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A Novel Quantitative Spectrophotometric Method for the Analysis of Gabapentin Hydrochloride

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Abstract

A simple Spectrophotometric method is developed for the determination of gabapentin hydrochloride because at present there is no official method for the analysis of gabapentin hydrochloride. The method is based on the reaction of carboxylic acid group of gabapentin hydrochloride with a mixture of potassium iodate (KIO_3) and iodide (KI) to form yellow colored product in aqueous medium at 25 ± 1º C. The reaction is followed spectrophotometrically by measuring absorbance at 450 nm. Under optimized experimental conditions, Beer's law is obeyed in the concentration range of 21.2 – 159.0 µg/mL. The method is validated with respect to accuracy, precision, limit of detection and limit of quantitation. Robustness testing is also conducted to evaluate the effect of minor changes to the absorbance of the product.

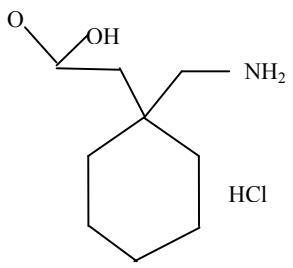
Keywords: *Spectrophotometer, Gabapentin Hydrochloride, Potassium Iodate, Potassium Iodide, Validation.*

Introduction:

Gabapentin hydrochloride is chemically known as *1 – (amino methyl) cyclohexane acetic acid hydrochloride* (Figure. 1). It is an intermediate product of Gabapentin which is a potent antiepileptic drug in adult patients who have not achieved adequate drugs control of partial seizures with these agents used alone or combination. It has a simple pharmacokinetic profile and is not protein bound. It is particularly useful in controlling secondarily generalized tonic – clonic seizures.

The molecular formula of gabapentin hydrochloride is $C_9H_{18}ClNO_2$. The molecular weight of gabapentin hydrochloride is 207.70 with CAS Registry number 60142 – 96 – 3.

Fig. 1. Structure of Gabapentin Hydrochloride**.**



Gabapentin Hydrochloride is used as an anticonvulsant (1-3). It increases GABA in the brain and binds with voltage – sensitive Ca^{2+} channels. It also prevents neuronal death.

There is no analytical method is developed till now for the determination of gabapentin hydrochloride. So there is a need of a method for the determination of gabapentin hydrochloride. Different analytical instruments such as High Performance Liquid Chromatography (HPLC) (4-5), Liquid Chromatography – Mass Spectrophotometry (LC – MS) (6 - 7), Gas Chromatography – Mass Spectrophotometry (GC – MS) (8), Gas Chromatography (GC) (9), Capillary Electrophoresis (CE) (10) are available for quantitative analysis of intermediates of different bulk drugs. The cost of these instrument are very high and requires long and tedious pretreatment of samples and laborious clean up procedures prior to analysis. Therefore, it is necessary to develop simple and sensitive analytical method for the determination of gabapentin hydrochloride. UV – VIS Spectrophotometry is low cost techniques

and do not requires any pretreatment of samples and laborious clean up procedures. It is easily available in pharmaceutical industry, hospitals and research laboratories. The present developed method is based on the reaction of carboxylic acid group of the intermediates with the mixture of potassium iodide and potassium iodate. The absorbance is measured at 450 nm against the reagent blank prepared simultaneously. The method is simple, sensitive, accurate and precise.

Materials and Methods

Apparatus

Spectral runs were made on UV 3000$^+$ UV – VIS Spectrophotometer (LABINDIA®, Mumbai, India) [Serial Number 17 – 1885 – 01 – 0016] with 1 cm matched glass cell.

Reagents

- Gabapentin Hydrochloride (Vardhman Chemtech Ltd, Mohali, Punjab, India) used as working standard.
- Potassium Iodate (KIO_3) was purchased from RFCL Limited (New Delhi, India)
- Potassium Iodide (KI) was purchased from RFCL Limited (New Delhi, India)

Standard Gabapentin Hydrochloride Solution

A stock solution of gabapentin hydrochloride (1062 µg/mL) was prepared by dissolving 50 mg gabapentin hydrochloride in 100 mL volumetric flasks with distilled water. The stock solution (1062 µg/mL) was used to prepare the working solutions by suitable dilutions with distilled water. The solutions were stable at least 10 days in room temperature.

