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DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR SIMULTANEOUS ESTIMATION OF VARENICLINE TARTRATE AND BUPROPION HYDROCHLORIDE

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ABSTRACT

A simple, sensitive, precise, rapid and accurate reverse phase high performance liquid chromatography (RP- HPLC) method was developed and validated for simultaneous estimation of Varenicline tartrate and Bupropion hydrochloride. The Chromatographic separation was achieved by using Cosmosil C18 (250 mm×4.6 mm, 5µ) as stationary phase and mobile phase consists of Methanol: phosphate buffer with pH 3.0 (65:35 v/v) with a flow rate of 1ml/min. The analysis was performed at ambient temperature and the eluent was monitored at 244 nm using UV detector. The retention time of Varenicline tartrate and Bupropion hydrochloride was found to be 3.0 min and 4.2 min respectively and the calibration curves were linear (r^2 = 0.999 and 0.998) over a concentration range of 10-50µg/ml for Varenicline tartrate and 100-500 µg/ml for Bupropion hydrochloride respectively. The Limit of detection (LOD) for Varenicline tartrate and Bupropion hydrochloride was observed to be 0.002µg/ml and 0.006µg/ml respectively, the limit of quantitation (LOQ) was found to be 0.006µg/ml and 0.018µg/ml respectively. The developed method was validated as per ICH guidelines using parameters like linearity, specificity, system suitability, precision, ruggedness, robustness, accuracy. All the validation parameters were found to be well within the acceptance criteria. Hence the proposed method can be used for the routine analysis of Varenicline tartrate and Bupropion hydrochloride in bulk and tablet dosage forms.

KEYWORDS: Varenicline tartrate, Bupropion hydrochloride, RP-HPLC, Simultaneous estimation. ICH guidelines.

INTRODUCTION

Varenicline tartrate is the first approved partial agonist nicotinic receptor which is used to treat smoking addiction. Specifically, it is a partial agonist of the α4/β2 subtype of the nicotinic acetylcholine receptor. Varenicline tartrate leads to the release of dopamine in the nucleus accumbens, because of its high-affinity partial agonist for the α4β2 nicotinic acetylcholine receptor subtype (nACH). Therefore, it has the capacity to reduce the feelings of craving and decreases the pleasurable effects of cigarettes which includes other tobacco products and withdrawal caused by smoking cessation.[1,2]

Bupropion is the hydrochloride salt of an amino ketone antidepressant. This agent does not inhibit MAO and compared to tricyclic antidepressants which is a weak blocker of neuronal uptake of serotonin and norepinephrine. Hence, the molecular mechanism of antidepressant effect of bupropion hydrochloride is not found clearly. Bupropion hydrochloride reduces the smoking addiction, cravings for nicotine and withdrawal symptom. Hence the effect is not due to anti-depressant effect. It also weakly inhibits the neuronal re-uptake of dopamine. But, bupropion hydrochloride alone is less effective than varenicline tartrate. [5,6]

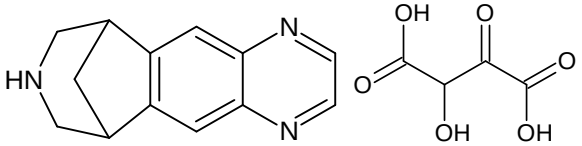
Bupropion hydrochloride potentiate the effects of varenicline tartrate when given in combination, it approximately doubles the chance of quitting smoking successfully. [10]

MATERIALS AND METHODS

Chemicals and Reagents

The reference samples of was obtained from SIGMA ALDRICH & was procured as a gift sample from M/s APOTEX. Acetonitrile, Methanol, and Water were of HPLC grade. Potassium dihydrogen phosphate & Disodium hydrogen phosphate used was of Analytical grade.

