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Validated Stability Indicating HPLC Method for Simultaneous Quantification of Trithioparamethoxy Phenylpropene and Chlorpheniramine Maleate in Tablet Forms

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A novel easy stability indicating high performance liquid chromatography method has been developed for the concurrent assessment of trithioparamethoxy phenylpropene in combination with chlorpheniramine maleate using Luna C8 column with UV detection at 224 nm. The mobile phase comprised of 0.02 N phosphate buffer (pH 5.5) and acetonitrile (55:45 v/v) was delivered with a flow rate of 1.5 mL/min. The method was linear over the concentration range 6.25-18.75 µg/mL (trithioparamethoxy phenylpropene) and 1.5-4.5 µg/mL (chlorpheniramine maleate). The limit of quantification was 1.57 µg/mL (trithioparamethoxy phenylpropene) and 0.969 µg/mL (chlorpheniramine maleate). The calculated recoveries were 100.083-100.287% (trithioparamethoxy phenylpropene) and 99.827-100.277% (chlorpheniramine maleate). Trithioparamethoxy phenylpropene and chlorpheniramine maleate were subjected to forced stress like acid hydrolysis, base hydrolysis, thermal and oxidation degradation. The drugs were found to degrade in the applied conditions. The degraded products were resolved effectively from the trithioparamethoxy phenylpropene and chlorpheniramine maleate. The suggested stability-indicating HPLC method can be used for the quantitative evaluation of trithioparamethoxy phenylpropene and chlorpheniramine maleate in bulk medications and tablet formulations.

Keywords: Trithioparamethoxy phenylpropene, Chlorpheniramine maleate, Hepasulfol AA, HPLC.

INTRODUCTION

Chlorpheniramine maleate is an antiallergic agent belonging to antihistamines drug category [1-5]. Histamines are produced in the body upon exposure of the body to allergens like pollen, house dust, animal dander, *etc*. This results in runny nose, watery eyes, blocked nose, skin rashes, sneezing, itching, *etc*. Chlorpheniramine maleate works by inhibiting histamine′s function, thus alleviating these symptoms.

Trithioparamethoxy phenylpropene belongs to the drug class called hepatoprotective agent [6-8]. Trithioparamethoxy phenylpropene protects the liver against hepatotoxic agents such as fatty acids, alcohol, *etc*. Trithioparamethoxy phenylpropene reduces blood cholesterol and thereby causes smooth blood flow. Trithioparamethoxy phenylpropene is also prescribed to allay dry mouth and constipation that occurs due to the use of tranquilizers.

A Hepasulfol AA tablet is a fixed dose combination drug which is manufactured and marketed by Franco Indian Remedies, Mumbai, India [9,10]. This tablet is a combination of chlorpheniramine maleate (3 mg) and trithioparamethoxy phenylpropene (12.5 mg). Few analytical methods including UV spectrophotometry [11], chemometry [11], fluorescence spectrophotometry [12] and HPLC [13] are proposed to quantify chlorpheniramine maleate alone in pharmaceutical samples. Any method not proposed to quantify trithioparamethoxy phenylpropene alone. According to literature review, no approaches are suggested to quantify chlorpheniramine maleate and trithioparamethoxy phenylpropene simultaneously in tablet forms.

The stability indicating approach is described as a quantitative method that would detect changes in the physical, chemical or microbiological properties of the drug or drug product over time [14-16]. This approach is precise and accurate so that active ingredient content can be determined precisely and accurately without intervention from degradants or impurities. Stability testing also provides information about mechanism of drug degradation, possible degradation products and interaction

between the drug and drug product excipients. Based on the facts a stability indicating method is necessary. According to online literature review, no stability indicating method to quantify chlorpheniramine maleate and trithioparamethoxy phenylpropene simultaneously in tablet forms was reported. Therefore, the aim of this study was to develop a stability indicating method using HPLC technique for the analysis of chlorpheniramine maleate and trithioparamethoxy phenylpropene in bulk and in its combined dosage form.

