanol, water and acetonitrile, buffer in different proportions were tried and finally methanol: Water (50:50 v/v), was selected as an appropriate mobile phase which gave good resolution and acceptable peak parameters for Cetirizine hydrochloride, Phenylpropanolamine hydrochloride, Paracetamol.

Preparation of mobile phase

Methanol : Water (50:50 v/v) was prepared, filtered through 0.2 µm membrane filter and sonicated in bath sonicator.

Selection of chromatographic parameters

a) Column: Hemsil C-18 (4.6 × 250 mm)
b) Mobile phase : Methanol: Water (50:50 v/v)
c) Flow rate : 1 ml/min
d) Detection Wavelength : 215 nm
e) Sample injector : 20 µl loop
f) Temperature : Ambient

Checking the resolution of three drugs

The column was equilibrated with the mobile phase (indicated by constant back pressure at desired flow rate). Mixed standard solution of Cetirizine hydrochloride, Phenylpropanolamine Hydrochloride and Paracetamol was injected to get the chromatogram. The retention times for the three drugs were found to be, Cetirizine hydrochloride (2.35±0.02 min) Phenylpropanolamine Hydrochloride (2.81±0.01min) Paracetamol (3.55±0.01 min). Chromatograms of Cetirizine hydrochloride, Phenylpropanolamine hydrochloride and Paracetamol are shown in Figures 2, 3 and 4. The baseline separation of mixture of the three drugs was confirmed by Figure 5.

Preparation of standard mixture

Accurately weighed quantities of Cetirizine Hydrochloride 1 mg, Phenylpropanolamine Hydrochloride 5 mg and Paracetamol 100 mg were transferred to separate 100 ml volumetric flask and dissolved in mobile phase and volume made up to mark. Dilution was done to get the final concentration of cetirizine Hydrochloride 10 µg /ml, Phenylpropanolamine Hydrochloride 50 µg/ml and Paracetamol 1 mg/ml.

Analysis of tablet formulation

Twenty Tablets, each containing 5 mg cetirizine hydrochloride, 25mg Phenylpropanolamine hydrochloride and 500 mg paracetamol were weighed and finely powdered. A quantity of powder equivalent to 100 mg paracetamol was weighed and transferred to 100 ml volumetric flask. 50 ml mobile phase was added to the same flask and shaken for 5 minutes. The volume was made up to 100 ml with mobile phase l. The solution was filtered using 0. 2 µ membrane filter paper. From the filtrate, appropriate dilution was done in mobile phase to get a solution of 1mg/ml of Paracetamol, 10 µg/ml of Cetirizine hydrochloride and 50 µg/ml Phenylpropanolamine hydrochloride. The chromatogram obtained is shown in Figure 6. The results obtained are shown in Table 2&3.

Brand : Cheston cold
Contents: Cetirizine Hydrochloride - 5mg
Phenylpropanolamine Hydrochloride - 25 mg
Paracetamol – 500 mg
Manufacturer: CIPLA Pharmaceuticals Ltd.

METHOD VALIDATION

Linearity

Suitable dilutions using mobile phase were made from the standard stock solutions containing 100 µg/ml of Cetirizine hydrochloride, 100 µg/ml of Phenylpropanolamine hydrochloride and 1000 µg/ml Paracetamol to prepare range of standard solutions containing five different concentrations of analytes. Five replicates per concentration were injected. The linearity of the relationship between peak area and concentration was determined by analyzing five standard solutions over the concentration range 10-50 µg/ml for Cetirizine hydrochloride and 20-100 µg/ml for Phenylpropanolamine hydrochloride and 200-1000 µg/ml Paracetamol. The results obtained are shown in Table 5, 6 and 7. Excellent correlation exists between response factor and concentration of drugs within the concentration range indicated above.

Precision

One set of three different concentrations of mixed standard solutions of cetirizine hydrochloride, Phenylpropanolamine hydrochloride and paracetamol were prepared. All the solutions were analyzed thrice, in order to record any Intra- day variations in the results. The result obtained for intra- day variations are shown in Table 8, 9 and 10. For Inter day variations study three different concentrations of the mixed standard were analyzed up to three days. The result obtained for Inter day variations are shown in Table 11 and 12, 13.

Accuracy

To check the accuracy of the method, recovery studies were carried out at three different levels 80%, 100%

and 120%. From the tablet powder blend, weight equivalent 100mg of Paracetamol, was weighed and transferred to 100 ml volumetric flask. Working standard of cetirizine hydrochloride, Phenylpropanolamine hydrochloride and Paracetamol was added equal to 80%, 100% and 120% of the equivalent weight of tablet taken in three different flasks. Mobile phase was then added and shaken vigorously for 10 min. Volume was then made up with mobile phase. The solutions are then filtered through 0.2 µm membrane filter paper. From these stock solutions further dilutions were prepared using mobile phase. The sample responses were obtained and drug concentrations of cetirizine hydrochloride, Phenylpropanolamine hydrochloride and Paracetamol were calculated by using above mentioned regression equations. The results obtained are shown in Table 14 and 15, 16.