Varenicline Tartrate

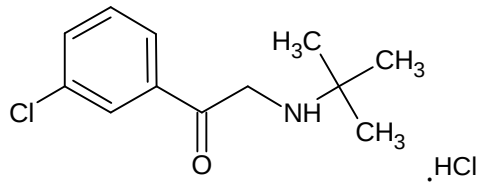


Category: Anti-smoking drug.

Molecular formula: $C_{17}H_{19}N_3O_6$

Molecular weight: 361.349g/mol

Bupropion Hydrochloride



Category: Anti-smoking and Anti-depressant.

Molecular formula: $C_{13}H_{19}Cl_2N$.

Molecular weight: 276.201g/mol.

Instrument and Chromatography Condition

The High Performance Liquid Chromatography consisted of SHIMADZU-SPD-20A prominence auto sampler fitted with UV Visible detector (SPD-20A) with SHIMADZU-LC-20AT pump. The chromatogram was recorded using LC Solution software. The Chromatographic separation was achieved by using Cosmosil C18(250 mm×4.6 mm, 5µ) stationary phase and mobile phase consists of Methanol : Phosphate buffer with pH 3.0 (65:35 v/v) with a flow rate of 1ml/min. The analysis was performed at ambient temperature and the eluent was monitored at 244 nm using UV detector.

Buffer Preparartion

Weighed accurately 3.4 g of Potassium dihydrogen orthophosphate and transferred into 500 ml of volumetric flask. Add small amount of HPLC grade water and shake it until it dissolves after that the volume made up to the mark with same HPLC water. The pH adjusted to 3 with orthophosphoric acid. Filter the above solution through the 0.45 µ membrane filter.

Preparation of Mobile Phase

Mobile phase was prepared by 500 ml of methanol and 500 ml of phosphate buffer were prepared separately. The pH was adjusted to 3 with orthophosphoric acid. And it is sonicated for 10 min and filtered through a 0.45 µ membrane filter.

Preparation of Standard Drug Solution

Accurately 10 mg of Varenicline tartrate & 100 mg of Bupropion hydrochloride were weighed into a clean and dry 100 mL volumetric flask separately dissolved with sufficient volume of diluent. The final volume was made up to 100 mL with diluent to give the solution containing 100 µg/mL of Varenicline tartrate & 1000 µg/mL of Bupropion hydrochloride.

Preparation of Working Standard Drug Solution

1 mL of standard stock solution of Varenicline tartrate and Bupropion hydrochloride was pipetted out into 10mL volumetric flask and further diluted with diluent to 10mL to get concentration of 10µg/mL of Varenicline tartrate and 100µg/mL Bupropion hydrochloride.

Determination

Wavelength for detection was selected by examining the resulted solution that consists of Varenicline tartrate & Bupropion hydrochloride in SHIMADZU UV- Spectrometer (UV-1800) instrument. The maximum absorbance for Varenicline tartrate & Bupropion hydrochloride was observed at 244 nm and hence 244 nm was selected as wavelength of detection.

Method Validation

The proposed method was validated in compliance with ICH guidelines for linearity, accuracy, precision, specificity, robustness, and system suitability parameters by the following procedures.

Linearity

Accurately 10 mg of Varenicline tartrate & 100 mg of Bupropion hydrochloride was weighed into a clean and dry 100 mL volumetric flask, dissolved with sufficient volume of diluent. The volume was made up to 100 mL with diluent to get the concentration of 100µg/mL for Varenicline tartrate & 1000 µg/mL of Bupropion hydrochloride.

Preparation of working standard solutions

The various concentration of working standard solutions of Varenicline tartrate & Bupropion hydrochloride was made by pipetting 1.0 mL, 2.0 mL, 3.0 mL, 4.0 mL and 5.0 mL from stock (I) separately into a series of 10mL volumetric flask and diluted to 10mL to get the final concentration of 10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL, 50 µg/mL of Varenicline tartrate and 100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL and 500 µg/mL of Bupropion hydrochloride solutions respectively.

