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Development of Pharmaceutical Technique for Antihypertensive Drugs

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ABSTRACT: Analysis of pharmaceutical compounds and newer drugs is commonly used in all the stages of drug discovery and development process. Gas chromatography is one of the sophisticated instrumental techniques. These analytical techniques provide more accurate and précised data for drug discovery and development. Modern pharmaceutical analysis is mainly dominated by costlier instrumental analysis. In present study focus is on developing new method of analysis to increase the specificity and sensitivity of a method. 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 anti-hypertensive drug. The Gas Chromatography system was used for method development and method validation was performed . 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 drug. 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.

I. INTRODUCTION

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. 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. If 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 sample. The high recovery and low relative standard deviation confirms the suitability of the method for purity of compound.

In present study GC-FID method was developed for determination of L-valine methyl ester hydrochloride.

II. MATERIALS AND METHODS

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). Nitrogen was used as a carrier gas with a constant flow rate. Sample was injected. Solutions of Isoluecine and L-valine methyl ester HCl were prepared in methanol. The area of all peaks were determined. Resolution between L-valine methyl ester HCl and Isoluecine methyl ester HCl should be more than 5.0.

III. OBSERVATIONS RESULTS AND DISCUSSION

Specificity: 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.

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 1. *Detection limit:*-Detection limit is about 40µg/ml. At DL level peaks are detected.
Quantification limit:-Quantitation limit is 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 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 Table 2. 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).

Table 1: Regression analysis of the calibration curve.

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 2: Data of system repeatability precision.

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 3: Recovery data.

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

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 3.
Solution stability:- The sample solution 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.

IV. CONCLUSIONS

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

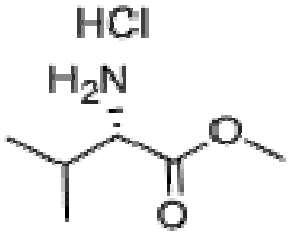
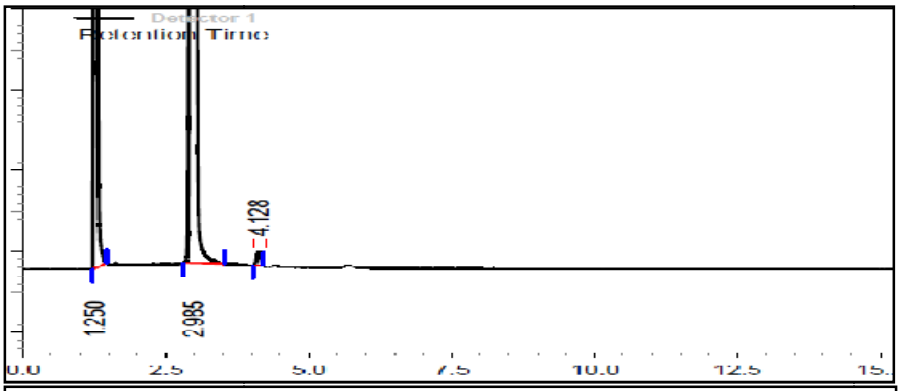


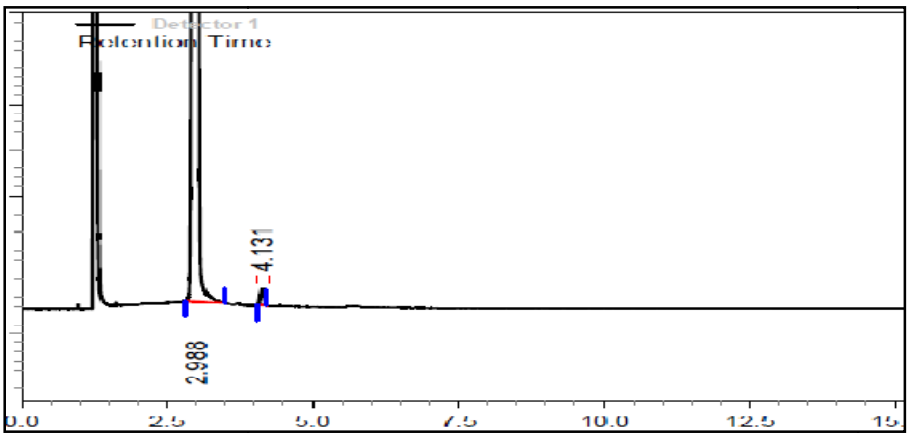
Fig. 1. (L-Valine Methyl Ester HCl Structure).



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	

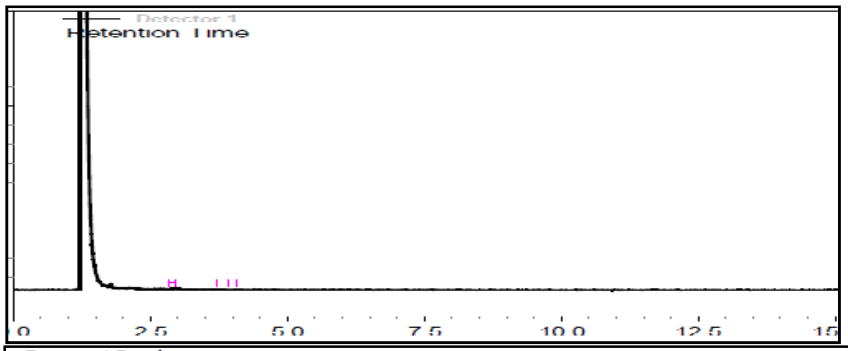
Fig. 2. (Chromatogram of sample with impurity for specificity).