Range

Cetirizine Hydrochloride : 10-50 µg/ml
Phenylpropanolamine Hydrochloride : 20-100 µg/ml
Paracetamol : 200-1000 µg/ml

Specificity

A blend of commonly used tablet excipients was treated as per developed procedure and the chromatogram showed no interfering peaks at retention time of the three drugs.

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase ratio and ambient temperature were altered. Variation of mobile phase ratio is seen to have greater impact on resolution than other parameters and hence should be meticulously controlled.

Mobile phase Ratio changes.
Methanol: water (70: 30)

The retention times for the three drugs were found to be:

Cetirizine hydrochloride : 2.30
Phenylpropanolamine hydrochloride : 2.31
Paracetamol : 3.50

Results and Discussion

One RP- HPLC method was developed for the Cetirizine Hydrochloride, Phenylpropanolamine Hydrochloride and Paracetamol ternary combination. In this methods methanol: water in the ratio 50:50 v/v was used as a mobile phase. Whereas no buffer solution was used in the method presented here which reduces the washing time of the column. Column used in this method as a Hypersil C18 method. The retention times for Cetirizine Hydrochloride, Phenylpropanolamine Hydrochloride and Paracetamol are 2.35 min, 2.81 min and 3.55 min respectively .In this method retention time is less. The developed method is suitable for the determination and quantification of the ternary combination of Cetirizine Hydrochloride, Phenylpropanolamine Hydrochloride and Paracetamol. A high percentage recovery shows that the method can be successfully used on a routine basis. The proposed method is simple, sensitive, rapid, specific and could be applied and stability monitoring of Cetirizine Hydrochloride, Phenylpropanolamine Hydrochloride and Paracetamol combinations.

References

1. Khopkar SM. Basic concepts of analytical chemistry. 2nd ed. New Delhi: New age International Ltd. Publishers: 1998. P: 1, 178-179.
2. Fifield FW, Kealey D. principles and practice of analytical chemistry. 5th ed. USA: Blackwell publishing: 2004. P: 2, 5-7.
3. Frank Settle. Handbook of Instrumental techniques for analytical chemistry. NJ: Prentice Hall PTR: 1997. P: 17, 19, 56, 57.
4. Beckett AH, Stenlake JB, editors. Practical pharmaceutical chemistry. 4th ed. Part II. New Delhi: CBS publishers and distributors: 1997. p: 143, 273- 278, 281-299.
5. Ohannesian L, Streeter, AJ. Handbook of Pharmaceutical Analysis. Vol. 117, Marcel Dekker Series: 2002. P: 219-221.
6. Thomas M. Analytical Chemistry by Open Learning, Ultraviolet and Visible Spectroscopy. 2nd ed. John Wiley and Sons. 1996. P: 131-132.
7. Skoog, Holler, Nieman. Molecular spectroscopy. In: Principles of instrumental analysis. 5th ed. Singapore: Thomson asia Pte. Ltd.: 2004. P: 342-344.
8. Willard HH, Merritt LL, Dean JA, Settle FA. High performance liquid chromatography: Methods and applications. In: Instrumental methods of analysis. 7th ed. New Delhi: CBS publishers and distributors: 2001. P: 614- 629.
9. Chatwal GR, Sham KA, High performance liquid chromatography. In: Instrumental methods of chemical analysis. 5th ed. Mumbai: Himalaya Publishing house: 2002. P: 2.626-2.636.
10. Weston A, Brown PR, High performance liquid chromatography, Separations in High performance liquid chromatography, instrumentation for HPLC. In: HPLC and CE – Principles and practice. USA: Academic Press: 1997. P: 1-4, 8-11, 24-26, 29-32, 71.

