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STABILITY INDICATING GREEN ANALYTICAL GAS CHROMATOGRAPHY METHOD FOR THE DETERMINATION OF DIPHENHYDRAMINE IN PURE AND MARKETED FORMULATIONS

T. S. Vishwas, B. M. Gurupadayya* and Rupshee Jain

Department of Pharmaceutical Chemistry, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Mysuru - 570015, Karnataka, India.

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Correspondence to Author:

B. M. Gurupadayya

Department of Pharmaceutical
Chemistry, JSS College of Pharmacy,
JSS Academy of Higher Education &
Research, Mysuru - 570015,
Karnataka, India.

E-mail: bmgurupadayya@jssuni.edu.in

ABSTRACT: The current work defines development of a simple, rapid and precise method based on gas chromatography coupled with Flame Ionization Detector (GC-FID) for the quantitative estimation of diphenhydramine (DPH) present in the marketed formulations following the principles of green analytical chemistry. The analysis was performed using Zebtron Phase: ZB-DRUG-1 column with Nitrogen as a carrier gas having a flow rate of 1 ml/min. The injection volume was 1 μ L and with the split ratio of 1:10. The retention time of the drug was found to be 4.5 minutes. The developed method was validated according to the ICH guidelines, proving the method to be sensitive towards the analyte with accuracy (mean recovery = 98%), linearity in the range of 2-10 μ g/ml (r^2 0.993), precision (intraday = 1.118% RSD, interday = 1.16 % RSD), LOD 0.2 μ g/ml and LOQ 0.5 μ g/ml. The degradation study showed that the drug remains stable in acid, base, neutral, oxidation, and photolytic method. The developed method can be effectively employed for the day to day analysis of DPH in pure and marketed formulations by industries and not so well equipped laboratories, as the method employs direct analysis of DPH without any derivatization technique by gas chromatography with simple flame ionization detection.

INTRODUCTION: Diphenhydramine (DPH) **Fig. 1** is an antihistamine which comes under the class of the first-generation antihistamine. It is a derivative of ethanolamine with sedative and anti-allergic properties and acts by binding and antagonizing histamine H₁ receptor¹. Diphenhydramine competitively inhibits the histamine-1 (H₁) receptor, thereby alleviating the symptoms caused by endogenous histamine on bronchial, capillary and gastrointestinal smooth muscles, mechanism of the CNS depressant effects of diphenhydramine is unknown².

The literature survey disclosed, titrimetric methods for the estimation of DPH has been described in European Pharmacopoeia³ and British Pharmacopoeia⁴ where HPLC has been described in United States Pharmacopoeia⁵. Several unofficial analytical techniques such as fluorometry⁵, spectrophotometry^{7, 8, 9, 10, 11, 12} and electrochemical analysis^{13, 14} has also been reported for the estimation of DPH in pharmaceutical preparations. Atomic absorption spectroscopy^{15, 16} and capillary electrophoresis^{17, 18, 19} was also used previously. Some of the chromatographic techniques such as HPLC^{20,21} LC-MS^{22, 23}, HPTLC^{24, 25} and GC^{22, 26, 27}. Comparison of other methods available for the estimation of DPH in references with the present developed method is as depicted in **Table 1**.

This paper is directed towards the development of a method for estimation of DPH present in the

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formulation and degradation study of DPH following the principles of green analytical chemistry²⁸. The method involves the direct analysis of DPH without any derivatization and use of no harmful chemicals which poses any harm to analytical chemists; it also minimizes the generation of analytical wastes during the process. The method was validated according to ICH guidelines²⁹.

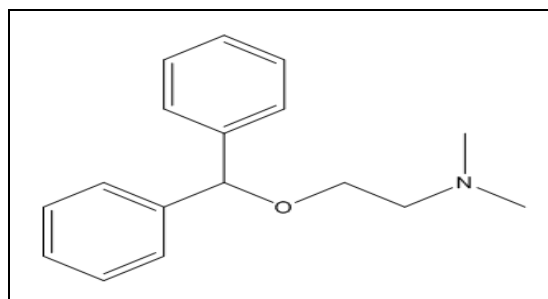


FIG. 1: DIPHENHYDRAMINE (DPH)

