IJPSR (2020), Volume 11, Issue 10 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 16 October 2019; received in revised form, 13 February 2020; accepted, 11 March 2020; published 01 October 2020

VALIDATION OF GAS CHROMATOGRAPHY (GC) METHOD FOR RESIDUAL SOLVENT IN BROMPHENIRAMINE MALEATE (API)

Sunil B. Lakhmapure [1], Seema Kothari [1] and Manohar V. Lokhande [*2]

Department of Chemistry [1], Pacific University, Udaipur - 313003, Rajasthan, India.
Department of Chemistry [2], Sathaye College, Mumbai - 400057, Maharashtra, India.

Keywords:

GC, Validation, Brompheniramine Maleate, Accuracy and Retention time

Correspondence to Author:
Dr. Manohar V. Lokhande

Department of Chemistry,
Sathaye College, Mumbai - 400057,
Maharashtra, India.

E-mail: manohar2210@gmail.com

ABSTRACT: Active Pharmaceutical ingredient (API) of pharmaceutical bulk drug Brompheniramine Maleate (API) was validation by gas chromatography. Aim for this article was to check the Residual Solvents in Brompheniramine Maleate (API) by Gas Chromatographic technique. To validate a Gas Chromatographic method for detection and Quantification of Residual Solvents Methanol, Isopropyl Alcohol, DMSO and -O-Xylene. This technique was developed accurately, and validation parameters such as Accuracy, Specificity, Precision, Linearity and Range, Limit of detection (LOD), Limit of quantitation (LOQ), ruggedness, robustness and system suitability testing are explained. Gas chromatograph equipped with FID detector and headspace injection and column 30m × 0.32mm ID × 1.8µm DB-624 capillary column with column temperature was 40 °C (hold 10 min) to 250 °C @ 40 °C/minutes, hold at 250 °C for 5 min. Specificity was retention time for Methanol (2.13), Isopropyl Alcohol (3.45), DMSO (13.90), and -O-Xylene (13.74). The recovery for Methanol % RSD was 2.49, Isopropyl alcohol 1.18, and O-Xylene 1.91. All validation parameters are used in the routine and stability analysis.

INTRODUCTION: The recent analytical techniques normally require specific analytical measurements at very low concentrations, through a variety of instruments. Normally, high-resolution separations have to be achieved with selective chromatographic methods past to analytical purposes [1]. Thus, the data of instrumentation are used in chemical analysis nowadays is of principal status to guarantee future growth in various fields of scientific work.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(10).5039-52

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(10).5039-52

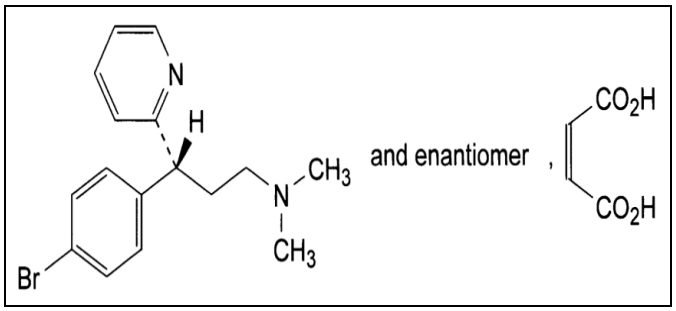
The best usage of instrumentation with more expressive data generation that can be interpreted constantly is probable only by the improved information of the values of the instrumentations used for measurements well as those utilized to attain several separations [2-3]. Brompheniramine Maleate is the maleate salt form of brompheniramine, an alkylamine derivative, and a histamine antagonist with anticholinergic and sedative properties. Brompheniramine maleate competes with histamine for the H-1 receptor.

This diminishes the actions of histamine on effector cells and decreases the histamine-mediated symptoms of an allergic reaction such as broncho-constriction, vasodilation, increased capillary permeability, and spasmodic contractions of

gastrointestinal smooth muscle. Brompheniramine Maleate is a white to almost white, crystalline powder [4]. It is soluble in water, freely soluble in ethanol (96 %), in methanol and in methylene chloride. Brompheniramine maleate is the maleic acid salt of brompheniramine. A histamine H-1 receptor antagonist, it is used for the symptomatic relief of allergic conditions, including rhinitis and conjunctivitis. It has a role as an anti-allergic agent. It contains a brompheniramine [5]. The present study deals with the validation of Residual solvent in Brompheniramine maleate by Cas Chromatography.

MATERIALS AND METHODS:

Materials:



Chemical Name: (3*RS*)-3-(4-Bromophenyl)-*N*,*N*-dimethyl-3-(pyridin-2-yl)propan-1-amine(*Z*)-butenedioate.

Molecular Formula: $C_{20}H_{23}BrN_2O_4$,

Molecular Weight: 435.3.

Limits for Residual Solvents: Methanol: NMT 3000 ppm, Isopropyl alcohol: NMT 5000 ppm and O-Xylene: NMT 2170 ppm.

Reagents: Methanol (AR Grade); Isopropyl alcohol (AR Grade); O-Xylene (AR Grade); Dimethyl sulfoxide (AR Grade) and Brompheniramine Maleate sample available from Supriya Lifescience, Mumbai.

Method: Following Instruments/equipment' sand reagent was used for the validation studies.

INSTRUMENTS / EQUIPMENT's

Instrument Name	Instrument Number	Make	Model Number
Analytical Balance	SLL/QC/50	Mettler	B247544075
GC – 05	SLL/QC/68	Agilent	7890B
GC- 02	SLL/QC/61	Shimadzu	GC-2010 plus

REAGENTS

Reagent Name	Batch Number	Purity	Make
Methanol	SC5P650110	99.8	Merck
o-Xylene	R112F15	99.0	Rankem
Isopropyl Alcohol	DI6P662898	99.5	Merck
Diluent (DMSO)	240433328kq	99.90	Finar
Brompheniramine Maleate sample	SLL/B/1217008	100.13	SLL

CHROMATOGRAPHIC CONDITION

Instrument	:	Gas chromatograph equipped with FID detector and headspace injection.
Column	:	30m × 0.32mm ID × 1.8µm DB-624 capillary column.
Column Temp.	:	40 °C (hold 10 minutes) to 250 °C @ 40 °C/minutes, hold at 250 °C for 5 min
Injector	:	250 °C/ 260 °C
Carrier Gas	:	Nitrogen @ 35cm/sec linear velocity
Total Flow	:	26 ml/min
Column Flow	:	2 ml/min
Purge Flow	:	3.0 ml/min
Split Ration	:	10.0
Head Space Parameters		
Incubation Temp.	:	95°C
Incubation Time	:	15 min
Syringe Temp.	:	115°C
Injection Volume	:	1.0 mL

Preparation of Standard Stock Solution: Transfer 1275µL isopropyl alcohol, 750µL methanol, and 505µL o-xylene into a 100.0 mL of the volumetric flask containing about 80 mL of Dimethyl sulfoxide (DMSO) & mix. Dilute up to the mark with DMSO and mix.

Preparation of System Suitability Solution: Take 5 ml of stock solution in a 100 ml volumetric flask and dilute up to the mark with DMSO.

Preparation of Test Solution: Take accurately about 500 mg of Brompheniramine Maleate 20ml Headspace Vial and add 5 ml of DMSO.