Determination

The working standard solutions of Varenicline tartrate from 10 µg/mL to 50 µg/mL and Bupropion hydrochloride from 100 µg/mL to 500 µg/mL were injected into a chromatograph at flow rate of 1ml/min. Retention time and peak area obtained were recorded and standard

calibration curve was plotted for Varenicline tartrate and Bupropion hydrochloride and linearity equation was derived. The correlation coefficient, % curve fitting were also calculated. The results obtained were shown in Table 1,2&3.

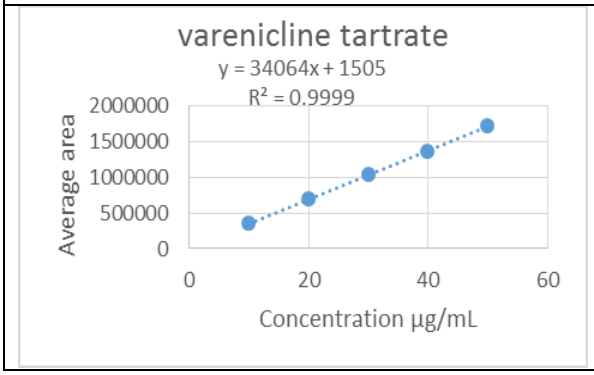
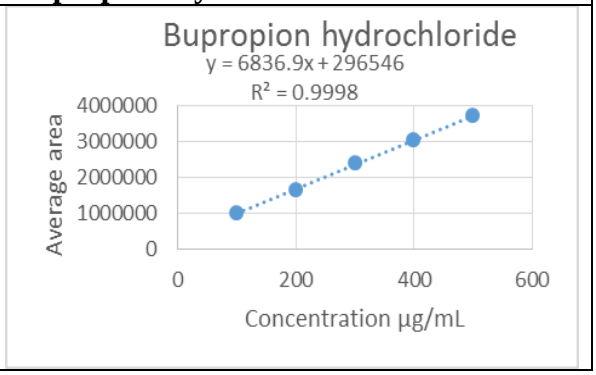
Table 1: Linearity Data for Varenicline Tartrate and Bupropion Hydrochloride.

Varenicline Tartrate		Bupropion Hydrochloride	
Concentration (µg/mL)	**Average Area**	**Concentration (µg/mL)**	**Average Area**
10 µg/Ml	340800	100 µg/mL	983207
20 µg/Ml	679899	200 µg/mL	1650415
30 µg/Ml	1032500	300 µg/Ml	2367676
40 µg/Ml	1359985	400 µg/Ml	3019829
50 µg/Ml	1703965	500 µg/Ml	3716949

Table 2: Linearity Report of Varenicline Tartrate And Bupropion Hydrochloride.

Parameters	**Varenicline Tartrate**	**Bupropion hydrochloride**	**Acceptance criteria**
Linearity Range	10 to 50 µg/ml	100 to 500 µg/mL	-
Regression Equation	y = 6836.9x + 296546	y = 34064x + 1505	-
Correlation Coefficient	0.9998	0.9999	**More than 0.999**
Intercept	296546	1505	
Slope	6836.9	34064	

Table 3: Standard Calibration Curves.

Varenicline Tartrate	**Bupropion Hydrochloride**
	

Accuracy

Preparation of sample stock solution

Twenty tablets each containing 10 mg of Varenicline tartrate and 100 mg of Bupropion hydrochloride was weighed and finely powered. Powder equivalent to 10 mg of Varenicline tartrate and 100 mg of Bupropion hydrochloride was taken and transferred into a clean, dry 100 mL volumetric flask. The powder was first dissolved in diluent and sonicated for 20 mins. The resulting mixture was then filtered through whatmann filter no 0.45 µ. The final volume of filtrate was made up to 100 mL with diluent.

Preparation of standard stock solution

Accurately weighed 10 mg of standard drug Varenicline tartrate and 100 mg of Bupropion hydrochloride was transferred into a clean, dry 100 mL volumetric flask and the volume was made up to 100 mL with diluent to get the concentration of 100 µg/mL of Varenicline tartrate and 1000 µg/mL of Bupropion hydrochloride.

