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RP-HPLC method for content of genotoxic 4-methoxybenzyl Chloride in Venlafaxine

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ABSTRACT

Venlafaxine, primarily available in dosage in Venlafaxine hydrochloride (VEN·HCl) form, is a discriminating serotonin and norepinephrine reuptake inhibitor (SNRI) anti-depressant drug. The 4-methoxybenzyl chloride (4-MBC) is a genotoxic impurity present in VEN·HCl. A reverse-phase high-performance liquid chromatography (RP-HPLC) method is developed for the determination of 4-MBC content this method is validated employing ICH (International Council on Harmonisation) guidelines. During the method development, Purospher STAR end-capped (250mmx4.0mm), 5µm HPLC column, and gradient profile for mobile phase with constant 1.0 mL/min flow rate are used. Mobile phase A is a buffer of pH 8.5 containing 0.1% v/v of liquid ammonia in water with adjusted pH using 10% orthophosphoric acid, whereas mobile phase B is acetonitrile. The injection volume, column oven temperature, and autosampler temperature were 50 µL, 55°C, and 5°C, respectively. The data is acquired at 225 nm wavelength. The method yields a well-separated peak of 4-MBC from Venlafaxine and its impurities. This method is linear in the range of LOQ to 150% level of specification concentration of impurity and the observed correlation coefficient is 0.999. The repeatability and intermediate precision are evaluated and the respective values of %RSD for 4-MBC content are 0.43 and 0.93. Robustness studies show insignificant change for the system suitability criteria like %RSD, theoretical plates, and tailing factor, their values being well within the acceptance limit. The new method for the quantification of genotoxic impurity 4-MBC is precise, sensitive, rugged, and accurate; it also qualifies all the criteria of stability, linearity, and robustness.

Keywords: Venlafaxine Hydrochloride, Anti-depressants, RP-HPLC Method Validation, 4-Methoxybenzyl chloride, Genotoxic impurity.

INTRODUCTION

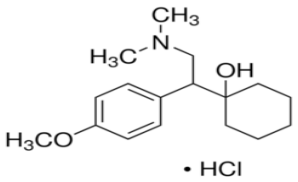
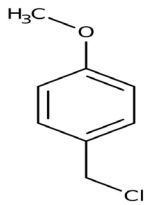
Depression is one of the common and disabling diseases, which has several socially and economically important implications. There are many anti-depressant[1] drugs available for the treatment of depression; however, their efficacy is limited due to toxic effects. These antidepressants may exert adverse effects like the risk for life-threatening arrhythmias, especially in patients who have a cardiac disease history. Moreover, anxiety and depression disorders are associated with

pervasive psychosocial, chronic, and occupational dysfunction, which are major reasons for disability, comparable to the disability associated with hypertension, rheumatoid arthritis, and diabetes[2]. A bicyclic phenylethylamine compound Venlafaxine (VEN) is a distinctive anti-depressant. The VEN has structural dissimilarities compared to other presently available anti-depressants. It acts by triggering neurotransmitter activity in the CNS.

The VEN drug is available as a racemic mixture of the (–) R and (+) S-enantiomer. While in literature survey exposed that approximately UV methods[3-8] and RP-HPLC methods[9-13] are described for the determination of impurities 1-[Cyano-(4-methoxyphenyl) methyl] cyclohexanol,1-[2-Amino-1-(4-methoxyphenyl) ethyl] cyclohexanol hydrochloride, O-Desmethyl, and N-Desmethyl Venlafaxine content in VEN·HCl API and dosage form[14]. The RP-HPLC method developed by R. Nageswara Rao and A. Narasa Raju *et al.,* has 30 min run time and the finalized LOQ for the respective impurities was between 0.22 ppm to 0.38 ppm[15]. In another article, the analytical method has achieved reliable sensitivity and quite lower LOQ (0.28 to 0.81 ppm), however with a longer run time (40 min)[16]. Although the 4-methoxybenzyl chloride (4-MBC) and its related compounds are genotoxic,[17,18] there is not any method reported for the estimation of content in VEN·HCl as an active pharmaceutical ingredient.