EXPERIMENTAL

The reference pure samples of trithioparamethoxy phenylpropene with 99.3% purity and chlorpheniramine maleate with 99.1% purity were received from Aurobindo Pharma Ltd., Hyderabad, India. Hepasulfol AA tablets labeled to have 3 mg of chlorpheniramine maleate and 12.5 mg of trithioparamethoxy phenylpropene were acquired from local pharmaceutical store. Water of HPLC class was acquired from Milli-Q water system. HPLC class acetonitrile, analytical class potassium dihydrogen phosphate, hydrochloric acid, orthophosphoric acid, hydrogen peroxide and sodium hydroxide were obtained from Qualigens Fine Chemicals Ltd., Mumbai, India.

Instrumentation and chromatography conditions: The method development and validation for the simultaneous analysis of trithioparamethoxy phenylpropene and chlorpheniramine maleate were performed on a Waters Alliance HPLC system integrated with degasser, auto sampler and UV detector. The separation followed by analysis of trithioparamethoxy phenylpropene and chlorpheniramine maleate was done in Luna C8 column (150 mm × 4.6 mm, 5 µm). The mobile phase composed of acetonitrile and 0.02 N KH_2PO_4 buffer (5.5 pH) in 45:55 (v/v) ratio. The mobile phase was filtered, sonicated and delivered at 1.5 mL/min flow rate. The column temperature and wavelength for detection was set at ambient temperature and 224 nm, respectively. The quantity of injection during analysis was 10 µL.

Preparation of standard solutions: Stock trithioparamethoxy phenylpropene (125 µg/mL) and chlorpheniramine maleate (30 µg/mL) solution was prepared. For this accurately weighed and transferred 12.5 mg of trithioparamethoxy phenylpropene and 3 mg of chlorpheniramine maleate into a 100 mL dry and clean volumetric flask. Added about 75 mL of diluent (mobile phase) and sonicated to dissolve completely. Make the volume to 100 mL mark with diluent.

Five calibration solutions of concentration range 6.25-18.75 µg/mL (trithioparamethoxy phenylpropene) and 1.5-4.5 µg/mL (chlorpheniramine maleate) were prepared by proper dilution of above stock solution (trithioparamethoxy phenylpropene 125 µg/mL and chlorpheniramine maleate 30 µg/mL) with diluent.

Working solutions with concentration for 12.5 µg/mL trithioparamethoxy phenylpropene and 3 µg/mL chlorpheniramine maleate for validation study were prepared by proper dilution of above stock solution (trithioparamethoxy phenylpropene 125 µg/mL and chlorpheniramine maleate 30 µg/mL) with diluent.

Sample solution preparation: Accurately weighed and transfer powder sample equivalent to 12.5 mg of trithioparamethoxy phenylpropene and 3 mg of chlorpheniramine maleate into a 100 mL dry and clean volumetric flask. Added about 75 mL of diluent (mobile phase) and sonicated to dissolve completely. Make the volume to 100 mL mark with diluent. This produces the stock solution with amount 125 µg/mL of trithioparamethoxy phenylpropene and 30 µg/mL of chlorpheniramine maleate. Further, for analysis, pipette 10 mL of above solution into a 100 mL volumetric flask and make the volume to 100 mL mark with diluent. Thus a test solution with amount 12.5 µg/mL of trithioparamethoxy phenylpropene and 3.0 µg/mL of chlorpheniramine maleate was prepared.

Calibration curve: Injected 10 µL aliquot of each calibration solution into the system. Measured the peak areas of trithioparamethoxy phenylpropene and chlorpheniramine maleate for all the calibration solutions. The calibration curve was constructed by plotting response against respective concentration of trithioparamethoxy phenylpropene and chlorpheniramine maleate. Using peak area and concentration data, regression equation was also determined.

Assay of trithioparamethoxy phenylpropene and chlorpheniramine maleate in tablets: About 10 µL aliquot of test solution prepared was injected three times into the system. The peak areas of trithioparamethoxy phenylpropene and chlorpheniramine maleate were measured. The content of trithioparamethoxy phenylpropene and chlorpheniramine maleate in tablets was quantified using calibration curve or regression equation.

Degradation studies: International conference on harmonization regulations were followed during degradation studies [17].

Acid hydrolysis: A 10 mL of tablet stock solution (125 µg/mL trithioparamethoxy phenylpropene and 30 µg/mL chlorpheniramine maleate) was pippetted into a 100 mL dry and clean volumetric flask. Then added 10 mL of 5 N HCl and refluxed for 60 min at 60 ºC. The solution was cooled to room temperature, neutralized with sufficient volume of 5 N NaOH and make volume to 100 mL mark with diluent. Filter the solution through 0.45 µm syringe filter.

