DEPARTMENT OF HEALTH & HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION

Public Health Service

Memorandum

Date . AUG 20 1999

2896 '99 AUG 24 P2:25

From Senior Regulatory Scientist, Regulatory Branch, Division of Programs & Enforcement Policy (DPEP), Office of Special Nutritionals, HFS-456

Subject 75-day Premarket Notification for New Dietary Ingredient

To Dockets Management Branch, HFA-305

New Dietary Ingredient:	Immuno-Rx, aka Munogen (containing *Lactobacillus bulgaricus*, and a dietary ingredient that consists of *Streptococcus pneumoniae*, *Neisseria catarrhalis*, *Streptococcus pyogenes*, *Haemophilus influenzae*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*)
Firm:	New Age Health Technologies, Inc.
Date Received by FDA:	June 10, 1999
90-day Date:	September 9, 1999

In accordance with the requirements of section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification for the aforementioned new dietary ingredient should be placed on public display in docket number 95S-0316 after September 9, 1999.

Robert J. Moore, Ph.D.

95S-0316

RPT 51

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Washington, DC 20204

AUG 20 1999

Mr. Beau Raines
Chairman
New Age Health Technologies, Inc.
3605 Stech Avenue
Suite 1128
Austin, Texas 78759

Dear Mr. Raines:

This letter is in response to your submission to the Food and Drug Administration (FDA) received on June 10, 1999, for a new dietary ingredient, made pursuant to 21 U.S.C. 350b(a)(2). Your letter notified FDA of your intent to market a product named "Immuno-Rx" or "Munogen" that contains killed, freeze-dried lysates and bacterial bodies of *Lactobacillus bulgaricus*, and a dietary ingredient that consists of *Streptococcus pneumoniae*, *Neisseria catarrhalis*, *Streptococcus pyogenes*, *Haemophilus influenzae*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*.

Under 21 U.S.C. 350b(a), the manufacturer or distributor of a dietary supplement that contains a new dietary ingredient that has not been present in the food supply as an article used for food in a form in which the food has not been chemically altered must submit to FDA, at least 75 days before the dietary ingredient is introduced or delivered for introduction into interstate commerce, information that is the basis on which the manufacturer or distributor has concluded that a dietary supplement containing such new dietary ingredient will reasonably be expected to be safe. FDA reviews this information to determine whether it provides an adequate basis for such a conclusion. Under section 350b(a)(2), there must be a history of use or other evidence of safety establishing that the new dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe. If this requirement is not met, the dietary supplement is deemed to be adulterated under 21 U.S.C. 342(f)(1)(B) because there is inadequate information to provide reasonable assurance that the new dietary ingredient does not present a significant or unreasonable risk of illness or injury.

FDA has carefully considered the information in your submission, and the agency has significant concerns about the product you intend to market. Those concerns are set forth below.

The product is not a dietary supplement as defined in 21 U.S.C. 321(ff).

Immuno-Rx is not a dietary supplement because it does not meet the statutory definition of a dietary supplement in that it does not contain a substance that is a "dietary ingredient" defined in 21 U.S.C. 321(ff)(1). It does not contain a substance that is a vitamin, mineral, herb or other botanical, amino acid, dietary substance for use by man to supplement the diet by increasing the total dietary intake, or a concentrate, metabolite, constituent, extract or combination of any ingredient described above. The ingredient in your product is not a vitamin, mineral, herb or other botanical, amino acid, or a concentrate, metabolite, constituent, extract or combination of these substances. The ingredient in your product also is not a "dietary substance" that increase the "total dietary intake" because it cannot reasonably be viewed as part of man's usual food or drink. The substance in your product, except for *L. bulgaricus*, is composed of pathogenic microorganisms. Pathogens are not substances that are food or that are used for food. Therefore, your product is not a dietary supplement.

The submission does not establish that a product containing the subject "new dietary ingredient" is reasonably expected to be safe.

Even if Immuno-Rx could meet the definition of a dietary supplement, it would violate other provisions of the Act. The evidence on which you rely does not support your conclusion that a dietary supplement containing the ingredient named above, when used under the conditions recommended or suggested in the labeling of your product, will reasonably be expected to be safe.

Your submission contained the results of several human and animal studies that you assert are adequate to evaluate the safety of ingested Immuno-Rx. The data from the animal studies you submitted are not adequate to evaluate the long-term safety of this product when used in humans. The studies are not appropriately designed nor of adequate length to provide the type of data necessary to evaluate the safety of a dietary ingredient intended to be consumed by humans. The data result in significant uncertainty in making dose comparisons and assessing the safety or hazards associated with human consumption of a dietary supplement containing this ingredient.

The human studies contained in the submission provide little support for concluding that chronic or long-term consumption of dietary supplements containing this ingredient will reasonably be expected to be safe in healthy people. The studies submitted were not designed nor intended to examine the adverse or toxicological effects of your product in healthy people; instead, the ingredient was used as a therapy in studies of persons with bronchitis or other pulmonary diseases. Such studies have limited utility for determining whether the long-term or repeated use of a substance as an ingredient in dietary supplements is safe.

Furthermore, the product is not adequately characterized to enable a careful examination of the potential hazards such a product might pose. There is no information in the submission regarding the potential presence of bacterial toxins in this product. More importantly, the specific strains of the microorganisms in the product are not identified. Consequently, it isn't possible to identify and critically evaluate risks that may be associated with the use of a lysate containing a particular organism. For example, Group A *Streptococcus* contain antigenic substances known to cross react with cardiac tissue and that may play a role in heart disease. The use of type a *H. influenzae* would raise different safety concerns than would the use of type b *H. influenzae*. Moreover, the information in the submission is not only inadequate for a determination of what risks may be associated with the use of this product as a dietary supplement, it indicates that the firm has not even considered such issues.

For the reasons discussed above, the information in your submission does not provide an adequate basis to conclude that Immuno-Rx, when used under the conditions recommended or suggested in the labeling of your product, will reasonably be expected to be safe. Therefore, even if your product were a dietary supplement (which as discussed above, it is not), it would be adulterated under 21 U.S.C. 342(f)(1)(B) as a dietary supplement that contains a new dietary ingredient for which there is inadequate information to provide reasonable assurance that such ingredient does not present a significant or unreasonable risk of illness or injury. Introduction of such a product into interstate commerce is prohibited under 21 U.S.C. 331(a) and (v).

Immuno-Rx appears to be a biological product as defined in 42 U.S.C. 262 [section 351 of the Public Health Service Act]

"[T]he term 'biological product' means a "virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product, or analogous product, or arsphenamine or derivative of arsphenamine (or any other trivalent organic arsenic compound), applicable to the prevention, treatment, or cure of a disease" 42 U.S.C. 262(see 21 CFR 600.3(h)). Your submission states that the product is to "enhance the body's immune system" and has a "stimulating effect on the immune system against a wide variety of non-specific maladies." It is to be used (by adults) daily for a period of 90 days followed by a six month regiment or daily (by children 3-16 years of age) for a period of 90 days. The submission contains information that the product is licensed with the National Drug Institute (Bulgaria) where it is used for the prevention and treatment of chronic and acute respiratory diseases in children and adults. The submission also contains reprinted articles from scientific journals and other sources that describe the use of the this product to treat or prevent diseases caused by pathogenic microorganisms. Based on the nature of the ingredients in your product, the effect of those ingredients on the body, and the information in your submission, your product appears to be a biological product as defined in 42 U.S.C. 262. A biological product must be licensed prior to being marketed in the United States. 42 U.S.C. 262.

Immuno-Rx also appears to be a drug under 21 U.S.C. 321(g)(1)(B).

The submission states that Immuno-Rx has a "stimulating effect on the immune system against a wide variety of non-specific maladies." The submission contains statements from foreign government officials that state that the product is "produced and used" for "the prophylaxis and treatment of chronic and acute respiratory diseases in children and adults." These statements also declare that the product is licensed with the National Drug Institute (Bulgaria). Based on the information in the submission, this product appears to be intended for use in the treatment, cure, prevention, mitigation, or diagnosis of disease. Therefore, the product appears to be a drug under 21 U.S.C. 321(g)(1)(B) and would be subject to regulation under the drug provisions of the Act.

In sum, your product is not a dietary supplement. Rather, it appears to be a biological product and drug that must be licensed, or otherwise preapproved, before being marketed in the United States.

Please contact us if you have any questions concerning this matter.

Sincerely,

Lynn A. Larsen, Ph.D.
Director
Division of Programs and Enforcement Policy
Office of Special Nutritionals
Center for Food Safety
and Applied Nutrition

65609

Rec'd
6/10/99
HFS-456

Office of Special Nutritionals (HFS - 450)
Center For Food Safety and Applied Nutrition
Food and Drug Administration
200 C. St. S.W.
Washington, D.C. 20204

Dear Sirs/Madams:

Please find the inclosed material for the Pre-Market Notification of a new dietary product known as Immuno-Rx and or Munogen.

You will find one original and two copies of such said material.

I hereby do certify that all materials presented here are true and are honest representations of this product to the best of my knowledge.

If you have need of further information, please contact me.

Yours truly,

Beau Raines
Chairman
New Age Health Technologies, Inc.
3605 Steck Ave. - Ste. 1128
Austin, TX. 78759
Ph. and Fax: (512) 794-0611

NAME OF

NEW DIETARY PRODUCT

TO BE INTRODUCED:

IMMUNO-RX AND/OR MUNOGEN

DISTRIBUTOR:

NEW AGE HEALTH TECHNOLOGIES, INC.

3605 STECK AVE. - STE. 1128

AUSTIN, TX. 78759

PHONE AND FAX: (512) 794-611

CONTACT: BEAU RAINES, CHAIRMAN

MANUFACTURER:

THE NATIONAL CENTER OF INFECTIOUS AND PARASITIC DISEASES

26 YANKO SAKAZOV BLVD.

SOFIA, 1504, BULGARIA

PHONE: 011-359-2-442-875

CONTACT: BOGDAN PETRUNOV, M.D.,DSc.