Procedure for Determination of Gabapentin Hydrochloride

Into a series 10 mL volumetric flasks, different volumes (0.2 – 1.5) mL of standard gabapentin hydrochloride (1062 µg/mL) solution corresponding to 21.2 – 159.0 µg/mL were pipetted. To each flask, 0.8 ml KIO_3 (4113 µg/mL) and 1.8 ml KI (9194 µg/mL) were added and diluted to volume with distilled water. The reaction was allowed to proceed at room temperature and absorbance was measured as a function of time at 450 nm against the reagent blank prepared simultaneously. The calibration curve was constructed by plotting the absorbance against the initial concentration of gabapentin hydrochloride. The content of gabapentin hydrochloride is calculated either from the calibration curve or corresponding regression equation.

Method Validation

Method validation is closely related to method development. When a new method is being developed, some parameters are already being evaluated during the 'development stage' while in fact this forms part of the 'validation stage'. The ICH guidelines achieved a great deal in harmonizing the definitions of required validation parameters, their calculation and interpretation. The international conference on the Harmonization of the Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) has harmonized the requirements in two guidelines (11 – 12). The first one summarizes and defines the validation characteristics needed for various types of test procedures, the second one extends the previous test to include the experimental data required and some statistical interpretation.

Accuracy

The closeness of agreement between a test result and the accepted reference value.

Precision

The closeness of agreement between independent test results obtained under stipulated conditions.

Specificity
The ability of the method to determine accurately and specifically the analyte of interest in the presence of other components in a sample matrix (that may be expected to be present in the sample matrix) under the stated conditions of the test (specificity = 100 % selectivity).

Limit of Detection (LOD)
The limit of detection is the point at which a measured value is longer than the uncertainty associated with it. It is the lowest concentration of analyte in a sample that can be detected but not necessary quantified.
$LOD = 3.3 \times S_O / b$
Where S_O and b are standard deviations and slope of the calibration line, respectively.

Limit of Quantitation (LOQ)
The lowest concentration or amount of analyte that can be determined quantitatively with an acceptable level of repeatability precision and trueness.
$LOQ = 10.0 \times S_O/b$

Linearity
The ability of the method to obtain test results proportional to the concentration of analyte (within a given range).

Linear Range
The range of concentrations or amounts of analyte over which the method gives test results proportional to the concentration of analyte or a linear calibration model can be applied with a known confidence level.

Ruggedness
The (intra – laboratory tested) behavior of an analytical process when small changes in the environmental and/or operating conditions are made.

Robustness
A measure of the capacity of the analytical procedure to remain unaffected by small but deliberate variations in method – performance parameters, which provides an indication of its reliability during normal usage.