TABLE 1: COMPARISON OF OTHER METHOD AVAILABLE FOR ESTIMATION OF DPH WITH PRESENT METHOD

Method/Reagents/Mobile phase (v/v)	Linearity	Method of analysis	Ref
Bromocresol green in a pH 3 buffer, chloroform	5–21µg/ml	Ion pair formation Flow injection spectrophotometry	7
Dichloromethane, patent blue, patent blue, methyl orange, bromocresol purple	2.0–10µg/ml	Ion pair formation Spectrophotometry	8
Cyclohexane, anhydrous sodium sulfate	500µg/ml	Stability assay UV spectrophotometry	9
Chloroform, alginic acid, cation-exchange resin, hydrochloric acid, ethanol	320µg/ml	Extraction, Purification Spectrophotometry	10
Hydrochloric acid	200-500 µg/ml	Second-Derivative UV Spectrophotometry	11
Potassium permanganate, sulphuric acid (0.1 and 2 M), Sodium hydroxide (1M)	2.5-20µg/ml	Spectrophotometry	12
Sodium tetraphenylborate, sodium phosphotungstate, ammonium reineckate, Bis (2-ethylhexyl)- sebacate, Polyvinylchloride	10 ⁻² -10 ⁻⁶ M	Diphenhydramine-plastic membrane sensor with potentiometry	13
Polyvinylchloride, graphite powder, o-Nitrophenyloctylether, dibutylphthalate, dioctylphthalate, dioctylsebacate, tricresylphosphate	1.0.10 ⁻² to 1.0.10 ⁻⁶ Mol/L	Carbon paste electrode using potentiometry	14
Bismuth (III) Nitrate Pentahydrate	10-100 µg/ml	Atomic Absorption Spectrometry	15
Mercury (II) iodide, potassium iodide, mercury atomic absorption standard solution	10-50µg/ml	Ion-associates formation, Indirect atomic absorption spectrometry	16
FE hydrochloride, DMF hydrobromide, DFH hydrochloride, acetonitrile, and sodium dihydrogen phosphate, sodium tetraborate	2.5 - 5.5µg/ml	Capillary electrophoresis	17
Sodium chloride, disodium hydrogen phosphate, sodium dihydrogen phosphate, methyl hydroxybenzoate	40-60µg/ml	Capillary zone electrophoresis	18
0.1M phosphate buffer ph: 4.5-acetonitrile (60:40 v/v)	5–80µg/ml	UV-Visible Detector HPLC	20
Acetonitrile and 10 mm ammonium formate buffer	0.01-1 µg/ml	LC-MS	23
Water with 0.1% formic acid and acetonitrile		LC-TOF/MS	
Ethyl acetate: methanol: Conc. ammonium hydroxide (85:10:5 v/v/v)	1-16µg/ml	UV detector HPTLC	24
Ethyl acetate: methanol: concentrated ammonium hydroxide (85:10:5 v/v/v)	4-16µg/ml	UV detector HPTLC	25
Chloroform and Nitrogen (30 ml/min)	1–5 mg/ml	GC-FID	26
Methanol and Nitrogen (1ml/min)	2-10µg/ml	Green analytical chemistry GC	Present method

MATERIALS AND METHODS:

Chemicals and Reagents: Diphenhydramine pure drug (API) was procured from TCI Chemicals (India) Pvt. Ltd., Tamil Nadu, India. HPLC analytical grade methanol was procured from specialties, private limited, Mumbai, India. The formulations were procured from the local pharmacy store.

GC (Analytical) Instrumentation and Chromatographic Conditions: Shimadzu GC-2014 Coupled with flame ionization detector (FID) was used for method development, validation, and degradation study of DPH. GC solution software was used to control the instrument. A 10 µL syringe made by Hamilton CO., Reno, Nevada, USA was used for the injection of samples.

Zebtron phase: ZB-DRUG-1 (dimethylpolysiloxane backbone with a special phenyl) column procured from Phenomenex private Ltd., Hyderabad, India, with an internal diameter 0.25 mm with a length of 30 meters was used for the analysis. The chromatographic conditions were as depicted in **Table 2**.

TABLE 2: CHROMATOGRAPHIC CONDITIONS

S. no.	Parameters	Values
1	Injection port	200°C with a split ratio 10:1 part
2	Column oven	Initially, 210°C, with a hold time of 1 min and then the temperature was increased to 280°C at a rate of 10°C/min Holding at 280°C for 2 min
3	Detector (FID)	280°C at 40msec sampling rate
4	Carrier gas	Nitrogen was used at 138.5 kPa pressure with the total flow rate of 14 ml/min and the column flow rate of 1ml/min
5	Injection volume	1 µl
6	Split ratio	1:10
7	Run time	10 min

Preparation of Solutions:

Preparation of DPH Standard Solutions: A Stock solution containing 100 µg/ml of DPH was prepared by using methanol as a diluent. Working standards having a concentration of 2, 4, 6, 8, 10 µg/ml was prepared by pipetting out 0.2, 0.4, 0.6, 0.8, 1 ml of stock to 5 different volumetric flasks and the volume was made up with methanol. All the solutions were sonicated for 5 min to remove the entrapped air.

