RESEARCH ARTICLE JPAR 2019

Journal of Pharmaceutical Advanced Research

(An International Multidisciplinary Peer Review Open Access monthly Journal)

Available online at: www.jparonline.com

Combined RP-HPLC methodology for the determination of Diphenhydramine hydrochloride, its impurities and preservatives in oral liquid formulation in a single run

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Received: 04.06.2019 Revised: 12.07.2019 Accepted: 06.08.2019 Published: 31.08.2019

ABSTRACT: Background: develop and validate a RP-HPLC chromatography for the simultaneous determination of Diphenhydramine hydrochloride, its related impurities, sodium methyl hydroxybenzoate and sodium propyl hydroxybenzoate in oral liquid dosage form. **Aim:** The present study was aimed to develop a new combined HPLC methodology for syrup formulation of DPH, its related impurities and preservatives in a single run. **Method:** The analysis was performed on a Waters HPLC system with a Phenomenex Kinetex C_{18} column (4.6 × 100 mm I.D., 2.6µm) and gradient elution consisting of sodium perchlorate with TFA as the buffer and acetonitrile with TFA the organic component as the mobile phase. The detection wavelength was 220 nm with an acquisition time of 40 min in which all the five impurities along with DPH and two preservatives were well separated. **Results:** The developed method was validated according to the ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the specified acceptance criteria. **Conclusion:** The proposed method was successfully applied to the oral liquid dosage form for routine analysis.

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Keywords: DPH – Diphenhydramine hydrochloride, Oral liquid, Methyl hydroxybenzoate, Propyl hydroxybenzoate.

INTRODUCTION:

Diphenhydramine hydrochloride (DPH) named as 2-(diphenylmethoxy)-N,N-dimethylethylamine hydrochloride, which is a first generation antihistamine, has been mainly used for the treatment of allergies and itchiness, insomnia, motion sickness, and extrapyramidal symptoms [1]. Additionally, DPH has significant anti-tussive activity [2]. The syrups containing DPH have been used as a cough suppressant for the control of cough due to colds or allergy [3]. Recently, the use of DPH in combination with other drugs has been reported as antiemetic for the prevention

of cisplatin-induced emesis in chemotherapy treatment. Furthermore, it has been used as sedative in dentistry for children and in local anesthesia [4-6]. DPH oral syrups or elixirs are available commercially. Several methods including capillary electrophoresis, atomic absorption spectrometry, fluorometry, flow injection analysis and spectrophotometry have been proposed for the determination of DPH in pharmaceutical preparations. Many chromatographic methods such as gas chromatography, liquid chromatography and high performance liquid chromatography (HPLC) have been used for the analysis of DPH in samples [7,8]. Currently, USP has detailed a RP-HPLC methodology for the determination of DPH and its impurities along with sodium benzoate as the preservative. The first method detailed specifies methodology for the estimation of DPH and sodium benzoate and the second method specified for the related impurities. When the current formulation was subjected to test with the USP 42 detailed methodology, impurities were found co-eluting with the preservatives - methyl hydroxybenzoate and propyl hydroxybenzoate. At this juncture, it was decided to modify the method to overcome the co-elution issues as well as develop a common method for the estimation of DPH, its related impurities and the preservatives which would reduce the cost in the long run for commercial batch analysis [9,10].

MATERIALS AND METHODS:

Chemicals and reagents:

Diphenhydramine, Methyl Hydroxybenzoate and Propyl Hydroxybenzoate working standards were used available in Oman Pharmaceutical Products L.L.C. Syrup formulation containing Diphenhydramine 14 mg/5ml was taken from the commercial batch manufactured at Oman Pharmaceutical Products L.L.C. HPLC grade Acetonitrile was procured from Merck Ltd. All other chemical reagents were of analytical grade.

Experimental:

The chromatographic condition for analysis of DPH in syrup by HPLC method is given in Table 1. The flow gradient of solvent system in HPLC column is given in Table 2.

Preparation of Solution A:

For the preparation of solution A 11.24 g/l of sodium perchlorate monohydrate was mixed with the distilled water. To the solution, 1 ml of trifluroacetic acid was added.

Table 1. Chromatographic conditions.

Parameters	Data
Column	Phenomenex Kinetex C_{18},100 x 4.6 mm, 2.6µ)
Injection volume	5 µl
Wavelength	220 nm
Column temp	35 °C
Sampler temp	10 °C
Retention time	For Diphenhydramine : about 18.5 min
Diluent	Acetonitrile : Water (18:82 % v/v)
Run time	40 min

Table 2. Flow gradient data of HPLC study.

Time	Flow	Solution C	Solution D
0	0.8	80	20
5	0.8	80	20
25	0.8	50	50
30	1	0	100
35	1	0	100
35.1	0.8	80	20
40	0.8	80	20

Preparation of Solution B:

For the preparation of solution B Acetonitrile and trifluroacetic acid were mixed in the ratio of (1000:1).

Preparation of Solution C:

For the preparation of solution C the solution A and B were mixed together in the ratio of 82:18.

Preparation of Solution D:

For the preparation of solution D the solution A and B were mixed together in the ratio of 50:50.

Standard stock solution A (Assay and Preservative):

About 50 mg of Diphenhydramine Hydrochloride working standard, 40 mg of Methyl Hydroxybenzoate working standard and 4 mg of Propyl Hydroxybenzoate working standard were weighed and taken in a 20 ml volumetric flask. To the flask 10 ml of diluent was added and mixed. The mixture was sonicated to dissolve the ingredients in diluent. The solution was diluted further and mixed well. In the standard stock solution A the concentration of Diphenhydramine Hydrochloride, Methyl Hydroxybenzoate and Propyl Hydroxybenzoate were 2500, 2000 and 200 ppm respectively [9].

Standard solution (Assay and Preservative):
About 10 ml of Standard stock solution A was diluted to 100 ml with diluent and mixed well. In the standard solution the concentration of Diphenhydramine Hydrochloride, Methyl Hydroxybenzoate and Propyl Hydroxybenzoate were 250, 200 and 20 ppm respectively [10].

