Dave Parth A.et al. / Vol6 / Issue 1 / 2016 / 34-42.

International Journal of Pharmaceutical Research & Analysis

e-ISSN: 2249 – 7781
Print ISSN: 2249 – 779X

www.ijpra.com

RP-HPLC METHOD DEVELOPMENT & VALIDATION FOR SIMULTANEOUS ESTIMATION OF TRITHIOPARAMETHOXYPHENYL PROPANE (ANETHOLE TRITHIONE) AND CHLORPHENIRAMINE MALEATE IN TABLET DOSAGE FORM

Dave Parth A*, Patel Ajay I, Vyas Amit J, Patel Ashok B, Chauhan Vipula B

Department of Quality Assurance, B. K. Mody Government Pharmacy College, Rajkot, Gujarat, India.

ABSTRACT

A Simple, precise and rapid RP-HPLC method was developed and validated for simultaneous Estimation of Trithiparamethoxyphenyl propene (Anetholetrithione) and Chlorpheniramine Maleate in tablet dosage form. Separation was achieved on Phenomenex Luna C18 (250mm × 4.6 mm, 5 µm) using an isocratic mobile phase Consisting of Acetonitrile and Mixture of 0.1% Ortho phosphoric acid & 0.1% triethylamine in water, (80:20,v/v). The analysis was performed at a flow rate of 1.0 ml / min. Detection was done by UV absorbance at 232 nm and the runtime was 8.0 minutes within which the compounds were separated. The retention time for Chlorpheniramine maleate and Anetholetrithione were found to be 2.42 min and 5.51 min respectively. The method was linear in the range of 20-100 µg/ml (r^2 = 0.9976) and 5-25 µg/ml (r^2 = 0.9988) for Anetholetrithione and Chlorpheniramine maleate respectively. The method was validated as per ICH guideline with respect to system suitability, Specificity, Linearity, accuracy, precision and robustness. Accuracy was assessed by the standard addition method. The recoveries were obtained in range of 99.56 -101.46% and 99.22-101.87% for CPM and ATT respectively. The repeatability was determined and %RSD for CPM and ATT were found to be 0.66% and 0.41% respectively. The intraday precision was determined and %RSD for CPM and ATT were found to 0.28-0.39% and 0.17-0.49 % respectively. The interday precision was determined and %RSD for CPM and ATT were found to be 0.34-0.73% and 0.54-0.75% respectively. The LOD and LOQ value for CPM was found to be 0.14 and 0.13µg/ml.

Keywords: Anetholetrithione, Chlorpheniramine maleate, Hepasulfol-AA® tablets, RP-HPLC.

INTRODUCTION

Anethole (anise camphor) is a derivative of phenypropene, a type of aromatic compound the occurs widely in nature and that is widely used as a flavouring substance. Anetholetrithione is an oltipraz analogue synthesis from Anethole by using sulfolane as a solvent. Anetholetrithione chemically is (5-[p-methoxyphenyl] 3H-1, 2-Dithiole-3-Thione) and used as hepatoprotective, Antitumor agent. Used in xerostomia,dryness of mouth and in combination with Chlorpheniramine maleate used as an antiallergic in case where anti-histamines alone is not giving proper action. e.g in the case Chronic urticaria, allergic dermatitis, food allergies hypersensitivity to the other drugs.It is not official in any pharmacopoeia. Literature review revealed several analytical method for estimation of Anetholetrithione from tablet RP-HPLC [1], in human plasma HPLC coupled with tandem mass spectroscopy [2], 4-hydroxy Anetholetrithione by HPLC-MS/MS, 4-hydroxy Anetholetrithione by HPLC in human plasma via enzymatic hydrolysis.

Chlorpheniramine maleate chemically is 2-[p-Chloro-a-[2-(dimethylamino)ethy]benzyl]pyridine maleate and widely used as anti-histaminics. It is official in indian

Corresponding Author:-Dave Parth A Email:- pdparthdave@gmail.com

and United states Pharmacopoeia. In literature review revealed several official methods titrimetry (non-aqueous) [3] Thin layer Chromatography, Gas Chromatography and in reported method several UV-Visible spectroscopy [4-6], RP-HPLC [7-10], HPLC-UV spectroscopy [11], Chemiluminescense [12], HPLC with Indirect conductometry [13].

The combination of these two drug is not official in any pharmacopoeia; hence no official method is available for their simultaneous estimation. Literature review revealed that there is no analytical method available for the simultaneous determination of Anetholetrithione and Chlorpheniramine maleate in combined dosage form.

There for an objective of this work is to develop and validated RP-HPLC method for simultaneous estimation of these two drugs.

MATERIALS AND METHODS

Chemicals and reagents

Anetholetrithione (99% purity) was procured from Synix labs, Hyderabad, India. Chlorphemiramine maleate (98% purity) and Maleic acid provided by he institute i.e. B.K.Mody govt. Pharmacy college, Rajkot. Hepasulfol-AA® manufactured by Franco-indianphamaceuticalsPvt Ltd, Mumbai, India and procured from local market of India. HPLC grade Acetonitrile and Water (Lobachemia Ltd, Mumbai) and Orthophosphoric acid (Finar chemicals Ltd), Triethylamine (Finar chemicals ltd)., Trifluoroacetic acid (S D Fine chem Ltd) have been used of AR grade.

