Separation and Determination of impurities in Chlorpheniramine Maleate and Dextromethorphan Hydrobromide in combined dosage form by using RP-HPLC.

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Abstract

It is unique, Robust, Rapid, Precise, Accurate, Selective and Reproducible RP-HPLC method for simultaneous determination of Chlorpheniramine Maleate, Dextromethorphan Hydrobromide and their related impurities in Cough syrup. This is unique method for determination of impurities in combined dosage form. The separation achieved using phosphate buffer pH 7.20 as mobile phases-A and Mixture of Acetonitrile and methanol as mobile phases on gradient method. The detection wavelength is 225 nm. Flow rate is 0.8mL/minute. Linearity was obtained in the range of LOQ to 150% of limit level concentration, 0.05 µg/mL-0.75 µg/ml for each Dextromethorphan HBr impurity A, B, C and D. The separation achieved on column YMC Triart C18, 250x 4.6 mm, 5µ. The column oven temperature was 40°C and injection volume was 50µL. The Correlation coefficient for all impurities found 1.000. According to ICH guideline Q2(R1) [20], the Method were validated. Using this Chromatographic method, the Impurities determination of both the drug can be achieved easily in single method.

Keywords: Related substance, Chlorpheniramine Maleate, Dextromethorphan HBr, Validation;

1. Introduction

Lot of methods are available for the determination of assay of Dextromethorphan Hydrobromide (DXM) and Chlorpheniramine Maleate (CPM) for individual and or in other combination [1-9,11,19]. At present there is no single method available which can determine the known impurities in single analytical method in combination DXM and CPM dosage form. But this is the unique method which is capable to determine the

impurities in combined dosage form in single injection. Chlorpheniramine Maleate and Dextromethorphan Hydrobromide are used in the treatment of dry cough.

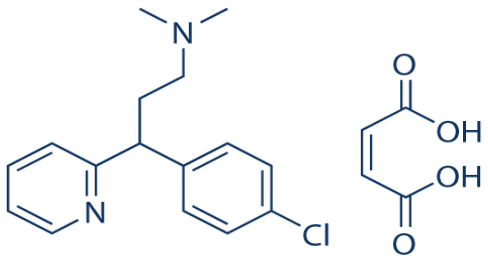
Dextromethorphan HBr (9S,13S,14S)-3-Methoxy-17-methylmorphinan hydrobromide is a levorphanol derivative and codeine analog commonly used as a cough suppressant. It exhibits antitussive activity and is devoid of analgesic or addictive property [12]

Chlorpheniramine Maleate [3-(4-Chlorophenyl)-N,N-dimethyl-3-(pyridin-2-yl)-propan-1-amine] is a histamine-H1 receptor antagonist indicated for the management of symptoms associated with upper respiratory allergies [13].

Dextromethorphan HBr and Chlorpheniramine Maleate combined syrup is used for the treatment of common cold, allergies, anaphylactic shock. It works by reducing the activity of cough center in the brain [14-15]

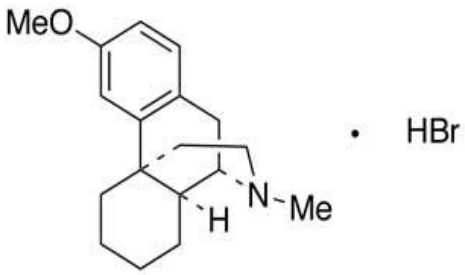
Chlorpheniramine Maleate and Dextromethorphan Hydrobromide individual monograph is official in USP and in Ph.Eur [10].

Figure 1. Structure of chlorpheniramine Maleate: $C_{16}H_{19}ClN_2.C_4H_4O_4$ [16]



a) Chlorpheniramine Maleate (CPM)

Figure 2. Dextromethorphan HBr: $C_{18}H_{28}BrNO_2$ [17]



b) Dextromethorphan Hydrobromide (DXM)

Figure. 1 & 2 Chemical structure of a) CPM b) DXM.

2. Materials and methods

➢ Equipment used

Waters liquid chromatographic system having UV/PDA detector was used for separation. YMC Triart C18, 250 mm X 4.6 mm, 5µ Column with flow rate 0.8 mL/min with gradient run was used to achieve the separation. Mettler Toledo AB265-S/FACT Balance and pH meter used for the work.