11. Fong GW, Lam SK, editors. Application of HPLC to dissolution testing of solid dosage forms. In: Hanson W. A., HPLC in the pharmaceutical Industry. New York: Marcel Dekker, Inc: 1991 P: 177.
12. ICH, Q2 (R1). Validation of analytical procedures: text and methodology International Conference on Harmonization. Geneva: 2005. P: 1- 13.
13. Nash RA, Wachter AH, editors. Validation of analytical methods process. In: Pharmaceutical process validation. 3rd Ed. New York: Marcel Dekker Inc.: 2003. P: 507- 524.
14. Gupta SC, Kapoor VK. Fundamentals of mathematical statistics. 9th ed. New Delhi: Sultan Chand and sons: 1994. P: 12.4-12.6.
15. Rao KV. Biostatistics- a manual of statistical methods for use in health, nutrition and anthropology. 1st ed. New Delhi: Jaypee Brothers' medical publishers: 1996. P: 155-157, 162-164.
16. Maryadele J. O'Neil, Ann Smith, editors. The Merck Index. White House Station. 13th ed. NJ: Merck and Co, Inc.: 2001, P: 86, 854, 11552, 1628.
17. James EFR., Anne BP, editors. Martindale: The extra pharmacopoeia, 28th ed., London: The pharmaceutical press: 1982. P: 600.
18. Indian Pharmacopoeia. Volume II. New Delhi: The controller of Publication: 1996.P:641.
19. British Pharmacopoeia. volume I. International edition, London: HMSO Publication: 2009. P-1197-1201, 4547-4551, 4697-4699.
20. Lakshmi sivasubramanian, k.s. lakshmi, reverse phase-high performance liquid chromatographic method for the analysis of paracetamol, cetirizine and pseudoephedrine from tablets, Der pharma chemica; 2009, (1):37- 46.
21. Gunawan Indrayanto, Ariani sunarto,Yenita adriani,simultaneous assay of phenylpropanolaminehydrochloride,caffeine,paracetamol,glyceryguaiacolatandchlorphenirami ne macerate in silabat tablet using HPLC with diode array detection , Journal of pharmaceutical and Biomedical analysis 13 (1995) 1555-1559.
22. J.D Fegade, R.P Bhole, I Sheik, R.Y Chaudhari, V, R Patil, Development and validation of reverse phase high performance liquid chromatographic method for simultaneous estimation of paracetamol and piroxicam in tablet, International journal of pharma tech research CODEN(USA):IJPRIF vol.1,no-2. P.no - 184-190, april-june 2009.
23. A. F. M. El Walilya, M. A. Koranya, A. El Indy and M. F. Bedairc, Spectrophotometric and high performance liquid chromatographic determination of cetirizine hydrochloride in pharmaceutical tablets, Journal of Pharmaceutical and Biomedical Analysis,Volume 17, Issue 3, July 1998, Pages 435- 442.
24. Chang-Soon, LeeYong-Ill, LeeYoung-Jae Yoo, The Detection Method of Phenylpropanolamine in Pharmaceuticals by HPLC, Department ofChemistry Graduate School, Changwon National University December 2001, 1- 43.
25. Goicoechea A. G., Lopez de alda M. J, Vila-jato J. L. A validated high-performance liquid chromatographic method for the determination of paracetamol and its major metabolites in urine, Journal of liquid chromatography ISSN 0148-3919 CODEN JLCHD8.
26. Knox JH, Jurand J, Determination of paracetamol and its metabolites in urine by high-performance liquid chromatography using reversed-phase bonded supports, PubMed will retrieve 4961 records. J Chromatogr. 1977 Nov 11; 142:651-70.
27. Wanda Bialecka, Anna Kulik,Teresa Kanieuska, Determination of active substances in antiallergic and antiphlogistic multicomponent prepration by HPLC ,drug research vol-59,no-1, P-9-14-2002.
28. Sun Ok Choi, Seok Ho Lee, Hak Soo Kong, Eun Jung Kim and Haeyoung park choo,Enantioselective determination of cetirizine in human urine by HPLC,springerlink date 31,octomber, 2008, P-178-179.
29. Sodhi R. A. ; Chawla J. L. ; Sane R. T. Simultaneous determination of paracetamol, ibuprofen and chlorzoxazone by hplc, hptlc and gc methods Indian Drug Manufacturers' Association, Bombay, INDE (1963) P-377-379.
30. Eustaquio, M. Blanco, R. D. Jee and A. C. Moffat Determination of paracetamol in intact tablets by use of near infrared transmittance spectroscopy Analytical Chimica ActaVolume 383, Issue 3, 15 March 1999, Pages 283-290 .
31. C. Celma J. A. Allué, J. Pruñonosa, C. Peraire and R. Obach Simultaneous determination of paracetamol and chlorpheniramine in human

plasma by liquid chromatography–tandem mass spectrometry Journal of Chromatography A Volume 870, Issues 1-2, 18 February 2000, Pages 77-86.

32. J. Macek P. Ptáek and J. Klíma Determination of cetirizine in human plasma by high-performance liquid chromatography Journal of Chromatography B: Biomedical Sciences and Applications Volume 736, Issues 1-2, 24 December 1999, Pages 231.

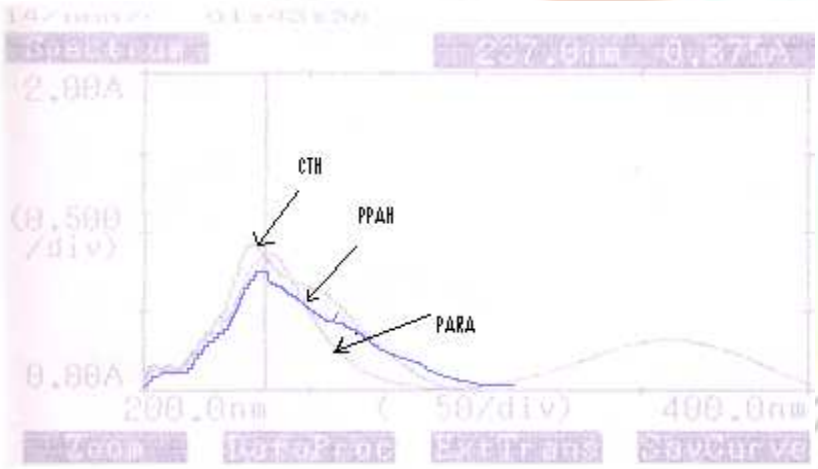


Fig. 1: Overlain spectra of Cetirizine Hydrochloride, Phenylpropanolamine Hydrochloride and Paracetamol