HPLC Instrumentation and chromatographic condition

The method was developed using Shimadzu HPLC (LC-10AT VP) Instrument equipped with photodiode array (PDA) detector. The Phenomenex Luna C-18 column,(250 mm × 4.6 mm, 5 µm) was used as stationary phase. Choromatographic separation was carried out using isocratic elution. Acetonitrilr: Mixture of 0.1% OPA and 0.1% TEA in water (80:20 %v/v) used as the mobile phase, delivered at a flow rate 1.0 ml/min. Injection volume was 20 µLand detectin has been carried out at 232nm. Data was integrated by LC Solution software.

Solutions

Combined Standard stock solution of mixture of Anetholetrithione and Chlorpheniramine maleate CPM (30 mg) and ATT (125 mg) were accurately weighed and transferred to a 100 ml volumetric flask, dissolved in sufficient quantity of Acetonitrile and then diluted up to the mark with mixture of 0.1% TEA and 0.1% OPA in water. The solution contain 1250 µg/ml of ATT and 300 µg/ml of CPM.

Working standard solutions –

10 ml aliquot of the solution was taken in a separate 100 ml volumetric flask and volume was adjusted to the mark with Mobile phase to produce solution contain 125 µg/ml ATT and 30 µg/ml of CPM. The solution was filtered through 0.45 µm Nylon 66 (N66) 47 mm membrane filter paper and first drop of filtrate were discarded.

Preparation of test solution from formulation

Twenty tablet were weighed and finely powdered. The powder equivalent to 60 mg of ATT and 14.4 mg of CPM was weighed accurately and mixed, diluted with acetonitrile (80 ml) in 100 ml volumetric flask, kept in sonicator for 2-3 min to get optimum dissolution of the active ingredient and diluted up to the mark with Water (0.1% TEA + 0.1% OPA) to produce 600 µg/ml of ATT and 144 µg/ml of CPM.

An aliquot of 2 ml from this solution was transferred in 10 ml volumetric flask and diluted up to mark with mobile phase to produce 125µg/ml of ATT and 30 µg/ml of CPM.

The solution was filtered using 0.45 µm Nylon 66 (N66) 47 mm membrane filter paper and first few drops of filtered were discarded.

Method validation

System suitability test

Analytical system performance before and/or during the analysis was evaluated by system suitability test. System suitability test are an integral part of method development and are performed to evaluate the behavior of the Chromatographic system such as Area, retention time, Resolution, plate number and tailing factor. For Chlorpheniramine maleate suitability test prepared the mixture solution 10 µg/ml for Chromatogram and observed the Area, Retetion time, Resolution, Theoretical plate and Tailing factor % RSD was calculated.

Linearity and construction of calibration curves (n=5)

The linearity response were determined by analyzing 5 independent levels of calibration curve in the range of 5-25 µg/ml (5, 10, 15, 20 and 25µg/ml) for CPM and 20-100 µg/ml (20, 40, 60, 80 and 100 µg/ml of ATT and 30 µg/ml of CPM. The plot of peak area against concentration was plotted. Correlation and regression line equation for CPM and ATT were calculated.

Accuracy (% Recovery) (n=3)

Accuracy studies were carried out to determine suitability and reliability of proposed method. It was carried out by the standard addition method in which, known amount of standards samples of CPM and ATT at 50%, 100% and 150% levels were added to the pre-analyzed sample. Known amount of standards solution of CPM (5, 10 AND 15 µg/ml) and ATT (20, 40 and 60 µg/ml) were added to a pre-quantified sample solution of CPM and ATT (10 and 40 µg/ml, respectively) the recovered amount of CPM and ATT were calculated at each level and %recovery was reported.

Precision

Repeatability (n=6)

From the working standard solution, an aliquot of 3.2 ml was transferred to a separate 10 ml volumetric flask and diluted up to mark with mobile phase such that it give the concentration of 10µg/ml of CPM and 40µg/ml of ATT. The solution was injected into the system. The peak area of CPM and ATT were observed. The procedure was repeated six time and % RSD was reported.

Intraday Precision (n=3)

From the working standard solution, aliquot of 1.6 ml, 3.2 ml and 4.8 ml were transferred to separate 10 ml volumetric flasks and diluted up to the mark with mobile phase to give the concentration of 5, 10 and 15 µg/ml for CPM and 20, 40 and 60 µg/ml for ATT. The solution were injected into the HPLC system and analyzed three time on the same day and %RSD was calculated.

Interday precision (n=3)

From the working standard solution, an aliquots of 1.6 ml, 3.2ml, and 4.8 ml were transferred to separate 10ml volumetric flask and diluted up to the mark mobile phase to give the concentration of 5, 10, and 15 µg/ml for CPM and 20, 40 and 60 µg/ml for ATT. The solution were injected into the HPLC system and analyzed three time on three different day and %RSD was calculated.

Specificity

In the case of assay, demonstration of specificity is to show that the procedure is unaffected by the presence of impurities or excipient. Specificity of an analytical method indicates that the analytical method is its able measure accurately and specificity the analyte of interest without any chromatograms of standard sample solution of CPM and ATT, Standard maleic acid, blank (mobile phase) and sample solution of CPM and ATT.