Base hydrolysis: A 10 mL of tablet stock solution (125 µg/mL trithioparamethoxy phenylpropene and 30 µg/mL chlorpheniramine maleate) and 10 mL of 5 N NaOH were pippetted into a 100 mL volumetric flask. The contents were refluxed up to 60 min with temperature adjusted at 60 ºC. Then the solution was cooled followed by neutralization by adding sufficient quantity of 5 N HCl. Make the volume in the flask to 100 mL mark with diluent. Filter the solution through 0.45 µm syringe filter.

Oxidative degradation: A 10 mL of tablet stock solution (125 µg/mL trithioparamethoxy phenylpropene and 30 µg/mL chlorpheniramine maleate) was pippetted into a 100 mL dry and clean volumetric flask. Then added 10 mL of 30% H_2O_2 and refluxed for 60 min at 60 ºC. The solution was cooled to room temperature make volume to 100 mL mark with diluent. Filter the solution through 0.45 µm syringe filter.

Thermal degradation: This was performed by placing the tablets in oven set at 105 ºC for about 48 h. After the degradation period, the solution was prepared as described above.

A 10 µL aliquot of degradation samples produced were injected into the sytem. The peak areas of trithioparamethoxy

phenylpropene and chlorpheniramine maleate were measured. The peak areas of degradation samples were compared with peak areas of control (undegraded sample) to assess the percent degradation and percent assay of trithioparamethoxy phenylpropene and chlorpheniramine maleate.

RESULTS AND DISCUSSION

Method optimization: The aim of this study was to develop a stability indicating method using HPLC technique for the analysis of chlorpheniramine maleate and trithioparamethoxy phenylpropene with good sensitivity, accuracy, selectivity and precision. Luna C8 column (150 mm × 4.6 mm, 5 µm) column with temperature adjusted to ambient temperature provided good resolution and peak shapes for trithioparamethoxy phenylpropene and chlorpheniramine maleate. The mobile phase with acetonitrile (45% volume) and 0.02 N KH_2PO_4 buffer of 5.5 pH (55% volume) delivered at 1.5 mL/min flow rate and wavelength detection set at 224 nm provided good sensitivity, good peak shape with relatively less retention time was obtained. Therefore, the same conditions were chosen as optimized conditions for the assay of trithioparamethoxy phenylpropene and chlorpheniramine maleate simultaneously. The retention times of trithioparamethoxy phenylpropene and chlorpheniramine maleate were 8.630 min and 2.329 min, respectively (Fig. 1). Theoretical plates (6528 for trithioparamethoxy phenylpropene; 7524 for chlorpheniramine maleate) and tailing factor (1.0 for trithioparamethoxy phenylpropene; 1.1 for chlorpheniramine maleate) were also found to be acceptable. These values proved the system suitability to analyze trithioparamethoxy phenylpropene and chlorpheniramine maleate simultaneously.

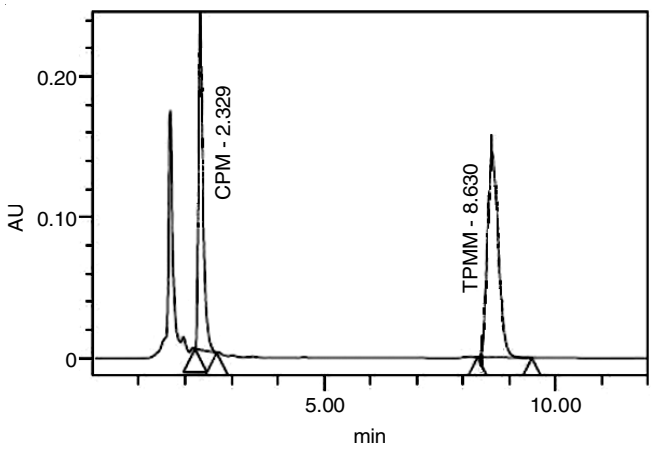

Fig. 1. Chromatogram of trithioparamethoxy phenylpropene (TPPM) and chlorpheniramine maleate (CPM)

Method validation: All experimental validity parameters are assessed in compliance with International conference on harmonization guidance for the proposed method [18].