Preparation of standard and sample mixture

Level I (80%)

Volume of 0.5 mL sample stock solution, 0.3 mL of standard solution was transferred to 10 mL volumetric flask and volume was made up to mark with diluent (three replicates).

Level II (100%)

Volume of 0.5 mL sample stock solution, 0.5 mL working standard stock solution was transferred to 10 mL volumetric flask and volume was made up to mark with diluent (three replicates).

Level III (120%)

Volume of 0.5 mL sample stock solution, 0.7 mL of working standard stock solution was transferred to 10 mL volumetric flask and volume was made up to mark with diluent (three replicates).

Determination

The resulting mixture was injected repeatedly into the chromatograph, the peak area and chromatogram obtained were recorded and the % recovery of standard Varenicline tartrate and Bupropion hydrochloride was calculated. The results obtained are presented in Table 4,5&6.

Table 4: Recovery Study Data for Varenicline Tartrate.

Level	Replicate	Std Conc. (µg/mL)	Sample Conc. (µg/mL)	Peak area	Total Conc found (µg/mL)	Amt of std. recovered (µg/mL)	% Recovery
80%	I	3	5	270098	7.925	2.925	97.514
	II	3	5	269101	7.896	2.896	96.539
	III	3	5	270979	7.951	2.951	98.375
100%	I	5	5	337802	9.912	4.912	98.241
	II	5	5	335897	9.856	4.856	97.123
	III	5	5	333692	9.791	4.791	95.829
120%	I	7	5	400336	11.747	6.747	96.385

	II	7	5	399390	11.719	6.719	95.988
	III	7	5	401327	11.776	6.776	96.800

Table 5: Recovery Study Data For Bupropion Hydrochloride.

Level	Replicate	Std Conc. (µg/mL)	Sample Conc. (µg/mL)	Peak area	Total Conc found (µg/mL)	Amt of std. recovered (µg/mL)	% Recovery
80%	I	30	50	778061	79.135	29.135	97.117
	II	30	50	769135	78.227	28.227	94.091
	III	30	50	787134	80.058	30.058	100.193
100%	I	50	50	979471	99.620	49.620	99.240
	II	50	50	973144	98.977	48.977	97.953
	III	50	50	976464	99.314	49.314	98.628
120%	I	70	50	1176255	119.635	69.635	99.478
	II	70	50	1156769	117.653	67.653	96.647
	III	70	50	1144878	116.443	66.443	94.919

Table 6: Report of Recovery Studies for Varenicline Tartrate & Bupropion Hydrochloride.

Level	Mean % Recovery of Varenicline tartrate	Mean % Recovery of Bupropion hydrochloride	Acceptance criteria
80%	**97.476**	**97.133**	**90-110%**
100%	**97.046**	**98.602**	**90-110%**
120%	**96.391**	**97.014**	**90-110%**

Precision

1 mL of standard solution of Varenicline tartrate and Bupropion hydrochloride was transferred into a 10 mL volumetric flask and final volume was then made up to 10 mL with diluent to get a concentration of 10 µg/mL of Varenicline tartrate and 100 µg/mL of Bupropion hydrochloride.

Determination

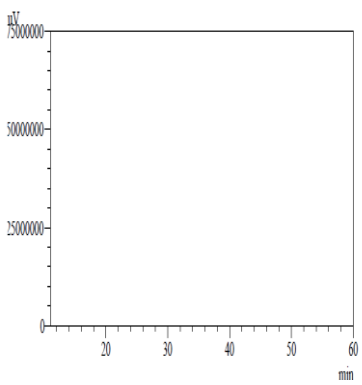
successive six injections of working standard solution (six replicates) were injected into a HPLC chromatograph, the peak area and chromatograms obtained were recorded. The % relative standard deviation was calculated for peak areas and retention time of replicates. The results and chromatogram obtained were shown in Table 7.