INTRODUCTION

This Pre-Market Notification will involve an introduction of a product known as **IMMUNO-RX** and/or **MUNOGEN.** The product is a combination of lactobacillus bulgaricus (which has been on the market for many years is already generally regarded as safe) and a product known in Europe as Respivax and in Latin America as Munostin.

Respivax(Munostin) is composed of killed and lyphilised lysates and bacterial bodies of six microbial species: Streptococcus pneumoniae, Streptococcus pyogenes, Staphylococcus aureus, Neisseria catarrhalis, Klebsiella pneumoniae, and Haemophilus influenzae.

IMMUNO-RX (MUNOGEN) is a combination of lactobacillus bulgaricus and Respivax(Munostin). The product is **inert** and contains no live or active ingredients. In some of the clinical investigations, the word "vaccine" is used to describe Respivax. A vaccine is defined as any substance which contains killed bacteria.

The clinical investigations that are present with this report are concerned with the safety and effectiveness of Respivax. Since lactobacillus bulgaricus is already generally regarded as safe, safety studies of it will not be presented.

The preparation Respivax(Munostin) was developed at The National Center of Infectious and Parasitic Diseases in Sofia, Bulgaria. It is also manufactured at that site. The preparation has been in existence since 1987 and has been consumed by millions of people in Europe and Latin America.

The product, IMMUNO-RX OR MUNOGEN, will be marketed as a compound concerned with proper immune function. It is not intended to treat, diagnose, or prevent any disease.

In this report the following will be presented: Certificates of Good Manufacturing, Stability Data and The Manufacturing Process, Animal Toxicity Studies, The Description of the Product, and Published Clinical Investigations.

DESCRIPTION

DESCRIPTION

IMMUNO-RX or **MUNOGEN** was developed at The National Center of Infectious and Parasitic Diseases in Sofia, Bulgaria in 1987. It consists of **killed** and freeze-dried lysates and bacterial bodies of the following species:

* LACTOBACILLUS BULGARICUS
* STREPTOCOCCUS PNEUMONIAE
* NEISSERIA CATARRHALIS
* STREPTOCOCCUS PYOGENES
* HAEMOPHILUS INFLUENZAE
* STAPHYLOCOCCUS AUREUS
* KLEBSIELLA PNEUMONIAE

The product is in the form of 50 mg. (adult acute dosage), 25 mg. (children acute dosage) and 10 mg. (maintenance daily adult dosage).

The amount of lysates and bacterial bodies are as follows:

50 mg. - 1.0 X 10 (9) of each of the bacterial strains

25 mg. - 0.5 X 10 (9) of each of the bacterial strains

10 mg. - 0.1 X 10 (9) of each of the bacterial strains

Other ingredients are as follows:

SUBSTANCE	**50 mg.**	**25 mg.**	**10 mg.**
Aerosil 200	6 mg.	3 mg.	1 mg.
Wheat Starch	65 mg.	51 mg.	37 mg.
Avicel pH 101	66 mg.	52 mg.	37 mg.
Kollidon K 25	10 mg.	7 mg.	3 mg.
Magnesium Stearate	3 mg.	2 mg.	1 mg.

CONDITIONS OF USE

Over 10 years of worldwide clinical research has shown that **IMMUNO-RX (OR MUNOGEN)** is a safe and natural way to enhance the body's immune system. It has a well established stimulating effect on the immune system against a wide variety of non-specific maladies.

RECOMMENDED ADULT INTAKE: Adults over 16 years old should take 1 tablet (50 mg.) daily with a full glass of water for a period of 90 days. After the 90 day period, one 10 mg. tablet can be taken daily for the proper maintenance of the immune system. A six month regimen of taking Immuno-RX (or Munogen) is all that is recommended for a healthy adult immune system.

RECOMMENDED CHILDREN INTAKE: Children from the ages of 3 to 16 should take one tablet (25 mg.) daily with a full glass of water for a period of 90 days. A 90 regimen of taking Immuno-RX (or Munogen) is all that is recommended for a healthy child's immune system.

Each box of Immuno-RX (or Munogen) will contain 30 tablets of 50 mg., 25 mg., or 10 mg.

Each tablets contains:
50 mg. - 1 x 10 (9) of each of the killed and freeze dried lysates and bacterial bodies of: Lactobacillus bulgaricus, Streptococcus pyogenes, Streptococcus pneumoniae, Staphylococcus aureus, Neisseria catarrhalis, Klebsiella pneumoniae, Haemophilus influenzae. Other ingredients: Aerosil 200, Wheat starch, Avicel pH 101, Kollidan K 25, Magnesium stearate.

25 mg. - 0.5 x 10 (9) of each of the killed and freeze-dried lysates and bacterial bodies of : Lactobacillus bulgaricus, Streptococcus pyogenes, Streptococcus pneumoniae, Staphylococcus aureus, Neisseria catarrhalis, Klebsiella pneumoniae, Haemophilus inflluenzae. Other ingredients: Aerosil 200, Wheat starch, Avicel pH 101, Kollidon K 25, Magnesium stearate.

10 mg. - 0.1 x 10(9) of each of the killed and freeze-dried lysates and bacterial bodies of: Lactobacillus bulgaricus, Streptococcus pyogenes, Streptococcus pneumoniae, Staphylococcus aureus, Neisseria catarrhalis, Klebsiella pneumoniae, Haemophilus influenzae. Other ingredients: Aerosil 200, Wheat starch, Avicel pH 101, Killidon K 25, Mangesium stearate.

The statements presented here have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure or prevent any disease.

A U.S.R.D.A. has not been established on this product.

If you are pregnant, lactating or have recently received a transplanted organ or tissue, you should consult your physician before using this product.

Store at room temperature and avoid excessive heat above 100 degrees Fahrenheit.

MADE IN BULGARIA AT THE NATIONAL CENTER OF INFECTIOUS AND PARASITIC DISEASES

CERTIFICATE

OF

GOOD MANUFACTURING

НАЦИОНАЛЕН ЦЕНТЪР ПО
ЗАРАЗНИ И ПАРАЗИТНИ БОЛЕСТИ
1504 София, бул. Янко Сакъзов 26, тел. 43 471,
факс 943 30 75; E-mail: 0449@main.infotel.bg

NATIONAL CENTER OF
INFECTIOUS AND PARASITIC DISEASES
1504 Sofia, 26, Yanko Sakazov Blvd., Phone +359 2 43 471,
Fax +359 2 943 30 75; E-mail: 0449@main.infotel.bg

ОСНОВАН-1881-FOUNDED

Certificate

We, the undersigned Assoc. Prof. Ilina Bineva, PhD, President of the Quality assurance committee and Prof. Rumen Manahilov, MD, Head of the Laboratory of quality control, certify herewith that the preparation "Munostin" is licensed with the NCA (National Drug Institute) and produced at the National Center of Infectious and Parasitic Diseases since 1988. The process of production is performed according to the guidelines of the good manufacturing practice. The Laboratory of quality control is an independent unit that tests and releases the starting substances, the intermediate and bulk products, packing and labeling materials and performs the analysis of the final product. QC approves the production records and takes care of the training of the personnel. QA approves the quality of the product and the manufacturing conditions.

The producer is liable to periodic inspections by NCA which confirm that the GMP requirements are met.

Prof. R. Manahilov
Head, Laboratory of Quality Control

I. Bineva
Assoc. Prof. I. Bineva, PhD
President, Quality Assurance Committee

НАЦИОНАЛЕН ЦЕНТЪР ПО
ЗАРАЗНИ И ПАРАЗИТНИ БОЛЕСТИ
1504 София, бул. Янко Сакъзов 26, тел. 43 471,
факс 943 30 75; E-mail: 0449@main.infotel.bg

NATIONAL CENTER OF
INFECTIOUS AND PARASITIC DISEASES
1504 Sofia, 26, Yanko Sakazov Blvd., Phone +359 2 43 471,
Fax +359 2 943 30 75; E-mail: 0449@main.infotel.bg

OCHOBAH-1881-FOUNDED

Munostin

Description

The preparation Munostin is produced at the National Center of Infectious and Parasitic Diseases since 1988. It is an immunostimulator, which enhances the natural resistance of the human body to a variety of infections. The cellular and humoral immunity is improved. Extensive toxicological studies of the preparation proved its safety. Large quantities of Munostin are produced and used in the country for the prophylaxis and treatment of chronic and acute respiratory diseases in children and adults.

The production is performed under the regulations of the good manufacturing practice and the product meets high quality standards.

The technology of production and the composition of the preparation ensure its innocuousness. No adverse reactions were registered over the years (1988-1999). Munostin may be used together with other medicines. No complaints from the patients or from the medics were communicated to the producer over the whole period of its use. The preparation is a safe means for the stimulation of the immune system to resist respiratory infections.

Prof. R.Manahilov, MD
Head, Laboratory of Quality Control

Assoc.Prof. I.Bineva, PhD
President, Quality Assurance Committee

НАЦИОНАЛЕН ЦЕНТЪР ПО ЗАРАЗНИ И ПАРАЗИТНИ БОЛЕСТИ
1504 София, бул. Янко Сакъзов 26, тел. 43471, факс *359 2 442 260

NATIONAL CENTER OF INFECTIOUS AND PARASITIC DISEASES
1504 Sofia, 26, Yanko Sakazov Blvd., tel. 359 2 43471, fax *359 2 442 260

Founded 1881

FREE SALE CERTIFICATE

Name of product: RESPIVAX

Packages: tablets of 50 and 25 mg packed by 10 in a blister, three blisters in a box

Expiry term: 30 months

Active ingredients: each tablet contains freeze-dried lysate and killed bacterial bodies of the following microbial species - Streptococcus pneumoniae, Streptococcus pyogenes, Neisseria catarrhalis, Haemophilus influenzae, Staphylococcus aureus, Klebsiella pneumoniae.

Approved indications: Oral immunotherapy and immunoprevention of non-specific respiratory diseases in children and adults suffering of recurrent and chronic infections of the respiratory system.

Manufacturer:
National Centre of Infectious and Parasitic Diseases
Sofia, Bulgaria

It is cerified that:
This product has been authorized to be placed on the market for use in Bulgaria or to be exported.