Procedure: Inject blank solution, and sample preparation (in duplicate) records the chromatogram for all injections.

Calculation:

PPM = Area of sample µl in standard × 5 × 5 × Purity of STD. × Density × 1000000 / Area of Standard × 100 ml × 100 × Wt. of Sample, mg × 100 STD

RESULTS AND DISCUSSION: The resultant peaks of DMSO solvent **Fig. 1**, methanol solvent **Fig. 2**, isopropyl alcohol **Fig. 3** and O-Xylene solvent, **Fig. 4** are given below.

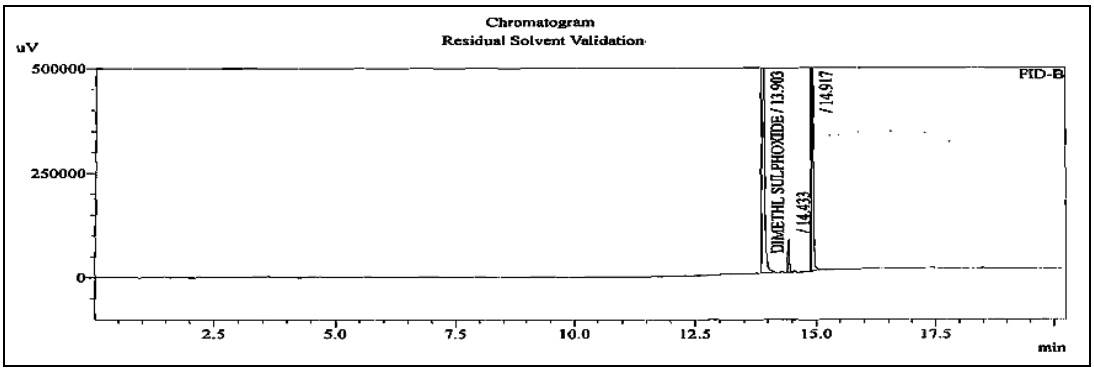


FIG. 1: CHROMATOGRAM OF DMSO SOLVENT

PEAK RESULTS

Peak	Name	RT	Area	Area %	Tailing factor	Resolution (USP)	No. of theoretical Plate (USP)
1	DMSO	13.903	18432543	78.764	0.997	-	793391
2	-	14.433	126168	0.539	1.299	10.113	1842483
3	-	14.917	8443444	20.697	1.287	11.497	2067091
Total	-	-	23402156	100.00	-	-	-

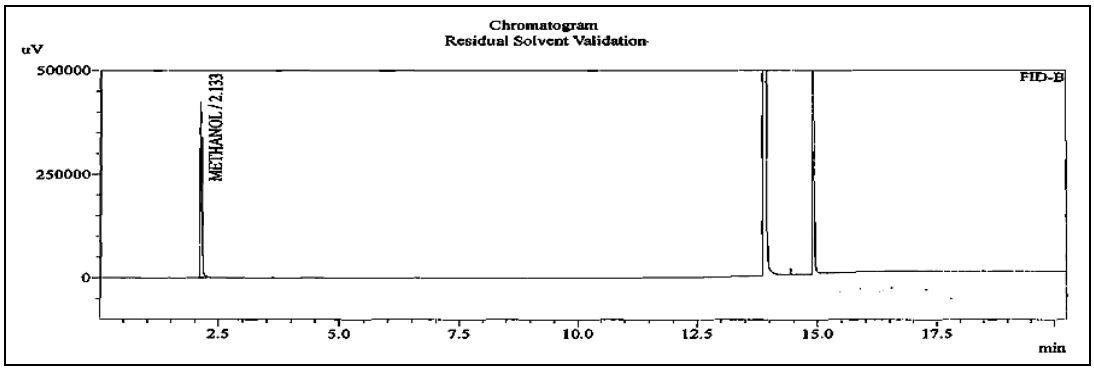


FIG. 2: CHROMATOGRAM OF METHANOL SOLVENT

PEAK RESULTS

Peak	Name	RT	Area	Area %	Tailing factor	Resolution (USP)	No. of theoretical Plate (USP)
1	CH_3OH	2.133	1184504	100.0	1.379	-	15881
Total			1184504	100.0	-	-	-

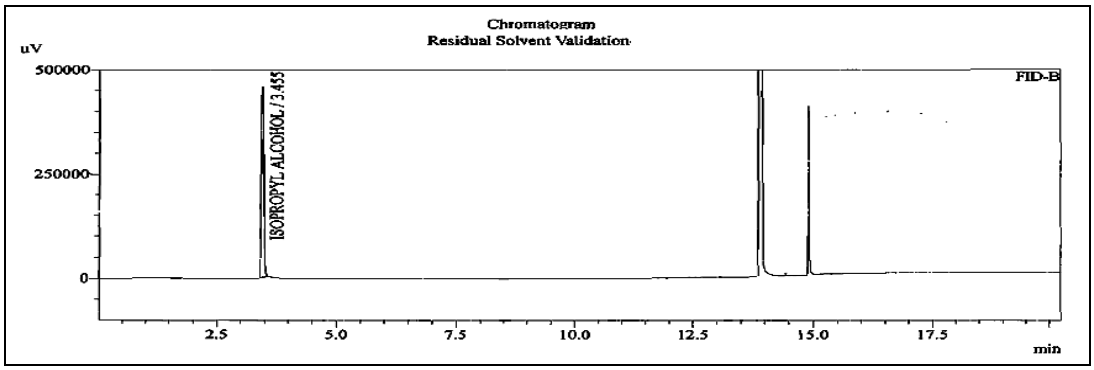


FIG. 3: CHROMATOGRAM OF ISOPROPYL ALCOHOL SOLVENT

PEAK RESULTS

Peak	Name	RT	Area	Area %	Tailing factor	Resolution (USP)	No. of theoretical Plate (USP)
1	Isopropyl Alcohol	3.455	1588229	100.0	1.300	-	22995
Total	-	-	1588229	100.0	-	-	-

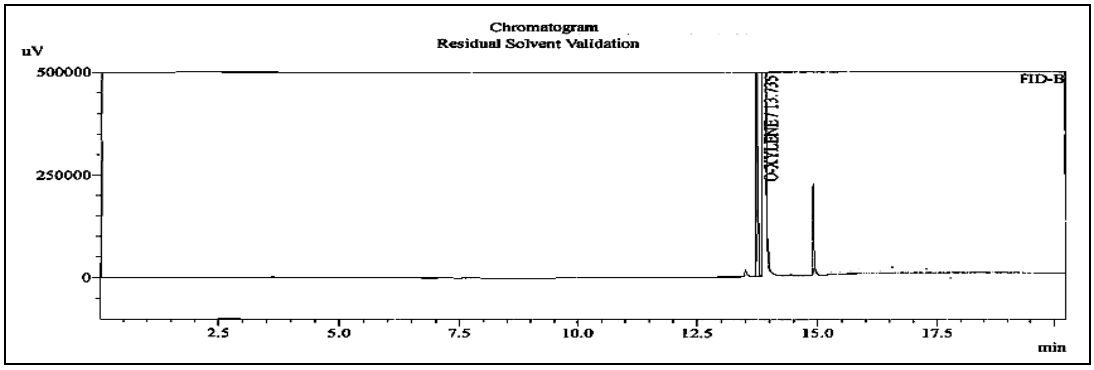


FIG. 4: CHROMATOGRAM OF O-XYLENE SOLVENT

PEAK RESULTS

Peak	Name	RT	Area	Area %	Tailing factor	Resolution (USP)	No. of theoretical Plate (USP)
1	O-Xylene	13.735	4311629	100.0	1.166	-	1431684
Total	-	-	4311629	100.0	-	-	-