Standard stock solution (RS):
About 25 mg of Diphenhydramine Hydrochloride was accurately weighed and put into a 100 ml volumetric flask. To the flask 50 ml of diluent was added. It was sonicated to dissolve the ingredient in diluents. The mixture was diluted up to the required volume and mixed well. In the standard stock solution the concentration of concentration of Diphenhydramine Hydrochloride was 0.25 mg/mL [11].

Impurity Stock Solution (RS):
About 2.5 mg of Diphenhydramine related compound A, Diphenhydramine related compound B, Diphenhydramine N-oxide, Benzhydrol, Benzophenone were weighed and kept in to a 10 ml volumetric flask. To the flask 5 ml of diluent was added and sonicated to dissolve. Finally, diluted to volume with diluent and mixed well. Each impurity was prepared in separate flask in concentration of 0.25 mg/ml [12].

Standard solution (RS):
About 1 ml of Standard stock solution for RS and 1 ml of Impurity Stock Solution were diluted to 100 ml with diluent and mixed well. The concentration of Diphenhydramine Hydrochloride was 0.0025 mg/ml.

System Suitability Stock Solution (RS):
About 1.25 mg of Diphenhydramine, Diphenhydramine related compound A and Diphenhydramine related comp B were accurately weighed in 10 ml volumetric flask. To the mixture 5 % of flask volume Acetonitrile was added and it was diluted with Diluent to make volume up to the mark. The one volume of solution was diluted with 10 volume of Dilute. The concentration of Diphenhydramine Hydrochloride was 0.0125 mg/ml [13].

System Suitability Solution (RS):
Each of Diphenhydramine, Diphenhydramine related compound A and Diphenhydramine related compound B were diluted with diluent from the standard stock and system suitability stock solution respectively. The concentration of Diphenhydramine Hydrochloride was 0.0025 mg/ml [14].

Sample preparation (Assay, Preservative and RS):
An amount Sample (25 mg - about 9 ml) was accurately transferred in to a 100 ml volumetric flask. To the flask about 40 to 50 ml of diluent was added and shaken well for 5 min. Finally, the contents of the flask was diluted up to the mark with diluent and mixed well. The resultant solution was filtered by using 0.45 µ PVDF filter. First 5 ml of filtrate was discarded. The concentration of Diphenhydramine Hydrochloride was 250 ppm [15].

Table 3a: System suitability (Assay and Preservative) for Diphenhydramine Hydrochloride.

Injection #	Area	Plate counts	Tailing factor
1	548472	30976	1.29
2	546553	30916	1.29
3	546162	31059	1.30
4	544579	30866	1.29
5	541479	31001	1.28
6	542246	30910	1.28
Mean	544915	30955	1.29
SD	2681		
%RSD	0.5%		

Table 3b: System suitability (Assay and Preservative) for Methyl Hydroxybenzoate.

Injection #	Area	Plate counts	Tailing factor
1	327740	14218	1.02
2	326665	14218	1.02
3	326987	14292	1.03
4	325676	14218	1.02
5	324028	14302	1.01
6	324750	14132	1.03
Mean	325974	14230	1.02
SD	1414		
%RSD	0.4%		

Table 3c: System suitability (Assay and Preservative) for Propyl Hydroxybenzoate.

Injection #	Area	Plate counts	Tailing factor
1	31671	30976	1.02
2	31593	30916	1.00
3	31823	31059	1.00
4	31470	30866	1.00
5	31318	31001	1.00
6	31330	30910	1.01
Mean	31534	30955	1.01
SD	199		
%RSD	0.6%		

RESULTS AND DISCUSSION:

The developed method for determination of Diphenhydramine was validated by using the following parameters.

System suitability (Assay, Preservative and RS):

System suitability followed the procedure described in the methodology and established the system suitability before starting the analysis. The standard solution is mentioned in Table 3a to 3c and 9a to 9g for related substances test. The data presented in Fig 1 to 4.

Table 9a. System suitability (RS).

System Suitability Solution parameter	Obs	Limits
Resolution - NLT 1.5 between Diphenhydramine Related Comp-B and Diphenhydramine Related Compound-A.	3.48	NLT 1.5
Resolution - NLT 1.5 between Diphenhydramine Related and Diphenhydramine Related Compound-A.	4.72	NLT 1.5

Obs – Observations.

Table 9b. System suitability of Diphenhydramine.

Inj	SA	PC	TF
1	5855	36134	1.01
2	5914	36103	0.98
3	5650	36832	0.99
4	5646	31172	0.99
5	5577	38091	1.00
6	5739	36714	0.99
Mean	5730	35841	0.99
SD	131.47		
%RSD	2.29		

Inj – Injection, SA – Standard area, PC – Plate count and TF – Tailing factor.

Table 9c. System suitability of Diphenhydramine Rel Comp-B.

Inj	SA	PC	TF
1	6387	30785	0.98
2	6360	30540	0.96
3	6282	30996	0.97
4	6442	30469	0.97
5	6139	31462	0.98
6	6497	30531	0.99
Mean	6351	30797	0.98
SD	127.03		
%RSD	2.00		

Inj – Injection, SA – Standard area, PC – Plate count and TF – Tailing factor.

Specificity (Assay, Preservative and RS):

There were no interfering peaks on the retention times of the APIs in the presence of excipients. Further, to demonstrate the specificity of the method, the sample had been subjected to acid, base, oxidation, thermal and photolytic degradation. This was evaluated by comparing the peak purity using Chromeleon software. The data has been presented in Table 4a to 4c for assay and Table 10, 11 for related substances. The relevant chromatograms have been presented in Fig 5 to 9.

Table 9d. System suitability of Diphenhydramine Rel Comp-A.

Inj	SA	PC	TF
1	5957	32775	1.00
2	5818	33346	1.01
3	5964	32851	0.99
4	5843	32890	0.98
5	6021	32234	0.99
6	5812	33553	0.99
Mean	5903	32942	0.99
SD	89.07		
%RSD	1.51		

Inj – Injection, SA – Standard area, PC – Plate count and TF – Tailing factor.

Table 9e. System suitability of Diphenhy. N-Oxide.

Inj	SA	PC	TF
1	9028	26661	0.99
2	9077	26789	0.97
3	8972	26983	0.99
4	8818	27002	1.00
5	8827	26381	1.00
6	8911	26872	0.99
Mean	8939	26781	0.99
SD	89.07		
%RSD	1.51		

Inj – Injection, SA – Standard area, PC – Plate count and TF – Tailing factor. Diphenhy – Diphenhydramine.