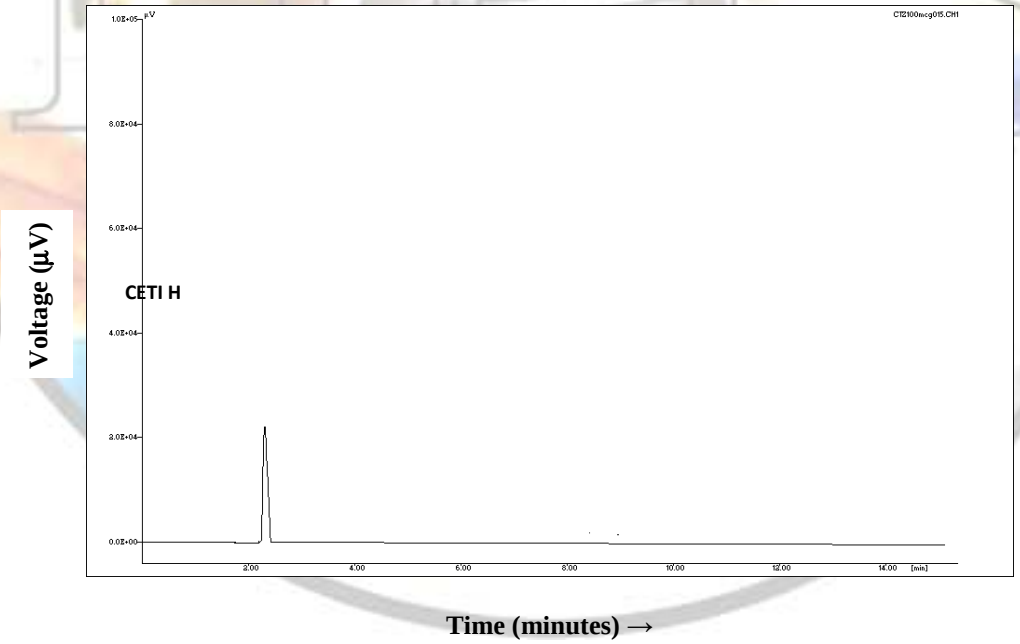


Fig. 2: Chromatogram of working standard Cetirizine Hydrochloride

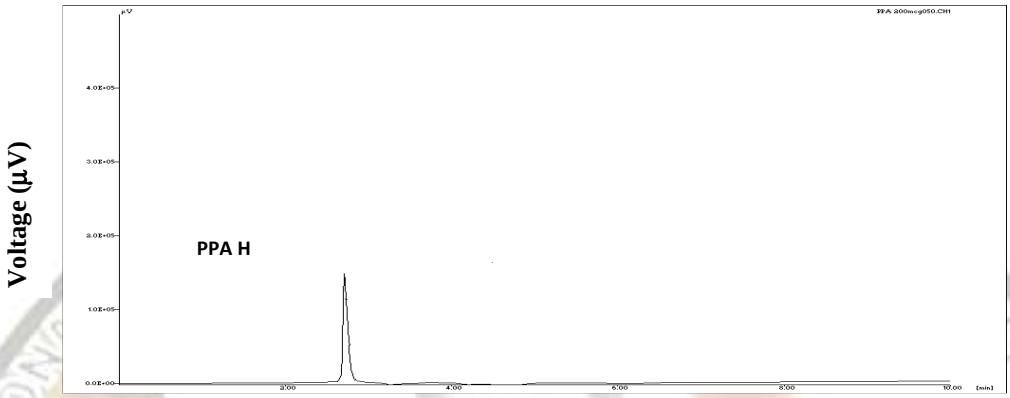


Fig. 3: Chromatogram of working standard Phenylpropanolamine Hydrochloride

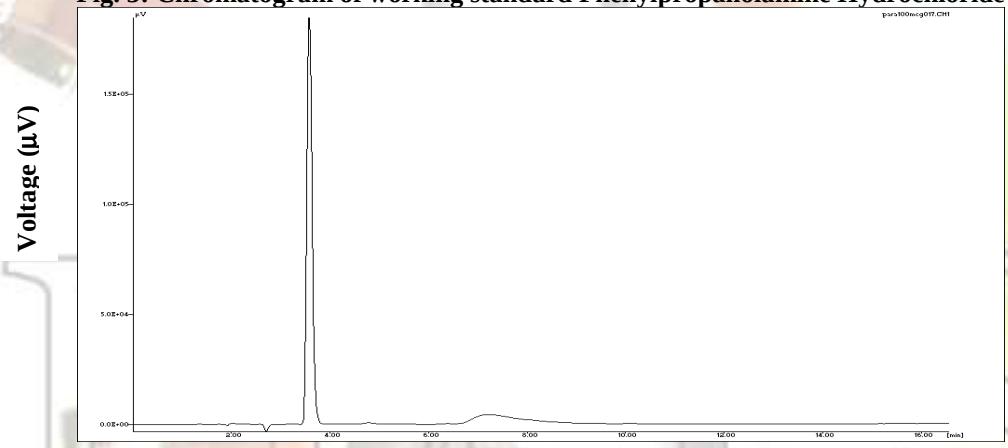


Fig. 4: Chromatogram of working standard Paracetamol

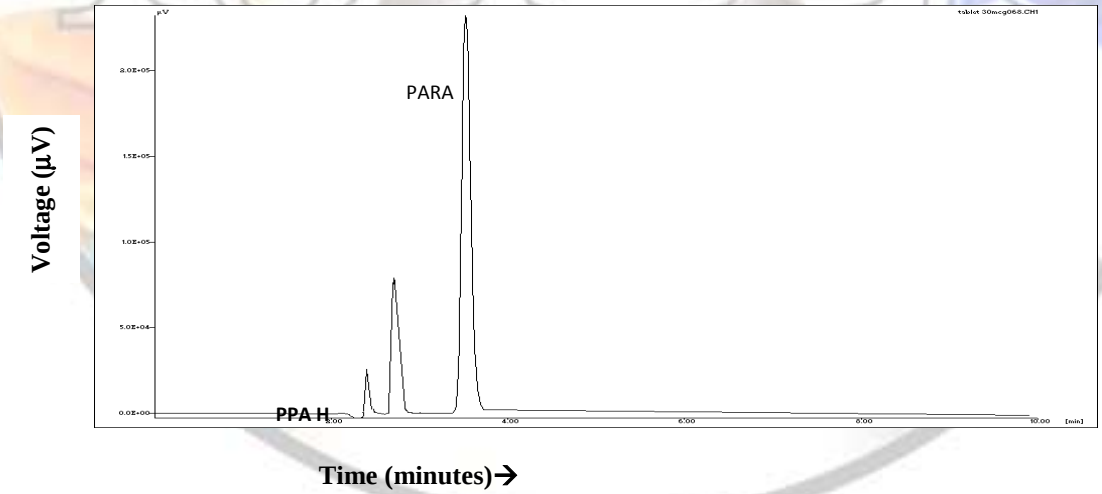


Fig. 5: Chromatogram of working standard mixture of Cetirizine Hydrochloride, Phenylpropanolamine Hydrochloride and Paracetamol