➢ Reagents and chemicals

Dextromethorphan Hydrobromide and Chlorpheniramine Maleate active ingredient received from Scientia Qualitek®. Navi Mumbai and syrup (Tossex DMR Syrup) with of 2mg of Chlorpheniramine Maleate and 10mg of Dextromethorphan Hydrobromide in each 5 mL of syrup Manufactured by ABBOTT INDIA LTD were purchase from local Pharmacy store. Chromatographic grade Solvents such as Acetonitrile, Methanol and water were manufactured by Merck and Rankem and same is used to perform the analysis.

➢ Chromatographic conditions

The combine sodium dihydrogen phosphate and disodium hydrogen phosphate with pH 7.20 buffer and mixture of Acetonitrile and Methanol 20: 80 v/v were used as mobile phase-A and B respectively. The

mixture water and Acetonitrile 85:15 v/v used as diluent. The detection wavelength fixed at 225nm. The column temperature kept 40°C and injection volume was 50µL.

- **Preparation of Mobile phase**

Weigh 2.85 g of disodium hydrogen phosphate and 2.75 g of sodium dihydrogen phosphate transfer in to 1000 ml of water, sonicate to dissolve. Adjust pH of the solution to 7.20 with triethylamine. Filter through 0.45µ nylon filter. This was used as mobile phase-A. Mixture of Acetonitrile and methanol in ratio 20:80 v/v was used as mobile phase-B.

Gradient:

Time in min	0	5	15	30	55	65	70	72	80
Mobile phase-A (%)	65	65	50	40	40	30	30	65	65
Mobile phase-B (%)	35	35	50	60	60	70	70	35	35

- **Diluent:**

Prepared Mixture of Water: Methanol (85:15 v/v)

- **Preparation of Standard solutions**

Weigh 40 mg of Dextromethorphan Hydrobromide and Chlorpheniramine Maleate and transfer into 200 ml of volumetric flask. Add

150 ml diluent sonicate to dissolve and make up to the mark with diluent. Dilute 5 ml of above solution to 200 ml with diluent. Final concentration will be 5 ppm for both CPM and DXM.

- **Preparation of Sample Solution**

Each 5 mL of syrup contains 2mg of CPM and 10 mg of DXM. Diluted 5 mL of syrup to 20 mL with diluent having final concentration 100 ppm of CPM and 500 ppm of DXM [18].

- **Enhancement of RP-HPLC method**

The Separation of Dextromethorphan HBr known impurities in Dextromethorphan Hydrobromide and Chlorpheniramine Malate achieved after various trials. Number of parameters evaluated to resolve Impurity-A, B, C and D such as various pH, Buffer, organic composition, Gradient. Lastly the suitable methodology established on combined phosphate buffer with pH 7.20 and mixture of Acetonitrile and Methanol using YMC Triart C18, 250x 4.6 mm, 5µ.

- **Validation of the RP-HPLC method**

Developed method has been validation using ICH Q2 (B) guidelines.

- **System suitability**

Six replicate injection of Standard solution of DXM and CPM, 5ppm each injected under the HPLC system. Calculated the %RSD, Plate count for chlorpheniramine peak. Refer Table 1 for results.

- **Specificity**

Specificity of method proved by injecting the Blank solution, Placebo solution, individual impurity solution, spiked sample solution and followed by performing the forced degradation study.

No peak observed due to the blank and placebo at RT of DXM and CPM. All impurities are well separated from each other.

Chromatogram of blank, placebo and sample shown in Figure 3(a), 3(b) and 3(c) respectively.

- **Linearity**

Demonstrated the linearity of developed method, CPM, DXM and impurity-A, B, C and D from LOQ to 150%. For CPM and DXM the linearity range is covered from 0.1ppm-7.6ppm and 0.25ppm-7.3ppm respectively. For the impurities A, B, C and D covered the linearity range from 0.5ppm-3.75ppm. Refer Table 2, 3 and 4 for Linearity of impurities, active CPM and DXM respectively.

- **Precision**

To determine the method precision, analyzed six Spiked samples. Evaluated the method precision by computing relative standard deviation. Refer Table 5 and 6 for Precision results.

- **Accuracy and Range**

The accuracy of the analytical method proved via preparing recovery samples by spiking known quantities of impurities from LOQ to 150% of targeted concentration to sample solution. Refer Table 7 for Accuracy and Range

- **Robustness**

Performed robustness study for pH of mobile phase i.e. 7.25±0.05. Refer Table 8 for results.