Table 9f. System suitability of Benzhydrol.

Inj	SA	PC	TF
1	6018	47069	0.99
2	5805	48134	0.97
3	6121	46643	0.98
4	6049	46492	0.99
5	5848	47455	1.01
6	6118	46685	1.04
Mean	5993	47080	1.00
SD	135.75		
%RSD	2.27		

Inj – Injection, SA – Standard area, PC – Plate count and TF – Tailing factor.

Linearity and range (Assay & Preservative & RS): Standard solutions containing Diphenhydramine Methyl Hydroxybenzoate, Propyl Hydroxybenzoate and known impurity were prepared.

Table 9g. System suitability of Benzophenone.

Inj	SA	PC	TF
1	5166	181492	0.96
2	5192	181164	0.95
3	5159	182647	0.97
4	5118	182688	0.96
5	5105	183437	0.95
6	5212	181614	0.96
Mean	5159	182174	0.96
SD	41.34	181492	0.96
%RSD	0.80		

Inj – Injection, SA – Standard area, PC – Plate count and TF – Tailing factor.

Then linearity was evaluated using the calibration curve to calculate coefficient of correlation, slope and intercept. In general, a value of correlation coefficient (r^2) > 0.999 is considered as the evidence of an acceptable fit for the data to the regression line.

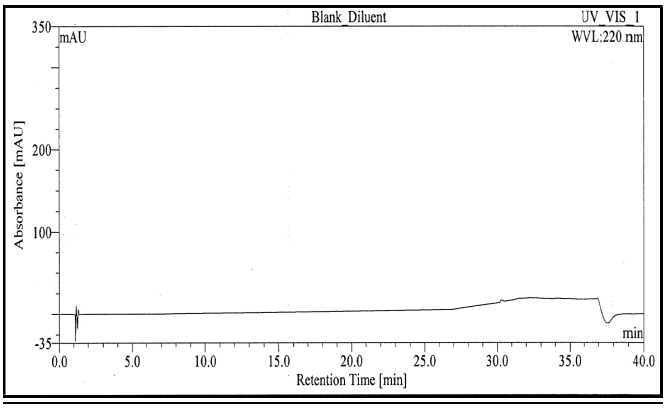

Fig 1. Reference chromatogram of Blank.

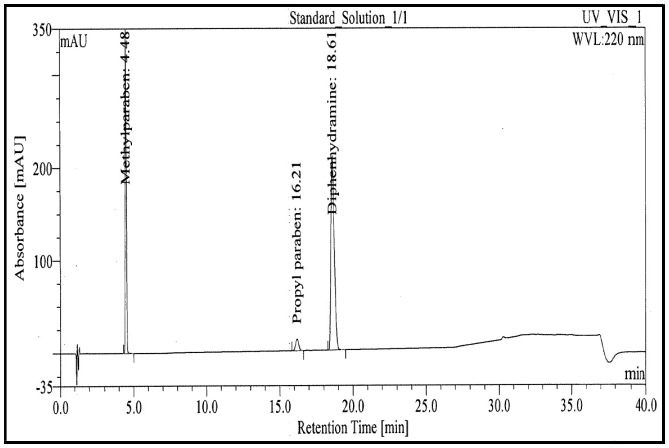

Fig 2. Reference chromatogram of Standard Solution (As and Preservative).

Linearity was determined by duplicate injections of Five different concentrations (50, 80, 100, 120 and 150 % of the target concentration). The average peak areas were plotted against concentrations. The data has been presented in Table 7a to 7c for assay and Table 14a to 14f for related substances. The corresponding linearity plots have been presented in Fig 10 to 18.

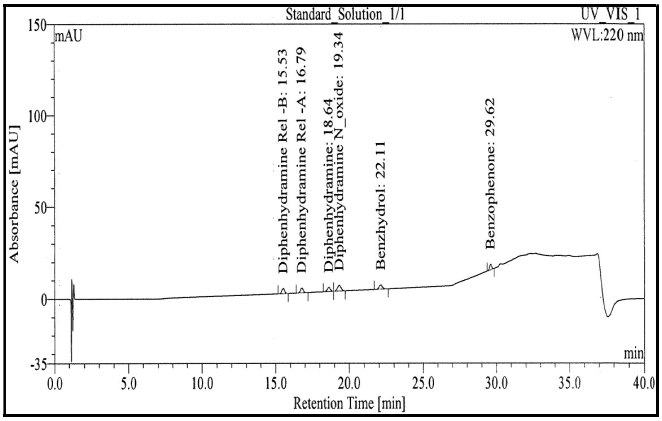

Fig 3. Reference chromatogram of Standard Solution (RS).

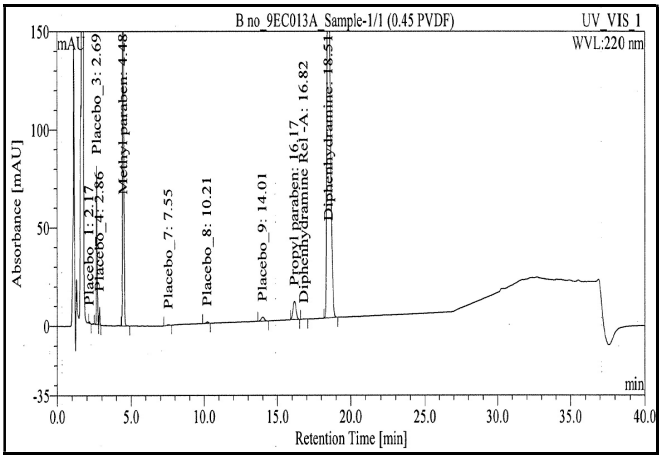

Fig 4. Reference chromatogram of As Such sample (Assay, Preservative and RS).

Table 4a. Forced degradation study for Diphenhydramine HCl.