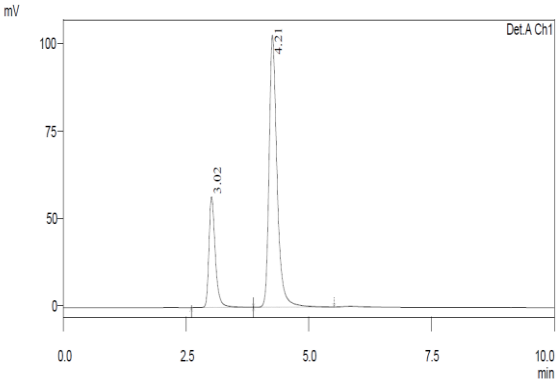
Table 7: Report of Precision for Varenicline Tartrate & Bupropion Hydrochloride.

Precision Parameters	% RSD Of Varenicline tartrate	% RSD Of Bupropion hydrochloride	Acceptance Criteria
System Precision	0.084	0.042	< 2.0%
Method Precision	0.232	0.220	< 2.0%
Intraday Precision	1.042	0.503	< 2.0%
Inter day Precision	0.288	0.287	< 2.0%

Specificity

The diluent, working standard of Varenicline tartrate and Bupropion hydrochloride were injected separately into the chromatograph to examine that the Varenicline tartrate and Bupropion hydrochloride peak is not affected by the mobile phase and diluent and the chromatogram was recorded and is presented in chromatogram 1-2.





Chromatogram of only diluent was taken to check the interference of diluent with the peaks of Varenicline tartrate and Bupropion hydrochloride at the retention time of respective drugs. There was no peak detected at retention time of Varenicline tartrate 3.02 min and Bupropion hydrochloride 4.21. so, proposed method is specific in nature.

LOD and LOQ

LOD and LOQ for Varenicline tartrate and Bupropion hydrochloride by this method were evaluated on the basis of signal-to-noise ratio method described in ICH guidelines. A signal-to noise ratio between 3 or 2:1 is generally considered acceptable for estimating the detection limit. A typical signal-to-noise ratio required for LOQ is 10:1. Using the proposed HPLC method, the LOD and LOQ values were calculated and are given in Table 8.

Table 8: Data For Lod And Loq Of Varenicline Tartrate & Bupropion Hydrochloride.

Parameter	Varenicline tartrate		Bupropion hydrochloride	
	Peak Area	Concentration in µg/mL	Peak Area	Concentration in µg/mL
LOD	990	0.002	523	0.006
LOQ	2802	0.006	2360	0.018

Robustness

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized parameters were made in chromatographic conditions like of flow rate, mobile phase ratio and wavelength. The effect of change in flow rate, mobile phase ratio and wavelength of detection on retention time and tailing factor were examined. The values obtained are mentioned in Table 9,10&11. The method was found to be unaffected by the small changes like ± 0.1 mL/min in flow-rate of mobile phase and change in mobile phase ratio from 55:45 to 50:50 & 60:40 and ± 5 nm in detection wavelength.

Table 9: Robustness Data of Varenicline Tartrate & Bupropion Hydrochloride With Change In Flow Rate.

Change in flow rate mL/min	Peak area* of Varenicline tartrate	% Assay	Peak area* of Bupropion hydrochloride	% Assay
0.9	342970	101.140	986812	100.868
1.1	340109	100.296	986159	100.802

Table 10: Robustness Data of Varenicline Tartrate & Bupropion Hydrochloride With Change In Mobile Phase.

Change in Mobile phase ratio v/v	Peak area* of Varenicline tartrate	% Assay	Peak area* of Bupropion hydrochloride	% Assay
60:40	334458	98.630	981595	100.335
70:30	342767	101.080	983575	100.538

Table 11: Robustness Data of Varenicline Tartrate & Bupropion Hydrochloride With Change In Wavelength.