TABLE 3: PREPARATION OF SAMPLE FOR DEGRADATION STUDIES

Types Degradation	Acid	Alkali	Neutral	Oxidation	Photolytic
Reagent used	1M, 5ml HCl	1M, 5ml NaOH	5ml water	5ml 30% H ₂ O ₂	5ml water
Condition	Keep in water bath for 30 min at 70°C			Keep in the UV chamber for 4 h	

RESULTS AND DISCUSSION:

System Suitability: After establishing the method conditions as described, the method was validated as per ICH guidelines for accuracy, precision, linearity, the limit of detection (LOD), the limit of quantitation (LOQ). Precision of the method was established by injecting the middle concentration 6 times intraday and inter-day. Accuracy, in terms of percentage recovery, was established by spiking the formulation at 80%, 100% & 120% (n=3). Linearity was established by injecting a series of dilutions in increasing concentrations (n=5). LOD & LOQ was established using standard deviation and slope. The selectivity of the method was

Preparation of Sample Solution (Formulations):

Syrup: Syrup solution having a concentration of 5.63 µg/ml was prepared by making necessary dilutions using methanol, and it was sonicated and filtered through syringe filter having a pore size of 0.20 µm.

Tablet: Powdered tablet equivalent to 25 mg of DPH was dissolved in methanol, and further dilutions were made to get a final concentration of 5 µg/ml. The solution was sonicated and filtered through a syringe filter, having a pore size of 0.20 µm.

Method Validation: The method was validated according to ICH guidelines for the following parameters: Linearity, accuracy, precision, the limit of detection (LOD) and limit of quantification (LOQ), and system suitability.

Preparation of Solution for Degradation Studies:

5 ml of the standard DPH solution was transferred into five, 25 ml volumetric flasks and treated with 5 ml of 1M Hydrochloric acid, 1M Sodium hydroxide, 30% Hydrogen peroxide, Water and UV rays respectively for 30 min.

The acid and alkali samples were neutralized. 5 ml of water was added to a UV stability sample and kept in a UV chamber for 4 h. The degradation studies for DPH areas depicted in **Table 3**.

assessed by comparing the retention time of standard and formulation; Quantitative analysis of DPH was performed under the established conditions. GC validation report of DPH is as depicted in **Table 4**. Blank, overlay, and standard chromatograms of DPH are shown in **Fig. 3, 4 & 5**, respectively.

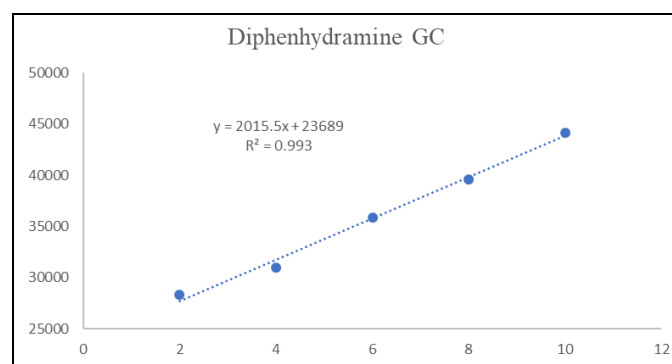
TABLE 4: GC VALIDATION REPORT OF DPH

Parameter	Value
Linearity	2-10 µg/ ml
Limit of detection (LOD)	0.2 µg/ml
Limit of Quantification (LOQ)	0.5 µg/ml
Recovery (%)	98.64%
Regression Coefficient	0.993
Retention time (min)	4.5 min

Linearity: The linearity of DPH was established by injecting 5 different concentrations, in the range of 2-10 µg/ml. The calibration curve was constructed by concentration vs. peak area. The regression equation ($r^2 = 0.993$) demonstrated good linearity of the method. The linearity of DPH is as depicted in **Table 5** and shown in **Fig. 2**