Table 1: Details of chromatogram of mixture

S/No.	Name	RT (min)	Area (µV.Sec)	Plates	Resolution	Asymmetry
1.	Cetirizine Hydrochloride	2.35	60250.31	2195.81	1.38	1.11
2.	Phenylpropanolamine Hydrochloride	2.81	635809.21	2069.34	2.97	1.08
3.	Paracetamol	3.55	4025150.33	7305.05	5.68	1.25

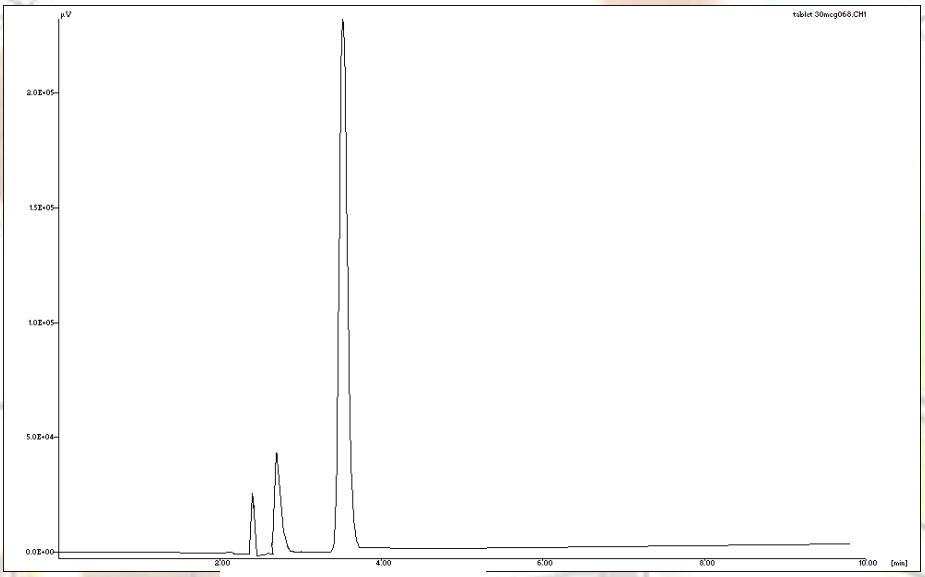


Fig. 6: Chromatogram of sample consisting Cetirizine Hydrochloride (2.34 min), Phenylpropanolamine Hydrochloride (2.81 min) and Paracetamol (3.55 min).