Specificity: International Council for Harmonization (ICH) defines specificity as "the ability to assess the analyte unequivocally in the presence of components which may be expected to be present. This reserves the use of 'specific' for those procedures that produce a response for a single analyte only. Our goal is to distinguish and quantify the response of the target compounds from the responses of all other compounds in **Table 1**. Analytical techniques that can measure the analyte response in the presence of all potential sample components should be used for specificity validation. It is not always possible to demonstrate that a single analytical procedure is specific for a particular analyte. In this case, a combination of two or more analytical procedures is recommended to achieve the necessary level of discrimination [6].

Identification: The retention time of the Brompheniramine maleate peak in the chromatogram of the sample preparation corresponds to that of the Brompheniramine maleate peak in the chromatogram of the Standard preparation. Compare the retention times obtained for Methanol, Isopropyl alcohol, and o-Xylene peaks. Also, inject Diluents (Blank). The data will be processed for Methanol, Isopropyl alcohol, and O-Xylene peaks. Check for the interference from diluents (dimethyl sulfoxide) at the retention time of main peaks of solvents, respectively.

TABLE 1: SPECIFICITY

S. no.	Name of sample	Retention time
1	Methanol	2.13
2	Isopropyl alcohol	3.45
3	o-Xylene	13.74
4	Diluent (DMSO)	13.90

Precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scattering) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision was considered at three levels, repeatability, intermediate, and reproductively precision [7].

A. System Precision: The system precision is checked by using a standard chemical substance to ensure that the analytical system is working properly. In this retention time and area of six determinations are measured, and % RSD should be calculated. In method precision, a homogenous sample of the single batch should be analyzed six times. This indicates whether a method is giving consistent results for a single batch. In this analysis, the sample six times and calculate known, unknown, and total impurities in the sample calculate the % RSD **Table 2**.

Experiment: Six replicate injections of the Standard preparation will be made into the GC using the method, and the standard deviation and relative standard deviation (% RSD) of the six replicate injections will be calculated and reported. The % RSD for NMT is 15%.

The % RSD should not be more than 15.0%. The RSD of system precision methanol is 48%, isopropyl alcohol 0.71%, and o-Xylene is 1.52%, respectively, and it meets acceptance criteria. Therefore, the GC method for the determination of methanol, isopropyl alcohol, and o-xylene in Brompheniramine Maleate API is precise.

TABLE 2: SYSTEM PRECISION

S. no.	Methanol		Isopropyl alcohol		o-Xylene	
	RT	Area	RT	Area	RT	Area
1	2.137	396531	3.45	396531	13.73	728120
2	2.137	395199	3.45	934893	13.73	734256
3	2.137	397713	3.45	939941	13.73	736282
4	2.137	392157	3.45	933148	13.73	724656
5	2.137	395806	3.45	947939	13.73	747489
6	2.137	396279	3.45	928737	13.73	714907
Average	2.137	395614	3.454	937342	13.73	730954
SD	0.000	1889.0	0.001	6647.0	0.000	11116.6
% RSD	0.01	0.48	0.02	0.71	0.001	1.52

B. Method Precision: method precision should be performed by determining % assay on six homogeneous samples at working concentration level on one single batch and determining the % RSD of assay value **Table 3**.

TABLE 3: METHOD PRECISION (SINGLE BATCH)

S. no.	Methanol		Isopropyl alcohol		o-Xylene	
	Area	Ppm	Area	Ppm	Area	ppm
Sample-1	1597	12	40574	214	BDL	BDL
Sample -2	1685	13	47380	250	BDL	BDL
Sample -3	1282	10	45674	241	BDL	BDL
Sample-4	1284	10	44387	234	BDL	BDL
Sample -5	1365	10	51285	271	BDL	BDL
Sample -6	1244	9	47306	250	BDL	BDL
Average		10.5	NA	243.3	NA	NA
Std. Dev.		1.4	NA	18.8	NA	NA
% RSD		13.18	NA	7.74	NA	NA

BDL-Below detection limit, NA – Not applicable

Experiment: Six sample preparations of Brompheniramine Maleate API are to be prepared and injected into the GC using the method as described under Methodology. Prepare six sample preparations, inject, and process as per the Methodology. Report the % RSD of each individual solvent present in the sample.

The % RSD should not be more than 15.0%. Therefore, the GC method for the determination of Methanol, Isopropyl alcohol, and o-Xylene in Brompheniramine Maleate API is reproducible.

Spike Study-1: Spike the Methanol, Isopropyl alcohol, and o-Xylene at a 100% concentration of specification level in the sample and inject and process as per the methodology. Separately standard preparation containing a concentration of 100% specification level of Methanol, Isopropyl alcohol, and o-Xylene are also to be prepared and injected and used for quantification of impurities in the sample **Table 4**. Prepare six sample preparations, inject, and process as per the Methodology. Report the % RSD of each individual solvent present in the sample.

TABLE 4: 100% SPIKE STANDARD SOLUTION

Sample Injection	% Recovery		
	Methanol	Isopropyl alcohol	o-Xylene
100% recovery -1	101.60	104.20	102.30
100% recovery-2	100.40	102.50	100.40
100% recovery -3	101.80	105.30	103.80
100% recovery -4	101.70	104.40	103.70
100% recovery -5	100.50	102.90	101.40
100% recovery -6	101.90	104.40	103.80
Average	101.27	104.00	102.17
% RSD	0.748	1.356	1.668

The RSD should not be more than 15.0% and mean of recovery, mean recovery should be in the range of 90.0% to 110.0% for 100% levels. The Mean Recovery is within limits. Therefore, the GC method for the determination of Residual solvents in Brompheniramine Maleate is accurate.

Spike Study 2:

Experiment: Spike the methanol, isopropyl alcohol, and o-xylene a 100% concentration of specification level in the sample and inject and process as per the methodology **Table 5**.

TABLE 5: 100% SPIKE STANDARD SOLUTION

Sample Injection	% Recovery		
	Methanol	Isopropyl alcohol	o-Xylene
100% recovery -1	99.00	101.40	99.30
100% recovery-2	101.60	104.10	102.70
100% recovery -3	98.40	102.30	99.60
100% recovery -4	100.80	103.30	99.80
100% recovery -5	100.90	103.30	100.60
100% recovery -6	100.80	104.40	101.00
Average	99.67	102.60	100.53
% RSD	1.707	1.340	1.872

Mean recovery should be in the range of 90.0% to 110.0% for 100% levels. The mean recovery is within limits **Table 6**. Therefore, the GC method for the determination of Methanol, Isopropyl alcohol, and o-Xylene Brompheniramine Maleate is accurate.