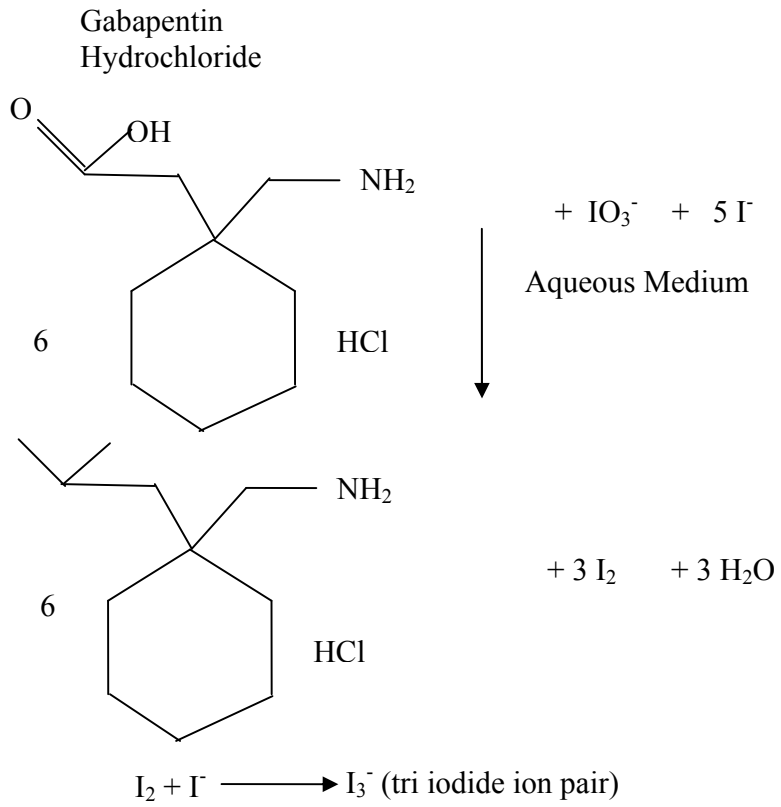
Results and Discussions

Reaction with a mixture of iodate and iodide
It has been reported in the literature (13) that iodine is formed as a result of the interaction of a mixture of iodide and iodate with inorganic or organic acid in accordance with the equation.

$$5\ I^- + IO_3^- + 6\ H^+ \rightarrow 3\ H_2O + 3\ I_2$$

In aqueous medium, the iodide ions react with the liberated iodine to yield triiodide ion ($I_2 + I^- \rightarrow I_3^-$) which detected in UV detector at 450 nm (Figure 2). We thought that this reaction would be helpful for developing a spectrophotometric method for determination of gabapentin hydrochloride as it contains – COOH group in its moiety. Keeping this in mind, a mixture of potassium iodide and iodate was allowed to react with gabapentin hydrochloride which yielded iodine. Then the liberated iodine reacted with the excess of iodide ion resulting in the formation of triiodide ion (**Scheme I**).

Optimization of reaction conditions
The different parameters affecting the development process were extensively studied to determine the optimum conditions for the assay procedures. The optimum values of the variables were maintained throughout the determination process.



Scheme I

Effect of the concentration of potassium iodate

The effect of the volume of potassium iodate (4113 µg/mL) on the absorbance of the product was studied in the range of 0.1 – 1.0 mL. The absorbance increases with the increase in the volume of potassium iodate and became constant at 0.6 mL. Further addition of KIO_3 does not change in the absorbance and therefore, 329.04 µg/mL KIO_3 was chosen as an optimum value (Figure 3).

Effect of the concentration of potassium iodide

The effect of the volume (9194 µg/mL) potassium iodide on the absorbance of the product was studied in the range of 0.1 – 2.0 mL, keeping the constant concentrations of gabapentin hydrochloride (10620 µg/mL) and 329.04 µg/mL KIO_3. The maximum absorbance was obtained with 1.6 mL; further addition caused no change on the absorbance. Thus, 1654.92 µg/mL potassium iodide (Figure 4) was used throughout the experiment.

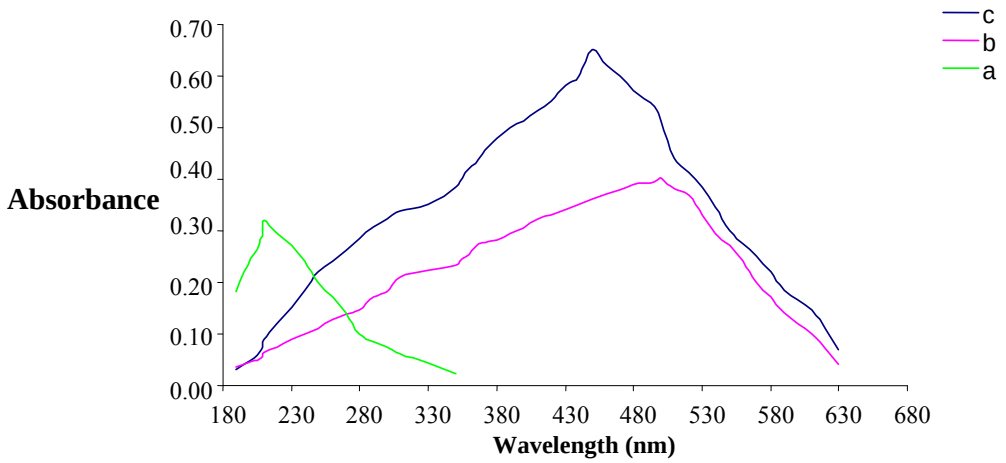


Fig. 2. Absorption spectra of (a) Gabapentin Hydrochloride 106.2 µg/mL in distilled water (b) Blank Solution: KIO_3 329.04 µg/mL and KI 1654.92 µg/mL in distilled water (c) Sample Solution: Gabapentin Hydrochloride 106.2 µg/mL + KIO_3 329.04 µg/mL and KI 1654.92 µg/mL in distilled water.