Manufacturing licence:
Tablets of 50 mg - licence No 1501869-89
Tablets of 25 mg - licence No 0050531-95

It is also certified that:
A. The manufacturing plant where Respivax is produced is subject to inspections at suitable intervals;
B. The manufacturer conforms to the requirements of good practices in the manufacture and quality control, as recommended by the World Health Organization, in respect of products to be sold within the country of origin or to be exported.

Sofia
10 August 1995

Prof.R.Manahilov
Head of the Laboratory
of Quality Control

НАЦИОНАЛЕН ЦЕНТЪР ПО ЗАРАЗНИ И ПАРАЗИТНИ БОЛЕСТИ
1504 София, бул. Янко Сакъзов 26, тел. 43471, факс *359 2 442 260

NATIONAL CENTER OF INFECTIOUS AND PARASITIC DISEASES
1504 Sofia, 26, Yanko Sakazov Blvd., tel. 359 2 43471, fax *359 2 442 260

Founded 1881

ANALYTICAL CERTIFICATE

R E S P I V A X

25 mg, batch No 010195
size of the batch - 28130 boxes

1. Appearance	passed
2. Colour	passed
3. Mean mass 0,139g	passed
4. Time of disintegration	passed
5. Water content 5,21%	passed
6. Content of aminoacids - 1,9mg/tablet	passed
7. Protein content - 3,5mg/tablet	passed
8. HPLC	identical
9 .Formaladehyde content - 0,007mg	passed
10.Microbial content	passed

It is certified herewith that RESPIVAX, 25 mg, batch 010195 meets the requirements of manufacturing licence No 0050531-95.

Prof.R.Manahilov
Head of the Laboratory
of Quality Control

Prof.P.Nenkov
Production Director

НАЦИОНАЛЕН ЦЕНТЪР ПО ЗАРАЗНИ И ПАРАЗИТНИ БОЛЕСТИ
1504 София, бул. Янко Сакъзов 26, тел. 43471, факс *359 2 442 260

NATIONAL CENTER OF INFECTIOUS AND PARASITIC DISEASES
1504 Sofia, 26, Yanko Sakazov Blvd., tel. 359 2 43471, fax *359 2 442 260

Founded 1881

ANALYTICAL CERTIFICATE

R E S P I V A X

50 mg, batch No 011294
size of the batch - 20150 boxes

1. Appearance	passed
2. Colour	passed
3. Mean mass 0,200g	passed
4. Time of disintegration	passed
5. Water content 7,4%	passed
6. Content of aminoacids - 4,1mg/tablet	passed
7. Protein content - 9mg/tablet	passed
8. HPLC	identical
9 .Formaline content - 0.045%	passed
10.Microbial content	passed

It is certified herewith that RESPIVAX, 50 mg, batch 011294 meets the requirements of manufacturing licence No 160186?-89.

Prof.R.Manahilov
Head of the Laboratory
of Quality Control

Prof.P.Nenkov
Production Director

STABILITY DATA

AND

MANUFACURING PROCESS

NCIPD
Quality control department

STABILITY REPORT

DOSAGE FORM: RESPIVAX E 25 mg

PRODUCT: RESPIVAX E

BATCH NUMBER: 021095

Indices	Specifications (standard)	initial	40 C				40 C relative humidity 75 %			
			1 month	2 months	3 months	6 months	1 month	2 months	3 months	6 months
Organoleptic properties										
Appearance	biconvex tablets	biconvex tablets	n. c.*	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.
Colour	yellow	yellow	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.
Odour	odourless to specific	odourless to specific	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.
Disintegration	up to 60 min.	25 min.	25	25	26	26	25	25	25	24
water content %	up to 10	3.74	3.78	3.69	3.62	3.66	3.75	3.76	3.76	3.78
content of aminoacids (mg /tablet)	1.0 – 3.0	1.67	1.73	1.71	1.66	1.70	1.70	1.74	1.72	1.69
content of protein (mg /tablet)	3.0 – 6.0	3.70	3.65	3.67	3.71	3.74	3.79	3.81	3.72	3.74
uniformity of mass %	$\overline{+}$ 7.5	+6.5	+6.5	+6.5	+6.4	+6.4	+6.5	+6.5	+6.55	+6.55
identity	complying	complying	complying	complying	complying	complying	complying	complying	complying	complying
microbial content per gram										
bacteria	not more than 10^2	complying	complying	complying	complying	complying	complying	complying	complying	complying
fungi	not more than 10^3	complying	complying	complying	complying	complying	complying	complying	complying	complying
safety	complying	complying	complying	complying	complying	complying	complying	complying	complying	complying

* --- no change

QUALITY CONTROL MANAGER :

/prof. R. MANAHILOV PhD , MD /

NCIPD
Quality control department

STABILITY REPORT

DOSAGE FORM: RESPIVAX E 2 mg

PRODUCT: RESPIVAX E

BATCH NUMBER: 200995

			40 C				40 C relative humidity 75 %			
Indices	Specifications (standard)	initial	1 month	2 months	3 months	6 months	1 month	2 months	3 months	months
Organoleptic properties										
Appearance	biconvex tablets	biconvex tablets	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.	nc.
Colour	yellow	yellow	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.	nc.
Odour	odourless to specific	odourless to specific	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.	nc.
Disintegration	up to 60 min.	31 min.	31	31	31	30	31	31	31	4
water content %	up to 10	4.50	4.50	4.58	4.52	4.45	4.51	4.54	4.53	4.78
content of aminoacids (mg /tablet)	1.0 – 3.0	2.12	2.10	2.18	2.16	2.15	2.17	2.20	2.19	2.69
content of protein (mg /tablet)	3.0 – 6.0	4.25	4.30	4.29	4.22	4.20	4.28	4.26	4.24	4.74
uniformity of mass %	± 7.5	+ 6.1	+ 6.1	+ 6. 15	+ 6.15	+ 6.1	+ 6.15	+ 6.15	+ 6.1	+ 6.55
identity	complying	complying	complying	complying	complying	complying	complying	complying	complying	complying
microbial content per gram										
bacteria	not more than 10^2	complying	complying	complying	complying	complying	complying	complying	complying	complying
fungi	not more than 10^3	complying	complying	complying	complying	complying	complying	complying	complying	complying
safety	complying	complying	complying	complying	complying	complying	complying	complying	complying	complying

• — no change

QUALITY CONTROL MANAGER :

/ prof. R.MANAHILOV , PhD , MD /

NCIPD
Quality control department

STABILITY REPORT

DOSAGE FORM: RESPIVAX E 25 mg

PRODUCT: RESPIVAX E

BATCH NUMBER: 040935

			40 C				40 C relative humidity 75 %			
Indices	Specifications (standard)	initial	1 month	2 months	3 months	6 months	1 month	2 months	3 months	6 months
Organoleptic properties										
Appearance	biconvex tablets	biconvex tablets	n. c.*	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.
Colour	yellow	yellow	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.
Odour	odourless to specific	odourless to specific	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.
Disintegration	up to 60 min.	28 min.	28	28	28	29	28	28	28	28
water content %	up to 10	6.1	6.1	6.0	6.05	6.05	6.1	6.1	6.15	6.15
content of aminoacids (mg /tablet)	1.0 – 3.0	2.45	2.40	2.46	2.49	2.47	2.44	2.48	2.49	2.42
content of protein (mg /tablet)	3.0 – 6.0	4.90	4.92	4.95	4.87	4.82	4.94	4.96	4.89	4.86
uniformity of mass %	$\pm$ 7.5	+6.2	+6.25	+6.22	+6.2	+6.2	+6.2	+6.2	+6.21	+6.2
identity	complying	complying	complying	complying	complying	complying	complying	complying	complying	complying
microbial content per gram										
bacteria	not more than 10^2	complying	complying	complying	complying	complying	complying	complying	complying	complying
fungi	not more than 10^3	complying	complying	complying	complying	complying	complying	complying	complying	complying
safety	complying	complying	complying	complying	complying	complying	complying	complying	complying	complying

* — no change

QUALITY CONTROL MANAGER :

/ prof. P. MANAHILOV ,PhD , MD /

NCIFD
Quality control department

STABILITY REPORT

DOSAGE FORM: RESPIVAX E 50 mg

PRODUCT: RESPIVAX E

BATCH NUMBER: 011095

Indices	Specifications (standard)	initial	40 C				40 C relative humidity 75 %			
			1 month	2 months	3 months	6 months	1 month	2 months	3 months	6 months
Organoleptic properties										
Appearance	biconvex tablets	biconvex tablets	n. c.*	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.
Colour	yellow	yellow	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.
Odour	odourless to specific	odourless to specific	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.
Disintegration	up to 60 min.	25 min.	25	25	25	24	25	25	24	24
water content %	up to 10	5.6	5.6	5.55	5.5	5.5	5.6	5.6	5.65	5.65
content of aminoacids (mg/tablet)	2.0 – 6.0	3.0	3.05	3.02	2.94	2.98	3.05	3.02	2.92	2.95
content of protein (mg/tablet)	6.0 – 12.0	7.0	7.09	6.96	6.92	7.01	6.91	6.98	6.96	7.04
uniformity of mass %	± 7.5	+ 6.9	+ 6.8	+ 6.85	+ 6.89	+ 6.96	+ 6.9	+ 6.9	+ 6.9	+ 6.9
identity	complying	complying	complying	complying	complying	complying	complying	complying	complying	complying
microbial content per gram										
bacteria	not more than 10^2	complying	complying	complying	complying	complying	complying	complying	complying	complying
fungi	not more than 10^3	complying	complying	complying	complying	complying	complying	complying	complying	complying
safety	complying	complying	complying	complying	complying	complying	complying	complying	complying	complying

* — no change

QUALITY CONTROL MANAGER :