TABLE 6: 100% OVERALL SPIKE STANDARD SOLUTION

Sample Injection	% Recovery		
	Methanol	Isopropyl alcohol	o-Xylene
Analyst -I			
100% recovery -1	101.60	104.20	102.30
100% recovery-2	100.40	102.50	100.40
100% recovery -3	101.80	105.30	103.80
100% recovery -4	101.70	104.40	103.70
100% recovery -5	100.50	102.90	101.40
100% recovery -6	101.90	104.40	103.80
Analyst -II			
100% recovery -1	99.00	101.40	99.30
100% recovery-2	101.60	104.10	102.70
100% recovery -3	98.40	102.30	99.60
100% recovery -4	100.80	103.30	99.80
100% recovery -5	100.90	103.30	100.60
100% recovery -6	100.80	104.40	101.00
Average	100.78	103.54	101.53
SD	1.110	1.120	1.682
% RSD	1.10	1.08	1.66

The % RSD should not be more than 15.0%. Therefore, the GC method for the determination of methanol, isopropyl alcohol, and o-xylene in Brompheniramine Maleate API is reproducible.

LOD and LOQ: The highest point of discovery is the time when deliberate esteem is bigger than the liability related to it. It is the most minimal centralization of analyte in an example that can be identified, be that as it may, not evaluated. The farthest point of location is oftentimes mistaken for the affectability of policy [8]. The affectability of an investigative strategy is the capacity of the technique to separate little contrasts in focus or mass of the test analyte. In down to earth terms, affectability is the incline of the adjustment bend that is gotten by plotting the reaction against the analyte focus or mass. In chromatography, as far as possible, is the infused sum that outcome in a top with size no less than a few times as high as the gauge commotion level. Moreover, this flag/clamor technique depicts three more policies (ICH) [9]. The point of the limit of Quantification [10] is the base infused sum that produces quantitative estimations in the objective system with satisfactory precision in chromatography, commonly requiring top statures 10 to 20 times higher than the gauge commotion **Fig. 5**. Various specimens with diminishing measures of the analyte are infused six times. It is similarly vital to evaluate other technique approval parameters, for example, accuracy, reproducibility, and precision, near the cutoff points of recognition and quantitation. The point of confinement of quantitation to represents both the farthest point of recognition **Table 7**.

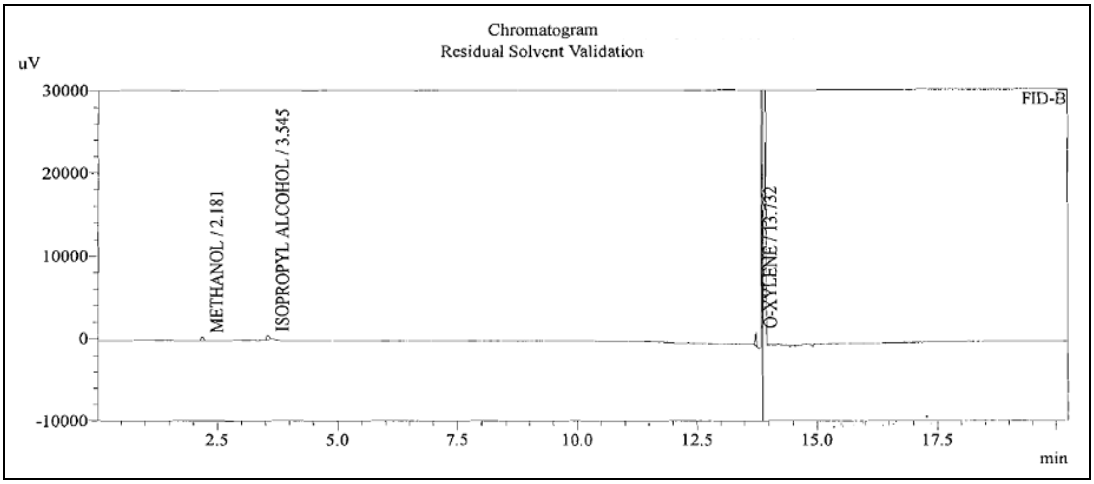


FIG. 5: GRAPH OF LIMIT OF DETECTION

PEAK RESULTS

Peak	Name	RT	Area	Area %	Tailing factor	Resolution (USP)	No. of theoretical Plate (USP)
1	Methanol	2.181	1319	21.702	1.511		11540
2	Isopropyl Alcohol	3.545	2159	35.516	1.307	13.803	14830
3	O-Xylene	13.732	2600	42.782	1.201	126.681	1532659
Total	-	-	6077	100.0	-	-	-

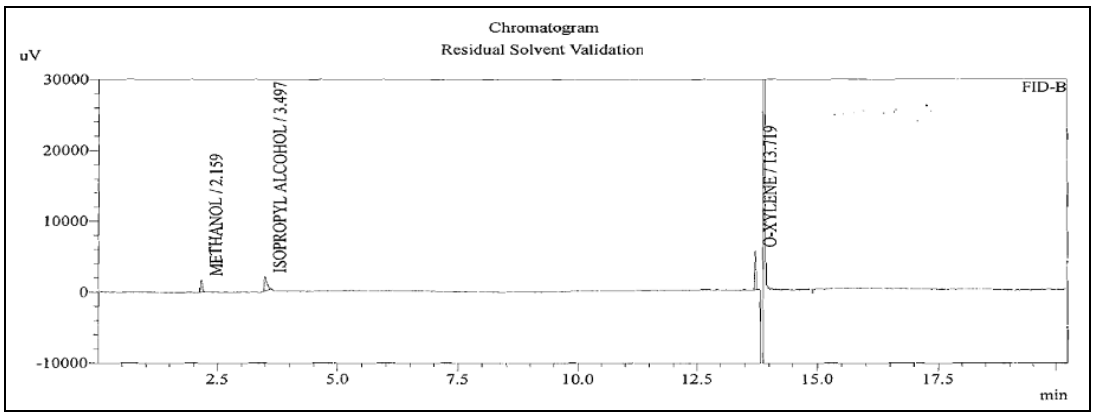


FIG. 6: GRAPH OF LIMIT OF QUANTIFICATION STANDARD

PEAK RESULTS

Peak	Name	RT	Area	Area %	Tailing factor	Resolution (USP)	No. of theoretical Plate (USP)
1	Methanol	2.159	5262	22.591	1.410	-	12569
2	Isopropyl Alcohol	3.497	8227	35.322	1.762	14.608	17347
3	O-Xylene	13.719	9802	42.087	1.221	133.509	1367146
Total	-	-	23290	100.0	-	-	-

Experiment:

Prediction of LOD: To determine LOD/LOQ and show precision at that level, **Fig. 6**.

Preparation of Stock Solution for LOD: (100 ppm): Take 10 mg (12.60 µl) of methanol, 10 mg (12.80 µl) of isopropyl alcohol, and 10 mg (11.35 µl) of o-xylene into 100 ml of the volumetric flask, containing 80 ml of DMSO & mix. Dilute up to the mark with DMSO and mix.