Sample Name	Assay %	Degra-Dation (%)	Peak Purity
As such (US)	99.8	-	-
ACD	93.4	6.4	1.00
ALD	95.9	3.9	1.00
PD	85.7	14.2	1.00
TD	96.8	3.0	1.00
PD	97.3	2.5	1.00

US - Unstressed sample, ACD - Acid degradation (0.1N HCl/1 h), ALD - Alkali degradation (5N NaOH/1 h), PD - Peroxide degradation (3 % w/v H_2O_2/30 min), TD - Thermal degradation (105°C/1Days) and PD - Photolytic Degradation (1.2Million).

Precision (Assay, Preservative and RS):

Precision was determined by preparing the standard and sample as per the methodology. The sample was prepared in six replicates and it was injected into the chromatograph. The percentage assay value of each preparation was calculated and finally the percentage RSD of the six replicate preparations was deduced. The data has been presented in Table 5a to 5c for assay and Table 12a to 12b for related substances.

Table 4b. Forced degradation study for Methyl Hydroxybenzoate.

Sample Name	Assay %	Degra-Dation (%)	Peak Purity
As such (US)	105.3	-	-
ACD	73.4	30.3	0.9997
ALD	94.5	10.3	0.9998
PD	95.2	9.6	0.9965
TD	80.9	23.2	0.9941
PD	95.0	9.8	0.9997

US - Unstressed sample, ACD - Acid degradation (0.1N HCl/1 h), ALD - Alkali degradation (5N NaOH/1 h), PD - Peroxide degradation (3 % w/v H_2O_2/30 min), TD - Thermal degradation (105°C/1Days) and PD - Photolytic Degradation (1.2Million).

Table 4c. Forced degradation study for Propyl Hydroxybenzoate.

Sample Name	Assay %	Degra-Dation (%)	Peak Purity
As such (US)	104.8		
ACD	65.5	37.5	0.9997
ALD	82.6	21.2	1.0000
PD	86.4	17.6	1.0000
TD	79.7	23.9	1.0000
PD	82.0	21.8	1.0000

US - Unstressed sample, ACD - Acid degradation (0.1N HCl/1 h), ALD - Alkali degradation (5N NaOH/1 h), PD - Peroxide degradation (3 % w/v H_2O_2/30 min), TD - Thermal degradation (105°C/1Days) and PD - Photolytic Degradation (1.2Million).

Table 10. Specificity of Standard and Sample Solutions (RS).

PP Diphenhydramine Hydrochloride	0.9995
PP Diphenhydramine RC-A	0.9997
PP Diphenhydramine RC-B	0.9997
PP Diphenhydramine N-Oxide	0.9998
PP Benzhydrol	0.9996
Peak Purity of Benzophenone	0.9999
PP Unspiked Sample	0.9999
PP Spiked Sample	0.9999

PP - Peak Purity and RC - Related Compound.

Table 11. Force Degradation (RS).

Sample Name	SA	PD	PP
Control (Sample)	535490	-	1.00
Acid Deg	509164	4.9	1.00
Alkali Deg	522358	2.5	1.00
Peroxide Deg	466851	12.8	1.00
Thermal Deg	527253	1.5	1.00
Photolytic Deg	530062	1.0	1.00

SA - Sample Area, PDeg – Percentage Degradation and PP - Peak Purity.

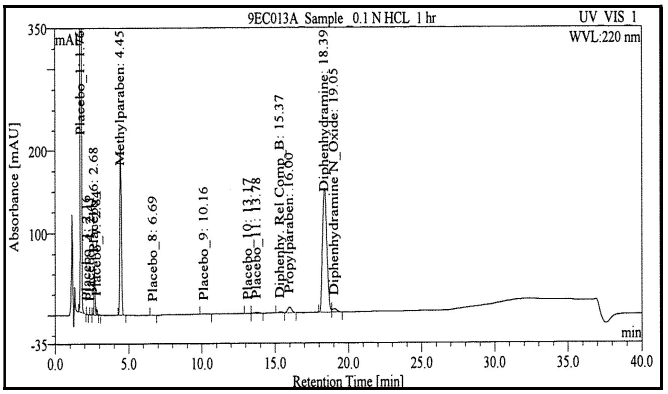

Fig 5. Reference chromatogram of Acid degradation (Assay, Preservative and RS).

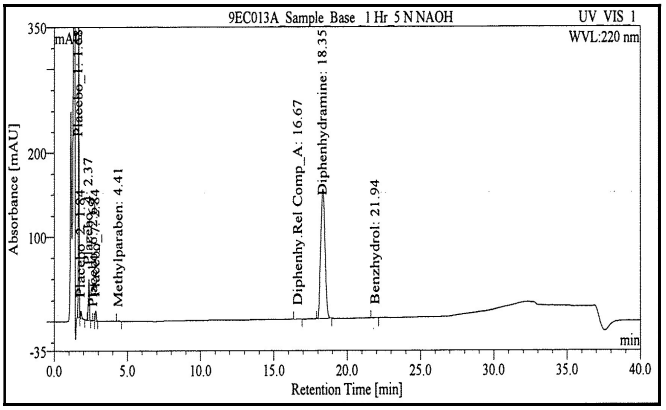

Fig 6. Reference chromatogram of Base degradation (Assay, Preservative and RS).

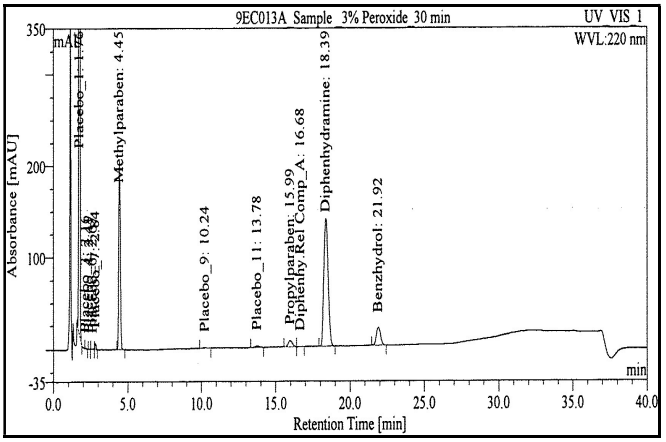

Fig 7. Reference chromatogram of peroxide degradation (Assay, Preservative and RS).