/ prof. R. MANAHILOV , PhD , MD /

NCIPD
Quality control department

STABILITY REPORT

DOSAGE FORM: RESPIVAX E 50 mg

PRODUCT: RESPIVAX E

BATCH NUMBER: 030995

Indices	Specifications (standard)	initial	40 C				40 C relative humidity 75 %			
			1 month	2 months	3 months	6 months	1 month	2 months	3 months	6 months
Organoleptic properties										
Appearance	biconvex tablets	biconvex tablets	n. c. •	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.
Colour	yellow	yellow	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.
Odour	odourless to specific	odourless to specific	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.
Disintegration	up to 60 min.	35 min.	35	35	35	35	35	35	35	35
water content %	up to 10	4.95	4.92	4.99	5.02	4.94	4.91	4.94	5.00	4.98
content of aminoacids (mg /tablet)	2.0 – 6.0	4.08	4.10	4.07	4.08	4.05	4.09	4.08	4.11	4.10
content of protein (mg /tablet)	6.0 – 12.0	9.95	9.88	9.90	9.98	9.92	9.92	9.96	9.95	9.93
uniformity of mass %	± 7.5	6.20	6.24	6.25	6.21	6.19	6.22	6.28	6.19	6.20
identity	complying	complying	complying	complying	complying	complying	complying	complying	complying	complying
microbial content per gram										
bacteria	not more than 10^2	complying	complying	complying	complying	complying	complying	complying	complying	complying
fungi	not more than 10^3	complying	complying	complying	complying	complying	complying	complying	complying	complying
safety	complying	complying	complying	complying	complying	complying	complying	complying	complying	complying

• — no change

QUALITY CONTROL MANAGER :

/ prof. R. MANAHILOV , PhD , MD /

NCIPD
Quality control department

STABILITY REPORT

DOSAGE FORM: RESPIVAX E 50 mg

PRODUCT: RESPIVAX E

BATCH NUMBER: 190995

Indices	Specifications (standard)	initial	40 C				40 C relative humidity 75 %			
			1 month	2 months	3 months	6 months	1 month	2 months	3 months	6 months
Organoleptic properties										
Appearance	biconvex tablets	biconvex tablets	n. c.*	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.
Colour	yellow	yellow	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.
Odour	odourless to specific	odourless to specific	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.	n. c.
Disintegration	up to 60 min.	32 min	32	32	32	32	32	32	32	32
water content %	up to 10	4.60	4.62	4.56	4.58	4.62	4.66	4.61	4.64	4.54
content of aminoacids (mg /tablet)	2.0 – 6.0	5.10	5.12	5.09	5.05	5.08	5.10	5.06	5.15	5.11
content of protein (mg /tablet)	6.0 – 12.0	9.32	9.26	9.29	9.31	9.34	9.36	9.33	9.30	9.35
uniformity of mass %	$\bar{+}$ 7.5	5.85	5.85	5.88	5.83	5.86	5.86	5.86	5.85	5.85
identity	complying	complying	complying	complying	complying	complying	complying	complying	complying	complying
microbial content per gram										
bacteria	not more than 10^2	complying	complying	complying	complying	complying	complying	complying	complying	complying
fungi	not more than 10^3	complying	complying	complying	complying	complying	complying	complying	complying	complying
safety	complying	complying	complying	complying	complying	complying	complying	complying	complying	complying

* — no change

QUALITY CONTROL MANAGER :

/ prof. R. MANAHILOV , PhD , MD /

Table 1.[a]

STABILITY DATA OF RESPIVAX

Indicators	Tablets after production	Tablets after 24 months
Appearance	Film coated tablets	Film coated tablets
Solubility	10 min	10 min
Total protein	3.80 mg/50 mg	3.75 mg/50 mg

In charge of the experiment: Dr. B.Dragulev, National Drug Institute. Time of testing: March 1985 and March 1988, at temperatures between 20°C and 25°C.

50 mg. batch No 3/ 86 - 08 86 - 0888

Table 1.[b]

STABILITY DATA OF RESPIVAX

Indicators	Tablets after production	Tablets after 24 months
Appearance	Film coated tablets	Film coated tablets
Solubility	10 min	10 min
Total protein	1.75 mg/25 mg	1.73 mg/25 mg

In charge of the experiment: Dr. B.Dragulev, National Drug Institute. Time of testing: March 1985 and March 1988, at temperatures between 20°C and 25°C.

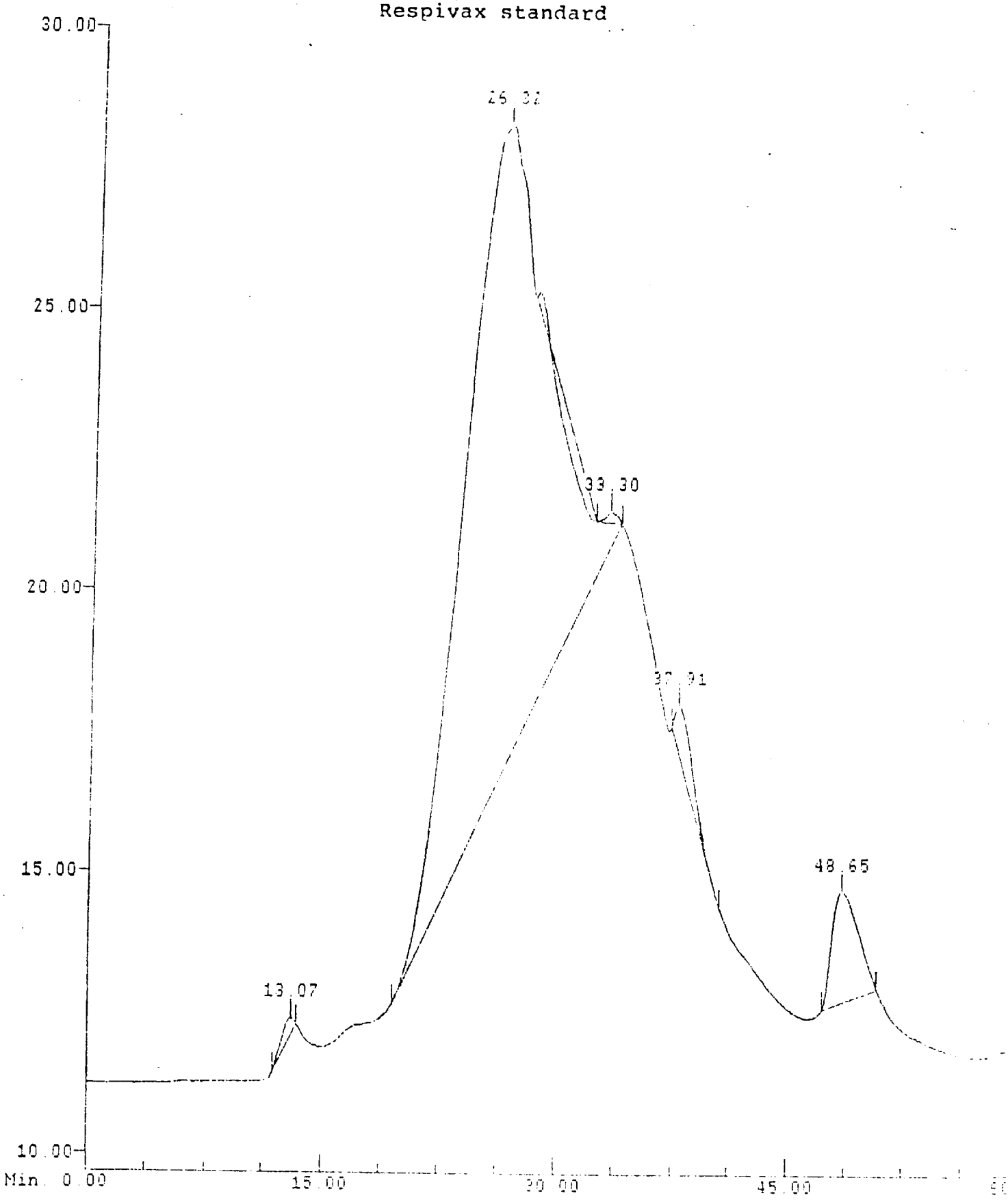
25 mg. batch No 2/ 87 - 03.87 - 03.89

INITIAL RAW AND AUXILIARY MATERIALS CONTENT IN A TABLET OF RESPIVAX

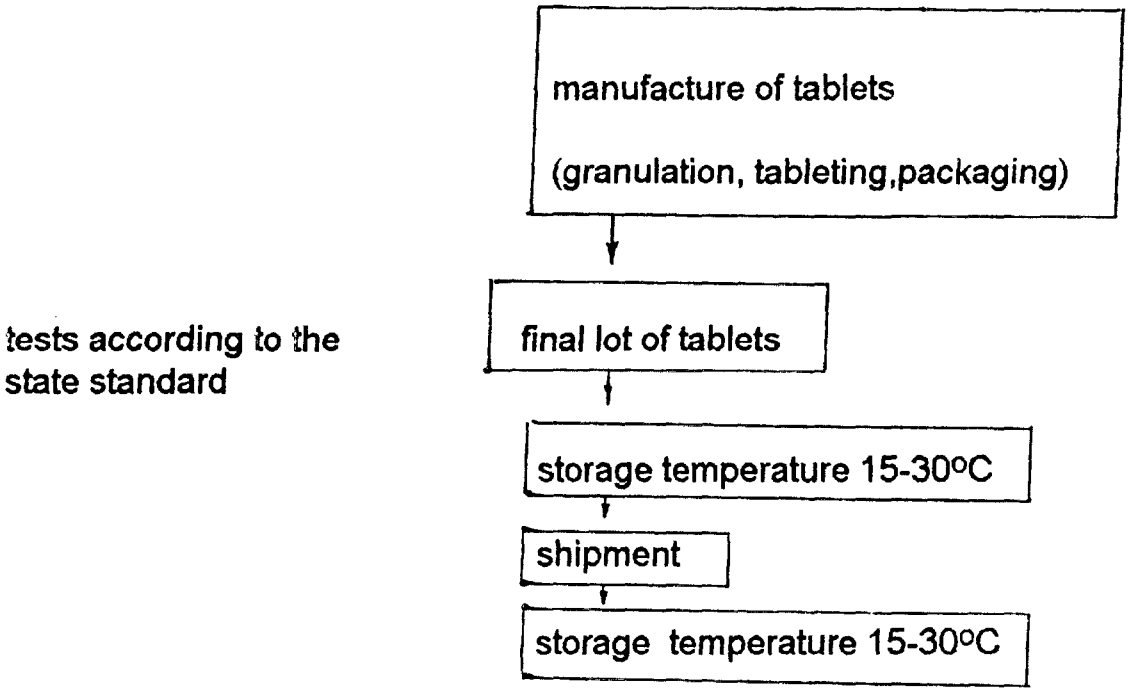
No.	Denomination	Content		Pharmacopoeia, National Standard (NS), company requirements
		50 mg tabl.	25 mg tabl.	
1.	RESPIVAX substance	50 mg	25 mg	NS 1681869-82.
2.	Wheat starch (Amylum tritici)	65 mg	45 mg	DAB-7
3.	Avicel pH 1.1	66.7 mg	5-.3 mg	"FMC"-USA
4.	Kollidon K-2[illegible] (Polyvinylpyrrolidone)	6 mg	5 mg	USP-XX
5.	Talc	4 mg	3 mg	DAB-7
6.	Magnesium stearate (Magnesium stearatum)	1.8 mg	1.2 mg	USP-XX, BP-80
7.	Kollidon CL 50-50	6 mg	3.5 mg	BASF"-FRG
8.	Aerosil 200	0.5 mg	3 mg	"Degussa"
9.	Ethyl alcohol (Spiritus aethylicus)	0.08 ml	0.06 ml	

