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Research Article

A STUDY OF THE BEHAVIOUR OF L – GLUTAMIC ACID IN THE COURSE OF AND AFTER γ – RAY TREATMENT

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ABSTRACT

The aim of current study is quality control of L – Glutamic acid in supplement mixture before and after treatment with γ – ray. Microbiological methods, included in European Pharmacopoeia were used for examination of microbial purity of substance L – Glutamic acid. Abnormal content ($1.5.10^{-4}$ g) of bacteria and contaminants were identified mostly as non patogenic bacilli of Subtilis group. Patogenic contaminants as Enterobacteriaceae and Staphylococcus aureus were not found. Resistency factors show moderate ray sensitivity of the microorganisms.

HPLC method was developed and applied and analytical parameters repeatability, limit of detection (LOD), limit of quantitation (LOQ) and linearity were studied and determined in accordance with ICH and European Pharmacopoeia requirements. For repeatability SD = 1.43, RDS = ± 0.44. The obtained LOD is 10 µg and LOQ is 40 µg. The correlation coefficient is found to be 0.99746 at SD = ± 3914.60 AU. There are no significant difference between content of L – Glutamic acid in supplement mixtures before (RDS = ± 0.44 %) and after γ – ray treatment (RDS = ± 0.082 %).

Keywords: L-Glutamic acid.

INTRODUCTION

Proteins and peptides are polymers of α – aminoacids. Aminoacids can be classified on lots of different features. According to the fact, whether or not human can acquire them through the diet, are recognized 3 types: nonessential, essential and conditionally essential aminoacids. Nonessential are produced by the human body either out of the essential or from normal proteins breakdown. Nonessential aminoacids include L – Alanine, L – Arginine, L – Aspartic acid, Asparagine, L – Cysteine, L – Glutamic acid, Glutamine, L – Glycine, L – Proline, L – Serine, L – Tyrosine. L – Glutamic acid (2 – Aminopentanedioic acid) (Fig. 1) is one of the most common nonessential aminoacids and is a major excitatory neurotransmitter in the human brain and in the spinal cord. L – Glutamic acid is necessary for proper cell functioning, but is considered as a nonessential aminoacid, because human body is able to produce it. Being one of the few nutrients able to pass through the blood – brain barrier, L – Glutamic acid supports brain function. L – Glutamic acid has the ability to detoxify brain and muscle cells by transforming all excess ammonia into the aminoacid Glutamine, which has antioxidant properties. As a chemical messenger in human brain, L – Glutamic acid is able to enhance a clarity of thinking, mental alertness, mood and intelligence and is applied to help for treatment of Parkinson's, fatigue, mental retardation, schizophrenia, muscular dystrophy and alcoholism. L – Glutamic acid is acting as an intermediary in the Kreb's cycle 1.

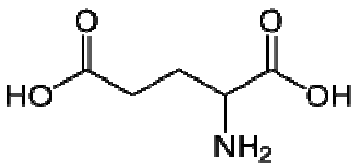

Fig. 1: Structure of L Glutamic acid.

There are different types of radiation – UV, γ. UV – radiation can be a health risk on the population 2 because causes sunburns, ageing of the skin and skin cancer. Sunscreens and sunblocks, included in skin care products, reduce UV – B – generated ROS 3. The effect γ – rays on survivor, morphological variation and chlorophyll mutation of some plants (Abelmoschus Moschatus) is also studied 4.

A crucial step in pharmaceutical production is sterilization. For sterilization (S) have been developed the following methods: dry heat S, pressured vapor S, ethylene oxide (EtO) S, formaldehyde S, gas plasma (H_2O_2) S, peracetic acid S, γ – radiation S and E – beam S. Each technique has aspects that make it suitable or unsuitable for the sterilization of a particular product 5.

Radiation sterilization of medical products is regulated by the following standards: EN 552 [6]; ISO 11137 [7]; ISO 11737 [8]; ISO 14937 [9].

In comparison with other methods for producing sterile products, the advantages of γ – radiation sterilization are:

1) better assurance of product sterility than filtration and aseptic processing

2) low – temperature process – preserving properties of materials

3) no residues (like EtO) or no radioactivity remain in the products

4) high penetrating power than E – beam.

5) simple validation process – only one process variable (exposure time or dose) needs to be controlled. Sterilization by EtO needs seven variables (temperature, time, pressure, vacuum, gas concentration, packaging and humidity) and steam sterilization needs six variables (temperature, time, pressure, vacuum, packaging and humidity) to be controlled 10.