Change in wavelength in nm	Peak area* of Varenicline tartrate	% Assay	Peak area* of Bupropion hydrochloride	% Assay
242	341908	100.827	983497	100.530
246	342078	100.877	986197	100.806

System Suitability

Six replicate of Varenicline tartrate & Bupropion hydrochloride sample containing were given to evaluate equipment, electronics, analytical operations and samples suitability.

Parameters calculated for system suitability were %RSD of retention time and area, number of theoretical plates and Resolution. The results are given in Table 12.

Table 12: Data for System Suitability Parameter For Varenicline Tartrate & Bupropion Hydrochloride.

Sr. No.	System Suitability Parameters	Varenicline tartrate	Bupropion hydrochloride	Acceptance Criteria
1.	**Resolution**	0.000	4.81	>2
2.	**Tailing Factor**	1.42	1.46	<2
3.	**Theoretical plates**	2435.34	3978.21	>2000

Ruggedness

Intermediate precision expresses the variations within laboratories variations: (different days, different analysts, different equipment etc.). The Intermediate precision was performed for Varenicline tartrate & Bupropion hydrochloride by different analyst on different instrument using different lot of column on different day. The % RSD for the same was calculated for Intermediate precision. The results are given in Table 13 & 14.

Table 13: Intermediate Precision Data of Analyst 1.

	Varenicline tartrate		Bupropion hydrochloride	
Replicates	**Peak Area***	**%Assay**	**Peak Area***	**%Assay**
1	340232	100.333	984167	100.598
2	341066	100.578	984712	100.654
3	340439	100.394	983932	100.574
4	342265	100.932	984094	100.591
5	341067	100.579	984656	100.648
6	340154	100.309	989566	101.150
Mean	**340871**	**100.521**	**985188**	**100.702**

***Average of six Determinations**

Table 14: Intermediate Precision Data of Analyst 2.

	Varenicline Tartrate		Bupropion Hydrochloride	
Replicates	**Peak Area***	**%Assay**	**Peak Area***	**%Assay**
1	343667	101.345	988645	101.056
2	343778	101.378	981545	100.330
3	342933	101.129	982767	10.455
4	342898	101.119	981987	100.375
5	340199	100.323	983413	100.521
6	344771	101.671	986567	100.843
Mean	**343041**	**101.161**	**984154**	**100.597**

***Average of six Determinations**

RESULT AND DISCUSSION

Optimized chromatography condition: Chromatographic conditions were screened for mobile phase composition, wavelength proportion and flow rate Finally, mobile phase of Methanol: Phosphate buffer with pH 3.0 (65:35v/w) was optimized to give symmetric peak with short runtime at UV detection wavelength of 244 nm and flow rate at 1mL/min was found to be appropriate with adequate separation between the two drugs. Chromatogram of Varenicline tartrate & Bupropion hydrochloride at optimized chromatographic condition was recorded, the runtime was 10 min and the retention times of Varenicline tartrate & Bupropion hydrochloride were found to be 3.01 and 4.2 min respectively.

CONCLUSION

The proposed HPLC method was found to be economical, simple, sensitive, accurate, precise, specific and robust and can be used for the routine analysis of Varenicline tartrate & Bupropion hydrochloride in industry and academia.

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REFERENCES

1. Varenicline tartrate [live search type of on internet] [cited Thu, Mar-1, 02:05:55 +5:30] availablefrom: https://www.drugbank.ca/drugs/DB01273, 2018.
2. Varenicline tartrate [live search type of on internet] [cited Thu, Mar-1, 07:05:55 + 3:30]availablefromhttps://en.wikipedia.org/wiki/Varenicline tartrate, 2018.
3. Varenicline tartrate [live search type of on internet] [cited Thu, Mar-1, 07:05:55 + 3:30]availablefrom:https://pubchem.ncbi.nlm.nih.gov/compound/Varenicline tartrate/ 9906942, 2018.
4. https://www.rndsystems.com/products/Varenicline tartrate-tartrate, 3754.
5. Bupropion HCl[live search type of on internet] [cited Sat,Mar-3, 03:00+5:00] availablefrom:https://pubchem.ncbi.nlm.nih.gov/compound/Bupropion hydrochloride/ 62884, 2018.