Bio-Rad Laboratories Series 800 HRLC System

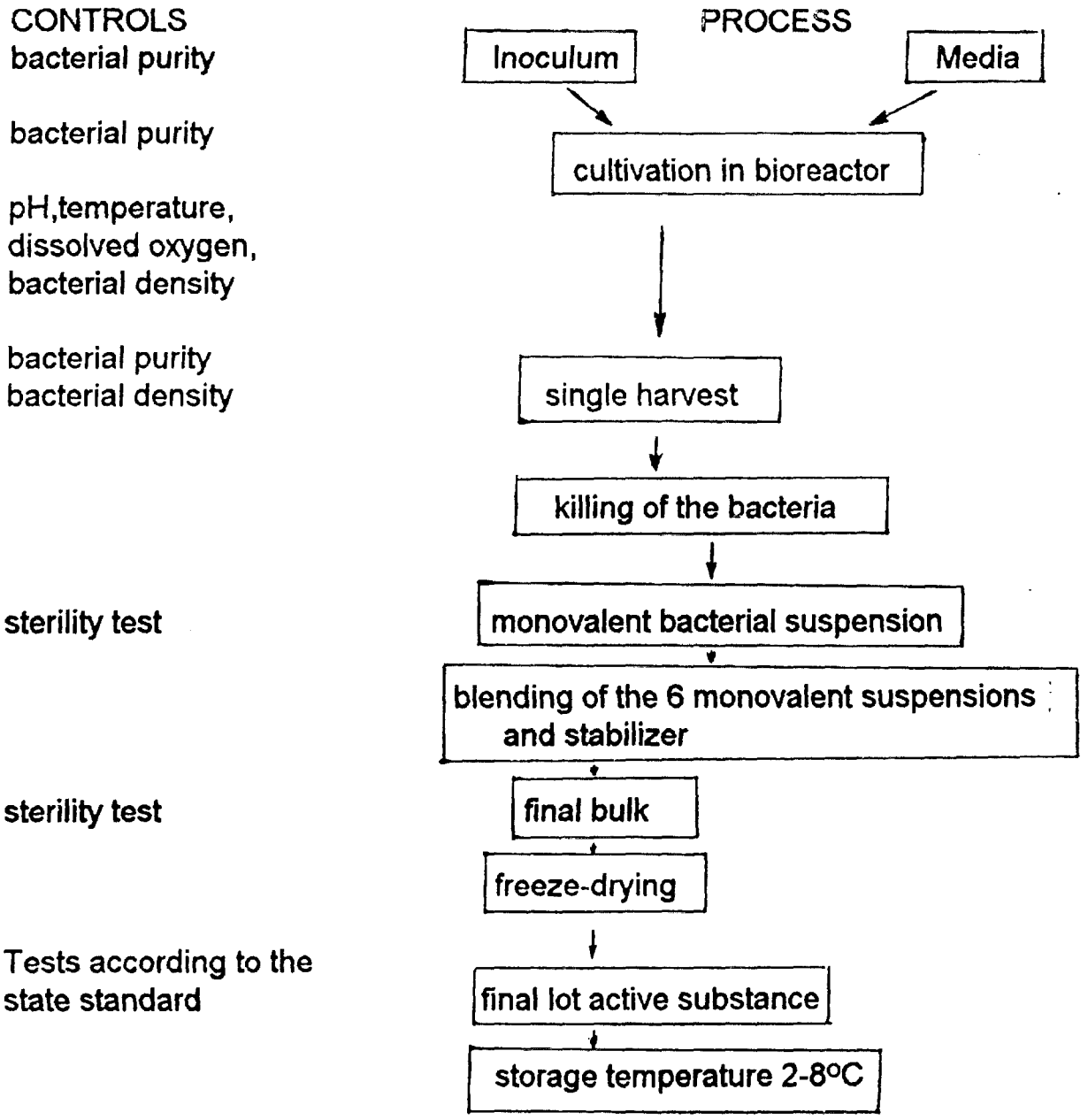
Respivax standard



PEN	AXIS	TYPE	NAME	DATE	INSTANCE	ENTRY	INJECT	DATA FILE
———	1	METHOD	JOJO	10-05-95	001			CHAN_1.ACQ

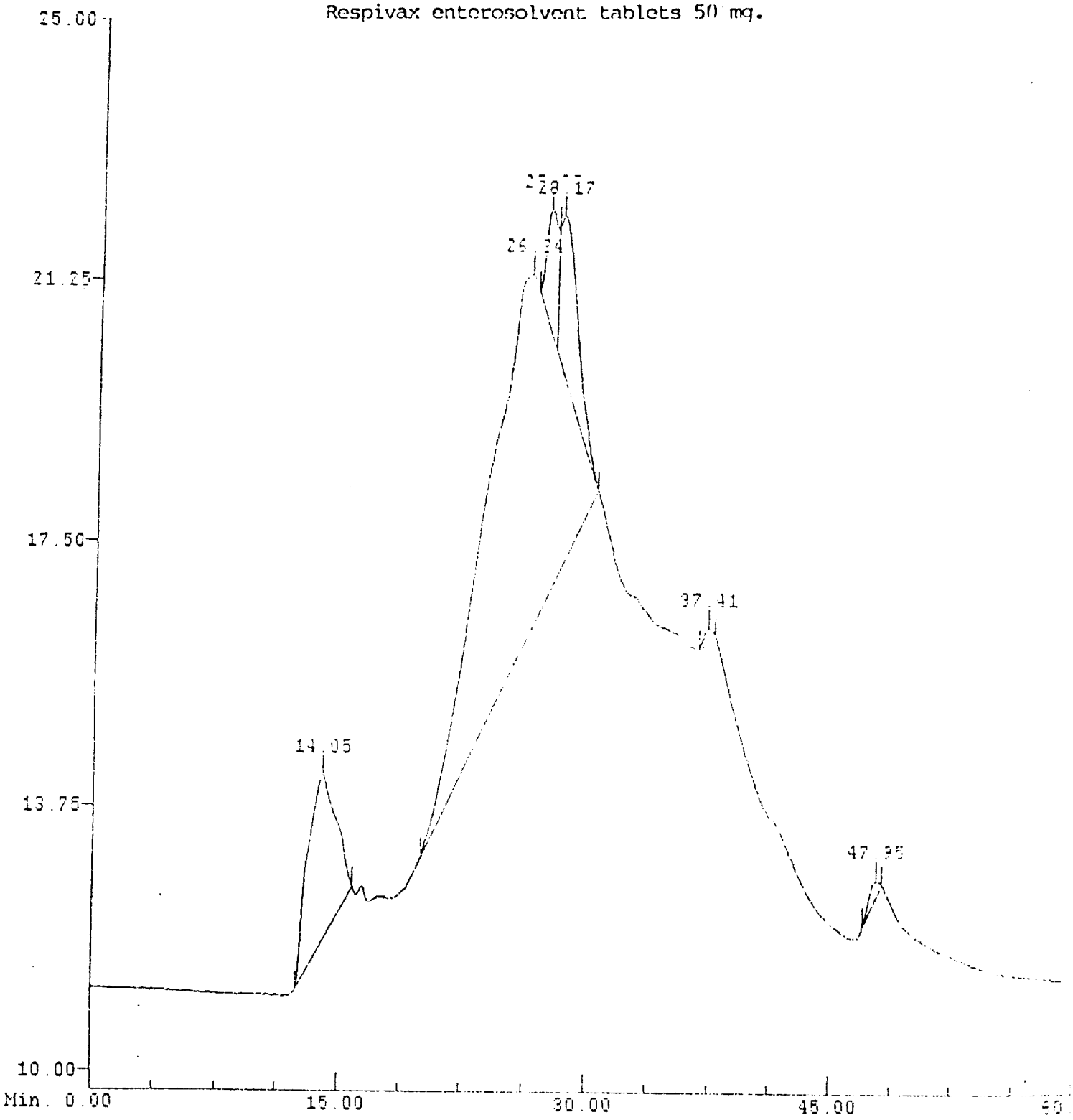

manufacture of tablets
(granulation, tableting,packaging)
final lot of tablets
tests according to the state standard
storage temperature 15-30°C
shipment
storage temperature 15-30°C

Flow chart of the Manufacturing Process



Bio-Rad Laboratories Series 800 HRLC System

Respivax enterosolvent tablets 50 mg.