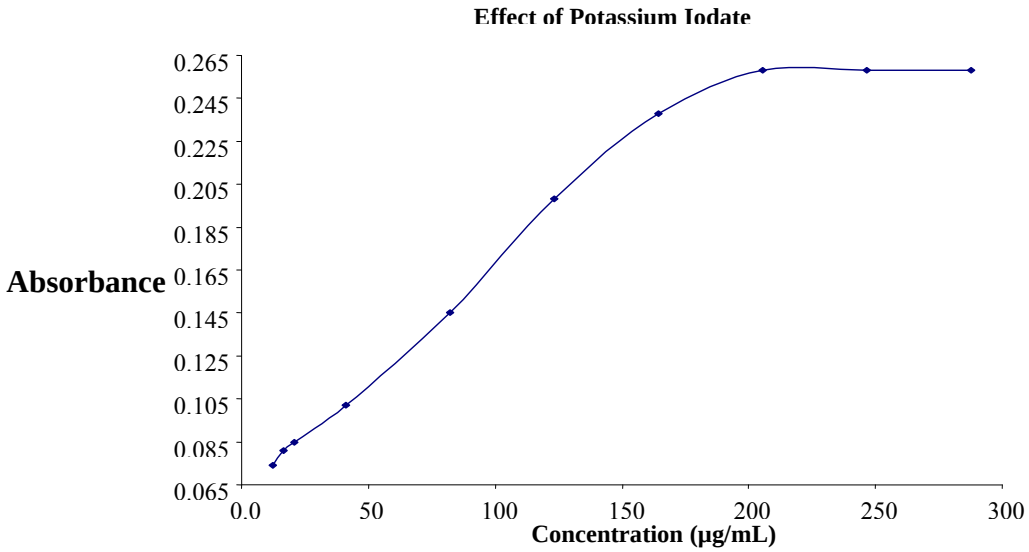


Fig. 3. Effect of the volume of KIO_3 (4113 µg/mL); keeping constant 106.2 µg/mL gabapentin hydrochloride and KI 1654.92 µg/mL.

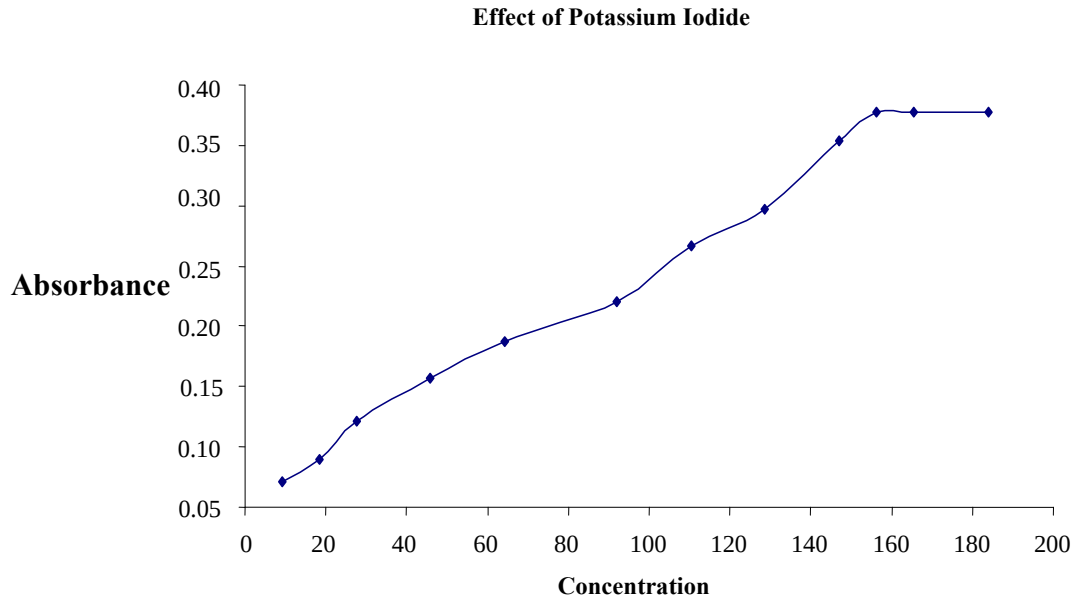


Fig. 4. Effect of the volume of KI (9194 µg/mL); keeping constant 106.2 µg/mL gabapentin hydrochloride and KIO_3 919.4 µg/mL).