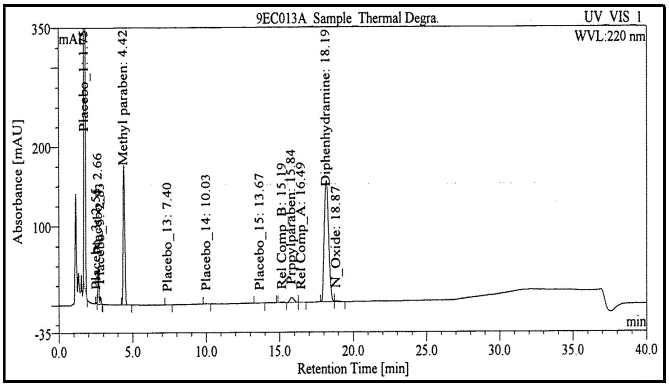

Fig 8. Reference chromatogram of Thermal degradation (Assay, Preservative and RS).

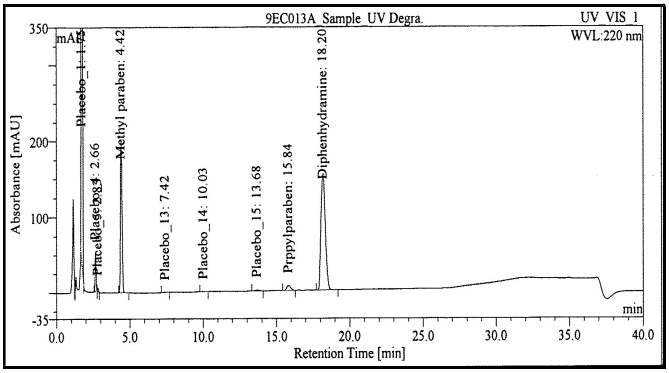

Fig 9. Reference chromatogram of UV degradation (Assay, Preservative and RS).

Table 7a: Linearity Data (Assay and Preservative) of Diphenhydramine Hydrochloride.

Level No.	Concentration (µg/mL)	Mean area
1	125.8478	276175
2	201.3565	437049
3	251.6956	550495
4	302.0347	657036
5	377.5434	831690
Slope		2204.051
Intercept		-4260.858
CC		0.9999
R^2		0.9997

Table 7b: Linearity Data (Assay and Preservative) of Methyl hydroxybenzoate.

Level No.	Concentration (µg/mL)	Mean area
1	99.4669	170779
2	159.1470	265485
3	198.9338	330341
4	238.7206	389109
5	298.4007	483621
Slope		2204.051
Intercept		-4260.858
CC		0.9999
R^2		0.9997

Table 7c: Linearity Data (Assay and Preservative) of Propyl hydroxybenzoate.

Level No	Concentration (µg/ml)	Mean area
1	10.850	16408
2	17.360	25727
3	21.700	32342
4	26.040	38671
5	32.550	48866
Slope		2204.051
Intercept		-4260.858
CC		0.9999
R^2		0.9997

Table 14a. Linearity data (RS) of Diphenhydramine.

Level No.	Conc. (µg/ml)	Mean area
1	1.2635	2591
2	2.0216	4575
3	2.5270	5667
4	3.0324	7045
5	3.7905	8493
Slope		2350.477
Intercept		-265.455
CC		0.9981
R^2		0.99

Table 14b. Linearity data (RS) of Diphenhydramine Rel Comp-B.

Level No.	Conc. (µg/ml)	Mean area
1	0.2448	684
2	0.3916	1132
3	0.4895	1379
4	0.5874	1711
5	0.7343	1978
Slope		2686.734
Intercept		61.590
CC		0.9951
R^2		0.99

Table 14c. Linearity of Diphenhydramine Rel Comp-A.

Level No.	Conc. (µg/ml)	Mean area of Diphenhydramine Related Comp. A
1	0.2527	549
2	0.4044	1068
3	0.5054	1207
4	0.6065	1677
5	0.7582	2059
Slope		2990.790
Intercept		-199.665
CC		0.9931
R^2		0.99

Table 14d. Linearity of Diphenhydramine N-Oxide.

Level No.	Conc. (µg/ml)	Mean area of Diphenhydramine N-oxide
1	3.6824	8870
2	5.8918	14660
3	7.3647	17688
4	8.8377	22213
5	11.0471	26703
Slope		2441.070
Intercept		48.956
CC		0.9983
R^2		0.99

Table 14e. Linearity of Benzhydrol.

Level No.	Conc. (µg/ml)	Mean area of Benzhydrol
1	2.486	5784
2	3.729	9146
3	4.972	10785
4	5.966	13907
5	7.458	18001
Slope		2398.692
Intercept		-282.433
CC		0.9933
R^2		0.99

Table 14f. Linearity of Benzophenone.

Level No.	Conc. (µg/ml)	Mean area of Benzophenone
1	0.5074	740
2	0.8118	1297
3	1.0148	1565
4	1.2177	1940
5	1.5222	2331
Slope		1570.042
Intercept		-18.648
CC		0.9976
R^2		0.99

Fig 10. Linearity plot of Diphenhydramine (Assay and Preservative).

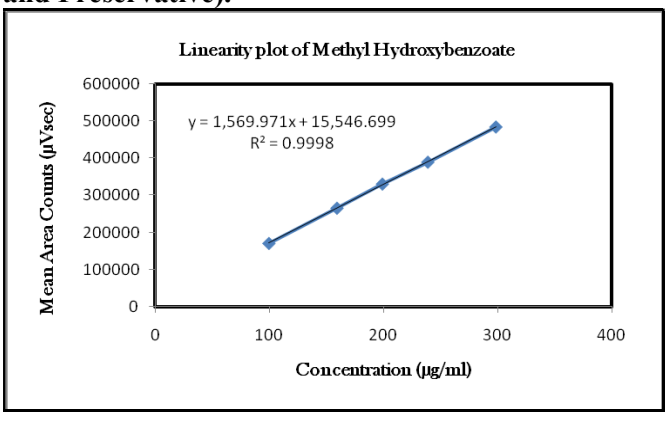

Fig 11. Linearity plot of Methyl hydroxybenzoate (Assay and Preservative).