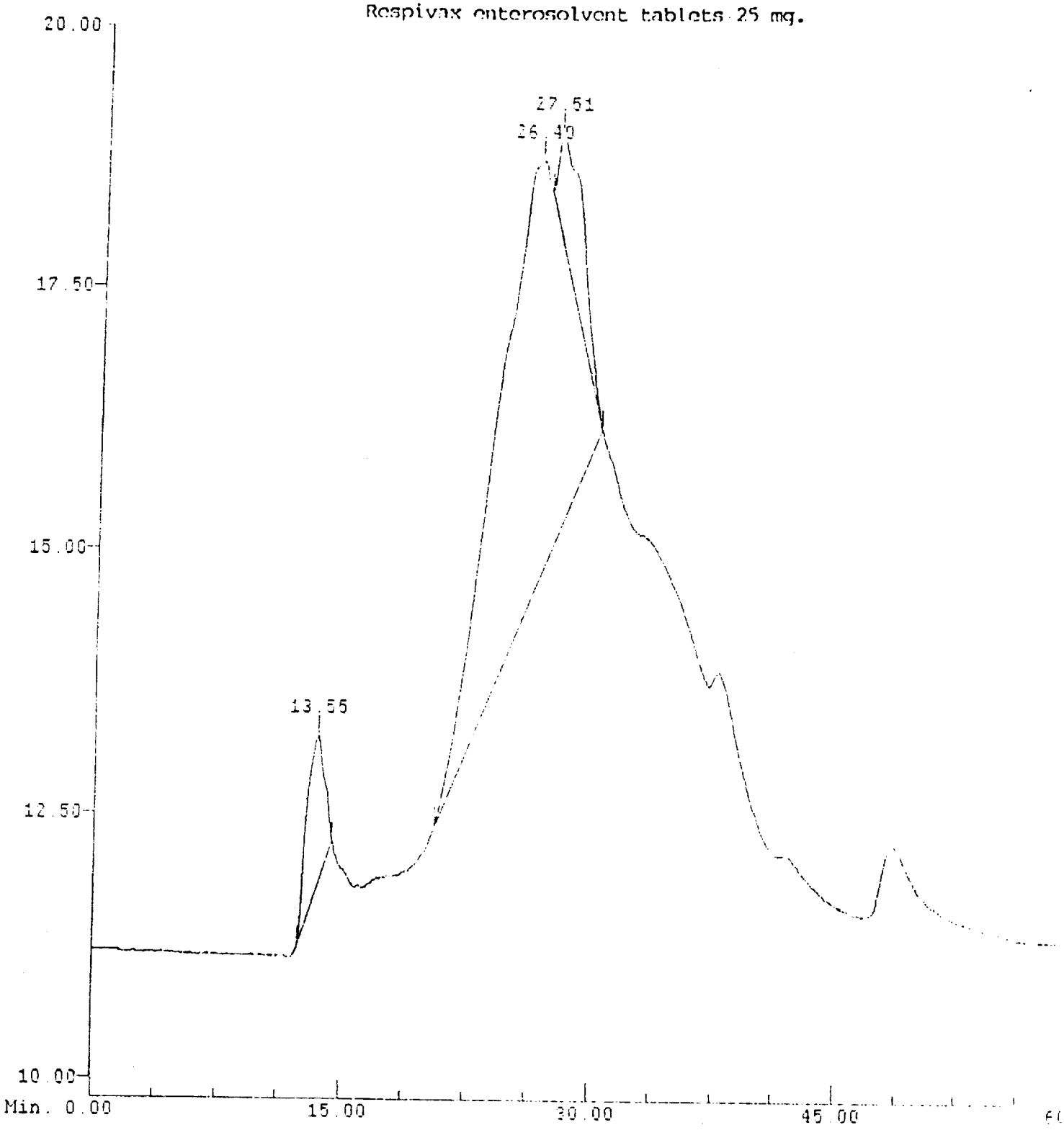


PEN	AXIS	TYPE	NAME	DATE	INSTANCE	ENTRY	INJECT	DATA FILE
————	1	METHOD	JOJO	10-05-95	003			CHAN_1.ACQ

Sample	RESPIVAX tabl. 50 mg ,100 E-6
Eluant A:	sodium phosfate buffer pH 6.8 0.1 M
Flow Rate	0.5ml/min
Column	tsk 250 gel filtration,300/7.8mm i.d.
Detector A	uv/vis 280nm,R =0.05

Bio-Rad Laboratories Series 800 HRLC System

Respivax enterosolvent tablets 25 mg.



PEN	AXIS	TYPE	NAME	DATE	INSTANCE	ENTRY	INJECT	DATA FILE
———	1	METHOD	JOJO	10-05-95	002			CHAN_1 ACQ

Sample	RESPIVAX tabl. 50 mg ,100 E-6
Eluant A:	sodium phosfate buffer pH 6.8 0.1 M
Flow Rate	0.5ml/min
Column	tsk 250 gel filtration,300/7.8mm i.d.
Detector A	uv/vis 280nm,R =0.05

Preparation of Respivax

The manufacturing process of Respivax is divided in three phases: production of the active ingredient , freeze - drying and manufacture of tablets.

Production of the active ingredient

The active ingredient of Respivax contains freeze-dried lysate and killed bacterial cells of 6 bacterial species(Streptococcus pneumoniae, Neisseria catarrhalis, Streptococus pyogenes, Haemophilus influenzae, Staphylococcus aureus, Klebsiella pneumoniae) in equal quantities. The strains used must possess the typical for the species taxonomic characteristics. For each cultivation new ampoule of the respecive strain seed lot is opened. After culture passages on hard and liquid nutrient media the bioreactor is inoculated with the inoculum and the cultivation of the bacterial strain is done under optimal for the strain conditions with respect to the nutrient medium, temperature, pH maintainance, regulation of the level of the dissolved oxygen. For each strain special technology for bioreactor cultivation is used. Each strain is cultivated separately.The necessary technological parameters are recorded automatically. The development of the bacterial population is followed by means of spectrophotometrical determination of density and the cultivation is terminated at the beginning of the stationary phase. Samples are taken every hour. Prior to killing the bacterial suspensions, the purity is studied by preparation according to Gram and culturing on solid nutrient media (blood agar or chocolate agar) and final bacterial density is determined. If presence of foreign microorganisms is detected ,the batch is discarded. After adding the inactivating agent and 3-day stay, the suspension is checked of viable microorganisms. A sample of the suspension is cultured on blood agar (H. influenzae on chocolate agar) and thyoglicolate broth. The cultivation lasts 5 and 14 days respectively at 37° C . The presence of living bacteria is not allowed. The monovalent bacterial suspensions are blended in ratio securing the presence of equal proportions of the six bacterial strains and dextrane with molecular mass of 40000 + 10000 is added to the mixture as stabilizer. The process is carried out in a clean room . The final bulk is checked for sterility by growing on blood agar . Presence of live bacterial cells should not be observed.

Freeze-drying

The freeze-drying is performed in a proper lyophilizer with computerized process management in sterile coditions and recording of the processes.

The produced active substance is tested according to the criteria ,set out in the respective state standard for Respivax active substance. The substance is

controlled chemically and bacteriologically and is released as ready for use after receipt of the protocol from the control laboratory.
The active substance is packed in soldered cotainers and is stored in dark dry premises at temperature 2-8° C.

Manufacture of tablets
The method of manufacture of Respivax tablets comprises two stages: granulation and tabletting.
Granulation
Materials for granulation:

Denomination	Content 50mg tabl.	25mg tabl
1.Respivax substance	50 mg	25mg
2. Wheat starch	65mg	45mg
3.Avicel pH 101	66,7mg	54,3mg
4.Kollidon K25	6,0mg	5mg
5. Ethyl alcohol 95%	0,08ml	0,06ml

The ingradients are homogenized in the dry state and the mixture is moisturized and granulated.

Tableting
Materials for tableting

Talc	4mg	3mg
Magnesium stearate	1,8mg	1,2mg
Kollidon CL 50-50	6mg	3,5mg
Aerosil 200	0,5mg	3mg

The materials are added to the granulate and the mixture is processed in homogenizer and tablets are made by rotation tableting mashine.
Tablets are analyzed by the control laboratory according to the criteria set out in the respective state standard for Respivax tablets. Tablets are put in PVC blisters and closed by aluminium foil.

Republic of Bulgaria
Ministry of Health

NATIONAL CENTER OF INFECTIOUS AND PARASITIC DISEASES

SOFIA

R E S P I V A X

PREPARATION FOR PERORAL IMMUNOPROPHYLAXIS AND IMMUNOTHERAPY OF NON-SPECIFIC INFECTIOUS DISEASES OF RESPIRATORY SYSTEM

S O F I A

1995

B. THE PROCEDURES OR ASSAYS EMPLOYED FOR QUALITY CONTROL

TECHNICAL REQUIREMENTS

Indices	Standard	Standard
	Tablets 25 mg	Tablets 50 mg
1. Apperance	Tablets with regular biconvex form, 7 mm in diameter.	Tablets with regular biconvex form, 8 mm diameter.
2. Colour	yellow	yellow
3. Odour	Odorless to slightly specific.	Odorless to slightly specific.
4. Disintegration in minutes	up to 60	up to 60
5. Average mass of 1 tablet in grams	0.129- 0.150	0.185-0.215
6. Uniformity of mass in %	±7.5	±7.5
7. Water in %	up to 10	up to 10
8. Content of aminoacids (mg/ tablet)	1.0- 3.0	2.0- 6.0
9. Identity	complying	complying
10. Content of protein (mg/ tablet)	3.0- 6.0	6.0- 12.0
11. Content of formaldehyde (mg/ tablet)	up to 0.1	up to 0.2
12. Microbial content -total number of microorganisms per gram -moulds and yeast-like fungi per gram -content of Enterobacteriacae p.aeruginosa, S. aureus -pathogenous microorganisms	 not more than 10^3 not more than 10^2 not allowed not allowed	 not more than 10^3 not more than 10^2 not allowed not allowed
13. Satety	complying	complying

Licenced **1681869- 88** **1682994- 88**

STATE STANDARD

Republic of Bulgaria

RESPIVAX

0050530- 95

Enteric coated tablets

50 mg

0050531- 95

Enteric coated tablets

25 mg

QUALITY COMMITTEE AT THE COUNCIL OF MINISTERS

Ratified by the Ministry of Health and Social Care

Deputy Minister:

Sofia

TO BE REGISTERED

To come into force from

06.01.95

Director:

The State Standards refer RESPIVAX enteric coated tablets 50 mg and enteric coated tablets 25 mg containing freeze-dried lysates and bacterial bodies of six microbial species (Streptococcus pneumoniae, Neisseria catarrhalis, Streptococcus pyogenes, Haemophilus influenzae, Staphylococcus aureus, Klebsiella pneumoniae), according to a ratified technological regulation.

