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Analytical Purity Method Development and Validation by gas Chromatography of L-valine Methyl Ester Hydrochloride for Production of Anti-hypertensive Drugs

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Abstract
Gas chromatography is the most widely used technique in pharmaceutical industry. Analytical chemistry research is largely driven by performance of sensitivity, selectivity, robustness, linear range, accuracy, precision. Validation is founded on but not specifically prescribed by regulatory requirements and is best viewed as an important and integral part of GMP (Good Manufacturing Practice). Gas chromatography method has been developed for L-valine methyl ester hydrochloride. It is used for production of anti-hypertensive drug. The Gas Chromatography system was used for method development and method validation with an auto injector and detection was performed by means of flame ionization detector (FID) with capillary column DB-624, 30m length, 0.53mm diameter and 1.0µm thickness. Nitrogen an inert gas was used as carrier gas. The method was validated for precision (system precision and method repeatability), recovery, linearity range, robustness and sample solution stability. The high recovery and low relative standard deviation confirms the suitability of the method for purity of L-valine methyl ester hydrochlride. It has been found from data of validation criteria that the proposed method has adequate reproducibility and specificity therefore suitable in pharmaceutical industry.
Key words: Validation, Gas chromatography, FID, GMP, Pharmaceutical industry.

INTRODUCTION

L-valine methyl ester Hydrochloride ((S)-2-AMINO-3-METHYL-BUTYRIC ACID METHYL ESTERHYDROCHLORIDE) is intermediate product for production of Valsartan (Anti-hypertensive drug).[1-2] L-Valine methyl ester structure was shown in Figure-I. Validation is a rapidly growing and evolving subject and is a requirement that has always made sense from both regulatory and quality perspective. It determined the quality purity of the final products.[3-4] Analytical methods rely on scrupulous attention to cleanliness, sample preparation accuracy and precision. A standard method for analysis of concentration involves the creation of calibration curve. In the concentration of elements of compound in a sample is too high for the detection range of a technique, it can simply be diluted in a pure solvent. If the amount in sample is below an instruments range of measurement, the method of addition can be used. In this method a known quantity of the elements or compound under study is added, and the concentration observed in the amount actually in the sample.[5-7] Analytical chemistry research is largely driven by performance of sensitivity, selectivity, robustness, linear range, accuracy, precision.[8-9] Validation is founded on but not specifically prescribed by regulatory requirements and is best viewed as an important and integral part of GMP (Good manufacturing Practice). The high recovery and low relative standard deviation confirms the suitability of the method for purity of compound.[10-11] Literature survey indicates that there is no GC-FID method available for the determination of L-valine methyl ester hydrochloride and thus we aimed to develop it. Gas chromatography (GC) is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition.[12-13] Principle of gas chromatography is similar to column chromatography typical uses of GC include testing the purity of a particular substance, or separating the different components of a mixture (the relative amounts of such components can also be determined).[14-15] In gas chromatography, the mobile phase (moving phase) is a carrier gas, usually an inert gas such as helium or an unreactive gas such as nitrogen. The stationary phase is a microscopic layer of liquid or polymer on an inert solid support, inside a piece of glass or metal tubing called a column.[16]

Experimental details, methods and materials

Gas chromatograph with split auto injector and flame ionised detector (FID) is used. Chemicals and Reagents Isoleucine methyl ester HCl Methanol (AR grade) and L-Valine methyl ester HCl are used. The GC system used for method development and method validation was with a auto sampler. The detection was performed by means of flame ionization detector (FID). DB-624, 30m length, 0.53mm diameter and 1.0µm thickness capillary column has been procured from Agilent technologies and used for the method development and method validation study. The column oven programme as follows: initial column oven temperature, 150°C hold for 15 min. The run time of analysis was 15 min. The injector and detector temperature was kept at 180°C and

200°C, respectively. Nitrogen was used as a carrier gas with a constant flow rate of 5.0ml/min. The split ratio was set at 1:50 deactivated open-glass tube liner packed with fused silica wool was employed. Sample was injected injection volume 0.5µL. Solutions of Isoluecine and L-valine methyl ester HCl were prepared in methanol. Inject system blank, QL and Sample solutions. Determine the area of all peaks in each solution. Disregard peaks observed in blank and report the results by area normalization. Resolution between L-valine methyl ester HCl and Isoluecine methyl ester HCl should be more than 5.0.