Limit of detection and limit of quantification

LOD and LOQ for the developed method were calculated using following Eq. (1) as per ICH guideline

$$LOD = 3.3\ \sigma/S \text{ and } LOQ = 10\ \sigma/S \quad \ldots\ldots\ldots\ldots (1)$$

Where the σ the standard deviation of the intercept and S is the slope of the calibration curve

Robustness

The robustness of method established by introducing small changes in various parameter like detection wavelength and flow rate. The change made in wavelength and flow rate were ± 2 mm (230, 232 and 234 nm), ±0.1 ml/min (0.9, 1, and 1.1 ml/min) respectively. The robustness of the developed method was calculated in terms of % RSD.

RESULT AND DISCUSSION

In RP-HPLC analysis the selection of the stationary phase depends on the chemical structures of the target molecules. Due to high carbon content and hydrophobic character of one drug i.e. Anetholetrithione, both the drug can be separated through C18 stationary phase. Objective of development and validation of RP-HPLC method for determination of Anetholetrithione and Chlopheniramine maleate should be accurate, precise, reproducible and robust. Both the drug should be well separated of active ingredients without interference excipients.

For the developed method mobile phase selected with a view to good selectivity along with short elutiontime. The combination of acetonitrile and mixture of 0.1% OPA and 0.1% TEA in water (80:20%v/v) was selected as mobile phase as it was found to be ideal to resolve CPM (Rt 2.422) and ATT (Rt 5.513) optimum resolution and good symmetry.

Method development and optimization

The first step in method development in to test all the published RP-HPLC mobile phase of both the drug. None of these mobile phase was able to achieve acceptable peak. Different parameter were manipulated to obtain sharp and symmetrical peak of Anetholetithione and Chlorpheniramine maleate, reduce the analysis time, enhance LOD and LOQ of the method and to satisfy HPLC system suitability parameters.

The chromatographic separation was started with C18 column as astationary phase and Acetonitrile: water (40:60 v/v) as a mobile phase pH adjusted 3.0 with TFA and maintaining the flow rate 1.0 ml/min, where retention time was acceptable but peak asymmetry was obtained. Mobile phase ratio was changed to Acetonitrile: water (60:40, v/v) with pH 4.0 but the peak asymmetry was obtained again. Then the mobile phase Acetonitrile: water mixture of 1.0 ml /min here good separation with resolution obtained. On the other hand different scanning wavelengths were tried (232, 261 and 284 nm) in order to enhance the sensitivity of the method where scanning at 232 nm gave considerable sensitivity for both the drugs.

After method optimization the chromatographic separation has been carried out using phenomenex Luna C18 column (250 mm× 4.6 mm, 5µm) and mobile phase of Acetonitrile: mixture of 0.1 % OPA and 0.1 %TEA in water (80:20 v/v) flow rate 1.0 ml/min with UV detection at 232 nm. Typical chromatogram (Fig. 3) shown good peak (CPM at 2.42 min and ATT 5.51 min).

Method validation

System suitability Testresult were within the acceptable range as shown in (table) indicated that the system is suitable for the intended analysis.

Linearity of a method reveals the linear relationship of response against the selected concentration of the analyte. For HPLC Method linear correlation was obtained between peak areas and concentrations of CPM in the range of 5-25 µg/ml and ATT in the range of 20-100 µg/ml. chromatograms for linearity range shown in (Fig. 4.8). The following regression equation was found by plotting the peak area (y) versus the concentration (x) expressed in µg/ml: for CPM y= 23,772.51x-30,604.68.with r^2: 0.9976 obtained for the regression line demonstrates excellent relationship between peak area and concentration of CPM and ATT. Data of regression analysis is summarized in Table 1 and Table 2 CPM and ATT respectively.

Accuracy was assessed by the standard addition method. Three replicate determination were performed at three different levels. The % recoveries for CPM and ATT was found to be 99.56-101.46 % and 99.22 -101.87% respectively. The accuracy data shown in Table 3 and Table 4 for CPM and ATT respectively.

Precision

Repeatability

The repeatability data for CPM and ATT were shown in Table 5. The % RSD for CPM and ATT was found to be 0.66 % and 0.41% respectively.

Intraday precision

The data for intraday precision for CPM and ATT is shown in Table 6. The % RSD of CPM and ATT was found to be 0.28-0.39% and 0.17-0.49% respectively.

Interday precision

The data for interday precision for CPM and ATT is shown in Table 7. The % RSD of CPM and ATT was found to be 0.34-0.73% and 0.54-0.75% respectively.

Specificity of the analytical method is its ability to measureaccurately and specifically the analyte of interest in the presence of sample matrix.