Linearity: The linearity for the proposed method was determined at five concentration levels ranging from 6.25-18.75 µg/mL for trithioparamethoxy phenylpropene and 1.5-4.5 µg/mL for chlorpheniramine maleate. The calibration curve was constructed by plotting response against respective concentration of trithioparamethoxy phenylpropene and chlorpheniramine maleate. The plots of peak area *versus* respective concentration were found linear in above said range with coefficient of correlation (r^2) 0.9998 and 0.9996 for trithioparamethoxy phenylpropene and chlorpheniramine maleate, respectively. The linearity was tested using regression analysis. Calculated slope and intercept for trithioparamethoxy phenylpropene and chlorpheniramine maleate are shown in Fig. 2.

Limits of quantification and detection: Limits of quantification and detection were used to test the sensitivity of the method. Limits of quantification and detection are calculated as below:

$$\text{Limit of detection} = \frac{\text{Regression line standard deviation}}{\text{Linearity plot slope}} \times 3.3$$

$$\text{Limit of quantification} = \frac{\text{Regression line standard deviation}}{\text{Linearity plot slope}} \times 10$$

The limits of detection and quantification were 0.52 µg/mL and 1.57 µg/mL for trithioparamethoxy phenylpropene and 0.32 µg/mL and 0.969 µg/mL for chlorpheniramine maleate, respectively. The values revealed that the system was sensitive for the evaluation of chlorpheniramine maleate and trithioparamethoxy phenylpropene.

Selectivity: Analysis was performed with working solution (trithioparamethoxy phenylpropene 125 µg/mL and chlorpheniramine maleate 30 µg/mL), placebo sample (contains only excipients) mobile phase blank (diluent without trithioparamethoxy phenylpropene and chlorpheniramine maleate). It was noticed after evaluation that there is no peak interference in placebo and mobile phase blank at the trithioparamethoxy phenylpropene and chlorpheniramine maleate region. The approach developed for the intended use was thus selective.

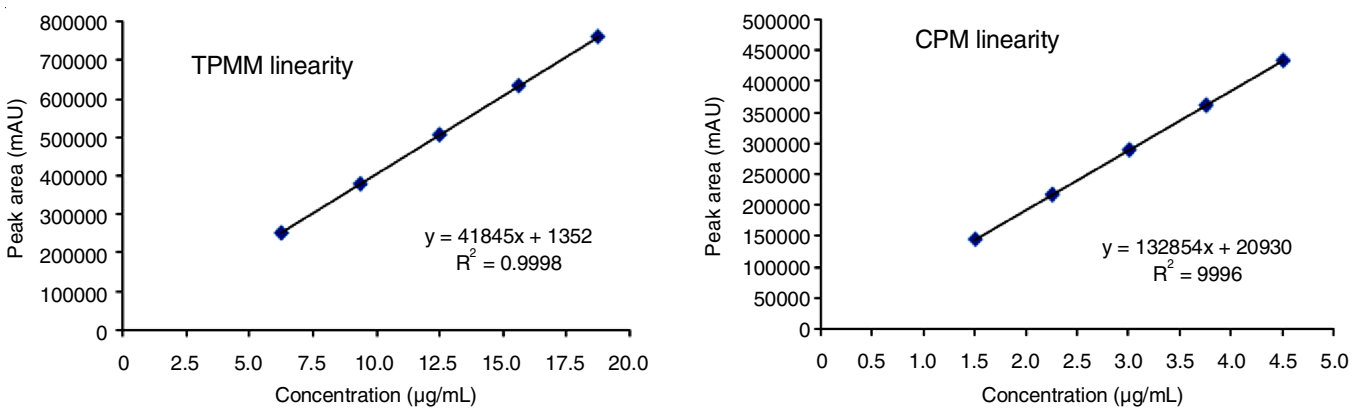

Fig. 2. Linearity plots of trithioparamethoxy phenylpropene (TPPM) and chlorpheniramine maleate (CPM)

Precision: Working solution (trithioparamethoxy phenyl propene 12.5 µg/mL and chlorpheniramine maleate 3.0 µg/mL) has been six times analyzed with proposed method on the same day for intraday accuracy. While for inter day precision, working solution (trithioparamethoxy phenylpropene 12.5 µg/mL and chlorpheniramine maleate 3.0 µg/mL) was analyzed on three different days. The relative standard deviation values were determined (Table-1). Low values of relative standard deviation (< 2%) depicted a high precision of the proposed method.