The preparation RESPIVAX is used in human medicine for oral immunoprophylaxis and immunotherapy of acute and chronic non-specific infections of respiratory system.

METHODS FOR ANALYSIS OF THE PREPARATION RESPIVAX - TABLETS

1. Appearance and colour

Appreciated visually.

2.Odour- appreciated organoleptically

3.Disintergration

The test is performed according to BP- 88 Apendix 12 B p.A141.

4.Mean mass and uniformity of mass

The mean mass of tablet is determined by a separate weighing of 20 tablets with accuracy up to 0.001 g. The average mass is calculated as arithmetical average. Deviations in the mass of two separate tablets admitted is up to $\pm$ 15 %. Deviations in the mean mass is up to $\pm$ 7.5 %.

5. Water Content Determination

It is performed by Carl Fisher's method.

6. Amino Acid Content Analysis

J.R.Spiess, D.C.Chambern, J.Biol.Chem., 191, 1951.

This method is based on formation of a complex between the amino acids and copper salts, staining of which is specrophotometrically measured at 620 mm.

Reactives used:

1. Copper chloride solution

   $CuCl_2.2H_2O$ - 28.0 g

   distilled water - up to 1000 cm^3

2. Phosphate solution

   $Na_3PO_4.12H_2O$ - 68.5 g

   distilled water - up to 1000 cm^3

3. Borate buffer

   $Na_2B_4O_7$ - 10.1 g

   distilled water - up to 1000 cm^3, pH 9.1-9.2

4. Copper sulphate suspension

Copper chloride - 20.0 cm^3 are poured into appropriate vessel. 40.0 cm^3 of phosphate solution /2/ are gradually added at continuous stirring. This solution is centrifuged for 5 min at 5000 rev/min. The sediment is suspended in 60.0 cm^3 of borate buffer /3/. Centrifugation is completed. This procedure repeats twice. Sediment is suspended in 100 cm^3 of borate buffer, 6.0 g of sodium chloride being added. This suspension is left for 4 days in a bottle with glass stopper and stored at +4°C. It can be used up to 1 month.

Specimen Preparation

Ten ground tablets are placed in a volumetric flask of 25 cm^3. Volume is supplemented with distilled water. After complete disintegration of the tablets, the specimen is transferred into an appropriate vessel and treated by ultrasound disintegrator for 2 min. It is centrifuged for 10 min at 5000 rev/min. A filter with magnitude of pores of 0.2 mkm is used for filtration.

Determination

5.0 cm^3 of the filtered specimen are mixed with 5.0 cm^3 of the copper sulphate suspension /4/. Mixture is well stirred. Then it is left for 5 min, after which is centrifuged for 5 min at 5000 rev/min. Supernatant, stained in blue, is transferred to a retord containing 0.200 g of L-alanin (analytic purity). 30 min later the extinction of the solution is assessed at 620 nm. A solution of 5.0 cm^3 of borate buffer /3/, 5.0 cm^3 of distilled water, 0.200 g of L-alanin, 6% of sodium chloride is used as control. 0.01 M of L-alanin solution is prepared (analytical purity). From it are prepared solutions with concentrations of 0.05 M, 0.025 M and 0.0125 M. Procedure with 5.0 cm^3 of each standard concentration is the same as that with the specimen. Extinctions are assessed at 620 nm. The data are graphically expressed and a standard curve is plotted.

According to the assessed extinction of the specimen, because a standard curve is used, the amount of amino acids is determined in mols. Amino acids content in a tablet is calculated by the following equation:

x. 89. 25. 0.1= mg / tabl.

x - amount of amino acids in mols

Accuracy of the method - ± 2%.

89 - molecule weight of L- alanin

25 - final volume of the sample

0.1 - coefficient

7. Separation by High Performance Liquid Chromatography (HPLC)

The separation is based on a chromatographic fractionation of the bacterial freeze-dried lysates included in one tablet and on its comparison with the chromatograms of standard freeze-dried lysates with same concentration.

Specimen Preparation

10 ground tablets are placed into 5.0 cm^3 of distilled water. Received suspension is treated by ultrasound desintegrator for 2 min and then is centrifuged for 10 min at 5000 rev/min. If the sedimentation is incomplete, procedure repeats. At last it is filtered through a membrane with magnitude of pores of 0.2 mkm.

Standard Preparation

Content of one ampoule with standard freeze-dried lysate is dissolved in 1.0 cm^3 of water. Processing is like the above described.

Analysis Performance

The chromatographic separation is done on a firm system for HPLC. Column for gel-filtration TSK-300/7.8 (or other appropriate column) is used. It is eluted with 0.1 M of phosphate buffer, pH 7.2 and sodium acid 0.2% as conservant. Under these conditions the optimal flow rate is 0.5 cm^3/min. 100 mkl of the standard and specimen each are injected.

Results Recording

Conclusions are drawn on the basis of comparison between chromatograms of both specimen and standard. Under the pointed out conditions, three fractions are established (record is made at 280 nm).

8. Protein Determination by Lowry

Materials:

1. Complex-forming reagent: prepared immediately before use by mixing the following 3 stock solutions A, B, and C in proportion 100:1:1, respectively.

Solution A: 2% (w/v) Na_2CO_3 in 0.1 N NaOH

Solution B: 0.5% (w/v) $CuSO_4.5H_2O$ in 1% Na citrate

Solution C: 50 ml of solution A is mixed with 1 ml of solution B (freshly made).

2. Folin reagent (commercially available): Used in concentration of 1 N.

3. Standards: A stock solution of standard protein (e.g., bovine serum albumin fraction V) containing 1 mg/ml protein in distilled water is used.

Stock solution: 8 ml of dH_2O are added to 2 ml of the initial solution. This solution is the starting one for the following dilutions. It contains 200 mkg/ml.

Stock solution	1.5	1.2	1	0.8	0.6	0.4	0.2	0.1

(ml)								
dH_2O (ml)	0.5	0.8	1	1.2	1.4	1.6	1.8	1.9
Protein concentration (mkg/ml)	150	120	100	80	60	40	20	10

Construction of Standard Curve

Each dilution is poured into test tubes of 0.4 ml. In all of them are added 2 ml of solution C. After shaking and 10 min in calm, 0.2 ml of Folin's reactive is added. Solutions are stirred and then left at room temperature for 30 min. The absorbtion is assessed at ^ = 750 nm ±2 nm.

Controls: 0.4 ml of dH_2O can be used instead of protein.

For building up a standard curve on abscise the protein quantity (in mkg) in the solution should be projected, and on the ordinate the corresponding absorbtion should be projected.

Determination

Ten ground tablets are suspended in dH_2O up to 25 ml. The resulting suspension is put under ultrasound disintegration for 2 min/15 000 Hz and then is centrifuged at 5 000 rev/min.

Supernatant is taken and diluted 50 times.

sample: 1 ml supernatant + 49 ml of dH_2O

Assessment of the obtained sample is analogous to that of the etalon dilutions for standard curve:

protein quantity in the tested sample in mg protein/tablet =

$$= \frac{C \cdot 1\,250 \cdot 0.1}{1\,000}$$

where

C - protein concentration (mkg/ml) in the tested sample, determined according to the standard curve.

1 250 - coefficient, product of the used dilutions (50.25)

0.1 - coefficient, ratio between the mass of 1 tablet to the mass of 10 tablets.

1 000 - coefficient for mkg in mg transition.

Duplicate sets of standards are needed with each group of assays. Unknown duplicates and triplicates are recommended.

9. Formaldehyde Determination is performed according to the Bulgarian State Standard 1674045- 92.

10. Microbiological content

It is performed according to the requirements of the European Pharmacopoeia 1983, V 2. 1. 8 and VIII 10.

11.Safety

It is determined by peroral introduction of 1 ground and suspended in 0.9 % saline tablet in volume not greater than 0.5 ml, per mouse. Use 10 white mice weighing 22.0 through 25.0 grams. It is required that neither of the animals should show symptoms of illness in the course of 7 days. If one or more animals die, repeat the test.

12. Storage, Transportation, Expiry Date

12.1. Product is packed and stored in dark, close, dry storehouses at moderate temperature in accordance with the Bulgarian State Standard (BSS) 3836-80.

*12.2. **Transportation***: at moderate temperature, according to BSS 3836-80.

*12.3. **Expiry date***: 30 (thirty) months from the date of tablet making. After a control testing conducted in the National Drug Institute , the expiry date can be prolonged only once for another 6 (six) months.

Analysis Performance

Chromatographic separation is done on a firm system for HPLC. A column for gel-filtration TSK-300/7.8 (or other appropriate column) is used. It is eluted with 0.1 M phosphate buffer, pH 7.2 and sodium azide 0.02%, as conservant. Under these conditions the optimal flow rate is 0.5 cm^3/min; 100 mkl of the standard and specimen each are injected.

Reading the Results

Conclusions are drawn on the basis of comparison between the chromatograms of the specimen and standard /detection at 280 nm/.

3.5. Microbial content- according to the European Pharmacopoeia 1983, V 2.1.8 and VIII 10

3.6. Safety- It is determined by peroral introduction of 50 mg substance ,dissolved in 0.9% saline in volume not greater than 0.5 ml, per mouse. Use 10 white mice weighing 22.0 through 25.0 grams. It is required that neither of the animals should show symptoms of illness in the course of 7 days. If one or more animals die, repeat the test.