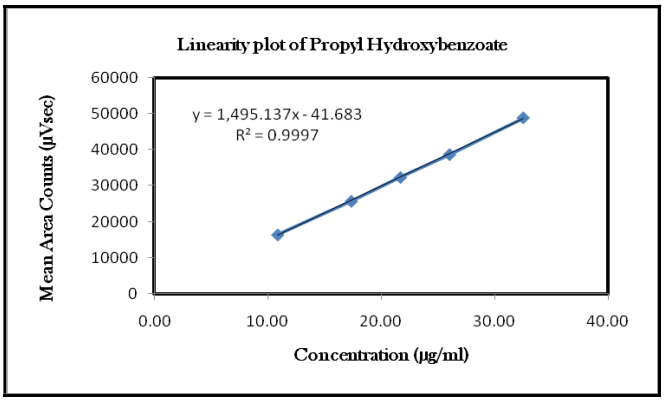

Fig 12. Linearity plot of Propyl hydroxybenzoate (Assay and Preservative).

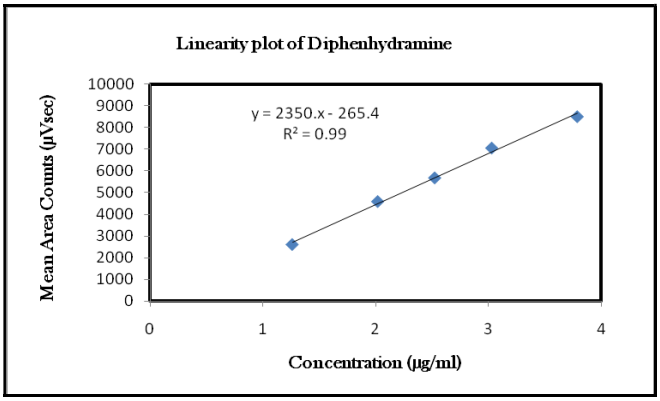

Fig 13. Linearity plot of Diphenhydramine (RS).

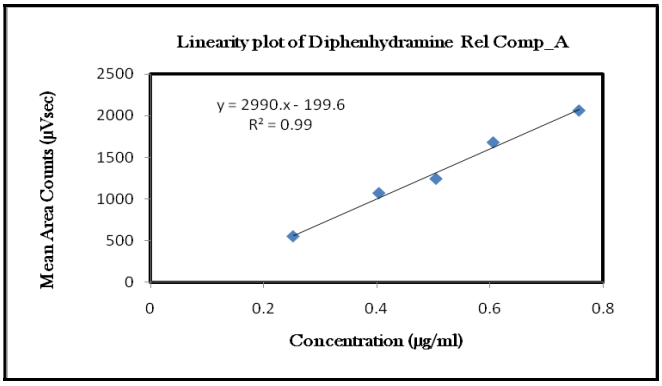

Fig 14. Linearity plot of Diphenhydramine related Compound A (RS).

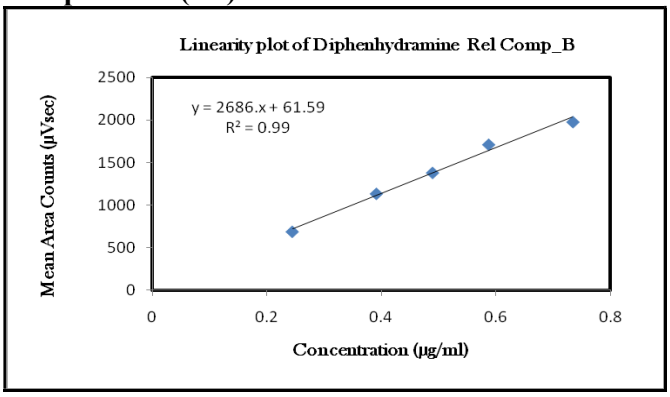

Fig 15. Linearity plot of Diphenhydramine related Compound B (RS).

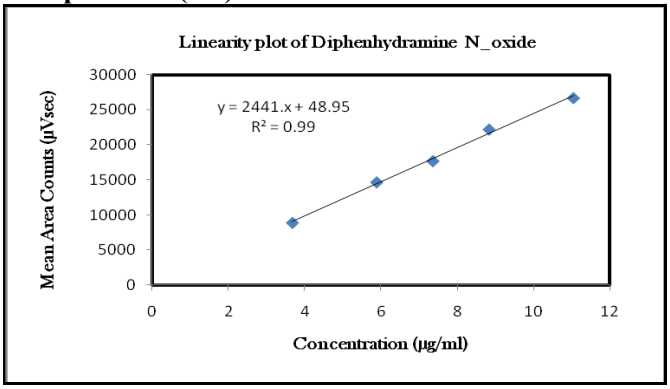

Fig 16. Linearity plot Diphenhydramine N-Oxide (RS).

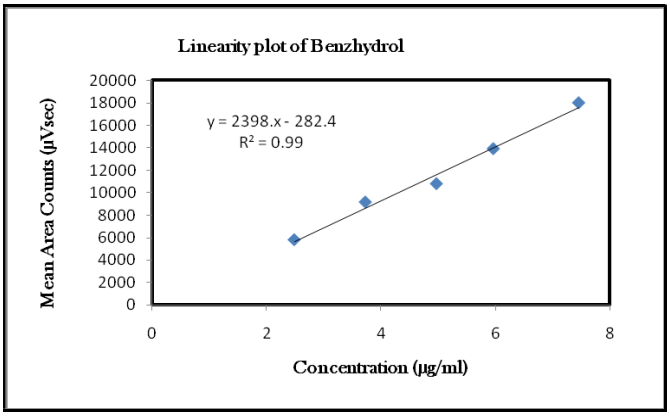

Fig 17. Linearity plot of Benzhydrol (RS).

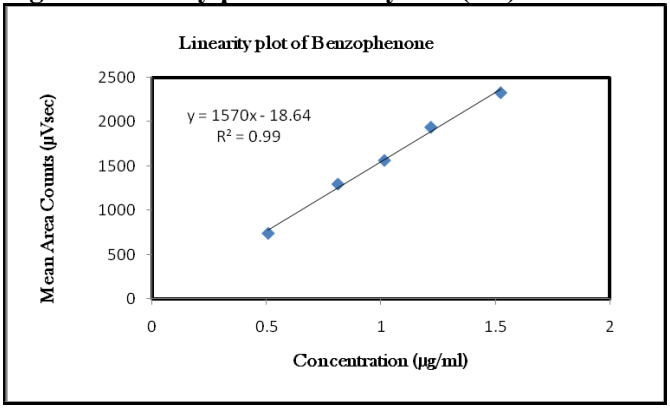

Fig 18. Linearity plot of Benzophenone (RS).