Observations results and Discussion

Specificity: The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

The specificity of the method was carried out by injecting the blank, System suitability and sample solution (un-spiked and spiked), determined the resolution factors between analyte peak (of L-Valine Methyl ester) and the nearest peak. Sample of L-Valine methyl ester HCl at about 200mg/mL spiked with about 0.2mg/mL of Isoleucine methyl ester HCl. Un-spiked will be injected once.

Linearity:- The linearity of an analytical procedure is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in sample within a given range. It should be established initially by visual examination of a plot of signals as a function of analyte concentration of content. If there appears to be a linear relationship, test results should be established by appropriate statistical methods (e.g., by calculation of a regression line by the method of least squares). In some cases, to obtain linearity between the response of an analyte and its concentration, the test data may have to be subjected to a mathematical transformation. Data from the regression line itself may be helpful for providing mathematical estimates of the degree of linearity. The correlation coefficient, y-intercept, slope of the regression line, and residual sum of squares should be submitted. Linearity curve was shown in Figure-VI and Regression analysis of the calibration curve was given in Table-I.

Detection limit:- The detection limit is a characteristic of limit tests. It is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. Thus, limit tests merely substantiate that the amount of analyte is above or below a certain level. The detection limit is usually expressed as the concentration of analyte (e.g., percentage, parts per billion) in the sample. Detection limit of L-Valine methyl ester HCl and Isoleucine methyl ester HCl are about 40µg/ml.

At DL level peaks are detected.

Quantification limit:- The quantification limit is a characteristic of quantitative assays for low levels of compounds in sample matrices, such as impurities in bulk drug substances and degradation products in finished pharmaceuticals. It is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. Quantitation limit of L-Valine methyl ester HCl and Isoleucine methyl ester HCl are about 100µg/ml. These QL solutions are injected in six replicate.

At QL level S/N ratio of peaks are more than 10.0

Precision:- The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of a homogeneous sample. The precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements. Precision may be a measure of either the degree of reproducibility or repeatability of the analytical method under normal operating conditions. The ICH documents recommend that repeatability should be assessed using a minimum of nine determinations covering the specified range for the procedure (i.e., three concentrations and three replicates of each concentration, or a minimum of six determinations at 100% of the test concentration).

The system precision for the method was assessed by three preparation of different concentration for L-valine methyl ester HCl standard about QL, 100% and 150% of nominal concentration and for Isoleucine methyl ester HCl standard about QL, 100% and 150% of nominal concentration. System repeatability RSD data is given in Tbale-II. Method repeatability was performed by injecting six different preparation of L-valine methyl ester HCl. If sample was not containing specified impurity (Isoleucine valine methyl ester HCl), again prepare six different solutions with spike 0.1 % Isoleucine methyl ester HCl (100% of specification level).

Recovery:- The ICH documents recommend that accuracy be assessed using a minimum of nine determinations over a minimum of three concentration levels, covering the specified range (i.e., three concentrations and three replicates of each concentration). Recovery data was given in Table-III.

Solution stability:- The sample solution prepared by spiking known concentration (0.1%) of L-valine methyl ester HCl with respect to sample concentration 200mg/mL in methanol was stored at 25 ± 2°C temperature conditions, and was injected into chromatographic system at different time intervals with fresh preparation. At each interval, the sample solution was found to be stable over a period of 36 hours.

Robustness:- To assess robustness of the method, the experimental conditions were deliberately altered and system suitability parameter was evaluated. Helium was used as a carrier gas with a constant flow rate 5.0 mL/min. To study the effect of flow rate on the resolution, the same was altered by 0.5 units that are from 4.5 to 5.5mL/min. The effect of column temperature was studied at 140°C and 160°C instead of 150°C. The effect of changing the split ratio by ±10% (1:45 and 1:55 instead of 1:50) was also studied. All the other chromatographic conditions were held constant as described above. In all the deliberate varied chromatographic conditions (flow rate, column temperature, and split ration), the all system suitability criteria were within the limits.

CONCLUSIONS:

The proposed GC methods provide simple, accurate and reproducible for purity and impurity profile of L-valine methyl ester HCl. The method was validated by testing its precision, linearity and recovery, limit of detection, limit of quantitation, robustness and specificity as per ICH guideline.

