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^-6 g) of bacteria and contaminants were identified mostly as non pathogenic 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 recognized 3 types: nonessential, essential and conditionally essential 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.

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, pressurized vapor S, ethylene oxide (EtO) S, Formaldehyde S, gas plasma [H2O] 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 S.

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 sterilization of thermolabile drugs is the high penetrating power 11. On the 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 pharmacological 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 (D0 value), which is defined as the radiation
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 subtilis, Staphylococcus aureus, Pseudomonas aeruginosa.

Determination of microbial contamination are used: 1) Method for quality control of L-Glutamic acid in supplement mixture containing 100 mg L-Glutamic acid, methanol HPLC grade, water R.

Validation of HPLC procedure

HPLC method for quality control of L-Glutamic acid in supplement mixture 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.
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 determined 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 1: Repeatability of samples from supplement mixture containing L-Glutamic acid 100 mg.

<table>
<thead>
<tr>
<th>N</th>
<th>Obtained amount of L-Glutamic acid (100 mg)</th>
<th>X</th>
<th>SD</th>
<th>RSD (± %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>102.20</td>
<td>101.5</td>
<td>1.4321</td>
<td>0.44</td>
</tr>
<tr>
<td>2</td>
<td>100.35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>103.30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>102.80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>100.20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>100.15</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sample N</th>
<th>Putted amount L-Glutamic acid (mg)</th>
<th>Obtained amount L-Glutamic acid before γ-ray treatment (mg)</th>
<th>Obtained amount L-Glutamic acid after γ-ray treatment (mg)</th>
<th>RSD = ± 0.44 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100.0</td>
<td>102.20</td>
<td>102.40</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>100.0</td>
<td>100.35</td>
<td>100.35</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>100.0</td>
<td>103.30</td>
<td>102.70</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>100.0</td>
<td>102.80</td>
<td>102.05</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>100.0</td>
<td>100.20</td>
<td>101.55</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>100.0</td>
<td>100.15</td>
<td>100.15</td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSION
Abnormal content (1.5±0.1 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.

REFERENCES