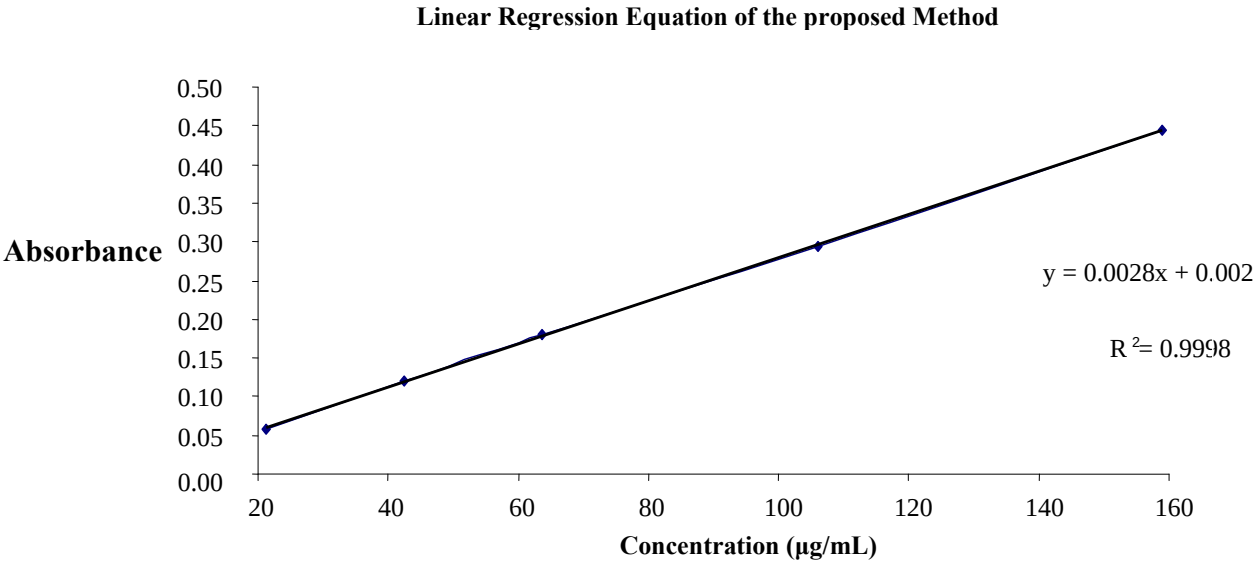


Fig. 5. Linearity and Linear Regression equation of the Proposed Method

Table 1. Summary of optical and regression characteristics of the proposed method

Parameters	Gabapentin Hydrochloride
Linear dynamic range (μg/mL)	21.2 – 159.0
Slope	0.0028
Intercept	0.002
Regression equation[a]	$Y = 2 \times 10^{-3} + 2.8 \times 10^{-3} X$
Correlation coefficient (r)	0.9998

Table 2. Summary of accuracy and precision results of the proposed method in pure form

Proposed methods	Amount (μg/mL)		RSD %	REC.	SAE[b]	C.L.[c]
	Taken	Found ± SD[a]				
Intra day assay	40.0	39.997 ± 0.125	0.313	99.993	5.59×10^{-2}	1.55×10^{-1}
	100.0	100.012 ± 0.101	0.101	100.012	4.52×10^{-2}	1.25×10^{-1}
	150.0	150.008 ± 0.091	0.061	100.005	4.07×10^{-2}	1.13×10^{-1}
Inter day assay	40.0	40.002 ± 0.162	0.405	100.005	7.25×10^{-2}	2.01×10^{-1}
	100.0	99.998 ± 0.125	0.125	99.998	5.59×10^{-2}	1.55×10^{-1}
	150.0	150.004 ± 0.111	0.074	100.003	4.96×10^{-2}	1.38×10^{-1}

[a] Mean for 5 independent analyses.
[b] SAE, standard analytical error.
[c] C.L., confidence limit at 95 % confidence level and 4 degrees of freedom (t = 2.776).