Table 5a. Method Precision Studies for Diphenhydramine HCl (Set I).

Sample Number	Diphenhydramine Hydrochloride	
	mg/ml	% Assay
1	2.805	100.2
2	2.804	100.1
3	2.804	100.1
4	2.794	99.8
5	2.810	100.4
6	2.754	98.4
Mean		99.8
SD		0.7
% RSD		0.7

Table 5b. Method Precision Studies for Methyl Hydroxybenzoate (Set I).

Sample Number	Methyl Hydroxybenzoate	
	mg/ml	% Assay
1	2.108	105.4
2	2.106	105.3
3	2.102	105.1
4	2.099	105.0
5	2.127	106.3
6	2.095	104.7
Mean		105.3
SD		0.6
% RSD		0.5

Table 5c. Method Precision Studies for Propyl Hydroxybenzoate (Set I).

Sample Number	Propyl Hydroxybenzoate	
	mg/ml	% Assay
1	0.210	104.9
2	0.209	104.7
3	0.209	104.6
4	0.209	104.7
5	0.210	105.1
6	0.210	104.9
Mean		104.8
SD		0.2
% RSD		0.2

Ruggedness (Assay, Preservative and RS):

Ruggedness of method was demonstrated by preparing the standard and sample as per the methodology by a different analyst on a different day, using a different column lot and using a different HPLC system. The sample was prepared in six replicates and injected into the chromatograph.

Table 6a. Ruggedness Data (Assay and Preservative) (Intermediate Precision Studies for Diphenhydramine Hydrochloride for Set II).

Sample Number	Diphenhydramine	
	mg/ml	% Assay
1	2.701	96.5
2	2.728	97.4
3	2.725	97.3
4	2.721	97.2
5	2.727	97.4
6	2.733	97.6
Mean		97.2
SD		0.4

The percentage assay value of each preparation was calculated and finally the percentage RSD of the six replicate preparations was deduced. The data has been presented in Table 6a to 6c for assay and Table 13a to 13d for related substances.

Table 6b. Ruggedness Data (Assay and Preservative) (Intermediate Precision Studies for Methyl Hydroxybenzoate for Set II).

Sample Number	Methyl Hydroxybenzoate	
	mg/ml	% Assay
1	2.020	101.0
2	2.007	100.4
3	2.032	101.6
4	2.021	101.1
5	2.023	101.1
6	2.027	101.4
Mean		101.1
SD		0.4
% RSD		0.4

Table 6c. Ruggedness Data (As and Preservative) (Intermediate Precision Studies for Propyl Hydroxybenzoate for Set II).

Sample Number	Propyl Hydroxybenzoate	
	mg/ml	% Assay
1	0.186	93.0
2	0.190	95.1
3	0.186	93.2
4	0.189	94.3
5	0.192	96.0
6	0.190	95.2
Mean		94.4
SD		1.2
% RSD		1.3

Accuracy (Assay, Preservative and RS):

Accuracy of the proposed method had been demonstrated by the recovery study that had been performed by the standard addition method at levels 50, 100 and 150 % of the target concentration. The data has been presented in Table 8a to 8c for assay and Table 14a to 14f for related substances.

CONCLUSION:

This intended study can be concluded as: the proposed method is economical, simple, ultrafast, sensitive and reliable and is found to be accurate, precise, specific, stability indicating, rugged . Hence it can be employed for the routine estimation of Diphenhydramine and its related impurities in Diphenhydramine Oral Solution dosage form.

ACKNOWLEDGEMENT:

Authors wish to thank the management of Oman Pharmaceutical Products Co. LLC, for providing library and laboratory facility to carry out this analytical method validation for the oral liquid formulation containing DPH and preservatives.

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Table 12a. Method Precision Study (un-spike)-SET-I.

Sample no	Rel Comp-B	Rel Comp-A	N-Oxide	Benzhydrol	Benzophenone	Total Imp
1	-	0.048	-	-	-	-
2	-	0.050	-	-	-	-
3	-	0.061	-	-	-	-
4	-	0.062	-	-	-	-
5	-	0.052	-	-	-	-
6	-	0.053	-	-	-	-
Mean	-	BQL	-	-	-	-
SD	-	-	-	-	-	-
% RSD	-	-	-	-	-	-

Table 12b. Method Precision Study (spike)-SET-I.

Sample no	Rel Comp-B	Rel Comp-A	N-Oxide	Benzhydrol	Benzophenone	Total Imp
1	0.202	0.205	3.132	2.401	0.428	6.37
2	0.207	0.205	3.170	2.403	0.427	6.41
3	0.207	0.212	3.090	2.344	0.430	6.28
4	0.182	0.209	3.151	2.410	0.442	6.39
5	0.204	0.213	3.126	2.389	0.439	6.37
6	0.197	0.216	3.263	2.425	0.446	6.55
Mean	0.200	0.210	3.155	2.395	0.44	6.40
SD	0.009	0.004	0.059	0.028	0.008	0.086
% RSD	4.5	1.9	1.9	1.2	1.8	1.3

Table 13a. Ruggedness Data (RS) - Method Precision Study (un-spike)-SET-II.

Sample no	Rel Comp-B	Rel Comp-A	N-Oxide	Benzhydrol	Benzophenone	Total Imp
1	-	0.049	-	-	-	-
2	-	0.038	-	-	-	-
3	-	0.060	-	-	-	-
4	-	0.037	-	-	-	-
5	-	0.030	-	-	-	-
6	-	0.060	-	-	-	-
Mean	-	BQL	-	-	-	-
SD	-	-	-	-	-	-
% RSD	-	-	-	-	-	-

Table 13b. Ruggedness Data (RS) - Method Precision Study (spike)-SET-II.