One of the most important advantage of γ – radiation in sterilization of thermolabile drugs is the high penetrating power 11. On the other hand, γ – radiation can cause degradation and changing of physicochemical properties and therapeutic effect of drugs 12.

The first aspect to consider when sterilizing with γ – radiation is product tolerance to the radiation. During use of this type of radiation, high – energy photons bombard the product, causing electron displacement within. These reactions generate free radicals, which aid in breaking chemical bonds. Disrupting microbial DNA renders any organisms that survive the process nonviable or unable to reproduce. These high – energy reactions also have the potential to disrupt bonds within the pharmaceutical formulation, to weaken the strength of packaging materials and to cause changes in color or odor in some materials. For these reasons, drug manufacturers should perform prequalification Dmax (maximum dose) testing, whereby the drug and it's packaging are subjected to a high dose of γ – radiation and then evaluated for stability and functionality 10.

The radiation resistance of a microorganism is measured by the decimal reduction dose (D_{10} value), which is defined as the radiation

dose (kGy), required to kill 90 % of the total number of microorganisms 13. The study of microbiological purity is important not only for drugs, but also for medicinal plants [14] and cosmetic products 15.

Parameters to characterize include potency, efficacy, stability, biocompatibility and chemical acceptability. Per guidelines under the International Conference on Harmonization (ICH), known as Technical Requirements for Registration of Pharmaceuticals for Human Use, it is recommended to use high – performance liquid chromatography (HPLC), mass spectrometry or gas chromatography to characterize and compare different analytical aspects of irradiated product versus nonirradiated product 16.

The use of food additives is not a modern – day invention, but their excessive and uncontrolled use increases each year. Food additives consumed in excessive amounts will be toxic. The process called risk assessment is connected with determination of the safe levels of chemicals in food by review of all information for the types of harmful effect of chemicals. The risk assessment is conducted by independent scientific advisory committees 14.

The contemporary requirements for microbial purity of aminoacids substances, when been produced in non – sterile environment, set the problems of the microbial determination of the product, as well as of the initial materials. The results of the routine tests show that very often the materials contain impurities over the required limits. γ – ray treatment may usefully reduce level of impurities in aminoacids used for the production of food supplements.

The sensitivity of different drugs to radiation has been studied: antibiotics [17]: Cefoperazone 18, Cefotaxime 19, Ceftazidime [20]; glycosides [21]; steroids: Hydrocortisone 22, Prednisolone [23]; anticancer drugs: Doxorubicin [24, 25]; Cyclophosphamide [26]; alkaloids [17]; antiemetic drugs [26]; Ketoprofen [27]; Metoprolol tartrate [28]; Metro – nidazole [29]; Salbutamol [30]; Sulfacetamide sodium [31]; Clinopodium vulgare L. 32.

For determination of L – Glutamic acid are described the following methods: I) HPLC with: 1) post – column derivatization with o – phthalaldehyde [33]: 2) ion – pairing reversed – phase with UV and evaporative light scattering detection [34]; 3) isocratic reversed – phase with fluorimetric detection [35, 36]; 3) mass spectrometry MS [37]; II) gas chromatography [38]; III) polarography [39]; IV) amperometry 40.

The aim of current study is the development and application of HPLC method for quality control of L – Glutamic acid in supplement mixture before and after treatment with γ – ray and the examination of microbial purity of substance L – Glutamic acid.

MATERIALS AND METHODS

Materials

Materials for microbiological methods

1. L – Glutamic acid substance.

2. Microorganisms: Bacillus subtillis, Staphylococcus aureus, Pseudomonas aeruginosa.

3. Buffered sodium chloride – peptone solution with pH = 7.

Materials for HPLC method

L – Glutamic acid substance, methanol, water

Methods

Microbiological methods

The following included in European Pharmacopoeia methods for determination of microbial contamination are used: 1) Method 2.6.12. Total viable aerobic count (for detection of bacteria: Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Candida albicans, Aspergillus niger); 2) Method 2.6.13. Tests for specified microorganism (for detection of bacteria, belonging to the family of Enterobacteriaceae) 41.

Preparation of test strain of microorganisms

Plates with casein soya bean digest agar were inoculated with 100 colony forming units respectively with the following microorganisms: Bacillus subtillis, Staphylococcus aureus, Pseudomonas aeruginosa. Plates were incubated for 18 – 24 h at 30 – 35 ^{0}C.

Sample preparation

10 g of L – Glutamic acid substance were dissolved in 10 ml buffered sodium chloride – peptone solution with pH = 7. Sample was filtered througth membrane filter with pore size 0.45 μm. Membrane filter was transferred to the surface of casein soya bean digest agar. Plates were incubated for18 – 24 h at 30 – 35 ^{o}C and sample were filtered. Filter was washed with 3 quantities of 100 ml buffered sodium chloride – peptone solution with pH = 7. The number of colony forming units were count.