The specificity was determined by the comparison of the chromatograms of

a) Standard sample solution of CPM and ATT (Fig.11 and Fig.12)
b) Standard solution of maleic acid (Fig.13)
c) Blank (mobile phase) (Fig. 14)
d) Sample solution of CPM and ATT (Fig.15)

LOD and LOQ were determined from intercept and slopes of linear regression curves. LOD for CPM and ATT was found to be 0.14 and 0.13 µg/ml respectively. Similarly LOQ for CPM and ATT was found to be 0.42 and 0.39µg/ml respectively. (Table 9)

Robustness The developed method was evaluated for Robustness. There were no significant changes were observed in the chromarographic pattern when the modification was made in the experiment condition, which indicate that the method was robust. The change was done in flow rate wavelength. %RSD for area was calculated which found to be less than 2. The change in flow rate data and change in wavelength data are shown in Table 10 and Table 11 respectively

Summary of validation parametersshown in Table 12.

Table 1. Linearity data for CPM

Conc.µg/ml)	Mean Area ± SD	% RSD
5	94826 ± 241.30	0.25
10	196326 ± 1899.13	0.97
15	327699.8 ± 1404.33	0.43
20	447522.8 ± 3184.58	0.71
25	563540.4 ± 2398.31	0.43

*(n=5)

Table 2. Linearity data for ATT

Conc.(µg/ml)	Mean Area ± SD	% RSD
20	1486554 ± 10773.61	0.72
40	2911993 ± 14330.55	0.49
60	4470725 ± 12526.18	0.28
80	5777670 ± 10514.36	0.18
100	6974473 ± 9155.183	0.13

*(n=5)

Table 3. Accuracy data for CPM

Level (%)	Target Conc. (µg/ml)	Std. Added (µg/ml)	Total Amount (µg/ml)	Amount found mean ± SD	% Recovery ±SD	% RSD
50	10	5	15	14.98 ± 0.16	99.56 ± 0.66	0.67
100		10	20	20.29 ± 0.11	101.46 ± 0.66	0.55
150		15	25	25.25 ± 0.21	100.99 ± 0.84	0.83

*(n=3)

Table 4. Accuracy data for ATT

Level (%)	Target conc (µg/ml)	Std. added (µg/ml)	Total amount (µg/ml)	Amount found Mean ± SD	% Recovery ± SD	% RSD
50	40	20	60	61.12 ± 0.35	101.87 ± 0.58	0.57
100		40	80	79.38 ± 0.75	99.23 ± 0.93	0.94
150		60	100	100.74 ± 0.72	100.74 ± 0.72	0.71

*(n=3)

Table 5. Reapetability data for CPM and ATT

Concentration of CPM(µg/ml)	Area of CPM	Concentration of ATT(µg/ml)	Area of ATT
10	198674	40	2955169
	198768		2964274
	198707		2965954
	195707		2983225
	197684		2974452
	196532		2987653
Mean Response	197678.66	Mean Response	2971787.83
SD	1299.01	SD	12298.67
% RSD	0.66	% RSD	0.41

**(n = 6)*

Table 6. Intraday precision data for CPM and ATT

CPM			ATT		
Conc. (µg/ml)	Mean Area ± SD	% RSD	Conc. (µg/ml)	Mean Area ± SD	% RSD
5	94860.67 ± 261.28	0.28	20	1479916 ± 7279.32	0.49
10	198349.76 ± 44.88	0.33	40	2918466 ± 5045.45	0.17
115	328254.31 ± 265.25	0.39	60	4477655 ± 10258.85	0.23

**(n = 3)*

Table 7.Interday precision data for CPM and ATT

CPM			ATT		
Conc. (µg/ml)	Mean Area ± SD	% RSD	Conc. (µg/ml)	Mean Area ± SD	% RSD
5	94627.33 ± 391.11	0.41	20	1473250 ± 8694.17	0.59
10	197983 ± 680.03	0.34	40	2928466 ± 15814.76	0.54
15	327587.7 ± 2402.26	0.73	60	4460988 ± 33510.85	0.75

**(n = 3)*

Table 8. Assay data for CPM and ATT (Hepasulfol-AA®)

CPM			ATT		
Amt. of CPM taken in mg	**Amt. of CPM found in mg**	**% content**	**Amt. of ATT taken in mg**	**Amt. of ATT found in mg**	**% content**
3	3.03	101	12.5	12.39	99.12
	2.99	99.67		12.47	99.76
	2.99	99.67		12.59	100.72
	2.99	99.67		12.56	100.48
	2.998	99.93		12.54	100.32
Mean amt. ± SD	2.99 ± 0.0173		Mean amt. ± SD	12.51 ± 0.0803	
Mean % Content ± SD	99.99 ± 0.5781		Mean % Content ± SD	100.08 ± 0.6424	
% RSD	0.58		% RSD	0.64	

**(n = 5)*

Table 9. LOD and LOQ data for CPM and ATT

Parameters	CPM	ATT
SD of the Y-intercept of the 5 calibration curves	1004.94	2689.18
Mean slope of the 5 calibration curves	23772.51	69207.58
LOD(µg/ml)	0.14	0.13
LOQ(µg/ml)	0.42	0.39

**(n = 5)*

Table 10. Results for change in flow rate

Flow rate (ml/min)	CPM (20 µg/ml)		ATT (80 µg/ml)	
	Mean area. ± SD	**% RSD**	**Mean area. ± SD**	**% RSD**
0.9	434367 ± 1821.53	0.42	4627780 ± 61581.58	1.33
1.0	447690 ± 2768.40	0.62	5805536 ± 64169.65	1.11
1.1	458031 ± 2013.25	0.44	4384878 ± 41396.01	0.94