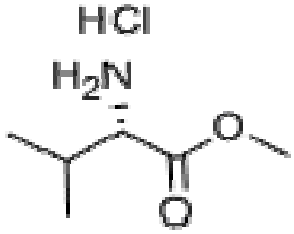
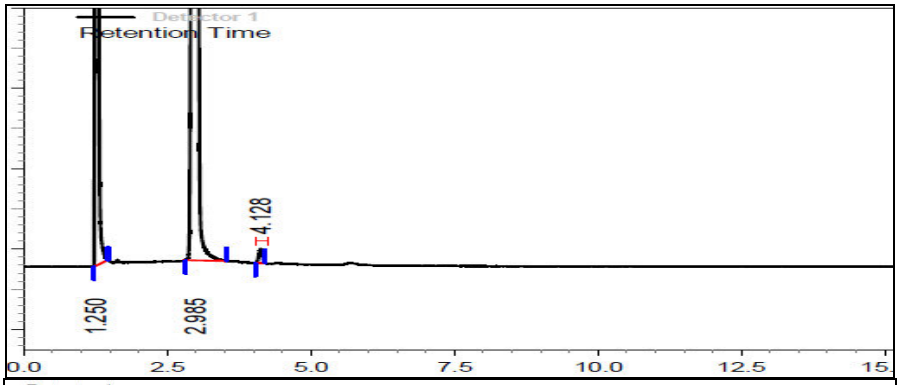


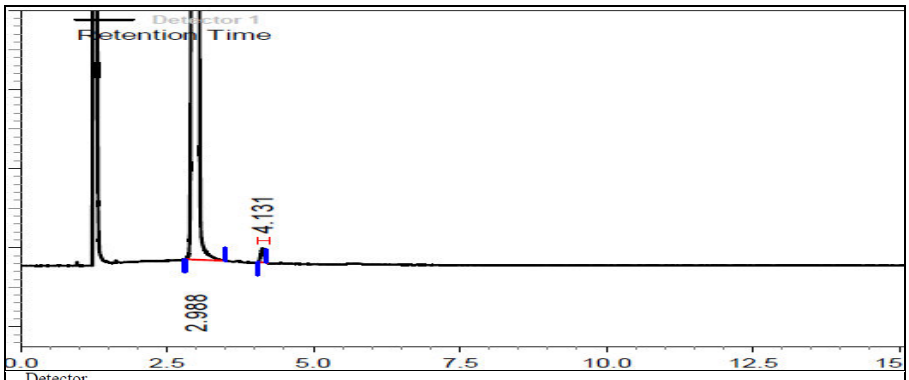
Figure-I (L-Valine Methyl Ester HCl Structure)



Detector 1 Results

Pk #	Name	Retention Time	Area	Area %	Resolution (USP)
1	Methanol	1.250	37014803	72.88	0.00
2	L-Valine methyl ester HCl	2.985	13755329	27.09	24.25
3	Isoleucine methyl ester HCl	4.128	15373	0.03	10.09
Totals			50785505	100.00	

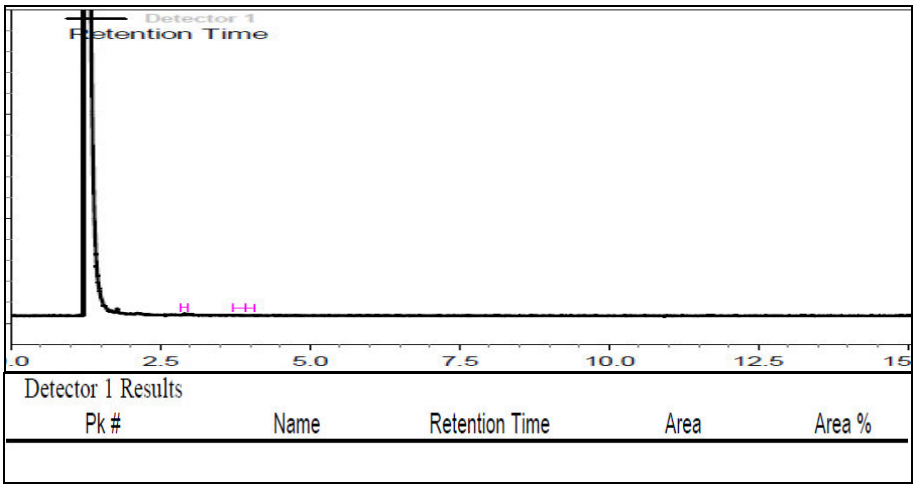
Figure-II (Chromatogram of sample with impurity for specificity)



Detector 1 Results

Pk #	Name	Retention Time	Area	Area %	Resolution (USP)	Theoretical plates (USP)	Asymmetry
1	L-Valine methyl ester HCl	2.988	14094968	99.90	0.0	12034	0.9
2	Isoleucine methyl ester HCl	4.131	14634	0.10	10.3	21487	1.0
Totals			14109602	100.00			

