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Analytical Method Development For Iso Propyl Alcohol Content In Lysine Monohydrate By GC-HS

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Abstract:

A simple, sensitive, accurate, robust headspace gas chromatographic method was developed for the quantitative determination of isopropyl alcohol in Lysine. The method validation was carried out with regard to the guidelines for validation of analytical procedures Q2 demanded by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH). All the validation characteristics were meeting the acceptance criteria. Hence, the developed and validated method can be applied for the intended routine analysis.

Introduction:

A method to determine methanol (MeOH) and isopropyl alcohol (IPA) was developed using static headspace sampling (HSS) with gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). The optimized HSS-GC and HSS-GC/MS methods were validated according to the parameters.
Isopropyl alcohol (IUPAC name propan-2-ol and also called isopropanol or 2-propanol) is a colorless, flammable organic compound with a pungent alcoholic odor.[1] As an isopropyl group linked to a hydroxyl group (chemical formula $(CH_3)_2CHOH$) it is the simplest example of a secondary alcohol, where the alcohol carbon atom is attached to two other carbon atoms. It is a structural isomer of propan-1-ol and ethyl methyl ether. They all have the formula C_3H_8O.

Isopropyl alcohol is miscible in water, ethanol, and chloroform, as it is an organic polar molecule. It dissolves ethyl cellulose, polyvinyl butyral, many oils, alkaloids, and natural resins.[2] Unlike ethanol or methanol, isopropyl alcohol is not miscible with salt solutions and can be separated from aqueous solutions by adding a salt such as sodium chloride. The process is colloquially called *salting out*, and causes concentrated isopropyl alcohol to separate into a distinct layer.[3] Isopropyl alcohol forms an azeotrope with water, which gives a boiling point of 80.37 °C (176.67 °F) and a composition of 87.7% by mass (91% by volume) isopropyl alcohol. It has a slightly bitter taste, and is not safe to drink.[4]

Lysine plays several roles in humans, most importantly proteinogenesis, but also in the crosslinking of collagen polypeptides, uptake of essential mineral nutrients, and in the production of carnitine, which is key in fatty acid metabolism. Lysine is also often involved in histone modifications, and thus, impacts the epigenome. The ε-amino group often participates in hydrogen bonding and as a general base in catalysis. The ε-ammonium group ($-NH^+_3$) is attached to the fourth carbon from the α-carbon, which is attached to the carboxyl (C=OOH) group.[5]. Lysine was first isolated by the German biological chemist Ferdinand Heinrich Edmund Drechsel in 1889 from the protein casein in milk.[6] He named it "lysin".[7] In 1902, the German chemists Emil Fischer and Fritz Weigert determined lysine's chemical structure by synthesizing it.[8].

Chemicals Used				
S.No	**Name of Chemical/Reagent**	**Grade**	**Make**	**B.No.**
01	Water	HPLC	JT Baker	0000126136
02	Iso Propyl alcohol	AR	Fisher scientific	1204700816

Typical Chromatographic conditions :

Column		
Type	Capillary ZB-624 (30m X 0.53 mm X3.0um)	
Colum Make (Part Number)	Phenomenex (7HK-G005-36)	
Carrier gas	Nitrogen	
Carrier flow	2.5 mL/min	
Injection Volume	…	
Split ratio	1:5	
Detector		
Type	FID	
Hydrogen flow	30 mL/min	
Air flow	300 mL/min	
Carrier gas/Make up Flow	30 mL/min	
Injector temperature	180ºC	
Detector temperature	250ºC	
Column pressure	Constant flow	
Oven program		
Rate	Temperature	Hold Time
	40ºC	5 min
20ºC/min	200ºC	2 min
Head space parameter		
Oven Temperature	80ºC	
Loop Temperature	85ºC	
Transfer Line Temperature	90ºC	
Vial equilibration Time	15min	
Pressurization time	0.2min	
Loop fill Time	0.2min	
Injection Time	1.0min	
GC Cycle Time	15 min	

Diluent :HPLC water used as diluent

Blank Preparation: Take 5.0 mL of HPLC water in 20.0 ml Head space vail and seal.

Sample Preperation : Take 100mg of sample in 20.0 ml Head space vail add 5.0ml of diluent and seal.