**(n = 3)*

Table 11. Results for change in wavelength

Wavelength (nm)	CPM (20 µg/ml)		ATT (80 µg/ml)	
	Mean area. ± SD	**% RSD**	**Mean area. ± SD**	**% RSD**
230	577853 ± 1998.15	0.35	5753454 ± 35235.42	0.61
232	445999 ± 3252.33	0.73	5805536 ± 48955.53	0.84
234	361192 ± 1018.62	0.28	5826102 ± 34411.38	0.59

**(n = 3)*

Table 12. Summary of Validation parameters

Sr. No.	Parameters	CPM	ATT
1	Linearity (n = 5)	5-25 µg/ml	20-100 µg/ml
2	Correlation coefficient	0.9988 %	0.9976 %
3	Accuracy (% Recovery) (n = 3)	99.56-101.46 %	99.22-101.87 %
4	Precision		
	Repeatability(n = 6)	0.66 %	0.41 %
	Intraday precision (n = 3)	0.28-0.39 &%	0.17-0.49 %
	Inter day precision (n = 3)	0.34-0.73 %	0.54-0.75 %

5	LOD(µg/ml)		0.14	0.13
6	LOQ(µg/ml)		0.42	0.39
7	Robustness	Change in flow rate	0.42-0.62	0.94-1.33
		Change in wavelength	0.28-0.73	0.59-0.84

Fig 1. Structure of Anetholetrithione

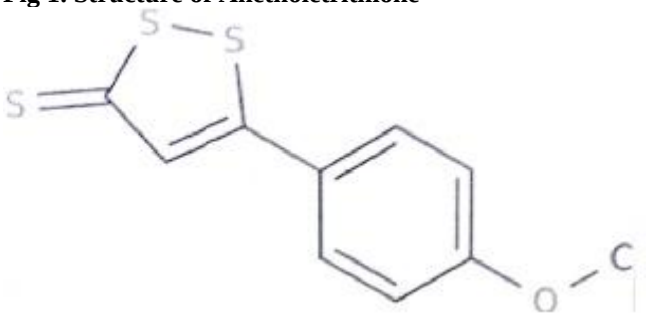


Fig 2. Structure of Chlopheniramine maleate

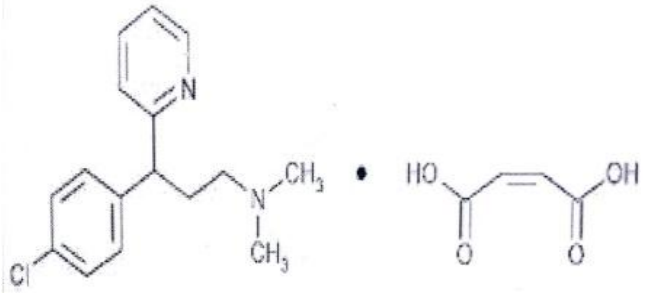


Fig 3. Chromatogram of optimized mobile phase

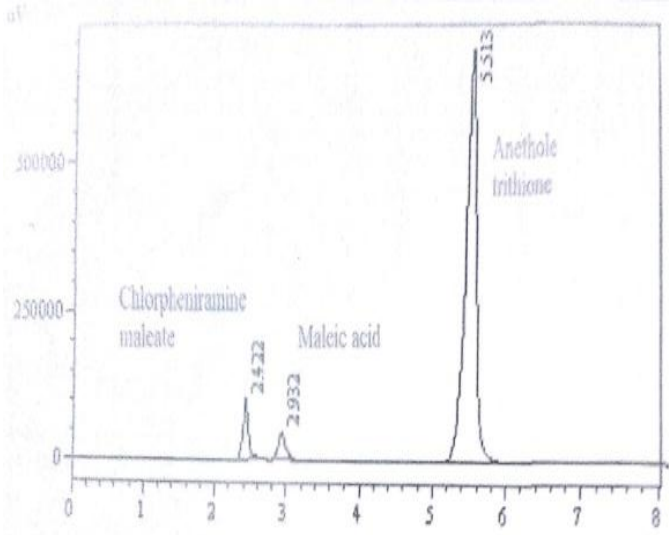


Fig 4. Chromatogram for standard solution of CPM(5µg/ml) and ATT (20 µg/ml)

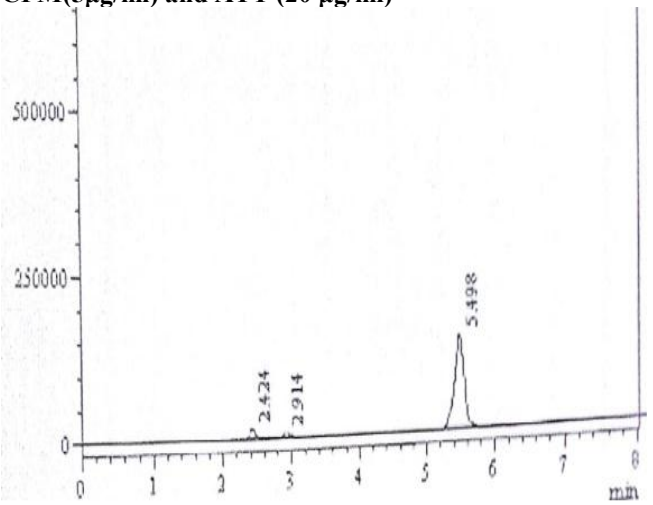


Fig 5.Chromatogram for standard solution of CPM(10µg/ml) and ATT (40µg/ml)