6. Bupropion HCl [live search type of on internet] [cited Sun, Mar-4, 05:00+2:00] available from: https://en.wikipedia.org/wiki/Bupropion HCl, 2018.
7. Bupropion HCl [live search type of on internet] [cited Fri, Mar-2, 03:00+5:00] available from:https://www.drugbank.ca/drugs/DB01156, 2018.
8. Koçak E, Altinöz S. Validation of Spectrophotometric Method to Quantify Varenicline Content in Tablets. Asian J Chem, 2013 Mar 1; 25(4): 1845.
9. Jorenby DE et al, Efficacy of Varenicline tartrate, a 42 Nicotinic acetylcholine receptor partial agonist, vs placebo or sustained–release Bupropion HCl for smoking cessation. JAMA, July 5, 2006; 296-11.
10. Rose JE, Behm FM. Combination treatment with Varenicline tartrate and Bupropion HCl in an adaptive smoking cessation paradigm. Am J Psychiatry, 2014 Nov 1; 171(11): 1199-205.
11. Bhattacharyya I, Bhattacharyya SP, Sen S. RP-HPLC method for the analysis of Bupropion HCl hydrochloride in pharmaceutical dosage form. Int J Pharm Pharm Sci., 2010; 1(2): 224-232.
12. Iche PP. Stress degradation studies of Bupropion HCl HCl and development of a validated UV spectrophotometric method in bulk and pharmaceutical dosage form. Int J Pharm Pharm Sci., 2013; 3: 103-7.
13. Thakar DS, Varghese A. Bioanalytical method development and validation of Bupropion HCl hydrochloride in rat plasma by RP-HPLC. Int J Pharm Biosci Technol, 2013; 1(1): 20-26.
14. Phani RC, Chaitanya D, Prasanthi B. RP-HPLC and spectrophotometric methods for the simultaneous estimation of Bupropion HCl hydrochloride and naltrexone hydrochloride. Int J Pharm Pharm Sci., 2015 Jul 1; 6(7): 2982.
15. Patel RB, Patel PU, Development and validation spectrophotometric method for simultaneous Determination of zonisamide and Bupropion HCl in synthetic mixture. World J Pharm. Res., 2017; 6(4): 1399-1409.
16. Pietras R, Szymanska K, Kondej M, Gladysz A, Kowalczuk D. Development and validation of the HPLC method for Varenicline tartrate Determination in pharmaceutical preparation. Current Issues in Pharmacy and Medical Sciences, 2017 Sep 26; 30(3): 142-6.
17. Kamali TV, Anandabaskaran T, high performance liquid chromatography validation of Varenicline tartrate in drug product. Asia Pac J Res, 2017; 1.

18. Kadi AA, Mohamed MS, Kassem MG, Darwish IA. A validated stability-indicating HPLC method for Determination of Varenicline tartrate in its bulk and tablets. Chem Cent J, 2011 Dec 1; 5(1): 30.
19. Channabasavaraj KP, Modiya JS, Sharath HM. Development and validation of RP-HPLC method for estimation of Varenicline tartrate tartrate in bulk drug and tablet dosage form. Int J Pharm Pharm Sci., 2011; 3: 59-61.
20. Alam MS, Bratty MA, Alzhami HA, Quitting smoking and quantification studies of available anti-smoking drugs. J.chem.pharm.res, 2016; 8(8): 1156-1164.
21. Pradeep S, Javali C. Analytical method development and validation for simultaneous estimation of ivermectin and fenbendazole in bulk dosage form by RP-HPLC. World J Pharm Pharm Sci., 2016 Feb 4; 5(4).