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			

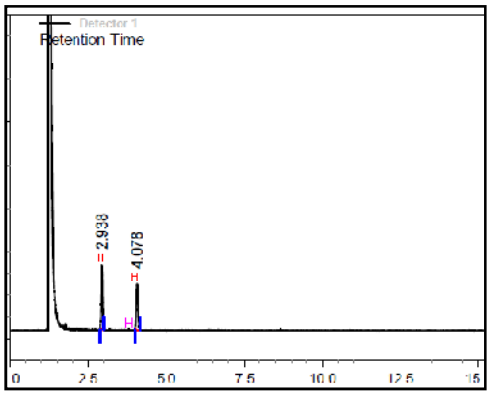
Fig. 3. (System suitability Chromatogram).



Detector 1 Results

Pk #	Name	Retention Time	Area	Area %

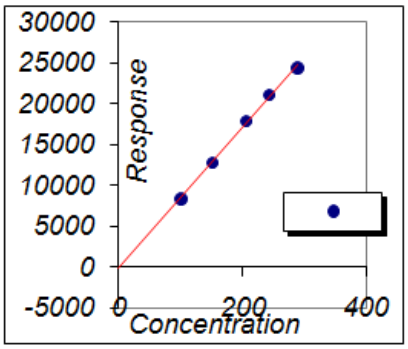
Fig. 4. {Blank (Methanol) Chromatogram}.



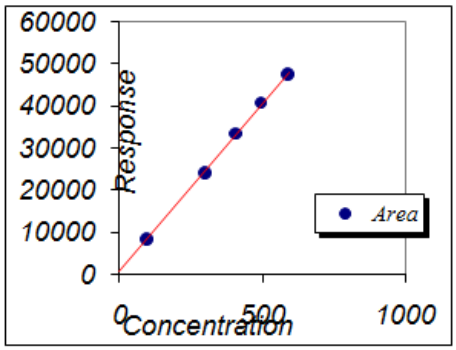
Detector 1 Results

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	

Fig. 5. (QL level Chromatogram).



Linearity of L-valine methyl ester HCl



Linearity of Isoleucine methyl ester HCl

Fig. 6. (Linearity curve for L-valine methyl ester HCl and L-Isoluecine HCl).

REFERENCES

[1]. www.chemicalbook.com
[2]. The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals, Thirteenth Edition, Merck & Co. Inc., 2001, 13:3453.
[3]. USP 32 <1225>: "Validation of Compendial Methods".
[4]. Guidance for Industry – Analytical Procedures and Method Validation (August-2000).
[5]. W.D. Snyder, L. Blumberg, in: P. Sandra, M.L. Lee (Eds.), Proceedings of the 14th International Symposium on Capillary Chromatography, Baltimore, MD, May 1991, p. 28.
[6]. WHO, Guidelines for Stability Testing of Pharmaceutical Products Containing Well Established Drug Substances in Conventional Dosage Forms, in WHO Expert Committee.
[7]. Willard Hobart. H., Merritt L.L., Dean John. A., Instrumental Methods of Analysis, 7th edition, CBS Publishers, 580-610.
[8]. International Conference on Harmonization of Technical Requirement for Registration of Pharmaceuticals for Human use, ICH harmonized tripartite Guideline, Validation of Analytical procedures Text and methodology Q2 (R1), 2005.
[9]. R. Jenke, "Chromatographic Method Validation: A review of Current Practices and Procedures. I. General Concepts and Guidelines", *J. Liq. Chrom. and Rel. Technol.*, vol. **19** (1996), pp. 719-736.
[10]. David M. Bliesner, Validating Chromatographic Methods, (John Wiley and Sons, 2006, p. 72).
[11]. F. David, B. Tienpont, P. Sandra, in: 23rd International Symposium on Capillary Chromatography, Riva del Garda, *Microcol. Sep.* **8** (1996) 353. 2000, E.06
[12]. Wikipedia, the free encyclopedia, en.wikipedia.org.
[13]. McDay, Jo Lisa, "Ecological method development for detecting N-nitrosodimethylamine in water using HPLC-PDAD " (2010).Masters Theses and Doctoral Dissertations. Paper 260. http://commons.emich.edu/theses/260
[14]. International Journal of Pharm Tech Research CODEN (USA): IJPRIF ISSN : 0974-4304 Vol. 3, No.2, pp 1083-1087, April-June 2011 Sathish Kumar Shetty et al /*Int. J. Pharm Tech Res.* 2011, **3**(2).
[15]. D.H. Desty, A. Goldup, W.T. Swanton, in: N. Brenner, J.E. Callen, M.D. Weis (Eds.), Gas Chromatography, Academic Press, New York, 1962, p. 105.
[16]. A. van Es, in: W. Bertsch, H. Frank, W.G. Jennings, P Sandra (Eds.), High Sarrow Bore Capillary Gas Chromatography, Hu¨thig, Heidelberg, 1992.