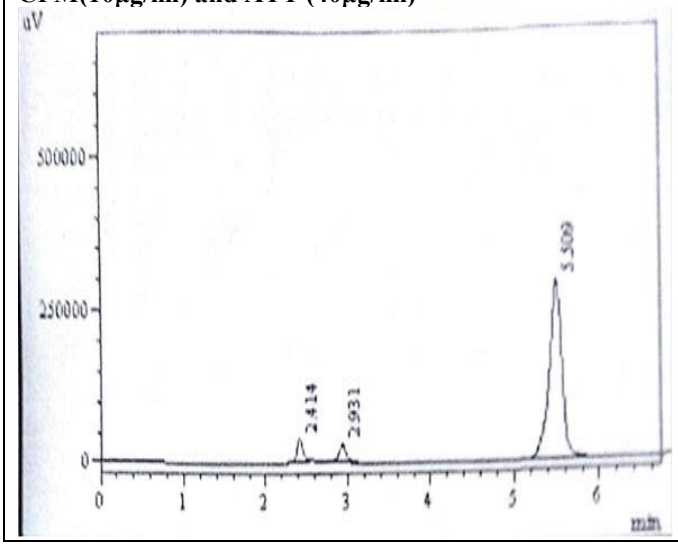


Fig 6. Chromatogram for standard solution of CPM(15µg/ml) and ATT (60µg/ml)

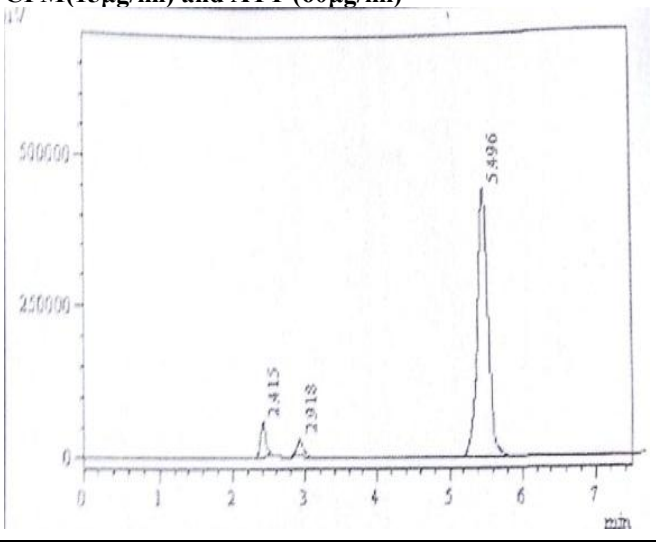


Fig 7. Chromatogram for standard solution of CPM(20µg/ml) and ATT (80µg/ml)

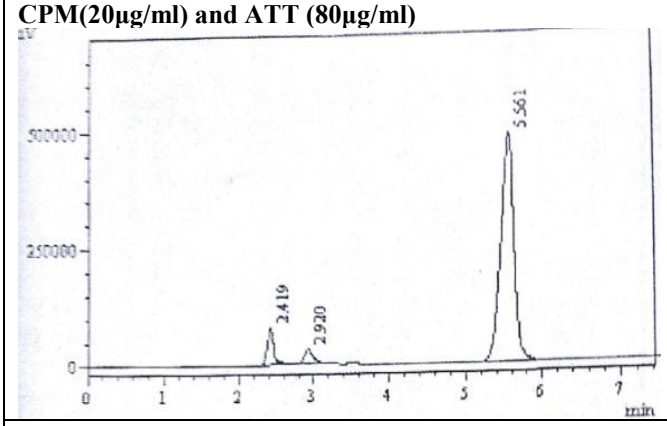


Fig 8. Chromatogram for standard solution of CPM (25µg/ml) and ATT (100µg/ml)