The current study involves the development and validation of a novel RP-HPLC method for the determination of 4-MBC content in VEN·HCl with superior sensitivity, minimal levels of detection, and quantification with accuracy. The structure of VEN·HCl and 4-MBC are depicted in Table 1. This analytical method was validated corresponding to ICH guidelines.

Table 1: The compound name and structure of VEN·HCl and 4-MBC

Name	Chemical Name	Chemical Structure	Type
VEN·HCl	1-[2-(dimethylamino)-1-(4-methoxyphenyl) ethyl] cyclohexan-1-ol; hydrochloride		API
4-MBC	1-(Chloromethyl)-4-methoxybenzene		Genotoxic impurity

EXPERIMENTAL

MATERIALS AND METHODS

The VEN·HCl and 4-MBC were procured from TCI Chemicals (India) Pvt. Ltd. Purified water and analytical grade liquid ammonia, o-phosphoric acid, and acetonitrile were acquired from Merck. The Waters HPLC system with UV as well as PDA detector and Empower 3 software for data acquisition were used. All the analytical instruments were standardized throughout method development and validation. The 0.1% v/v liquid ammonia solution in purified water is treated as a mobile phase-A, while mobile phase B is acetonitrile. The mixture of purified water and acetonitrile with a ratio of 50:50 is used as a diluent for the standard and sample preparation. The 0.16 ppm of 4-MBC solution and 40,000 ppm of VEN·HCl solution were used as a standard solution and test solution.

Analytical Method development

The HPLC technique has been the choice for the separation of various drugs and related impurities in pharmaceutical analysis. The VEN·HCl and 4-MBC both are quite polar, therefore reverse phase chromatography technique is selected for method development. The VEN·HCl and its 4-MBC

impurity show absorption maxima near 225 nm (see. Fig. 1), therefore this wavelength is preferred for analyte detection throughout method development. During method development HPLC columns having 4.6 mmx250 mm lengths and 5 µm particle size with different carbon load (C18 and C8) are used, however, they yield broad peaks. While Purospher STAR (C8) end capped (4.0 mmx250 mm), 5µ column yield a well separated sharp peak shape for the 4-MBC and unknown impurities. However, the 4-MBC impurity elute simultaneously with VEN.

To separate the 4-MBC and VEN peak the carbon loading of column is better from C8 to C18. All known and unknown impurities are appropriately separated from each other and from VEN with good peak shapes, when Purospher STAR (C18) end capped column is used. Among different column oven temperatures (35, 40, 50 and 55°C) better chromatogram is observed at 55°C. The other parameters such as sample temperature (5°C), injection volume (50 µL) and flow rate (1.0 mL/min) are decided by performing various experiments. Method development in chromatographic conditions for VEN·HCl is listed in Table 2.

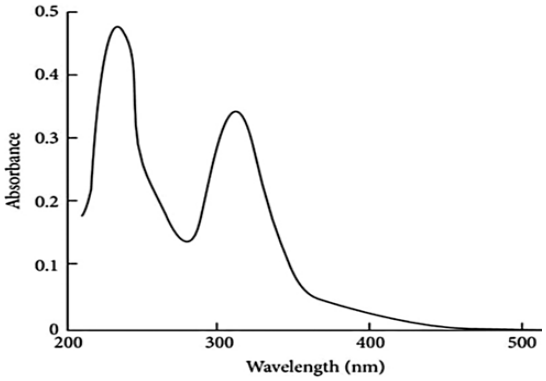


Fig. 1. The UV-Visible spectra of 1.5 ppm solution of 4-MBC in water:acetonitrile (50:50)

Table 2: Chromatographic states for the content of 4-MBC method of VEN·HCl

Instrument	HPLC equipped with UV/PDA detector, an injector, pump and recorder
Column	Purospher STAR end capped (250mm X 4.0mm), 5µm (C18)
Wavelength	225 nm
Flow rate	1.0 mL min^{-1} (Gradient program)
Injection volume	50 µL.
Column oven temperature	55°C
Sample cooler temperature	5°C
Run time	60 min