Table 2: Details of chromatogram of sample containing Cetirizine hydrochloride, Phenylpropanolamine Hydrochloride and Paracetamol

S/No.	Name	RT (min)	Area (µV .Sec)	Plates	Resolution	Asymmetry
1.	Cetirizine Hydrochloride	2.34	60350.82	2167.93	2.28	1.11
2.	Phenylpropanolamine Hydrochloride	2.81	636950.45	2059.34	2.87	1.06
3.	Paracetamol	3.55	4026350.23	7315.05	5.78	1.35

Table 4: Analysis of Tablet Formulation

S/No.	Amount present in (mg/tab)			Amount found in * (mg/tab)			% of Label claim*		
	CET	PPA	PAR	CET	PPA	PAR	CET	PPA	PAR
1	5	25	500	4.99	24.95	499.84	99.83	99.81	99.96

* Denotes average of five determinations.

Table 5: Linearity of Cetirizine hydrochloride

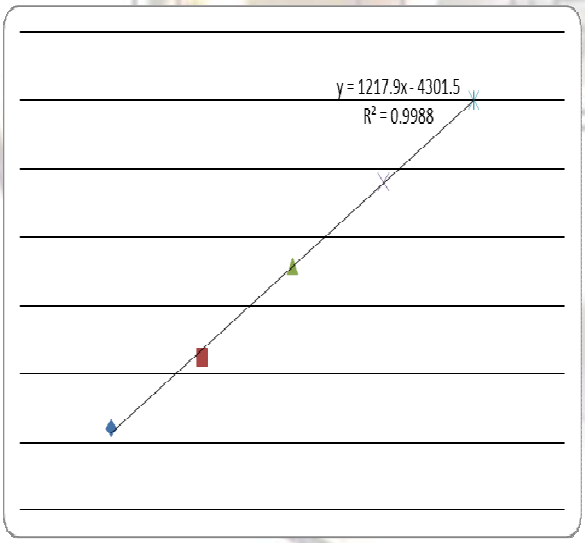
Standard ⇨ Concentrations	10 µg/ml	20 µg/ml	30µg/ml	40 µg/ml	50 µg/ml
Replicates ⇩	Peak Area				
1	60100.58	111230.78	179800.51	240400.11	300600.11
2	59997.98	113456.67	180100.71	239800.34	301000.31
3	60240.54	111070.22	177855.68	240500.24	299850.10
4	60434.32	112122.28	176878.31	241441.12	300550.98
5	59850.81	112400.48	180200.24	239190.90	300450.71
Mean	60124.84	112056.10	178967.09	240266.54	300490.44
SD	224.1310	966.6501	1508.1923	840.6008	414.5099
% RSD	0.3727	0.8626	0.8427	0.3498	0.1379

Regression Equation: y =1217.x – 4301, Coefficient of correlation: 0.998

Table 6: Linearity of Phenylpropanolamine Hydrochloride

Standard ⇨ Concentrations	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml
Replicates ⇩	Peak Area				
1	473457.73	588337.83	687019.81	787020.34	884606.89
2	464550.80	585443.71	689113.79	783931.55	894708.11
3	483640.38	574650.23	691331.45	761833.41	873818.28
4	474539.11	578349.49	701433.32	781727.88	885671.13
5	471340.23	591433.06	712410.48	779130.97	883382.01
Mean	473505.65	583642.86	696261.77	778728.83	884437.27
SD	6867.0	6979.62	10587.04	9879.63	7431.65
% RSD	1.4502	1.20	1.5205	1.2686	0.8402

Regression Equation: y = 5084.x + 37823, Coefficient of correlation: 0.997



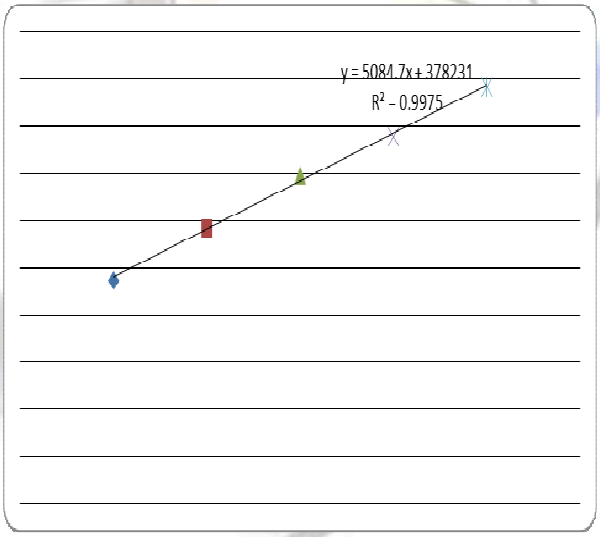


Fig. 7: Calibration Curve of Cetirizine Hydrochloride Fig. 8: Calibration Curve of Phenylpropanolamine Hydrochloride