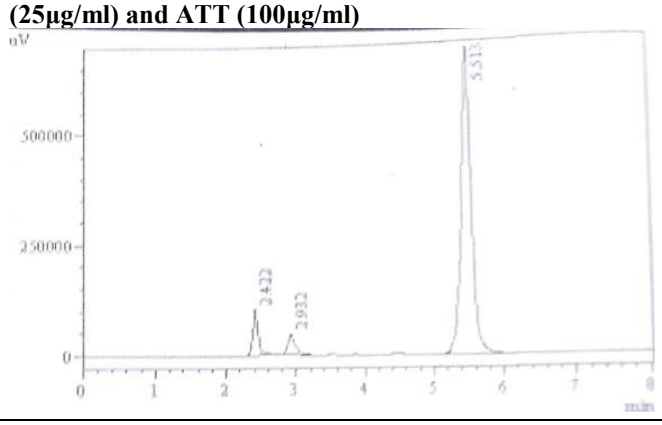


Fig 9. Calibration curve for CPM

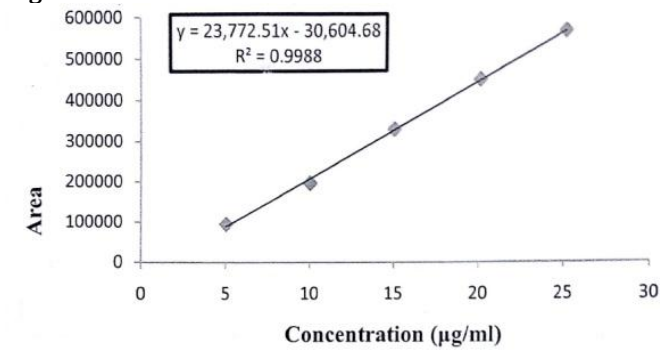


Fig 10. Calibration curve for ATT

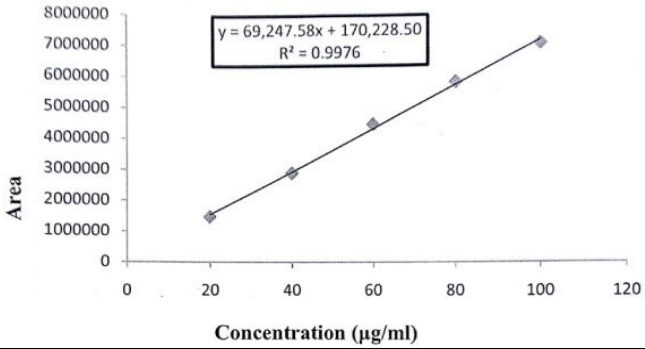


Fig 11. Chromatogram for standard solution of CPM (25µg/ml)

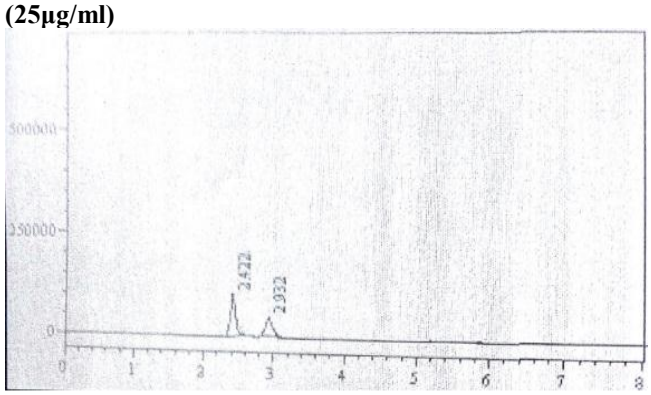


Fig 12. Chromatogram for standard solution of ATT (100µg/ml)

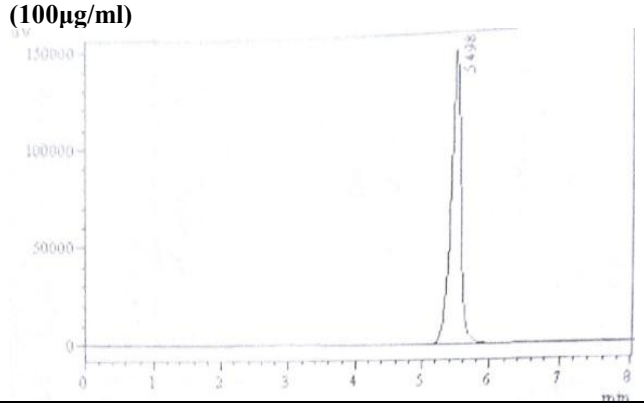


Fig 13. Chromatogram for standard solution of Maleic acid (100µg/ml)