The ramp program with combination of 0.1% ammonia buffer of pH 8.5 (mobile phase A) and Acetonitrile as an organic solvent (mobile phase B) is used. At the start composition of mobile phase A and B in 95:5 ratios have been employed, which is then steadily changed to 80:20 up to 15 minute. Then mobile phase proportion is gradually altered to 40:60 up to 45 minutes. Finally, composition of mobile phase A and B is brought to original values of 95:5 up to 50 min and sustained till 60 minute.

RESULTS AND DISCUSSION

The method validation is performed as per ICH guideline[19-21]. Each parameter in the method validation study is examined below.

Specificity

Specificity signifies the capacity of analytical method to assess the analyte response in the occurrence of its impurity. The specificity studies can be carried out by spiking identified impurity with its restrict concentration and demonstrating that the results are unchanged due to the presence of any known impurity. Selectivity overlay chromatogram for blank, standard preparation, unspiked and spiked test preparation is shown in Fig. 2. Blank run does not show any interfering peak at the retention time of 4-MBC as well as VEN·HCl peaks in chromatogram. The peak due to VEN·HCl is well separated from 4-MBC peak. The peak pureness measures for 4-MBC peak around 16 min are according to the expectations. The %relative standard deviation of six replicates of standard preparation is 0.96%. Theoretical plates and tailing factor in standard preparation of 4-MBC are 12826 and 1.14, respectively.

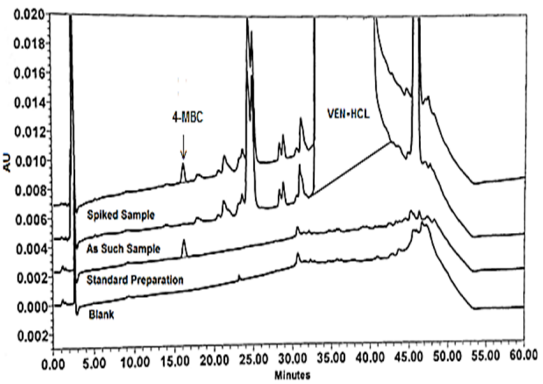


Fig. 2. Selectivity overlay chromatograph for blank, standard preparation (4-MBC), as such sample (VEN·HCl) and spiked sample (4-MBC in VEN·HCl)

Limit of detection and Limit of quantitation

The limit of detection (LOD) and the limit of quantitation (LOQ) are found from the signal-to-noise ratios being 3:1 and 10:1, respectively. The LOQ concentration (0.052 ppm) of 4-MBC is determined by injecting various concentration levels (10 to 120 %) of standard solution. The precision studies for LOD and LOQ are performed by injecting six replicates of each concentration of 4-MBC. The % RSD of these six replicate solutions is 4.56 and signal to noise ratio is 15.8. The LOD concentration is 0.016 ppm and the detected signal-to-noise ratio is 5.7. The reported LOQ concentration in the present method is 30% (i.e. 0.052%) low down than the earlier methods reported in the sources[22].

Linearity and Range

Linearity of method is established from a calibration plot of detector response versus concentration of the 4-MBC impurity. Thus, the linearity of existing HPLC method is determined from LOQ level to 150% of requirement limit concentration (0.052 ppm to 0.242 ppm of 4-MBC impurity). The linearity data for LOQ, 50, 75, 100 and 150% concentrations of standard preparation is specified in Table 3. The least squares linear regression analysis for the peak area and concentration was performed and the correlation coefficient value of 0.999 was obtained. This shows that the data points remain close to the straight line in different concentration levels in the linearity range. Thus, as is seen from Fig. 3 and correlation coefficient it is inferred that the current HPLC method is linear.