- **Forced degradation study**

Forced degradation study perform on sample and placebo. Forced degradation study performed on Acid, Base, Peroxide, Thermal and Photolytic degradation. Refer Table 9 for results.

- **Assay of Marketed Formulations**:

Performed analysis in triplicate. Calculated the amount of both drugs. Refer Chromatogram given in Figure 4. And for results refer Table 10.

3. Results: All the results are summarized below.

- **System suitability**

Table 1: System suitability of CPM and DXM

Parameter	CPM	DXM
Tailing factor (T)	1.5	1.4
Theoretical Plate (N)	25067	12321
%RSD of Area	1.6	3.7

- **Specificity**

Figure 3(a). Chromatogram of Blank

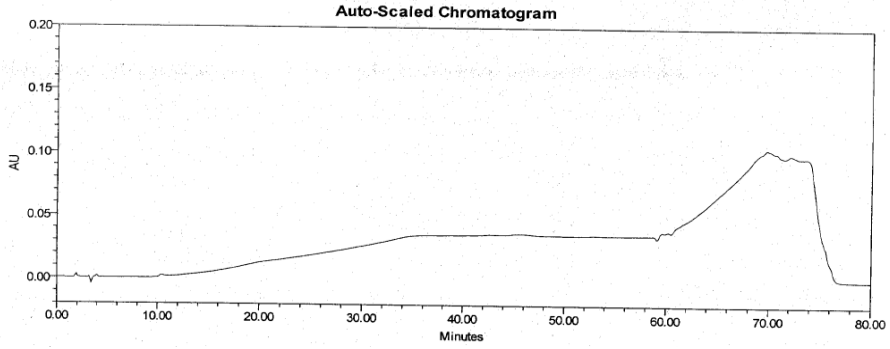


Figure 3 (b). Chromatogram of Placebo