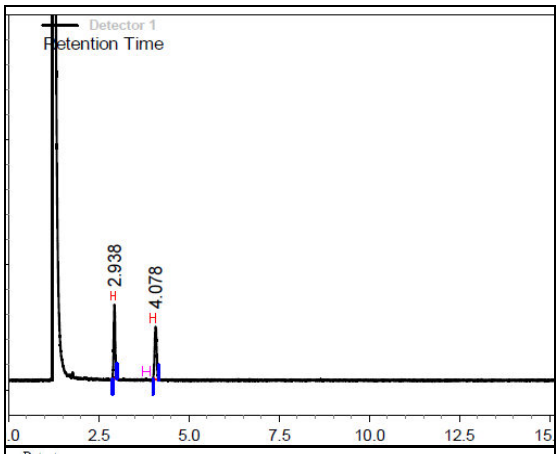
Figure-III (System suitability Chromatogram)



Detector 1 Results

Pk #	Name	Retention Time	Area	Area %

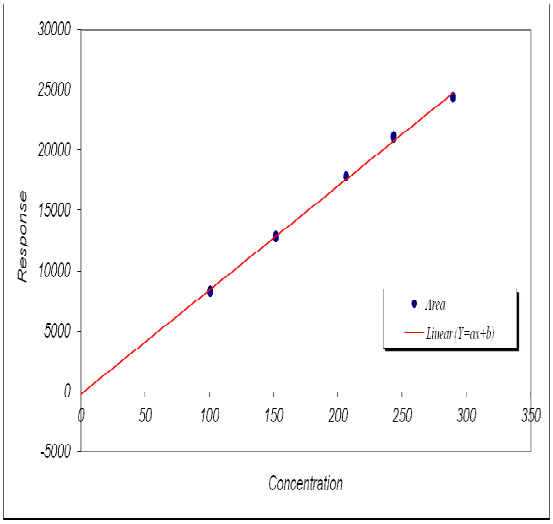
Figure-IV {Blank (Methanol) Chromatogram}

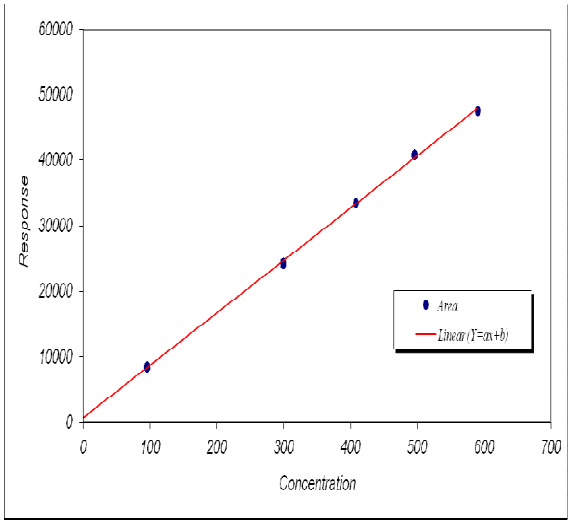


Detector 1 Results

Pk #	Name	Retention Time	Area	Area %	s/n
1	L-Valine Methyl Ester HCl	2.938	8315	50.37	36
2	Isoleucine methyl ester HCl	4.078	8193	49.63	26
Totals			16508	100.00	

Figure-V (QL level Chromatogram)





Linearity of L-valine methyl ester HCl Linearity of Isoleucine methyl ester HCl

Figure-VI (Linearity curve for L-valine methyl ester HCl and L-Isoluecine HCl)

L-Valine methyl ester HCL		**Isoleucine methyl ester HCL**	
Parameters	**Results**	**Parameters**	**Results**
Intercept value at 0.1%	3.9%	Intercept value at 0.1%	0.7%
Slop	79692	Slope	85866
Intercept	653	Intercept	131
Correlation factor r^2	0.999	Correlation factor r^2	0.998

Table-I Regression analysis of the calibration curve

Precision-System Repeatability			
L-Valine methyl ester HCL		Isoleucine methyl ester HCL	
Concentration level	% RSD	Concentration level	% RSD
QL	0.9%	QL	2.6%
100%	2.4%	100%	2.3%
150%	1.2%	150%	1.1%

Table-II Data of system repeatability precision

Accuracy/Recovery			
L-isoluecine methyl ester HCl	QL	100%	150%
Spike amount mg/ml	0.1022	0.2051	0.3026
Average Recover amount mg/ml	0.1054	0.203	0.2945
% recovery	103.1	99	97.3

Table-III Recovery data

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