Validation of proposed method

Validation of analytical procedures is a vital aspect not just for regulatory purposes, but also for their efficient and reliable long – term application. The ICH guidelines achieved a great deal in harmonizing the definitions of required validation parameters, their calculation and interpretation.

Under the optimum experimental conditions, the absorbance – concentration plot for the proposed method was found to be rectilinear over the range of 21.2 – 159 μg/mL (Figure 5). It was found that the absorbance is stable for at least two days at room temperature. Linear regression analysis of calibration data gave the regression equations cited in Table 1 with correlation coefficients close to unity. The within day precision assays were carried out through replicate analysis (n = 5) of gabapentin hydrochloride corresponding to 40, 100 and 150 μg/mL. The interday precision was also evaluated through replicate analysis of the pure samples for five consecutive days at the same concentration levels as used in within day precision. The results of these assays are reported in Table 2**.** As can be seen from Table 1 that Recovery values for intraday and interday precision were in the range of 99.960 to 100.038 % and RSD values for intraday and interday precision were in the range of 0.031 to 0.115 %. The accuracy was ascertained by recovery studies using the standard addition method. The results are summarized in Table 2. As can be seen from Table 2 that the recovery and RSD values for the proposed method were in the range of 99.993 to 100.012 % and 0.061 to 0.405 %.

References

1. K. C. Cundy, T. Annamalai, L. Bu, J. D. Vera, J. Estrela, W. Luo, P. Shirsat, A. Torneros, F. Yao , J. Zou, R. W. Brret, and M. A. Gallop. XP13512 [(±) – 1- ([(α – Isobutanoyloxy) J. carbonyl] aminomethyl) – 1 – cyclohexane Acetic Acid], A Novel Gabapentin Prodrug: II. Improved Oral Bioavailability, Dose Proportionality, and Colonic Absorption Compared with Gabapentin in Rats and Monkeys, Pharmacol. Experim. Therap 311: 324 (2004).
2. http: www.epochem.com/products/pharm/60142-96-3.htm.
3. http:// www.tocris.com/dispprod.php?ItemId=2038.
4. R. S. Gujral, S. M. Haque, and S. Kumar. A novel method for the determination of Pregabalin in bulk pharmaceutical formulations and human urine samples, African. J. Pharm. Pharmacol 3: 327 – 334 (2009).
5. R. S. Gujral, and S. M. Haque. Development and Validation of a New HPLC Method for the Determination of Gabapentin, Int. J Biomed. Sci 5: 63 -69 (2009).
6. U. Mandal, A. K. Sarkar, K. V. Gowda, S. Agarwal, A. Bose, U. Bhaumik, D. Ghosh, and T. K. Pal. Determination Pregabalin in Human Plasma Using LC – MS – MS, Chromatogra. 67: 237 – 243 (2008).
7. R. Oertel, N. Arenz, J. Pietsch, and W. Kirch. Simaltaneous determination of three anticon – vulsants using hydrophilic interaction LC – MS, J. Sep. Sci 32**:** 238 – 243 (2009).
8. D. C. R. Borrey, K. O. Godderis, V. I. L. Engerlrelst, D. R. Bernard, and M. R. Langlois. Quantitative determination of vigabatrin and gabapentin in human serum by gas chromatography – mass spectrometry, Clin. Chim. Acta 354: 147 – 151 (2005).
9. M. M. Kushnir, J. Crossett, P. I. Brown, and F. M. Urray. Analysis of gabapentin in serum and plasma by solid phase extraction and gas chromatography mass spectophotometry for therapeutic drug monitoring. Anal. Toxicol 23:1 – 6 (1999).
10. F. M. Lin, H. S. Lin, H. S. Kou, S. M. Wu, S. H. Chen, and H. L. Wu. Capillary electrophoresis analysis of gabapentin and vigabatrin preparations as ofloxacin derivatives, Anal. Chim. Act 523:9 – 14 (2004).
11. International Conference on Harmonization of technical requirements for the registration of pharmaceuticals for Human Use (ICH) Q2A. Validation of Analytical Methods (Definitions and Terminology) (October 1994).
12. ICH: Q2B Analytical Validation Methodology (November 1996).
13. F. Feigl, Spot tests in organic analysis. Chapter 3, Preliminary (Exploratory tests), 6th ed., Elsevier publishing company, Amsterdam, 1960, pp. 117 – 118.