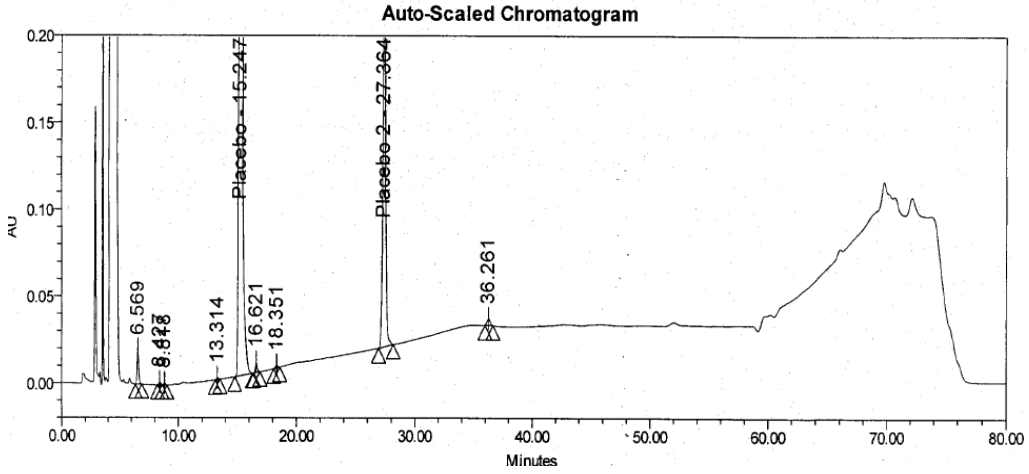


Figure 3 (c). Chromatogram of Sample

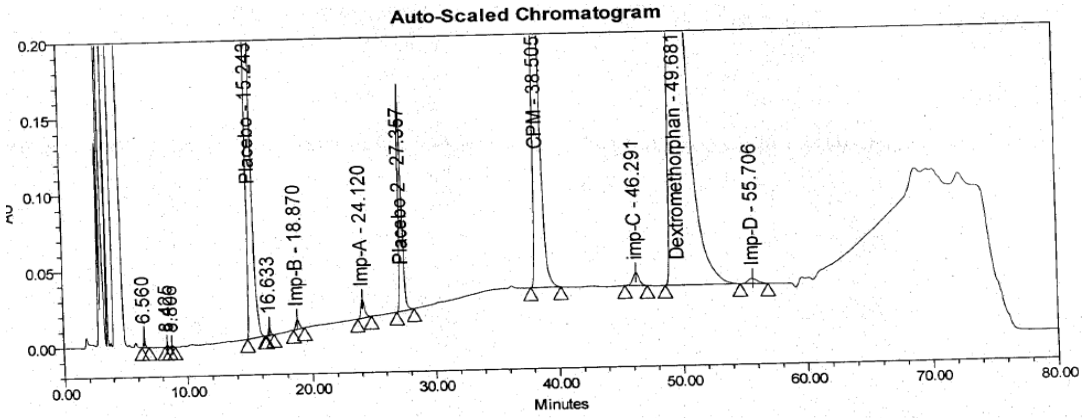


Figure 3. Chromatogram of Chlorpheniramine Maleate and Dextromethorphan HBr

Table 2:. Linearity results for Impurities

Linearity level	Conc. in ppm				Peak area			
	Imp-A	Imp-B	Imp-C	Imp-D	Imp-A	Imp-B	Imp-C	Imp-D
LOQ Level	0.498	0.498	0.498	0.498	47806	27174	70507	40462
100%	2.492	2.492	2.492	2.492	238715	136597	331086	205650
150%	3.738	3.738	3.738	3.738	354186	203465	491049	308709
Correlation coefficient (r)					1	1	1	1
				Slope	94671.55	54454.11	129879.2	82796.96
				Intercept	1252.276	289.5846	6271.482	-745.649
				Y-intercept	0.5	0.2	1.9	-0.4

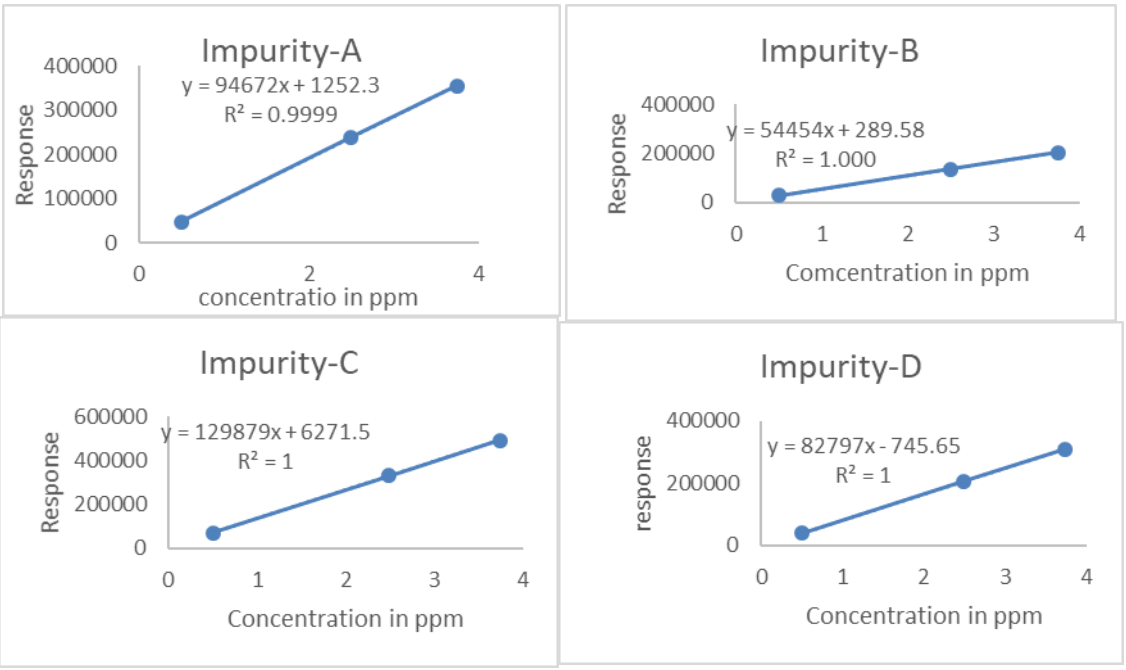
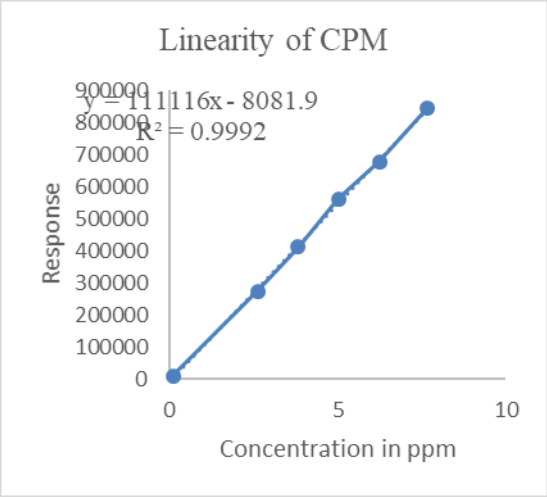