TABLE 7: PREDICTION DILUTION SOLUTION FOR LOD

Sample name	Amount of Prediction LOD stock solution transferred (mL)	Volume made up with DMSO (ml)	Conc. in ppm
LOD solution-1	0.5	50	1
LOD solution-2	1.0	50	2
LOD solution-3	1.5	50	3
LOD solution-4	2.0	50	4
LOD solution-5	2.5	50	5
LOD solution-6	3.0	50	6
LOD solution-7	3.5	50	7
LOD solution-8	4.0	50	8
LOD solution-9	4.5	50	9
LOD solution-10	5.0	50	10

Determine the predicated LOD & LOQ from the above prediction solution. RSD for LOD to NMT is 15%, and RSD for LOQ to NMT is 15%.

Linearity and Range: ICH defines linearity of an analytical procedure as its ability (within a given range) to obtain test results that are directly proportional to the Curve concentration (amount) of analyte in the sample. Linearity may be demonstrated directly on the test substance (by dilution of a standard stock solution) or by separately weighing synthetic mixtures of the test product components [11]. The first is to plot the deviations from the regression line versus the concentration or versus the logarithm of the concentration if the concentration ranges over's several decades, **Table 8**. For linear ranges, the deviations should be equally distributed between positive and negative values.

Experiment: Prepare six different concentrations of isopropyl alcohol, methanol, and o-xylene concentration values LOQ level, 50, 80, 100, 120, and 150% of the working levels. Prepared concentration at each level should be analyzed in duplicate, from the responses obtained for each conc. level, (y- value) should be plotted against conc. (x- value) using a least-squares of test results versus analyte conc. from regression data, calculate the following parameters:

Preparation of Linearity Stock Solution: Take 1275 µL of isopropyl alcohol, 750 µl of methanol and 505 µl of o-xylene into 100.0 mL of the volumetric flask containing about 80 mL of dimethyl sulfoxide and mix. Dilute to the mark with dimethyl sulfoxide and mix properly.

TABLE 8: DILUTIONS FOR LINEARITY

Linearity solution	Standard Stock solution added ml	Vol. in ml with int. standard
LOQ	Prepared as per LOQ std.	
50 %	2.5 ml	100.0ml
80 %	4.0 ml	100.0ml
100 %	5.0 ml	100.0ml
120 %	6.0 ml	100.0ml
150 %	7.5 ml	100.0ml

A least-square fit graph of the individual area counts against the concentration of methanol **Fig. 7**, isopropyl alcohol **Fig. 8,** and o-xylene **Fig. 9** will be plotted and the correlation coefficient, slope, and intercept reported **Table 9**. The range of the analytical method in concentration (µg per ml) will be reported. Correlation coefficient: Not less than 0.99.

TABLE 9: LINEARITY AND RANGE

Sample name	Response Area		
	Methanol	Isopropyl alcohol	o-Xylene
LOQ std. solution	5340	8065	6590
Lin 50%	181210	431131	326349
Lin 80%	303106	727528	552089
Lin 100%	382742	918011	693309
Lin 120 %	467601	1131751	863822
Lin 150 %	586030	1413163	1070710
Correlation coefficient	0.999	0.999	0.999

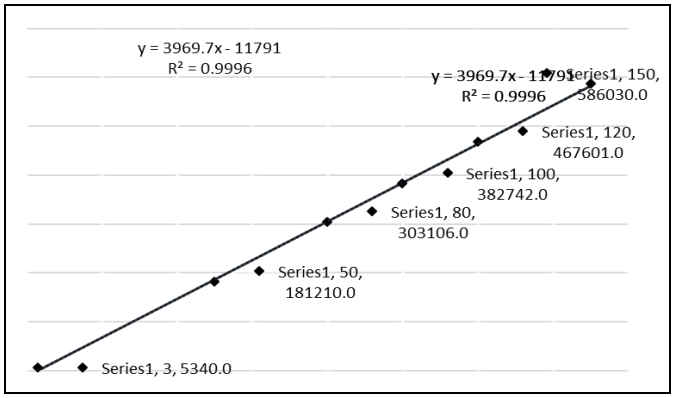


FIG. 7: LINEARITY GRAPH OF METHANOL

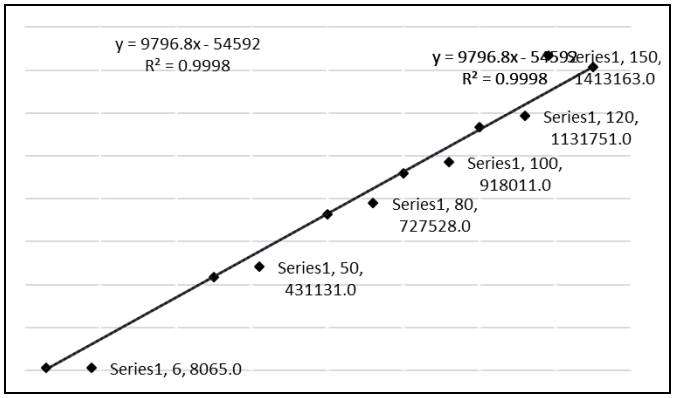


FIG. 8: LINEARITY GRAPH OF ISOPROPYL ALCOHOL

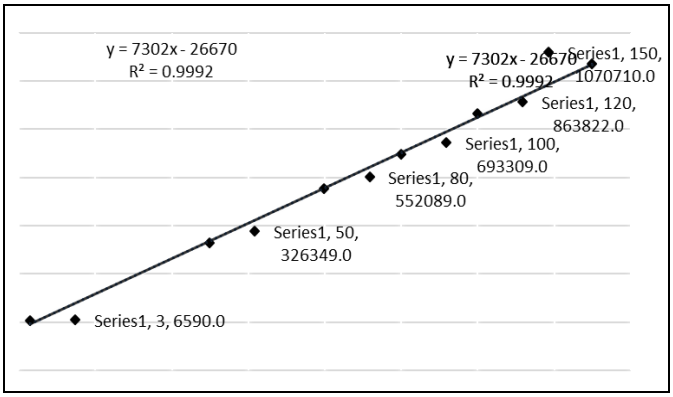


FIG. 9: LINEARITY GRAPH OF O-XYLENE

The correlation coefficient should not be less than 0.99. The correlation coefficient meets acceptance criteria. Therefore, the GC method for the determination of methanol, isopropyl alcohol, and o-xylene is linear.

Accuracy (Recovery): ICH defines the accuracy of an analytical procedure as the closeness of agreement between the actual conventional value or an accepted reference value and the value found. Efficiency can also be described as the extent to which test results generated by the method and the true value agree **Table 10**. The true value for accuracy assessment can be obtained in several ways.

Experiment: Brompheniramine Maleate API will be spiked with methanol, isopropyl alcohol (IPA),

and o-Xylene at three different levels 80%, 100%, 120%, and 150% of specification limit of solvents in triplicate (in total nine determinations). They will be prepared according to the sample preparation mentioned below.

Preparation of Solvent Recovery Stock Solution: Take 1275 µL of isopropyl alcohol, 750µl of methanol, and 505µl of o-xylene into 100.0 mL of the volumetric flask containing about 80 mL of dimethyl sulfoxide and mix. Dilute to the mark with dimethyl sulfoxide and mix properly.

TABLE 10: PREPARATION OF 80, 100, 120 AND 150% STANDARD SOLUTION

Standard solution	Standard Stock solution added ml	Vol. in ml with dimethyl sulfoxide
80 %	4.0 ml	100.0ml
100 %	5.0 ml	100.0ml
120 %	6.0 ml	100.0ml
150%	7.5 ml	100.0ml

Preparation of Sample without Spiking (Control Sample): Take accurately about 500 mg of Brompheniramine Maleate in 20ml Headspace Vial and add 5 ml of dimethyl sulfoxide.