TABLE 5: LINEARITY OF DPH

Concentration (µg/ml)	Peak area
2	28321
4	30967
6	35871
8	39587
10	44166

**FIG. 2: CALIBRATION CURVE OF DPH****TABLE 7: ACCURACY STUDY OF DPH**

Level of recovery	Amount of formulation (µg/ml)	Amount of pure drug (µg/ml)	Total amount of drug (µg/ml)	Peak area	Diff.	% recovery	Level of recovery	Mean
80%	5.6	4.5	10.1	65112	33252	98.11	80%	98.74%
	5.6	4.5	10.1	65503	31859	99.3		
	5.6	4.5	10.1	65347	33488	98.8		
100%	5.6	5.6	11.2	67329	33191	98.01	100%	98.57%
	5.6	5.6	11.2	67564	33426	98.7		
	5.6	5.6	11.2	67695	33557	99.02		
120%	5.6	6.7	12.32	69734	33280	98.19	120%	98.63%
	5.6	6.7	12.32	69897	33439	98.66		
	5.6	6.7	12.32	70031	33573	99.06		

Analysis of Sample:

Syrup: According to the label claim of the formulation every 5 ml of syrup contains 14.08 mg of DPH. 5 ml of syrup was taken in a 25 ml volumetric flask, and the volume was made up to 25 ml with methanol. Then 0.1 ml of the diluted solution was taken in a 10 ml volumetric flask and diluted to 10 ml with methanol to get a final concentration of 5.63 µg/ml. The % of label claimed drug found by GC-FID method was found to be 98.43%, which has been depicted in **Table 11**. The chromatogram of syrup sample is shown in **Fig. 6**

Precision: The precision was established by injecting the 6 replicates of middle concentration on the same day (intra-day) and the consecutive day (inter-day). The % RSD values were found to be less than 2% as depicted in **Table 6**.

TABLE 6: PRECISION STUDY OF DPH

Precision studies	Intraday	Interday
Conc. (µg/ml)	6	6
Average (n=6)	36195	35588
Standard deviation	404.683	414.5563
% RSD	1.118063	1.164866

Accuracy: The accuracy, in terms of percentage recovery was established by spiking the formulation at 80%, 100% & 120% and injecting each spiked sample 3 times (n=3) finding out the amount of the drug recovered. The mean percentage recovery was found to be (mean) 98.64% as depicted in **Table 7**.

Limit of Detection and Limit of Quantitation: LOD and LOQ was calculated with the aid of standard deviation (σ) and slope (s) by using the formula $LOD = 3.3 \sigma/s$ $LOQ = 10 \sigma/s$, LOD and LOQ of DPH was found to be 0.2 µg/ml and 0.5 µg/ml respectively as depicted in **Table 4**.

Tablet: According to the label claim of the formulation each tablet contains 25 mg of DPH. Weight of 10 tablets was noted, and then the tablets were crushed using mortar and pestle. Quantity of the tablet equivalent to 25 mg of DPH was transferred to a 25 ml volumetric flask, and the volume was made up with methanol. Necessary dilutions were made to get a final concentration of 5 µg/ml. The % of label claimed drug found by GC-FID method was found to be 98.08%, which has been depicted in **Table 8**. The chromatogram of tablet sample is shown in **Fig. 7**

Degradation Studies: After subjecting the drug solution for respective degradation environment as depicted in **Table 2**, the drug solution is neutralized, filtered through a syringe filter, and

injected to GC. The percentage degradation of DPH in acid, alkali, neutral, oxidation, and photolytic environment was found to be less than 11% as depicted in **Table 9**.

TABLE 8: ANALYSIS OF FORMULATION

Formulation	Drug	Label Claim (mg)	Amount found (mg)	% label claim
Benadryl (syrup)	Diphenhydramine	Each 5 ml syrup contains 14.08 mg of DPH	13.86	98.43
Benadryl (tablets)		Each tablet consists of 25 mg of DPH	24.52	98.08

TABLE 9: RESULTS OF DPH DEGRADATION STUDIES

Degradation	Acid	Alkali	Neutral	Oxidation	Photolytic
% degradation	7.45	7.16	5.94	6.78	10.52