Table 7: Linearity of Paracetamol

Standard ⇨ Concentrations	200 µg/ml	400 µg/ml	600 µg/ml	800 µg/ml	1000 µg/ml
Replicates ⇩	Peak Area				
1	810550.30	1612960.11	2398950.13	3009080.23	3997830.10
2	812569.89	1614850.32	2412850.50	3008450.78	4012340.19
3	811450.78	1598970.09	2399733.31	3010345.65	4103450.32
4	813543.67	1613456.89	2425830.38	3009678.90	3999950.52
5	809340.09	1611567.90	2397633.10	3109789.89	4011860.62
Mean	811490.90	1610361.10	2406999.50	3029469.10	4025086.40
SD	1650.03	6475.02	12187.65	44906.19	44308.60
% RSD	0.2033	0.4020	0.5063	1.4823	1.1008

Regression Equation: y = 3923.x +22792, Coefficient of correlation: 0.996

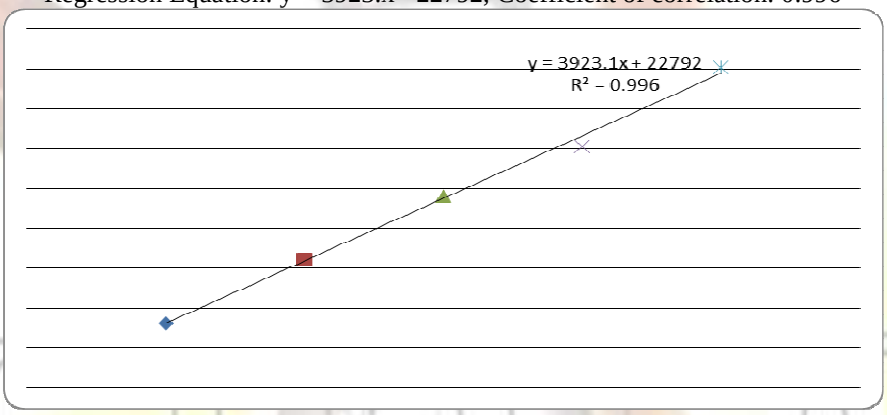


Fig. 9: Calibration Curve of Paracetamol

Table 8: Intra-day variability of cetirizine Hydrochloride

Conc. (µg/ml)	Peak area			Mean	SD	% RSD
	Trial 1	Trial 2	Trial 3			
10 µg/ml	60489.98	58858.78	61356.46	60235.07	1268.20	2.1054
20 µg/ml	122265.34	120276.78	118421.34	120321.2	1922.38	1.5977
30 µg/ml	176878.54	181001.93	178366.11	178748.86	2088.17	1.1682

Table 9: Intra-day variability of Phenylpropanolamine Hydrochloride

Conc. (µg/ml)	Peak area			Mean	SD	% RSD
	Trial 1	Trial 2	Trial 3			
20 µg/ml	463457.73	471646.89	468321.21	467808.6	4118.57	0.8803
40 µg/ml	588457.56	588234.78	587934.89	588209.1	262.28	0.0445
60 µg/ml	688019.31	687023.43	682233.30	685758.7	3093.41	0.4510

Table 10: Intra-day variability of Paracetamol

Conc. (µg/ml)	Peak area			Mean	SD	% RSD
	Trial 1	Trial 2	Trial 3			
200 µg/ml	811560.70	812450.89	810456.45	811489.3	999.13	0.123
400 µg/ml	1612560.89	1614356.67	1616342.45	1614420.0	1891.58	0.117
600 µg/ml	2412345.90	2416789.87	2413543.61	2413543.61	2299.33	0.095

Table 11: Inter-day variability of cetirizine Hydrochloride

Conc. (µg/ml)	Peak area			Mean	SD	% RSD
	Day 1	Day 2	Day 3			
30 µg/ml	178779.56	177001.93	178366.11	178049.20	930.22	0.5224
40 µg/ml	240311.35	239915.49	238850.11	239692.30	755.75	0.3153
50 µg/ml	299560.9	298988.12	299767.78	299438.9	403.89	0.1389

Table 12: Inter-day variability of Phenylpropanolamine Hydrochloride

Conc. (µg/ml)	Peak area			Mean	SD	% RSD
	Day 1	Day 2	Day 3			
60 µg/ml	688019.31	687023.43	682233.30	685758.7	3093.41	0.4510
80 µg/ml	787130.40	786950.31	787239.20	787040.40	127.34	0.0161
100 µg/ml	884551.20	884850.11	885020.01	884807.10	237.35	0.0268

Table 13: Inter-day variability of paracetamol

Conc. (µg/ml)	Peak area			Mean	SD	% RSD
	Day 1	Day 2	Day 3			
600 µg/ml	2412345.90	2416789.87	2413543.61	2413543.61	2299.33	0.095
800 µg/ml	3012967.90	3101567.89	3115345.76	3076627.20	55559.30	1.805
1000 µg/ml	4012345.89	4015671.12	4030453.98	4019490.30	9639.27	0.239