Preparation of Sample with Spiking: Weigh 500 mg of Brompheniramine Maleate in 20 ml Headspace vial and add 5 ml of 80% std. solution. Inject 1.0 ml using Headspace instruments. Calculate the residual solvents of methanol, isopropyl alcohol, and o-xylene. Apply correction if required (Prepared in triplicate) same procedure for 100%, 120%, and 150%.

Data Evaluation: For each level and each replicate, the following will be calculated:

(i) Amount added in mg

(ii) % Recovery = Amount recovered/Amount added × 100.

The Mean, Standard deviation, and RSD % will be computed for the twelve determinations and reported along with the above (i) and (ii).

(iii) Amount of recovered calculation formula

PPM = Area of Sample µl in standard × 5 × 5 × Purity of STD. × Density × 1000000 / Area of Standard × 100 ml × 100 × Wt. of Sample, mg × 100 STD

Brompheniramine Maleate was spiked with methanol, isopropyl alcohol, and o-xylene at three different levels 80%, 100%, 120%, and 150% of the specifications in triplicate (in total twelve determinations) and preceded according to the sample preparation described in methodology **Table 11, 12** and **13**.

TABLE 11: RECOVERY OF METHANOL

Sample no.	Amount added (mg)	Amount recovered (mg)	% Recovery
Acc. 80% -1	2368	2163	91.30
Acc. 80% -2	2368	2194	92.70
Acc. 80% -3	2368	2180	92.10
Acc. 100% -1	2960	2885	97.40
Acc. 100% -2	2960	2827	95.50
Acc. 100% -3	2960	2932	99.10
Acc. 120% -1	3552	3382	95.20
Acc. 120% -2	3552	3438	96.80
Acc. 120% -3	3552	3426	96.50
Acc. 150% -1	4440	4297	96.80
Acc. 150% -2	4440	4320	97.30
Acc. 150% -3	4440	4285	96.50
	Mean		95.60
	SD		2.378
	% RSD		2.49

TABLE 12: RECOVERY OF ISOPROPYL ALCOHOL

Sample no.	Amount added (mg)	Amount recovered (mg)	% Recovery
Acc. 80% -1	3958	4077	103.00
Acc. 80% -2	3958	4032	101.90
Acc. 80% -3	3958	4118	104.00
Acc. 100% -1	4948	5132	103.70
Acc. 100% -2	4848	5008	101.20
Acc. 100% -3	4948	5144	104.00
Acc. 120% -1	5937	6141	103.40
Acc. 120% -2	5937	6180	104.10
Acc. 120% -3	5937	6161	103.80
Acc. 150% -1	7421	7719	104.00
Acc. 150% -2	7421	7781	104.80
Acc. 150% -3	7421	7862	105.90
	Mean		103.70
	SD		1.226
	% RSD		1.18

TABLE 13: RECOVERY OF O-XYLENE

Sample no.	Amount added (mg)	Amount recovered (mg)	% Recovery
Acc. 80% -1	1760	1782	101.30
Acc. 80% -2	1760	1761	100.10
Acc. 80% -3	1760	1813	103.00
Acc. 100% -1	2200	2223	101.00
Acc. 100% -2	2200	2163	98.30
Acc. 100% -3	2200	2232	101.50
Acc. 120% -1	2640	2693	102.00
Acc. 120% -2	2640	2716	102.90
Acc. 120% -3	2640	2692	102.00
Acc. 150% -1	3300	3444	104.40
Acc. 150% -2	3300	3460	104.90
Acc. 150% -3	3300	3453	104.70
	Mean		102.20
	SD		1.953
	% RSD		1.91

The mean recovery should be in the range of 90.0% to 110.0% for 80%, 100%, and 150% levels.

The mean recovery for all components is within limits. Therefore, the GC method for the determination of residual solvents in brompheniramine maleate is accurate.

Ruggedness: Ruggedness is not addressed in the ICH documents -4.5 [12-13]. Its definition has been replaced by reproducibility, which has the same meaning. Ruggedness is defined by the USP as the degree of reproducibility of results obtained under a variety of conditions, such as different laboratories, analysts, instruments, environmental conditions, operators, and materials [14]. Ruggedness is a measure of the reproducibility of test results under normal, expected operational conditions from laboratory to laboratory and from analyst to analyst **Table 14**.

Experiment: Six sample preparations of Brompheniramine Maleate API are to be prepared and injected into the GC using the different column on a different day and injected into a different GC using the method and spike the samples at a 100% concentration of specification level in the sample and inject and process as per the methodology. Separately standard preparation containing a concentration of 100% specification level of methanol, isopropyl alcohol, and o-xylene are also to be prepared and injected and used for quantification of impurities in the sample.

Prepare six sample preparations, inject, and process as per the Methodology. Report the % RSD of each impurity present in the sample.

TABLE 14: RUGGEDNESS

S. no.	Methanol		Isopropyl alcohol		o-Xylene	
	RT	Area	RT	Area	RT	Area
1	3.60	1502154	5.90	3946492	14.47	3119833
2	3.60	1501629	5.90	3951167	14.48	3092359
3	3.60	1481989	5.90	3894289	14.48	3062407
4	3.60	1503850	5.90	3936368	14.47	3107441
5	3.60	1530561	5.90	4024126	14.48	3196964
6	3.60	1500660	5.90	3924030	14.48	3104531
Average	3.60	1503473.8	5.90	3946078.7	14.48	3113922.5
SD	0.000	15546.8	0.000	43313.0	0.005	45121.8
% RSD	0.00	1.03	0.00	1.10	0.036	1.45

The RSD should not be more than 15.0%. The RSD of system precision methanol is 1.03%, isopropyl alcohol is 1.10, and o-xylene is 1.45%, respectively, and it meets acceptance criteria. Therefore, the GC method for the determination of methanol, isopropyl alcohol, and o-xylene in Brom-pheniramine Maleate API is precise.

Robustness: ICH defines the robustness of an analytical procedure as a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters. Robustness refers to a change of parameters and stress to test a particular analytical method. The importance of demonstrating robustness in a particular analytical method is to meet regulatory and manufacturing standards [15].

Three sample preparations of Brompheniramine Maleate API are to be prepared and injected into the GC using the method and spike the three samples at a 100% concentration of specification level in sample and inject and process as per the methodology. Separately standard preparation containing a concentration of 100% specification level of methanol, isopropyl alcohol, and o-xylene **Table 15** are also to be prepared and injected and used for quantification of impurities in the sample. The blank solution, system suitability solution, and sample preparation will be injected into GC under different chromatographic conditions as mentioned below.

1. Change in flow rate (± 2%)
2. Change in oven temperature.

Experiment: Spike the three samples at a 100% concentration of specification level in the sample and inject and process as per the methodology. Separately standard preparation containing a concentration of 100% specification level of methanol, isopropyl alcohol, and o-xylene are also to be prepared and injected and used for quantification of impurities in the sample. A) As such a method, B) Change temperature and C) Change in flow rate (±2%).