Accuracy: Accuracy was performed on tablet sample solution spiked with pure trithioparamethoxy phenylpropene and chlorpheniramine maleate at 50, 100 and 150% levels. The percent recovery values of trithioparamethoxy phenylpropene and chlorpheniramine maleate at each level were determined (Table-2). Good values of recovery (close to 100%) depicted a high accuracy of the proposed method.

Robustness: Robustness of the method was determined by evaluating working solution (trithioparamethoxy phenylpropene 12.5 µg/mL and chlorpheniramine maleate 3.0 µg/mL) with small intentional alterations in mobile phase pH, flow rate and acetonitrile ratio in mobile phase. Such changes did not adversely affect the assay of trithioparamethoxy phenylpropene and chlorpheniramine maleate as obvious from the low relative standard deviation values (< 2%). This suggested robustness of the method (Table-3).

Stability of working standard and table sample solutions: So as to check the stability of standard and sample solutions, both solutions were analyzed over a phase of 24 h at room temperature. For standard and sample solutions, the retention time and peak area of trithioparamethoxy phenylpropene and chlorpheniramine maleate remained approximately identical. There was no noticeable degradation within the time specified, so both solutions remained stable for at least 24 h, which was enough to complete the entire analytical process.

Degradation studies: The chromatograms of trithioparamethoxy phenylpropene and chlorpheniramine maleate after degradation process are shown in Fig. 3a-d. Trithioparamethoxy phenylpropene and chlorpheniramine maleate degradation was observed in all applied conditions. Table-4 summarizes the percent recovery and percent degradation of trithioparamethoxy phenylpropene and chlorpheniramine maleate after degradation. Well resolution of degradation products from trithioparamethoxy phenylpropene and chlorpheniramine maleate peak shows that the method is stability indicating and specific to analyze trithioparamethoxy phenylpropene and chlorpheniramine maleate.

Assay of trithioparamethoxy phenylpropene and chlorpheniramine maleate in Hepasulfol AA tablets: Applicability of proposed method was checked by analyzing the content of trithioparamethoxy phenylpropene and chlorpheniramine maleate in Hepasulfol AA tablets. The data from analysis of Hepasulfol AA tablets are shown in Table-5. The average amount of trithioparamethoxy phenylpropene and chlorpheniramine maleate in hepasulfol tablet was 12.526 mg and 3.022 mg

TABLE-1
PRECISION OF TRITHIOPARAMETHOXY PHENYLPROPENE AND CHLORPHENIRAMINE MALEATE

Drug	Amount analyzed (µg/mL)	Intra-day precision			Inter-day precision		
		Mean found (µg/mL)	SD	RSD (%)	Mean found (µg/mL)	SD	RSD (%)
Trithioparamethoxy phenylpropene	12.5	12.499	0.041	0.332	12.479	0.033	0.268
Chlorpheniramine maleate	3	3.008	0.012	0.427	2.994	0.009	0.320

TABLE-2
ACCURACY OF TRITHIOPARAMETHOXY PHENYLPROPENE AND CHLORPHENIRAMINE MALEATE

Level spiked	Amount of drug (mg)			Recovery (%)	Mean recovery (%)	RSD (%)
	In tablet	Added	Total found			
Trithioparamethoxy phenylpropene						
50%	12.5	6.25	18.849	100.53	100.260	0.072
	12.5	6.25	18.716	99.82		
	12.5	6.25	18.831	100.43		
100%	12.5	12.5	24.940	99.76	100.287	0.137
	12.5	12.5	25.060	100.24		
	12.5	12.5	25.215	100.86		
150%	12.5	18.75	31.422	100.55	100.083	0.155
	12.5	18.75	31.294	100.14		
	12.5	18.75	31.113	99.56		
Chlorpheniramine maleate						
50%	3	1.5	4.512	100.27	100.227	0.016
	3	1.5	4.493	99.85		
	3	1.5	4.525	100.56		
100%	3	3	5.972	99.54	99.873	0.019
	3	3	6.011	100.18		
	3	3	5.994	99.9		
150%	3	4.5	7.475	99.66	99.827	0.035
	3	4.5	7.526	100.35		
	3	4.5	7.460	99.47		