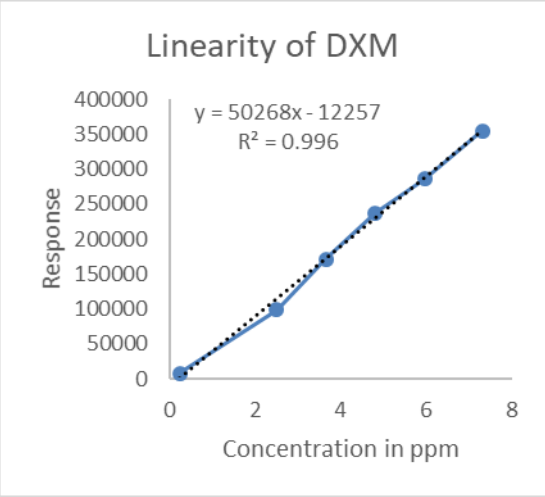
Table 3:. Linearity results for Chlorpheniramine maleate

Level	Conc. In ppm	Area
LOQ	0.101	8831
50%	2.614	273083
75%	3.821	412200
100%	5.027	562087
125%	6.234	677446
150%	7.642	844540

Table 4: Linearity results for Dextromethorphan HBr

Level	Conc. In ppm	Area
LOQ	0.250	7375
50%	2.501	99064
75%	3.655	171622
100%	4.810	237621
125%	5.964	286483
150%	7.311	355396





➢ **Precision**

Table 5: Result of Precision study for Impurities

Acceptance Criteria		Results				Limit
		Chlorpheniramine maleate		Dextromethorphan HBr		
		IMP-A	IMP-B	IMP-C	IMP-D	
% impurities	1	99.7	99.5	98.1	97.8	85.0% to 115.0%
	2	100.7	99.5	95.8	95.2	
	3	98.6	101.2	96.3	99.3	
	4	100.2	100.1	93.8	97.7	

	5	100.3	100.1	92.3	95.7	
	6	100	99	92.5	99.7	
	Mean	99.9	99.9	94.8	97.6	
%RSD of six preparation		0.73	0.76	2.44	1.9	% RSD for six samples should not be more than 15.0%.

Table 6: Result of Precision study for CPM and DXM

Method Precision	**CPM in (%)**	**DXM in (%)**
Preparation-1	0.540	0.522
Preparation-2	0.542	0.530
Preparation-3	0.574	0.507
Preparation-4	0.532	0.498
Preparation-5	0.522	0.491
Preparation-6	0.552	0.491
Average	0.544	0.506
%RSD	3.3	3.2

- **Accuracy and Range**

Table 7: Result for CPM, DXM and its Impurities

Acceptance Criteria		**Results**				**Limit**
		Chlorpheniramine maleate		**Dextromethorphan HBr**		
Tailing factor for the peak		1.1		NA		NMT 2.0
Area ratio of two injection of standard		0.99		0.99		0.90 to 1.10
% recovery at	Level	Imp-A	Imp-B	Imp-C	Imp-D	85.0% to

50% level	LOQ_1	99.3	98.0	101.7	101.7	115.0%
	LOQ_2	102.0	100.0	102.6	98.3	
	LOQ_3	102.8	98.8	101.4	96.5	
	LOQ_4	98.4	100.4	100.4	93.2	
	LOQ_5	99.6	96.4	100.6	95.2	
	LOQ_6	98.1	102.5	100.9	91.1	
	Average	100.0	99.4	99.7%	96.0	
% recovery at 100% level	100%-1	99.7	99.5	98.1	97.8	85.0% to 115.0%
	100%-2	100.7	99.5	95.8	95.2	
	100%-3	98.6	101.2	96.3	99.3	
	100%-4	100.2	100.1	93.8	97.7	
	100%-5	100.3	100.1	92.3	95.7	
	100%-6	100.0	99.0	92.5	99.7	
	Average	99.9	99.9	94.8	97.6	
recovery at 150% level	150%-1	99.1	99.9	94.9	100.8	85.0% to 115.0%
	150%-2	98.8	99.1	93.1	96.8	
	150%-3	98.5	98.6	93.2	95.4	
	Average	98.8	99.2	93.7	97.7	
% RSD of % recovery at LOQ Level		1.93	2.12	1.26	3.90	NMT 15.0%
% RSD of % recovery at 150%		0.30	0.66	1.08	2.90	NMT 15.0%