TABLE 15: OVERALL RSD OF ROBUSTNESS STUDY

100% spike sample			
	Methanol	**Isopropyl alcohol**	**o-Xylene**
As such method	101.60	104.20	102.30
	100.40	102.50	100.40
	101.80	105.30	103.80
Change in temperature	103.30	107.80	106.60
	102.40	106.50	103.10
	103.20	106.60	102.80
Change in floe plus	101.60	102.50	101.00
	100.20	102.70	100.00
	100.10	102.30	99.40
Change in flow minus	99.50	104.30	103.80
	101.50	105.30	103.70
	100.30	103.60	101.50
Average	101.33	104.47	102.37
SD	1.243	1.849	2.032
% RSD	1.23	1.77	1.99

The % RSD should not be more than 15%. Robustness of the methods done on the same instruments with a change in temperature and carrier gas flow. Therefore, the GC method for the determination of methanol, isopropyl alcohol, and o-xylene in Brompheniramine Maleate robust. Methanol, isopropyl alcohol, and o-xylene will be calculated & reported along with Standard deviation.

System Suitability: The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) was formed to provide a uniform set of guidelines for international use by the pharmaceutical industry.

1. Resolution: The resolution factor between any two peaks should not be less than 2.0.

2. Relative Standard Deviation: Not more than 15%, a standard solution of each standard.

3. Tailing Factor: Should not be greater than 2 for each peak of interest

%RSD of six replicates injections, retention time and area for methanol, isopropyl alcohol, and o-xylene. Standard solution and was recorded as per method on every day **Fig. 9, 10,** and **Table 16**.

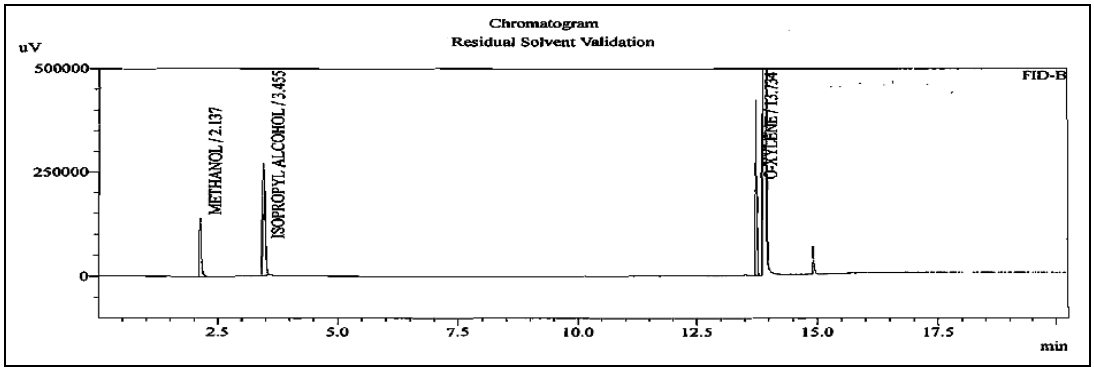


FIG. 9: CHROMATOGRAM OF STANDARD SOLUTION

PEAK RESULTS

Peak	Name	RT	Area	Area %	Tailing factor	Resolution (USP)	No. of theoretical Plate (USP)
1	Methanol	2.137	396531	19.211	1.506		15382
2	Isopropyl Alcohol	3.455	939396	45.512	1.394	16.485	23104
3	O-Xylene	13.734	728120	35.216	1.203	150.697	1457700
Total	-	-	2064047	100.0	-	-	-

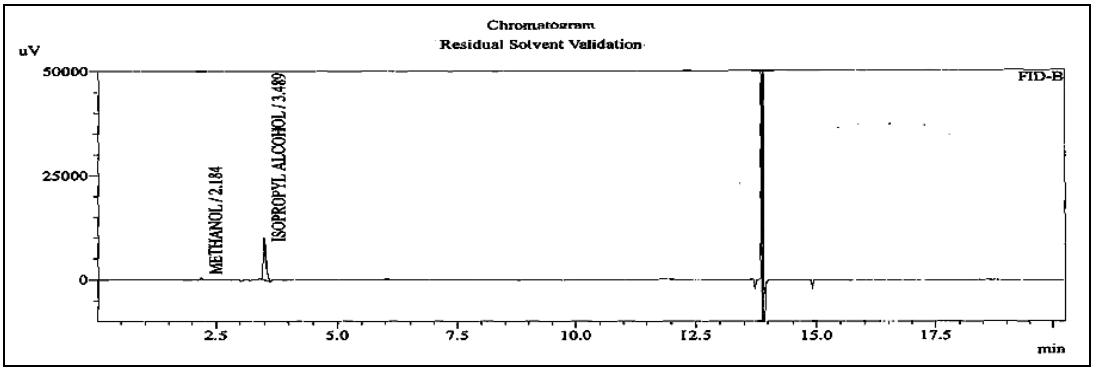


FIG. 10: CHROMATOGRAM OF SAMPLE SOLUTION

PEAK RESULTS

Peak	Name	RT	Area	Area %	Tailing factor	Resolution (USP)	No. of theoretical Plate (USP)
1	Methanol	2.184	1597	3.767	1.032		11345
2	Isopropyl Alcohol	3.489	40574	96.213	1.979	14.228	18941
Total	-	-	42171	100.0	-	-	-

RT= Retention Time, RSD = Relative standard deviation

The relative standard deviation should not be more than 15%. And tailing and experimentation factor should not more than 2, and resolution should not less than 2. System suitability complies with **Table 17, 18, 19,** and **20**.

TABLE 17: ACCEPTANCE CRITERIA

Validation Parameter	Acceptance Criteria
Specificity and Selectivity	Results should be comparable with respect to Retention time.
Precision	
System Precision	RSD should not be more than 15%
Method Precision	RSD should not be more than 15%
Spike study	Mean of recovery should be in the range of 90.0 to 110.0 % for 100 % levels.
Limit of detection and Limit of quantification	RSD should not be more than 15.0%. For experimental limit of detection.
Linearity and Range	Correlation coefficient should not be less than 0.99
Recovery (Accuracy)	Mean of recovery should be in the range of 90.0 to 110.0 % for 50 to 150 % levels.
Ruggedness (Intermediate precision)	RSD should not be more than 15%
Robustness	RSD should not be more than 15%
System suitability	Resolution: Resolution factor between any two peaks should not be less than 2.0 Relative standard deviation: Not more than 15%, standard solution of each standard. Tailing Factor: Should not be greater than 2 for each peak of interest.