Table 2[a]

HEMAGGLUTINATION OF ANTIBODIES TITRE AGAINST SHEEP ERYTHROCYTES IN MICE, TREATED WITH RESPIVAX STORED FOR 24 MONTHS AT ROOM TEMPERATURE

Test groups of 10 white mice treated with:	**Antibody titre after administration of antigen sheep erythrocyte**		
	Primary antibody response		**Secondary antibody response**
	7th day	**14th day**	**7th day**
RESPIVAX, perorally, 7 days before and 7 days after Ag	1:128	1:512	1:2048
RESPIVAX, perorally, 7 days after administration of Ag	1:128	1:256	1:1048
RESPIVAX, s.c., 7 days before and 7 days after Ag	1:512	1:2048	1:4096
RESPIVAX, s.c. 7 days after administration of Ag	1:128	1:512	1:1044
Control group, injected with Ag only	1:8	1:32	1:64

Ag - antigen. Each mouse is injected i.v. with 0.2 ml of 50% suspension of sheep erythrocytes. On the 28th day after first administration the antigen is injected again in order the secondary antibody response to be followed up. RESPIVAX is administered in a daily dose of 2 mg/0.5 ml of physiological solution perorally or s.c.

Batch 3/86 - 08.86 to 08.88.

Table 3[a]

RESULTS, OBTAINED FROM THE STUDY ON PROTECTIVE EFFECT OF RESPIVAX, STORED FOR 24 MONTHS AT ROOM TEMPERATURE, PERORALLY ADMINISTERED TO WHITE MICE INFECTED WITH Staphylococcus aureus Sg 511

Test groups of mice* treated with:	**Number of dead mice on:**											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12 day**
RESPIVAX, 5 mg / 0,5 saline perorally, 10 days before infection	3	1	1	1	2	-	-	1	1	-	-	-
Physiological sol. perorally, 10 days before infection	8	2	-	-	-	-	-	-	-	-	-	-

* Each test group is of 10 white mice with body mass between 15 g and 18 g.
RESPIVAX 50mg - batch 3/86 - 08.86 to 08.88

B

STATE STANDARD

0050532- 95

Republic of Bulgaria	**R E S P I V A X SUBSTANCE**	QUALITY COMMITTEE AT
		THE COUNCIL OF MINISTERS
Ratified by the Ministry of Health and Social Care		TO BE REGISTERED
Deputy Minister:		**To come into force from 06.01.95**
Sofia		Director:

The State Standard refers RESPIVAX active substance containing freeze-dried lysates and bacterial bodies of six microbial species (Streptococcus pneumoniae, Neisseria catarrhalis, Streptococcus pyogenes, Haemophilus influenzae, Staphylococcus aureus, Klebsiella pneumoniae), according to a ratified technological regulation.

The preparation RESPIVAX is intended for oral immunoprophylaxis and immunotherapy of acute and chronic non-specific infections of respiratory system.

2. REQUIREMENTS FOR ACCEPTANCE AND MODE OF GETTING SAMPLES

According to Bulgarian State Standard 3836-80.

3. ANALYTICAL TEST METHODS

3.1. Appearance and colour - appreciated visually.

3.2. Odour - appreciated organoleptically.

3.3. Water content determination - performed by the method

- of Carl Fisher.

3.4. Fractionation on molecule mass by High Performance Liquid Chromatography /HPLC/.

It is based on chromatographic fractionation of bacterial freeze-dried lysates, included in the tablet and on comparison with the chromatograms of standard freeze-dried lysates with same concentration.

Specimen Preparation

500 mg of the substance are placed in 5 cm^3 of distilled water. This suspension is treated for 2 min by ultrasound desintegrator and centrifuged for 10 min at 5000 rev/min. In a case of incomplete sedimentation, procedure is done again. Suspension is filtered through membrane with magnitude of the pores of 0.2 mkm.

Standard Preparation

Ampoule content of standard freeze-dried lysate is dissolved in 1.0 cm^3 of water. Procedure is the same as the above described.

1. TECHNICAL REQUIREMENTS

Indices	Standard
1. Appearance	Porous substance
2. Colour	White to pale brown
3. Odour	Specific
4. Water content in %	up to 10
5. Identity	Identical
6. Microbial content	
-total number of micro-organisms per gram	not more than 10^3
-moulds, yeast-like fungi per gram	not more than 10^2
-content of Enterobacteriaceae, P.aeruginosa, S.aureus	not admitted
7.Satety	complying

Licenced 1681870- 88

ANIMAL

TOXICITY STUDIES

Table 2[B]

HEMAGGLUTINATION OF ANTIBODIES TITRE AGAINST SHEEP ERYTHROCYTES IN MICE, TREATED WITH RESPIVAX STORED FOR 24 MONTHS AT ROOM TEMPERATURE

Test groups of 10 white mice treated with:	**Antibody titre after administration of antigen sheep erythrocyte**		
	Primary antibody response		**Secondary antibody response**
	7th day	**14th day**	**7th day**
RESPIVAX, perorally, 7 days before and 7 days after Ag	1:256	1:512	1:4096
RESPIVAX, perorally, 7 days after administration of Ag	1:128	1:512	1:2048
RESPIVAX, s.c., 7 days before and 7 days after Ag	1:256	1:1024	1:2048
RESPIVAX, s.c. 7 days after administration of Ag	1:256	1:512	1:2048
Control group, injected with Ag only	1:16	1:32	1:64

Ag - antigen. Each mouse is injected i.v. with 0.2 ml of 50% suspension of sheep erythrocytes. On the 28th day after first administration the antigen is injected again in order the secondary antibody response to be followed up. RESPIVAX is administered in a daily dose of 2 mg/0.5 ml of physiological solution perorally or s.c.
Batch 2/87 - 03.87 to 03.89.

Table 3B

RESULTS, OBTAINED FROM THE STUDY ON PROTECTIVE EFFECT OF RESPIVAX, STORED FOR 24 MONTHS AT ROOM TEMPERATURE, PERORALLY ADMINISTERED TO WHITE MICE INFECTED WITH Staphylococcus aureus Sg 511

Test groups of mice* treated with:	Number of dead mice on:											
	1	2	3	4	5	6	7	8	9	10	11	12 day
RESPIVAX, 5 mg / 0,5 saline perorally, 10 days before infection	2	1	2	1	2	-	1	1	-	-	-	-
Physiological sol. perorally, 10 days before infection	8	1	1	-	-	-	-	-	-	-	-	-

* Each test group is of 10 white mice with body mass between 15 g and 18 g.

RESPIVAX 25mg - batch 2/87 - 03.87 to 03.89.

SUB- CHRONIC TOXICITY IN RATS

30 rats of the Wistar race, weighing 180-200g each were used. 20 of them were fed per os through probe with 0,5g/1ml saline of Respivax daily in the course of 4 weeks. One dose per rat is equivalent to 5000 daily human doses. The remain 10 rats were fed with 1ml saline on the same scheme.

The rats were controlled daily with regard to their general state, weight, feeding, morbidity and mortality. Their haematological indices were examined every week /erythrocytes, leukocytes, haemoglobin and differential counting/. The animals were sectioned under light ether narcosis on the second day after the last feeding. A macroscopic view was made and material for histological and electron-microscopic examination of the main parenchymal organs /lung, liver, spleen and brain/ was collected.

As a result from the studies no changes in the general state /weight, feeding, morbidity and mortality/ of the animals fed with Respivax was observed compared to those of the control group. The haematological indices were within the limits of norm in both groups. No pathological changes were observed in the examined through histological and electron-microscopic studies. It was seen demonstrative histological and electron-microscopic changes in the immunocompetent organs /lymph nodes, spleen/ proving the immunostimulatory effect of Respivax.

SUB- CHRONIC TOXICITY IN RABBITS

15 rabbits of the "Chinchilla" race were used, weighing 1,500- 1,600g each. 10 of them were fed through probe with Respivax 2g/2ml saline per os daily in the course of 4 weeks. The dose of Respivax is equivalent to about 2,000 daily human doses. 5 rabbits were fed with 2ml saline on the same scheme. All rabbits were controlled daily regarding their general state, feeding and weight, and blood probes were taken for determination of the main haematological indices /erythrocytes, leukocytes, haemoglobin and differential counting/ each month.

The rabbits were sectioned after treatment with 300mg hexobarbital sodium intracutaneously on the second day after the last feeding. A macroscopic view was made of the sectioned animals and material was collected for histological and electron- microscopic examination of their parenchymal organs /lung, liver, spleen and brain/.

Compared with those of the control group, no changes in the general state, weight and behaviour of the animals fed with Respivax were observed. The haematological indices were in limits of norm in both groups. No pathological changes were observed in the organs examined through histological and electron- microscopic studies. It was seen demonstrative histological and electron- microscopic changes in the immunocompetent organs /lymph node, spleen/ proving the immunostimulatory effect of Respivax.

CHRONIC TOXICITY IN RABBITS

35 rabbits of the "Chinchilla" race were used weighing 1,500-1,600g each fed per os through probe with Respivax as follows: 10 of them with a dose of 0,5g/2ml saline, 10 of them with a dose of 1mg/2ml saline and 10 of them with a dose of 2mg/ml saline daily in the course of 12 months. The remaining 5 rabbits were fed with 2ml saline of the same scheme.

The rabbits were controlled daily regarding their general state, feeding and weight, and blood probes were taken for determination of their main haematological indices /erythrocytes, leukocytes, haemoglobin and differential counting/weekly.

The rabbits were sectioned after treatment with 300mg hexobarbital sodium intracutaneously on the second day after the last feeding. A macroscopic view was made of the sectioned animals and material was collected for histological and electronmicroscopic examination of their parenchymal organs /lung, liver, spleen and brain/.

In comparison with the control group/ 5 rabbits treated with 2ml saline daily per os in the course of 12 months/, no changes in the general state, wight and behaviour of the animals fed with Respivax were observed. The haematological indices were in the limits of norm in both groups. No pathological changes were found in the organs examined through histological and electron-microscopic studies. It was seen demonstrative histological and electron-microscopic changes in the immunocompetent organs /lymph nodes, spleen/ proving the immunostimulatory effect of Respivax.