Standard stock solution Preperation : Dissolve 100mg of Iso propyl alcohol standard in 100ml volumetric flask which is already contain 20ml of diluent,dilute up to the mark with diluent.

Standard Preperation: Take 10ml from above stock solutionin 100ml volumetric flask which is already contain 20ml of diluent,dilute up to the mark with diluent

Solvent name	**Limit**
Iso propyl alcoholcontent	Not more than 5000 ppm

Evaluation of system suitability:

Inject blank and standard preparations using the above GC-HS parameters. Record the chromatograms and evaluate. The system is suitable for analysis if and only if,

- The percentage relative standard deviation for the peak area of Iso propyl alcoholshould be not more than 10.00% for six replicate injections of standard solution.
- The percentage relative standard deviation for the peak area of Iso propyl alcohol should be not more than 10.00% for seven replicate (Through out run) injections of standard solution.

Procedure:

If system suitability passes, Inject sample solution preparations and record the chromatograms. Identify the Iso propyl alcoholpeak in the sample solution based on the retention times obtained in standard chromatogram and note down the area response.

S. No.	compound name	Approximate Retention time (minutes)
1	Iso propyl alcohol	6.7

Order of injections:

Name of the preparations	No. of injections	Purpose
Blank	2	Blank
Standard solution	6	For system suitability and Iso propyl alcohol content calculation
Blank	1	Blank
Sample preparation	1	Estimation of Iso propyl alcoholin sample
Sample preparation	1	Estimation of Iso propyl alcohol in sample
Standard solution	1	For system suitability

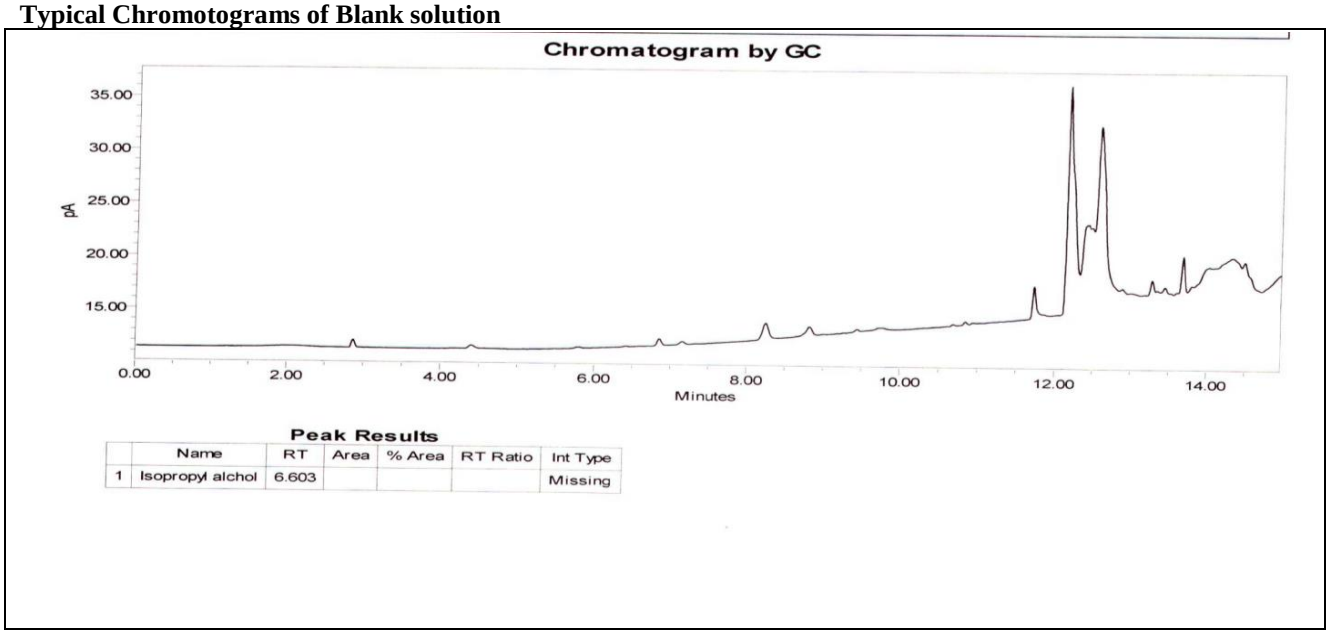
Calculation:

Calculate the Iso propyl alcoholcontent with individual injection and report as per the formulae given below

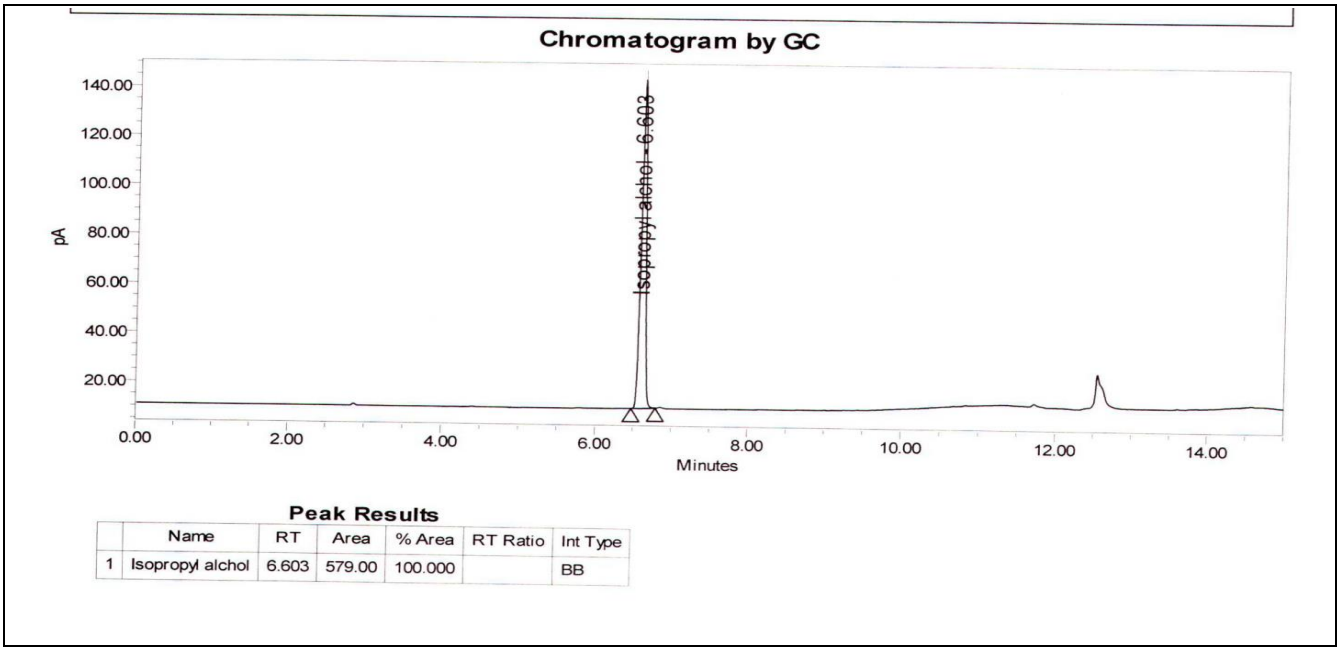
$$\text{Iso propyl alcoholcontent(in ppm)} = \frac{AT}{AS} \times \frac{WS}{WT} \times \frac{105P1}{100} \times 10^6$$

Where,
AT = Peak area response of Iso propyl alcoholpeak in sample preparation.
AS = Average peak area response of Iso propyl alcoholpeak in standard preparation.
WT = Weight of sample in mg.
WS = Weight of Iso propyl alcoholstandard in mg.
P1 = Purity of Iso propyl alcoholacid standard.