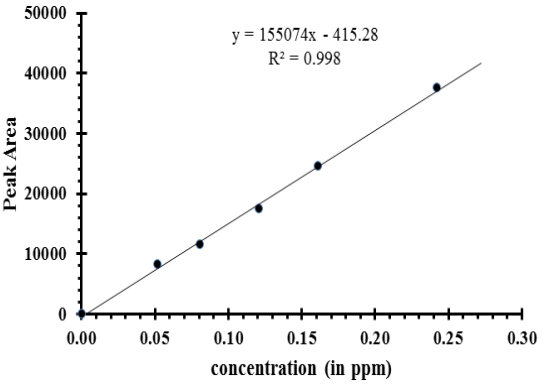


Fig. 3. Linearity graph of 4-MBC range from the LOQ to 150% of concentration level

Table 3: Linearity data for 4-MBC from LOQ to 150 % concentration limit

Linearity levels	Conc. in ppm	Conc. w.r.t. test in ppm	Area		Average Area (n=2)
			Run 1	Run 2	
Linearity-1(LOQ level)	0.052	1.300	8334	8016	8175
Linearity level-2 (50%)	0.081	2.025	11615	11434	11525
Linearity level-3 (75%)	0.121	3.025	17591	17509	17550
Linearity level-4 (100%)	0.161	4.025	24997	24009	24503
Linearity level-5 (150%)	0.242	6.050	37960	37318	37639
	Correlation Coefficient			0.999	
	Slope			157493	
	%Y Intercept			-823.343	

Precision

Precision study achieved when the entire analytical method is confirmed. It involves the repeatability, intermediate precision and reproducibility studies. The system precision is carried out using six replicates of 4-MBC standard preparation and chromatograms are recorded. The system fitness criteria such as tailing factor, theoretical plates and %RSD are calculated. The %RSD, tailing factor and theoretical plates results in system precision study are 1.41, 1.11 and 9091, respectively. Comparative data of 4-MBC in spiked test preparation in repeatability and intermediate precision are explained in Table 4. The repeatability of 4-MBC has been achieved by injecting six separate spiked test preparations. The content of 4-MBC and %RSD of spiked test sample acquired are determined. The intermediate precision is estimated mentioned in earlier chapters. The %RSD for content of 4-MBC of repeatability and intermediate precision study is 0.43 and 0.93, respectively. The overall %RSD of twelve spiked test preparations (six separately for repeatability and intermediate precision) is 4.27.

Accuracy

Accuracy is a measure of nearness between real value and value observed. The accuracy study of 4-MBC is performed in triplicate at LOQ, 50, 100 and 150% of the requirement

limit (4 ppm) in the test preparation. The observed % recoveries of 4-MBC are in between 103.26 to 111.47%. The accuracy values for the content of 4-MBC are provided in Table 5.

Table 4: Relative data for %of 4-MBC in repeatability as well as intermediate precision study

Tests	4-MBC (in ppm)		
	Repeata-bility		Intermediateprecision
Spiked test 1	4.18		3.87
Spiked test 2	4.20		3.89
Spiked test 3	4.20		3.88
Spiked test 4	4.17		3.91
Spiked test 5	4.22		3.87
Spiked test 6	4.20		3.80
Mean (n=6)	4.19		3.87
SD (n=6)	0.018		0.040
%RSD (n=6)		0.43	0.93
Mean (n=12)		4.03	
SD (n=12)		0.172	
%RSD (n=12)		4.27	

Table 5: %Recovery values of 4-MBC impurity

Test preparations	LOQ	50 % level	100 % level	150 % level
1	109.24	109.20	103.85	103.94
2	109.66	107.95	103.26	103.82
3	111.47	107.95	104.87	103.31
Average (n=3)	110.12	108.22	103.99	103.69
SD (n=3)	1.19	0.72	0.81	0.33
%RSD (n=3)	1.08	0.81	0.78	0.32

Robustness

The robustness of the analytical method is performed by purposefully changing of experimental circumstances such as column oven temperature (±2°C), flow rate (by ±10%) and pH of buffer solution (±0.2), Actual flow rate of 1.00 mL/min is altered to 0.9 mL/min and 1.1 mL/minute. Likewise, the column oven temperature is altered from 55°C in the innovative method to 53 and 57°C, pH of the buffer solution is changed from 8.50 in the original method to 8.30 and 8.70. In all the above cases the retention times differ by ±0.6 min matched to actual retention times. In all deliberate varied chromatographic requirements observed are not significant for the system suitability criteria like tailing factor and theoretical plates. %RSD and overall %RSD of n=8 samples (Six from repeatability and 2 from each robustness study) are calculated (Table 6) and found to be within acceptance value.