Sample no	Rel Comp-B	Rel Comp-A	N-Oxide	Benzhydrol	Benzophenone	Total Imp
1	0.201	0.217	3.058	2.373	0.359	6.21
2	0.202	0.202	3.114	2.402	0.378	6.30
3	0.224	0.229	3.070	2.305	0.361	6.19
4	0.187	0.250	3.034	2.283	0.352	6.11
5	0.209	0.194	3.084	2.407	0.377	6.27
6	0.216	0.221	3.185	2.390	0.388	6.40
Mean	0.207	0.219	3.091	2.360	0.37	6.25
SD	0.013	0.020	0.053	0.053	0.014	0.15
% RSD	6.3	9.1	1.7	2.2	3.8	2.4

Table 13c. Precision & Ruggedness comparison (Un-spike): SET- I and II.

Sample ID#	Rel Comp-B		Rel Comp-A		N-Oxide		Benzhydrol		Benzophenone	
	SET-I	SET-II	SET-I	SET-II	SET-I	SET-II	SET-I	SET-II	SET-I	SET-II
1	-	-	0.048	0.049	-	-	-	-	-	-
2	-	-	0.050	0.038	-	-	-	-	-	-
3	-	-	0.062	0.060	-	-	-	-	-	-
4	-	-	0.062	0.037	-	-	-	-	-	-
5	-	-	0.053	0.030	-	-	-	-	-	-
6	-	-	0.054	0.060	-	-	-	-	-	-
Mean	-	-	BQL	BQL	-	-	-	-	-	-
SD	-	-	-	-	-	-	-	-	-	-
% RSD	-	-	-	-	-	-	-	-	-	-
Overall Mean	-		-		-		-		-	
Overall SD	-		-		-		-		-	
Overall % RSD	-		-		-		-		-	

Table 13d. Precision and Ruggedness comparison (spike): SET- I and II.

Sample ID#	Rel Comp-B		Rel Comp-A		N-Oxide		Benzhydrol		Benzophenone	
	SET-I	SET-II	SET-I	SET-II	SET-I	SET-II	SET-I	SET-II	SET-I	SET-II
1	0.202	0.201	0.205	0.217	3.132	3.058	2.401	2.373	0.428	0.359
2	0.207	0.202	0.205	0.202	3.170	3.114	2.403	2.402	0.427	0.378
3	0.207	0.224	0.212	0.229	3.090	3.070	2.344	2.305	0.430	0.361
4	0.182	0.187	0.209	0.250	3.151	3.034	2.410	2.283	0.442	0.352
5	0.204	0.209	0.213	0.194	3.126	3.084	2.389	2.407	0.439	0.377
6	0.197	0.216	0.216	0.221	3.263	3.185	2.425	2.390	0.446	0.388
Mean	0.200	0.207	0.210	0.219	3.155	3.091	2.395	2.360	0.435	0.369
SD	0.009	0.013	0.004	0.020	0.059	0.053	0.028	0.053	0.008	0.014
% RSD	4.5	6.3	1.9	9.1	1.9	1.7	1.2	2.2	1.8	3.8
Overall Mean	0.203		0.214		3.123		2.378		0.402	
Overall SD	0.011		0.015		0.063		0.044		0.036	
Overall % RSD	5.4		7.0		2.0		1.9		9.0	

Table 8a. Accuracy of Diphenhydramine Hydrochloride.

Sl. No.	Level	Sample Area	Amount recovered (mg)	Amount added (mg)	% Recovery	
1	50%-1	1610045	125.60	125.50	100.1	Avg:100.2%
2	50%-2	1608157	125.50	125.50	100.0	SD:0.2
3	50%-3	1614624	126.00	125.50	100.4	%RSD:0.2%
4	100%-1	3185890	248.50	251.00	99.0	Avg:100.0%
5	100%-2	3204762	250.00	251.00	99.6	SD:1.2
6	100%-3	3258514	254.20	251.00	101.3	%RSD:1.2%
7	150%-1	4876696	380.40	376.50	101.0	Avg:101.5%
8	150%-2	4914162	383.40	376.50	101.8	SD:0.4
9	150%-3	4905574	382.70	376.50	101.6	%RSD:0.4%
	Overall Mean				100.5	
	Overall SD				1.0	
	Overall RSD (%)				1.0	

Table 8b. Accuracy of Methyl Hydroxybenzoate.

Sl. No.	Level	Sample Area	Amount recovered (mg)	Amount added (mg)	% Recovery	
1	50%-1	951619	100.10	99.70	100.4	Avg:100.6%
2	50%-2	950668	100.00	99.70	100.3	SD:0.4
3	50%-3	956569	100.70	99.70	101.0	%RSD:0.4%
4	100%-1	1865043	196.20	199.40	98.4	Avg:99.3%
5	100%-2	1878859	197.70	199.40	99.1	SD:1.1
6	100%-3	1903313	200.30	199.40	100.5	%RSD:1.1%
7	150%-1	2826446	297.40	299.10	99.4	Avg:97.1%
8	150%-2	2855842	300.50	299.10	100.5	SD:0.4
9	150%-3	2859581	300.90	299.10	100.6	%RSD:0.4%
	Overall Mean				100.5	
	Overall SD				2.9	
	Overall RSD (%)				2.9	

Table 8c. Accuracy of Propyl Hydroxybenzoate.

Sl. No.	Level	Sample Area	Amount recovered (mg)	Amount added (mg)	% Recovery	
1	50%-1	1	10.10	10.20	99.0	Avg:99.7%
2	50%-2	2	10.10	10.20	99.0	SD:1.2
3	50%-3	3	10.30	10.20	101.0	%RSD:1.2%
4	100%-1	1	20.10	20.40	98.5	Avg:99.5%
5	100%-2	2	20.20	20.40	99.0	SD:1.3
6	100%-3	3	20.60	20.40	101.0	%RSD:1.3%
7	150%-1	1	30.90	30.60	101.0	Avg:100.7%
8	150%-2	2	30.50	30.60	99.7	SD:0.9
9	150%-3	3	31.00	30.60	101.3	%RSD:0.8%
	Overall Mean				99.9	
	Overall SD				1.1	
	Overall RSD (%)				1.1	

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Conflict of Interest: None
Source of Funding: Nil
Paper Citation: Das P*, Prajapati M, Maity A. Combined RP-HPLC methodology for the determination of Diphenhydramine hydrochloride, its impurities and preservatives in oral liquid formation in a single run.. J Pharm Adv Res, 2019; 2(8): 607-620.