Typical Chromotograms of Blank solution

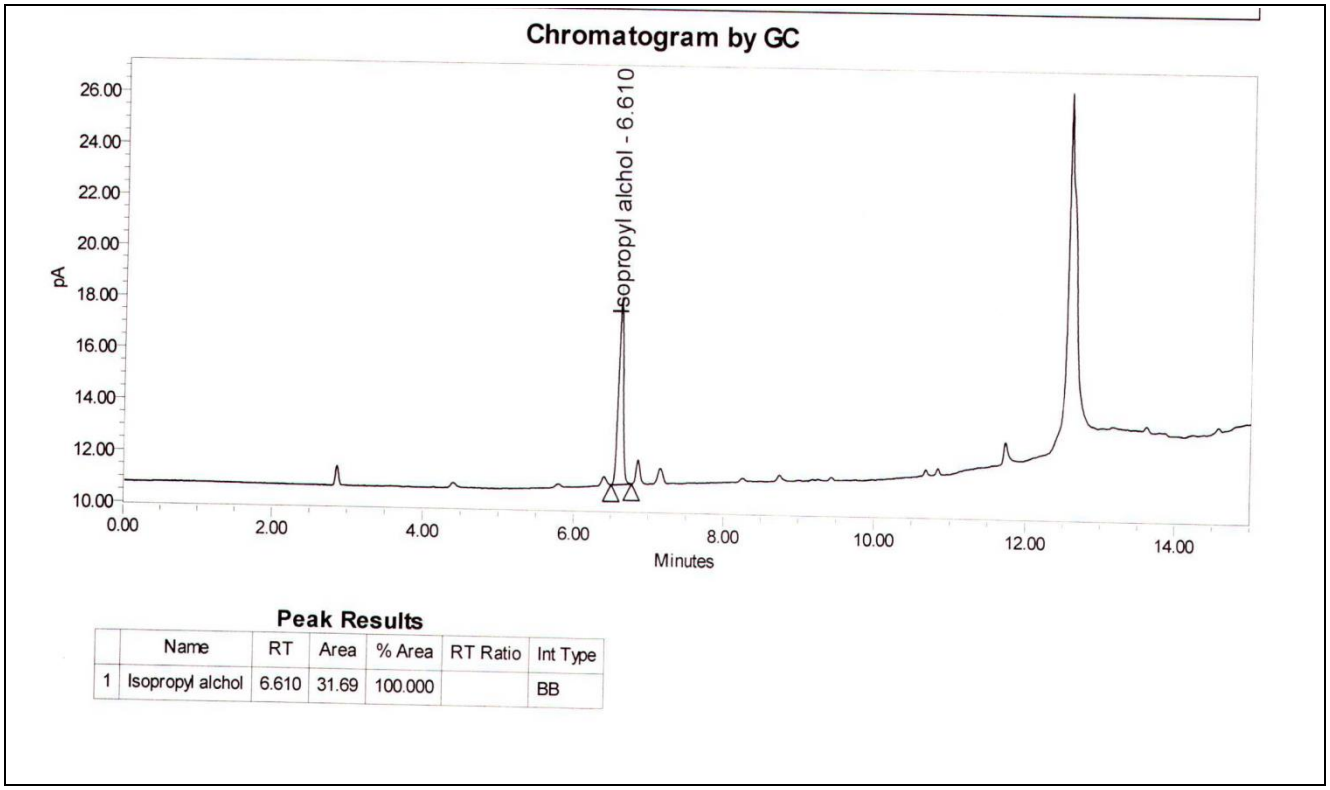


Tipical Chromotogram of Standard solution:



	Name	RT	Area	% Area	RT Ratio	Int Type
1	Isopropyl alchol	6.603	579.00	100.000		BB

Tipical Chromotogram of Sample solution:



	Name	RT	Area	% Area	RT Ratio	Int Type
1	Isopropyl alchol	6.610	31.69	100.000		BB

Determination of Limit of quantitation (LOQ):