Solution Stability

The solution stability of standard preparations as well as sample solution and spiked test solution are performed on hourly basis 6,12,18 and 24 hat the self-controlled room temperature (25°C). The %recovery of 4- MBC in standard solutions as well as spiked test preparation is calculated. The % RSD of standard preparation from initial, 6, 12, 18 and 24 hours are 0.27, 0.89, 0.76, 0.57, and 0.25%, respectively. The cumulative %RSD of 4-MBCin spiked test preparation against initial preparation is 2.68, 1.96, 1.93, and 1.74%. The %Recovery of 4- MBC in spiked test preparation against initial preparation are 96.29, 97.28,100.00 and 99.50%. Also, there is no significant change in the tailing factor (0.9 to 1.0) and theoretical plates (12456 to 12528) up to 24 hours. This reveals that the standard preparation and test preparation are stable for up to 24 h at controlled room temperature.

Table 6: Comparative % 4-MBC value of repeatability and robustness

Parameter	Flow rate (mL/min)		Column oven temp		pH of buffer	
	0.9	1.1	53°C	57°C	8.30	8.70
Tailing Factor	1.11	1.13	1.21	1.17	1.05	1.13
Theoretical plates	9635	9521	9452	9532	9841	9601
%RSD for (n=6) of standard	0.38	1.47	0.38	0.61	3.40	1.30
Mean (n=8) test solution	4.15	4.14	4.12	4.14	4.11	4.11
SD (n=8) test solution	0.13	0.15	0.19	0.14	0.22	0.23
%RSD for (n=8) test solution	3.17	3.54	4.55	3.44	5.39	5.67

Mobile phase stability

The mobile phase stability at the monitored temperature is executed for 24 hours. After 24 h both A and B mobile phases look is clear, and haziness is not detected. The %RSD of 4-MBC standard solutions on 24 h is observed to be 0.25%. The system suitability parameters (a) tailing factor (0.9 to 1.0) and (b) theoretical plates (12456 to 12528) are comparable with initial data. Therefore, the mobile phase stored at the monitored room temperature is stable for up to 24 hours.

CONCLUSION

Venlafaxine is a discriminating serotonin and norepinephrine reuptake inhibitor antidepressant.

The RP-HPLC method is established for the determination of 4-MBC content in VEN·HCl and this method is validated operating ICH guidelines. The method was developed using Purospher STAR end-capped (250 mm x 4.0 mm), 5 µm column, and using gradient profile for the mobile phase. Mobile phase A contains 0.1% v/v of Liquid ammonia in water as a buffer of pH 8.5 with orthophosphoric acid, whereas mobile phase B is the acetonitrile. The 1.0 mL min^{-1} total flow rate, 50 µL injection volume, 55°C column oven temperature, and sample cooler temperature 5°C are set in this method. The eluted compounds were examined at 225 nm. The 4-methoxybenzyl chloride (4-MBC) peak is well separated from Venlafaxine and its impurities. The method is accurate, precise as well as linear in the limit of LOQ to 150% level with respect to stated concentration limit. [LOQ (0.052 ppm to 0.242 ppm) to 150%] Furthermore, the LOQ is lower for this method. Compared to 0.22 ppm reported in the literature. The solution and mobile phase are steady for up to 24 hours. The existing method is precise, accurate, sensitive, and rugged; it also succeeds all the norms of robustness, linearity, as well as stability.

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Conflict of interest

The author declare that we have no conflict of interest.

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