Table 14: Recovery Studies of Cetirizine Hydrochloride

	Expected Concentration of Sample		
	Level of Recovery		
	80 %	100 %	120 %
	18 µg/ml	20 µg/ml	22 µg/ml
Replicate 1	110381.11	120700.61	130800.45
Replicate 2	110531.33	120830.20	130920.30
Replicate 3	111003.14	120599.13	130610.50
Mean	110638.50	120710.00	130777.10
SD	324.57	115.52	156.22
% RSD	0.2933	0.1057	0.1194
conc. found (µg/ml)	18.57	19.89	22.90
% Recovery	100.84	99.76	100.91
Mean % Recovery	100.50		

Table 15: Recovery Studies of Phenylpropanolamine Hydrochloride

	Expected Concentration of Sample		
	Level of Recovery		
	80 %	100 %	120 %
	90µg/ml	100µg/ml	110µg/ml
Replicate 1	840950.31	884890.31	932840.39
Replicate 2	839998.91	884930.13	932771.81
Replicate 3	840731.01	884810.80	932560.44
Mean	840560.10	884877.10	932724.20
SD	498.20	60.76	145.92
% RSD	0.0592	0.0068	0.0156
conc. found (µg/ml)	90.10	98.89	110.91
% Recovery	100.62	99.12	100.89
Mean % Recovery	100.21		

Table 16: Recovery Studies of Paracetamol

	Expected Concentration of Sample		
	Level of Recovery		
	80 %	**100 %**	**120 %**
	800 µg/ml	1000 µg/ml	1200 µg/ml
Replicate 1	7006920.33	7995943.23	8996830.33
Replicate 2	7005870.10	7996930.20	8995110.40
Replicate 3	7007110.29	7995110.12	8996980.11
Mean	7006633.60	7995994.50	8996306.90
SD	667.97	911.12	1038.98
% RSD	0.0095	0.0113	0.0115
conc. found (µg/ml)	798.07	1010.12	1205.45
% Recovery	99.62	100.68	100.08
Mean % Recovery		100.12	

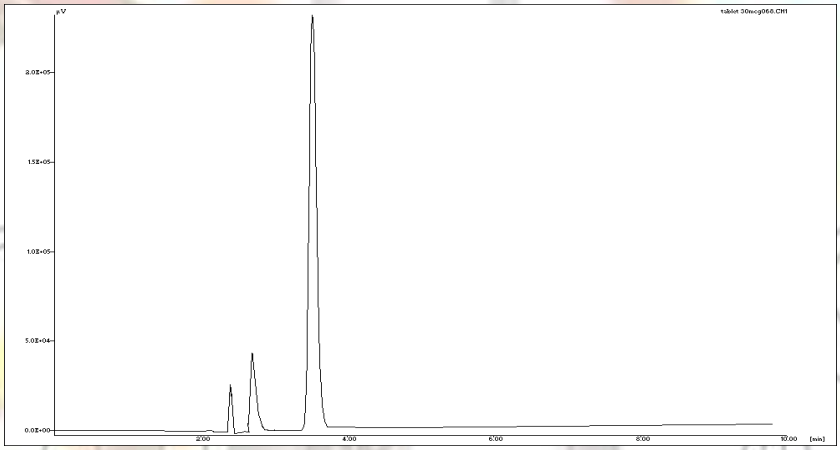


Fig. 10: No Interfering Peaks Chromatogram of sample consisting Cetirizine Hydrochloride (2.34 min), Phenylpropanolamine Hydrochloride (2.81 min) and Paracetamol (3.55 min)

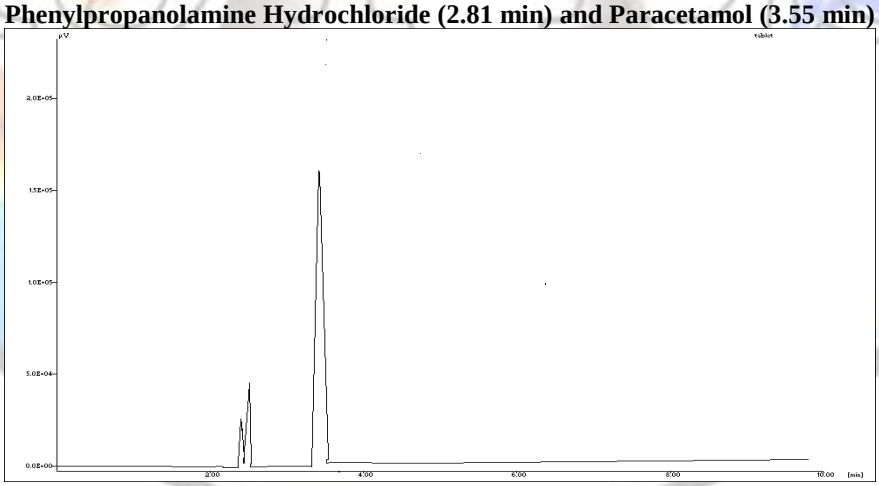


Fig. 11: Mobile ratio changes Chromatogram of sample consisting Cetirizine Hydrochloride (2.30 min), Phenylpropanolamine Hydrochloride (2.31 min) and Paracetamol (3.50 min)