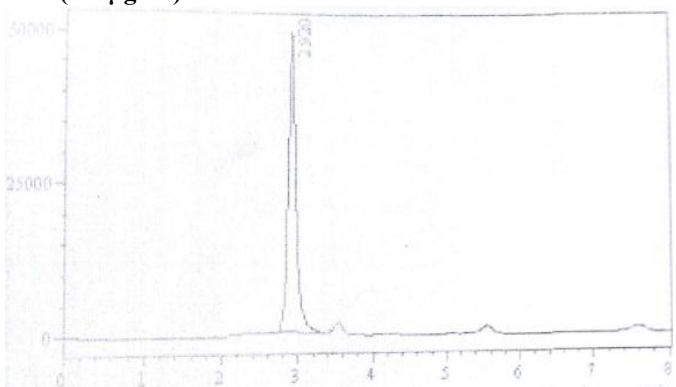


Fig 14. Chromatogram for blank

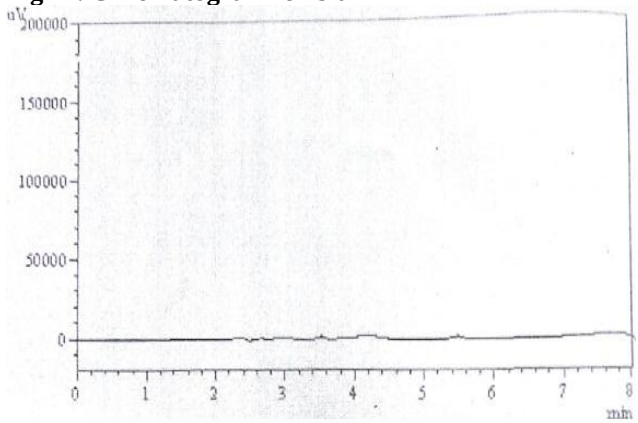
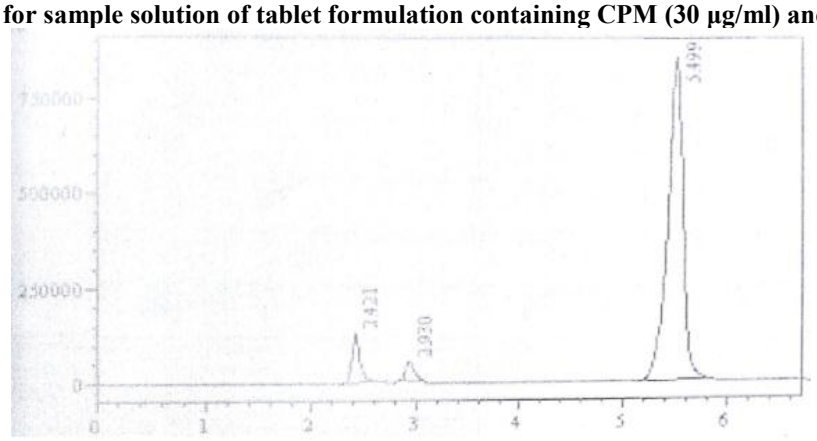


Fig 15. Chromatogram for sample solution of tablet formulation containing CPM (30 µg/ml) and ATT (125 µg/ml)



CONCLUSION

A valid and fast RP-HPLC method for simultaneous estimation of Trithioparamethoxyphenyl propene (Anetholetrithione) and Chlorpheniramine maleate in tablet dosage form is established. The developed method is simple, rapid, precise, accurate, sensitive, specific, and robust. Developed method can be used as quality-control tool for routine quantitative analysis of Anetholetrithione and chorpheniramine maleate.

ACKNOWLEDGEMENT: None

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Rong-qin Z. RP-HPLC Determination of AnetholeTrithione Tablets. *Medical information,* 2010,10.
2. Zunjian Z, *et al*. Determination of Anetholetrithione in human plasma using High performance liquid chromatography coupled with tandem mass spectrometric detection. *AnalyticaChimicaActa,* 594, 2007, 274–278.
3. Li W. HPLC determination of 4-hydroxy-anethole trithione in plasma via enzymatic hydrolysis and its application to bioequivalence study. *J Pharm Biomed Anal*, 47(3), 2008, 612-617.
4. Indian Pharmacopoeia. New Delhi: Government of India, ministry of health and family welfare, 2, 2008, 301-303.
5. USP. Asian Ed., United state Pharmacopoeial convention, Inc., Rockville, MD, 2007, 1726.
6. Koladiya R. UV Spectrophotometric methods for estimation of Chlorpheniramine Maleate (CPM) in pharmaceutical dosage form by absorption maxima method and area under curve. *Resear. Journ. Of Pharmaceu.,Biolog. And Chemi. Sciences*, 4(2), 2013, 760.
7. Kumarapu SK, "Method development and validation of UV Spectroscopic method for simultaneous estimation of Chlorpheniramine maleate and Diethylcarbamazine citrate in combined tablet dosage forms *Interna. Jour. of pharma. And bio scien*, 4(3), 2013, 216-223.
8. Sawant R. Mathematical and Multiwavelength Spectrophotometric Methods for Simultaneous Estimation of Paracetamol, Phenylephrine HCl, Chlorpheniramine maleate and Caffeine. *Intern. Jour. of pharma. Fronti. Research*, 1(2), 2011, 31-38.
9. Bhaskar D, "Development and validation of RP-HPLC method for simultaneous estimation of chlorpheniramine maleate and diethylcarbamazine citrate in pharmaceutical dosage forms, *Asian Journal of Pharma.& Clinical Research*, 7(3), 2014, 98.
10. Sehrawat R. Development and validation of RP-HPLC method for simultaneous estimation of phenylephrine hydrochloride and chlorpheniramine maleate in pharmaceutical dosage form. *Internationale pharmaceutica sciencia*, 3(2), 2013, 91-95.
11. Yin SF, Qui ZY. Simultaneous determination of ibuprofen and Pseudoephedri hydrochloride and Chlorpheniramine maleate in the compound buluoweimanami tablets. *HPLC*, 30(7), 2010, 1703-1704.
12. Maria A. Simultaneous Determination of Chlorpheniramine Maleate and Dexamethasone in a Tablet Dosage Form by Liquid Chromatography. *Journal of Associa. of office. Anal*. Chemi. international,88(6), 2005, 1677-1683.
13. Al-shaalan N, "Determination of phenylephrine hydrochloride and chlorpheniramine maleate in binary mixture using chemometric-assisted spectrophotometric and high-performance liquid chromatographic-UV methods. *Journal of Saudi chemical society*, 4(1), 2010, 15-21.