TABLE-3
ROBUSTNESS OF TRITHIOPARAMETHOXY PHENYLPROPENE (TPMM) AND CHLORPHENIRAMINE MALEATE (CPM)

Value tested	Concentration of TPMM (µg/mL)		RSD (%)	Concentration of CPM (µg/mL)		RSD (%)
	Taken	Found		Taken	Found	
Alternation in pH						
5.4	12.5	12.495		3	2.997	
5.5	12.5	12.508	0.257	3	2.956	0.725
5.6	12.5	12.556		3	2.965	
Alternation in flow rate						
1.4	12.5	12.495		3	3.005	
1.5	12.5	12.445	0.404	3	3.012	0.338
1.6	12.5	12.546		3	2.992	
Alternation in acetonitrile ratio						
40	12.5	12.564		3	3.019	
45	12.5	12.459	0.463	3	2.996	0.384
50	12.5	12.554		3	3.006	

TABLE-4
DEGRADATION DATA OF TRITHIOPARAMETHOXY PHENYLPROPENE (TPMM) AND CHLORPHENIRAMINE MALEATE (CPM)

Stress conditions	Peak area (mAU)		Degradation (%)		Drug remained after degradation (%)	
	TPMM	CPM	TPMM	CPM	TPMM	CPM
Control	507550	288951	–	–	–	–
Acid	441521	263385	13.009	8.848	86.991	91.152
Base	452092	257522	10.927	10.877	89.073	89.123
Peroxide	417586	254189	17.725	12.030	82.275	87.970
Thermal	445624	257373	12.201	10.928	87.799	89.072

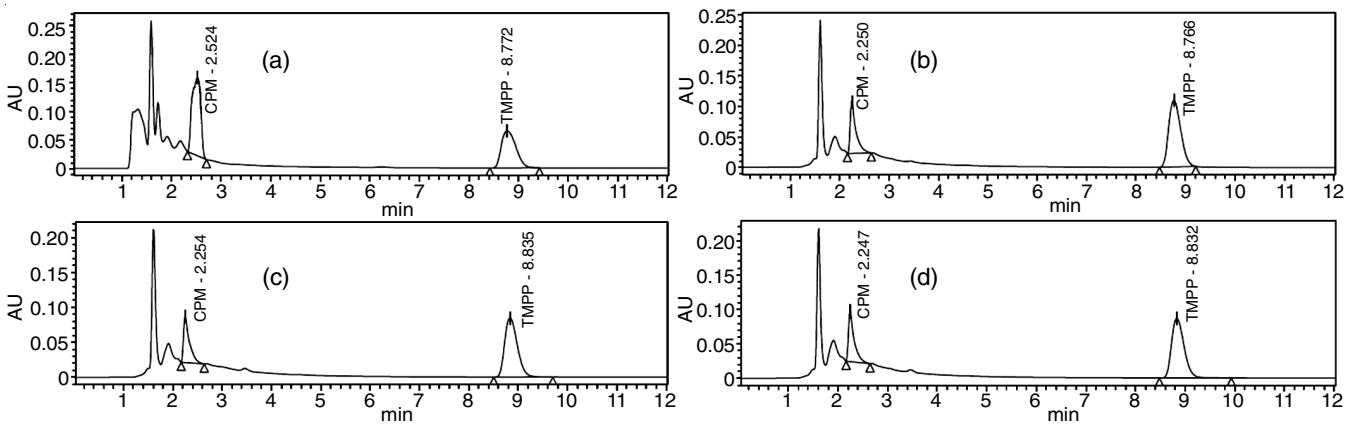

Fig. 3. Chromatogram of trithioparamethoxy phenylpropene (TPPM) and chlorpheniramine maleate (CPM) degraded by (a) acid (b) base (c) peroxide and (d) thermal

TABLE-5
ASSAY RESULT OF TABLET FORMULATION

Drug	Label strength (mg)	Amount found (mg)*	Assay (%)	RSD (%)
TPMM	12.5	12.526	100.208	0.029
CPM	3.0	3.022	100.744	0.188

TPMM = Trithioparamethoxy phenylpropene; CPM = Chlorpheniramine maleate; *Average of three values

equivalent to 100.2085 and 100.744% of the label claim. The percent recovery and relative standard deviation were calculated and falls inside the requirements for the assay.