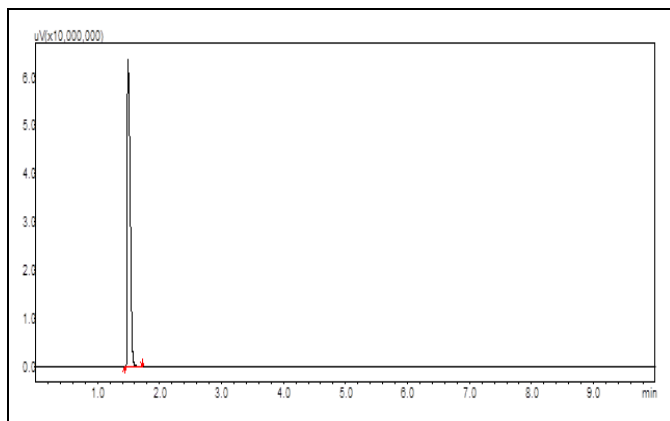


FIG. 3: BLANK CHROMATOGRAM

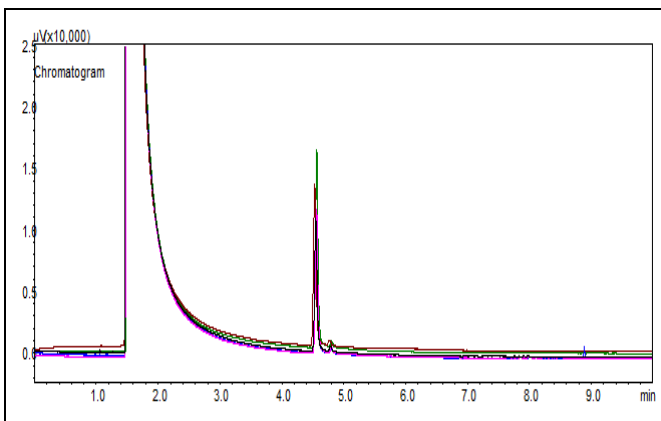


FIG. 4: OVERLAY CHROMATOGRAM OF DPH

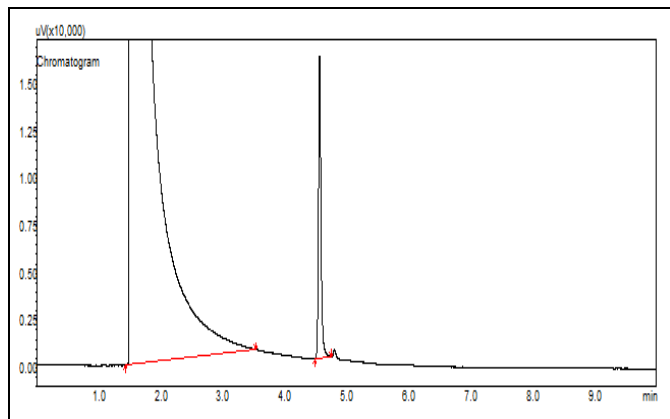


FIG. 5: STANDARD (10 µg/ml) CHROMATOGRAM OF DPH

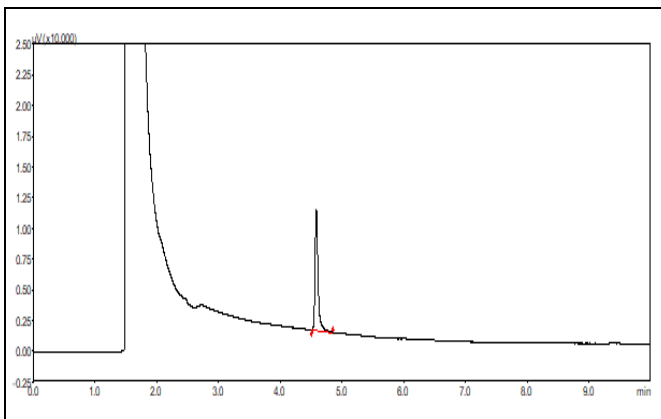


FIG. 6: CHROMATOGRAM OF SYRUP (DPH)

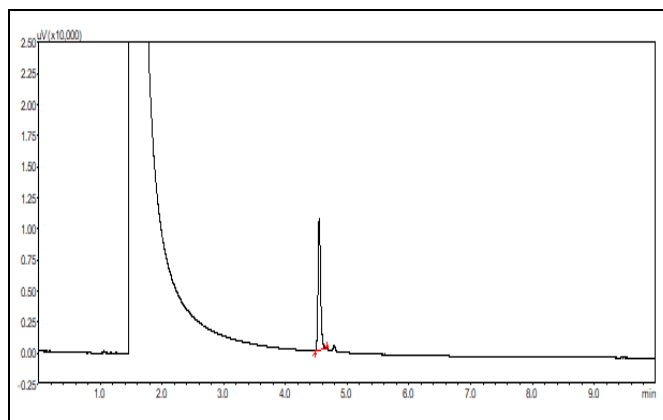


FIG. 7: CHROMATOGRAM OF TABLET (DPH)

CONCLUSION: A simple, fast, accurate, sensitive, cost-effective and environmentally friendly method has been developed for the estimation of DPH in pure form and formulations along with the stability study of DPH following the principles of green analytical chemistry. This method can be used by industries and laboratories which are not very well equipped for the routine estimation of DPH present in formulations as the method employs direct analysis of DPH without any derivatization by gas chromatography with simple flame ionization detection.

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CONFLICT OF INTEREST: Nil

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