HPLC method

Chromatographic system

The chromatographic procedure was carried out using: Liquid chromatograph Shimadzu LC – 10 Advp equipped with 4.6 x 250 mm column RP – 18, ODS Spherisorb with particle size 5 μm; detector SPD 10 AVvp – UV – VIS with fixed analytical wavelengths.

Chromatographic conditions

Isocratic mobile phase, prepared by mixing of filtered and degassed methanol : water = 50 : 50 v/v; column temperature 25 ^{o}C; flow rate: 1.0 ml/min; 210 nm analytical wavelength.

Reagents

L – Glutamic acid CRS, supplement mixture containing 100 mg L – Glutamic acid , methanol HPLC grade, water R.

Standard preparation

100.00 mg L – Glutamic acid CRS were dissolved in water R and were diluted to 100.0 ml with a solvent mixture – mobile phase.

Test preparation

100.00 mg from the tested substance were dissolved in water and were diluted to 100.0 ml with mobile phase. 1 ml from the obtained solution was dissolved with the same solvent mixture to obtain solutions with different concentrations.

RESULTS AND DISCUSSION

The results from the tests for determining the existence of microbial impurities show abnormal content ($1.5.10^{-4}$ g) of bacteria and contaminants were identified mostly as non patogenic bacilli of Subtilis group. Impermissible (patogenic) contaminants as Enterobacteriaceae, Staphylococcus. aureus were not found. Resistency factors show moderate ray sensitivity of the microorganisms, which are comparatively low sterilizing doses. Efficiency of the integral (total) dose of 10 Kgy was confirmed by the microorganism impurity tests after treatment and this dose guarantees a stable sterilizing coefficient.

Validation of HPLC procedure

HPLC method for quality control of L – Glutamic acid in supplement mixtures was developed and validated in accordance with ICH and European Pharmacopoeia criteria. Analytical parameters specificity, repeatability, limit of detection, limit of quantitation and linearity were studied and determined.

Specificity in respect of reagents

"Placebo" solution containing all reagents without active substances was prepared. There are no peaks in the chromatogram obtained from this solution with Rt of L – Glutamic acid.

Repeatability

Six (6) equal solutions from homogenous samples containing L – Glutamic acid were analyzed by HPLC method. Standard deviation (SD) and relative SD (RSD) were found. The results are presented on Table 1.

Table 1: Repeatability of samples from supplement mixture containing L – Glutamic acid 100 mg.

N	Obtained amount of L – Glutamic acid (100 mg)	$\bar{X}$	SD	RSD (± %)
1.	102.20			
2.	100.35	101.5	1.4321	0.44
3.	103.30			
4.	102.80			
5.	100.20			
6.	100.15			

Limit of detection

10 µg for L – Glutamic acid, established on the base of ratio noise/signal – 1 : 3.

Limit of quantitation

40 µg for L – Glutamic acid, established on the base of ratio noise/signal – 1: 10.

Linearity

The analytical parameter linearity was studied in concentration ratio 10 µg – 150 mg. The accordance between the area of peaks, measured in absorption units (AU) and concentrations in g/ml is proportional in the mentioned interval. The correlation coefficients is found to be 0.99746 at SD = ± 3914.60 AU.

The identification and assay of L – Glutamic acid in supplement mixtures before and after γ – ray treatment were determinated and compared. The results are shown on Table 2. There are no a significant difference between the putted quantity and obtained values for L – Glutamic acid concentration in the supplement mixture. At applied chromatographic conditions related substances and other impurities are not observed.

Table 2: Results from HPLC assay test for supplement mixture containing L – Glutamic acid 100 mg before and after γ – ray treatment

Sample N	Putted amount L – Glutamic acid (mg)	Obtained amount L – Glutamic acid before γ – ray treatment (mg)	Obtained amount L – Glutamic acid after γ – ray treatment (mg)
1.	100.0	102.20	102.40
2.	100.0	100.35	100.35
3.	100.0	103.30	102.70
4.	100.0	102.80	102.05
5.	100.0	100.20	101.55
6.	100.0	100.15	100.15
		RSD = ± 0.44 %	RSD = ± 0.082 %

CONCLUSION

Abnormal content ($1.5.10^{-4}$ g) of bacteria is determined, without patogenic microorganisms. There are no significant difference between content of L – Glutamic acid in supplement mixtures before and after γ – ray treatment.

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