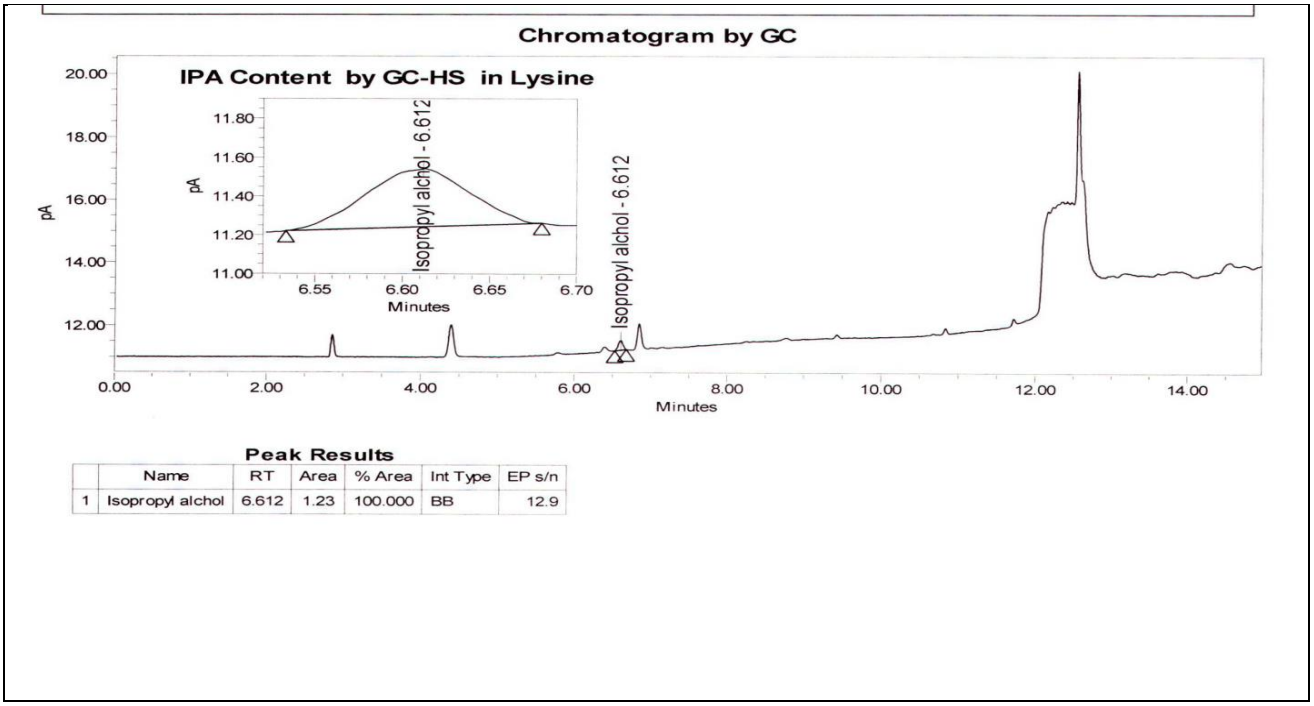
Compound Name	**Iso propyl alcohol**
Weight taken (mg)	**99.3**
Conc.(µg/mL)	0.1986
LOQ with respect to sample conc. (ppm)	10.0
Signal to Noise ratio	12.9:1
Reported LOQ (ppm)	**10.00**

As shown in the above table, the S/N ratio values (LOQ values) is obtained as about 12.9:1 for Iso propyl alcohol(10.0 ppm).

The acceptance criteria are met/criterion is met.

See chromatogram below.

Typical chromatogram of Limit of quantitation:



	Name	RT	Area	% Area	Int Type	EP s/n
1	Isopropyl alchol	6.612	1.23	100.000	BB	12.9

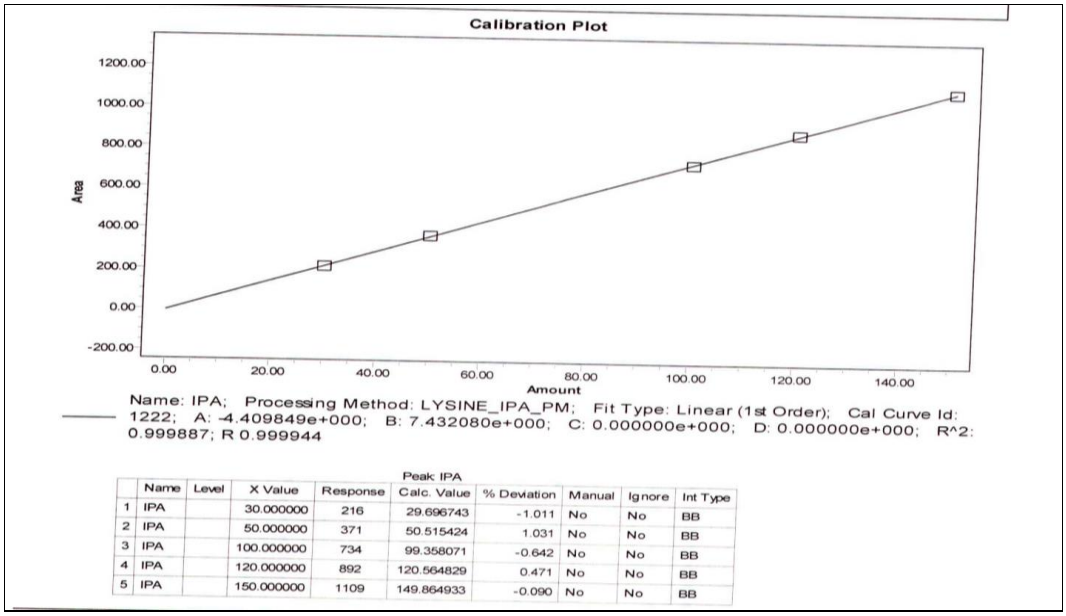
Linearity:

The linearity of the GC-HS method was demonstrated for Iso propyl alcoholsolutions ranging from 50.0% to 150.0% of the specification limit.
Results obtained are shown in below table and drawn the linearity graph for peak area versus concentration for Monochloro acetic acid to calculate slope, intercept and correlation coefficient values.

Acceptance criteria:

- No apparent non-linearity should be observed graphically for Iso propyl alcohol.
- The correlation coefficient (R) should be NLT 0.9900.
- Report the slope and intercept values.

Linearity Graph for Monochloro acetic acid:



Peak: IPA

	Name	Level	X Value	Response	Calc. Value	% Deviation	Manual	Ignore	Int Type
1	IPA		30.000000	216	29.696743	-1.011	No	No	BB
2	IPA		50.000000	371	50.515424	1.031	No	No	BB
3	IPA		100.000000	734	99.358071	-0.642	No	No	BB
4	IPA		120.000000	892	120.564829	0.471	No	No	BB
5	IPA		150.000000	1109	149.864933	-0.090	No	No	BB

Accuracy:

The accuracy was performed on samples spiked with known amount of Iso propyl alcohol.The inherent amount of the individual compound was taken into account.
The results have been calculated as recovery rate:
Recovered result x 100 / (adherent analyte +spiked analyte).
The Specification Level concentrations were performed:

Acceptance criteria:

- Report percentage recovery and percentage relative standard deviation for each level. The percentage recovery calculated should be in the range of 80.0% to 120.0%.

Level	Theoretical conc. (ppm)	Measured conc. (ppm)	% Recovery
50.0%	2500	2414	96.6
100.0%	5000	4797	95.9
120.0%	6000	5726	95.4
150.0%	7500	7266	96.9

Batch results:

S.No	Batch number	Result(ppm)
1	100/17	286
2	102/17	252
3	103/17	196

Observation:

1) The peak shape ofIso propyl alcoholwas good.
2) The base line drift was less.

Conclusion:

The chromatographic conditions are giving clear resolution for all the Impurities in Lysine monohydrate. Hence the same chromatographic conditions shall be adopted for routine testing for the analysis for Iso propyl alcoholcontent in Lysine monohydrate.

References:

1. "Isopropanol". PubChem. Archived from the original on 12 February 2019. Retrieved 10 February 2019.
2. Doolittle, Arthur K. (1954). The Technology of Solvents and Plasticizers. New York: John Wiley & Sons, Inc. p. 628.
3. Jump up to:[a] [b] The Merck Index (10th ed.). Rahway, NJ: Merck & Co. 1983. p. 749. ISBN 9780911910278.
4. Logsden, John E.; Loke, Richard A. (1999). "Propyl Alcohols". In Kroschwitz, Jacqueline I. (ed.). Kirk-Othmer Concise Encyclopedia of Chemical Technology (4th ed.). New York: John Wiley & Sons, Inc. pp. 1654–1656. ISBN 978-0-471-41961-7.
5. Lysine. The Biology Project, Department of Biochemistry and Molecular Biophysics, University of Arizona.
6. Drechsel E (1889). "Zur Kenntniss der Spaltungsprodukte des Caseïns" [[Contribution] to [our] knowledge of the cleavage products of casein]. Journal für Praktische Chemie. 2nd series (in German). **39**: 425–429
7. Drechsel E (1891). "Der Abbau der Eiweissstoffe" [The disassembly of proteins]. Archiv für Anatomie und Physiologie (in German): 248–278.
8. Fischer E, Weigert F (1902). "Synthese der α,ε – Diaminocapronsäure (Inactives Lysin)" [Synthesis of α,ε-diaminohexanoic acid ([optically] inactive lysine)]. Berichte der Deutschen Chemischen Gesellschaft (in German). **35** (3): 3772–3778.