Conclusion

This study presents a simple stability indicating HPLC method for the simultaneous assessment of trithioparamethoxy phenylpropene and chlorpheniramine maleate in tablets and raw bulk samples. The method was linear in stated range and sensitive enough. The developed method proved as selective, accurate, precise and robust. The degradation products generated during forced stress studies are well separated from the trithioparamethoxy phenylpropene and chlorpheniramine maleate peaks demonstrating the specificity and stability indicating power of the proposed method. The method proposed could be applied with success to the evaluation of marketed products of trithioparamethoxy phenylpropene and chlorpheniramine maleate combined tablet formulation. No interference was observed because of excipients.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. G.A. Jalbani, K. Aamir, A.M. Shaikh, M.A. Unar, R. Ashraf and F.M. Soomro, *J. Pak. Med. Assoc.*, **54**, 556 (2004).
2. J.M. Serra-Grabulosa, M. Sánchez-Turet and C. Grau, *Rev. Neurol.*, **34**, 1178 (2002).
3. W. Xu, S. Xia, J. Pu, Q. Wang, P. Li, L. Lu and S. Jiang, *Front. Microbiol.*, **9**, 2643 (2018); https://doi.org/10.3389/fmicb.2018.02643
4. P. Assanasen and R.M. Naclerio, *Clin. Allergy Immunol.*, **17**, 101 (2002).
5. F. Kalpaklioglu and A. Baccioglu, *Antiinflamm. Antiallergy Agents Med. Chem.*, **11**, 230 (2012); https://doi.org/10.2174/1871523011202030230
6. S. Sharma, Trithioparamethoxy Phenylpropene. 1mg.com. Accessed on October 2019. Available at: https://www.1mg.com/generics/trithioparamethoxy-phenylpropene-211206.
7. Trithioparamethoxy Phenylpropene - Uses, Side-effects, Reviews, and Precautions. Tabletwise. Accessed on October 2019. Available at: https://www.tabletwise.com/medicine/trithioparamethoxy-phenylpropene.
8. M.D. Sadullah and V. Chauhan, Trithioparamethoxy Phenylpropene information. Myupchar. Accessed on October 2019. Available at: https://www.myupchar.com/en/medicine/trithioparamethoxy-phenylpropene-p37141776#benefits.
9. A.A. Hepasulffol, Health potlio. Accessed on October 2019. Available at: http://healthpotli.com/productdetails.aspx?q=P00006863.
10. A.A. Hepasulfol, 12.5 mg/3 mg Tablet. 1mg.com. Accessed on October 2019. Available at: https://www.1mg.com/drugs/hepasulfol-aa-12.5-mg-3-mg-tablet-120158.
11. P. Valderrama, A.L. Romero, P.M. Imamura, I.R.S. Magalhães, P.S. Bonato and R.J. Poppi, *Anal. Bioanal. Chem.*, **397**, 181 (2010); https://doi.org/10.1007/s00216-010-3472-7
12. S.N.H. Azmi, S.S.K. Al-Hadhrami, B.M.R. Al-Marhoubi, S.S.S. Al-Sulaimi and Z.D.S. Al-Shamoosi, *J. Mol. Liq.*, **243**, 750 (2017); https://doi.org/10.1016/j.molliq.2017.08.081
13. M. Karikalan, M.G.R. Priya and P. Shanmugapandiyan, *Int. J. Pharma BioSci.*, **7**, 240 (2016).
14. G. Ngwa, *Drug Deliv. Technol.*, **10**, 56 (2010).
15. R. Singh and Z. Rehman, *J. Pharm. Educ. Res.*, **3**, 54 (2012).
16. S.B. Kanthale, S.S. Thonte and B.B. Supekar, *Int. J. Pharm. Sci. Res.*, **10**, 1625 (2019).
17. International Conference on the Harmonization, ICH Harmonized Tripartite Guideline, Stability Testing of New Drug Substances and Products Q1A (R2) (2003).
18. International Conference on the Harmonization, ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2 (R1) (2005).