TABLE 18: SUMMARY REPORT OF METHANOL

Validation Parameter	Acceptance Criteria		Results
Specificity	No interference		Meets Acceptance Criteria
System suitability	% of RSD Area – NMT 15%		0.478
	% of RSD for RT – NMT 15%		0.011
	Tailing Factor – NMT 3		1.50
	Resolution – NLT 2		--
LOD	Experimental observed PPM		1
LOQ	Experimental observed PPM		3
Linearity and Range	Correlation NLT 0.98		0.999
	Method Precision		
As such	% RSD of Sample -NMT 15%		13.18
With spike	% RSD of Sample -NMT 15%		0.784
Accuracy / Recovery	% Recovery –	Level	
	Between 90.0 % to 110.0 %	80%	92.03
		100%	97.33
		120%	96.17
		150%	96.87
Ruggedness	Cumulative RSD NMT 15% for 100% recovery (analyst)		1.10
Robustness	Over all RSD NMT 15% for 100% recovery (Normal and + flow change + temperature)		1.23

TABLE 19: SUMMARY REPORT OF ISOPROPYL ALCOHOL

Validation Parameter	Acceptance Criteria	Results
Specificity	No interference	Meets Acceptance Criteria
System suitability	% of RSD Area – NMT 15%	0.709
	% of RSD for RT – NMT 15%	0.019
	Tailing Factor – NMT 3	1.39
	Resolution – NLT 2	16.485
LOD	Experimental observed PPM	1
LOQ	Experimental observed PPM	3

Linearity and Range	Correlation NLT 0.98		0.999
	Method Precision		
As such	% RSD of Sample -NMT 15%		7.74
With spike %	% RSD of Sample -NMT 15%		1.356
	% Recovery –	Level	
	Between 90.0 % to 110.0 %	80%	102.97
Accuracy / Recovery		100%	102.97
		120%	1103.77
		150%	104.90
Ruggedness	Cumulative RSD NMT 15% for 100% recovery (analyst)		1.08
Robustness	Over all RSD NMT 15% for 100% recovery (Normal and + flow change + temperature)		1.77

TABLE 20: SUMMARY REPORT OF O- XYLENE

Validation Parameter	Acceptance Criteria		Results
Specificity	No interference		Meets Acceptance Criteria
System suitability	% of RSD Area – NMT 15%		1.521
	% of RSD for RT – NMT 15%		0.001
	Tailing Factor – NMT 3		1.20
	Resolution – NLT 2		150.697
LOD	Experimental observed PPM		2
LOQ	Experimental observed PPM		6
Linearity and Range	Correlation NLT 0.98		0.999
	Method Precision		
As such	% RSD of Sample -NMT 15%		Below detection limit
With spike %	% RSD of Sample -NMT 15%		1.668
Accuracy / Recovery	% Recovery –	Level	
	Between 90.0 % to 110.0 %	80%	101.47
		100%	100.27
		120%	102.30
		150%	104.67
Ruggedness	Cumulative RSD NMT 15% for 100% recovery (analyst 1 + analyst 2)		1.66
Robustness	Over all RSD NMT 15% for 100% recovery (Normal and + flow change + temperature)		1.99

CONCLUSION: This procedure is to validate by Gas Chromatography (GC) method of analysis for detection and Quantification of Residual Solvents methanol, isopropyl alcohol, and o-xylene in Brompheniramine Maleate. This method is applicable for quantifying & monitoring the traces of residual solvents methanol, isopropyl alcohol, and o-xylene simultaneously on a routine basis using Gas chromatograph in Brompheniramine Maleate. The recovery of % RSD for Methanol was 2.49, Isopropyl Alcohol 1.18, and O-Xylene 1.91. The residual solvent test method is validated for specificity, the limit of detection, the limit of quantification, linearity, and range, precision, recovery, ruggedness, and robustness are found to be meeting the pre-determined acceptance criteria. The validated method is Specific, LOD, LOQ, Linear, Precise, Accurate, Rugged, and Robust for Residual solvents of Brompheniramine Maleate. On the basis of the above, it was concluded that the developed method of assay for candidate drug was validated as per the ICH guideline

ACKNOWLEDGEMENT: The author, thanks to Mr. Satish Wagh, MD, Dr. Saloni Wagh, and Dr. Prashant Zate, Supriya Life Science Ltd, Mumbai for their cooperation and help to carry out this research work.

REFERENCES:

1. Sethi PD: HPLC, Quantitative Analysis of Pharmaceutical Formulation. CBS Publishers and Distributer. New Delhi. 1996: 3-35
2. Shaikh KA, Patil SD and Devkhile AB: Development and validation of a reversed-phase HPLC method for simultaneous estimation of ambroxol hydrochloride and Azithromycin in tablet dosage form. Journal of Pharmaceutical and Biomedical Analysis 2008; 48: 1481-84.
3. ICH Q3D Guideline, Impurities in New Drug Substances, Step- 5 version 2014.
4. Simons FE, Frith EM and Simons KJ: The pharmacokinetics and antihistaminic effects of brompheniramine. Journal of Allergy and Clinical Immunology 1992; 70(6): 458-64.
5. Fischer J and Ganellin CR: Analogue-based Drug Discovery. John Wiley & Sons, 2006: 546-50.
6. Prasad YR, Rajasekhar KK, Shankarananth V, Keerthisikha P, Ravindra A and Sujitha MM: RP-HPLC

method for the estimation of Tramadol in bulk and capsule dosage form., Journal of Pharmacy and Research 2006; 4(3): 886-87.
7. Kishore VS, Kumar PJ, Chakravarthy TK, Naik SV, Reddy MH and Babu NE: Development and validation of RP-HPLC method for quantitative analysis voglibose in pure and pharmaceutical formulations. International Journal of Pharmaceutical chemistry and Biochemical Science 2013; 3: 336-41.
8. Rajput M, Dahiya M, Kumari P, Kalra K, Aggarwal M and Khandal RK: Method development and validation for determination of voglibose in tablet formulation using LC-MS/MS., European Journal of Chemistry 2011; 8: 1770-83.
9. Zate PB, Kothari S and Lokhande MV: Confirmation and Quantification of Genotoxic Impurity 2-Dimethyl-aminoethyl chloride hydrochloride (DMC HCl) by GCMS in Chlorpheniramine/Chlorphenamine Maleate., IOSR Journal of Applied Chemistry 2017; 10(7): 21-26.
10. Wagh SS, Kothari and Lokhande MV: Quantification of (4-Bromophenyl) {Pyridine-2-Yl} Acetonitrile Impurity (4-BPPA) by HPLC in Brompheniramine Maleate Active Pharmaceutical Ingredient. IOSR Journal of Applied Chemistry 2017; 10(7): 27-32.
11. Maurya CP and Lokhande MV: Characterization and validation of impurities related to Pharmaceutical Bulk Drug (API) by using some analytical techniques. International Journal of Pharmaceutical Sciences and Research 2017; 8(8): 3325-40.
12. ICH, Validation of Analytical Procedure: Methodology (Q2B), International Conference on Harmonization, Geneva 1996.
13. ICH: International conference on harmonization, validation of analytical procedures; text and methodology Q2 (R1) 2005.
14. Lee H, Riu M and Kim E: A single residue method for the determination of chlorpropamide in representative crops using HPL Chromatography, J Korean Society Applied Biological Chemistry 2013; 56(2): 181-86.
15. Santos Delgado MJ, Rubio BS and Polo-Diez LM: Stability studies of carbamate pesticides and analysis by gas chromatography with flame ionization and nitrogen-phosphorus detection. Journal of Chromatography-A 2001; 921(2): 287-96.

How to cite this article:
Lakhmapure SB, Kothari S and Lokhande MV: Validation of gas chromatography (GC) method for residual solvent in brompheniramine maleate (API). Int J Pharm Sci & Res 2020; 11(10): 5039-52. doi: 10.13040/IJPSR.0975-8232.11(10).5039-52.