- **Robustness**

Table 8: Robustness Results (checked for placebo peak interference as critical parameter)

Parameter	**Change**	**Placebo interference at CPM, DXM and Impurities**
Wavelength (225nm)	223nm	No Interference

	227nm	No Interference
Column Temperature (40°C)	38°C	No Interference
	42°C	No Interference
pH (7.20)	pH- 7.00	No Interference
	pH- 7.40	No Interference

- **Forced degradation**

Table 9: Forced degradation Results for CPM and DXM

Condition	**%Degradation of Sample**	**Peak purity***
Acid 5N, 2 mL 60°C for 3 Hours	0.08	Pass
Base 5N, 2 mL 60°C for 3 Hours	2.83	Pass
Peroxide, 30%, 2mL at RT for 3 Hours	18.5	Pass
Thermal 80°C for 24 Hours	0.07	Pass
Photolytic degradation 1.2 MLH	0.05	Pass

*Peak purity confirmed using PDA detector empower software i.e Purity threshold>Purity Angle.

- **Assay of Marketed Sample**

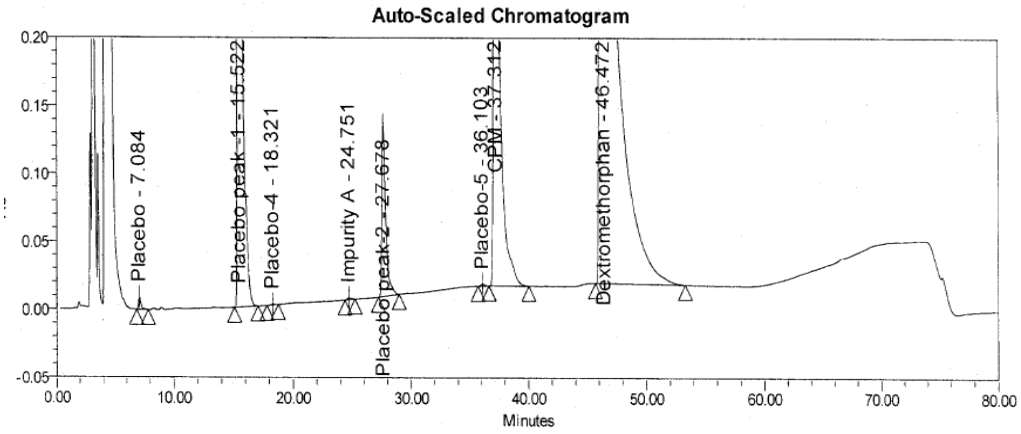


Figure 4. Chromatogram of sample having CPM and DXM

Table 10. Result of Marketed Sample

%Impurities	%
Impurity-A	BDL
Each impurity B, C & D	ND
Unknown impurity	0.05%
Total	0.05%

4. Discussion

Different trial has been conducted to achieve the separation between impurities. Impurity-C and D are more sensitive to Organic concentration in mobile phase and pH. However, number of different trails on different buffer, different mobile phase composition, pH, Gradient, different pH has been conducted and finally the separation achieved on Phosphate buffer pH 7.20 and mixture of Methanol and acetonitrile in the ratio 20:80v/v was found suitable for better separation, further different type of column used but finally the good separation occurs on YMC Triart C18, 250x 4.6 mm, 5µ. Wavelength 225nm found suitable for detection.

5. Conclusion

The developed method has been validated and based on result obtained in the validation for determination of impurities in presence of both CPM and DXM is found suitable. The validated method found selective,

precise, accurate and robust. At present it is unique single run method for determination of present of impurities in both the drug combination i.e. CPM and DXM. ICH guideline referred for validation of above method. The marketed sample analysed and for workability of above method and found suitable.

6. Abbreviation's

HPLC **–** High performance liquid chromatography

CPM – Chlorpheniramine Maleate

DXM – Dextromethorphan Hydrobromide.

7. Acknowledgements

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8. Conflicts of interest

No any conflicts.

9. References

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