

ATTACHMENT 5

II. Pharmaceutical/technical documentation

II.A. Pharmaceutical

1. Name of the Product: HUMET_®-R syrup, 300 ml

Product for macro and micro element supplementation

2. Qualitative and quantitative composition of the product with INN of the active ingredient(s) and excipients:

	Name of the active ingredients	Quantity/ 300 ml	Unit	Reference standards
1.	Humic acid	2.25	g	manufacturer's stand. specification
2.	Potassium	1.10	g	BP
3.	Magnesium	450	mg	BP/USP/DAB
4.	Iron	420	mg	USP/Ph. Eur.
5.	Zinc	300	mg	Sigma for anal. use
6.	Manganese	90	mg	Sigma for anal. use
7.	Copper	60	mg	USP
8.	Vanadium	15	mg	Sigma for anal. use
9.	Cobalt	6	mg	Sigma for anal. use
10.	Molybdenum	5.25	mg	Sigma for anal. use
11.	Selenium	3.75	mg	Sigma for anal. use

3. Description of the manufacturing process

Before disclosure, the peat is to be crushed through a screen with 1 cm aperture. The peat is then to be processed during 48 hours in a mixing vessel, enamelled or made of acid-resisting steel, in a 1% (with regard to the total quantity of the reaction mixture) aqueous solution of K-pyrophosphate, in the proportion of 4:1 to the mass of the peat. After the sedimentation (24 hours) the upper colloidal solution phase is to be removed by suction. The temperature of the material is to remain between 15-45 °C during the whole process.

The sediment remaining after the first decantation is to be processed for a second time in a 1% K-pyrophosphate solution (analogous to the first phase of disclosure). The joint solution phase of the two decantation sections is to be set to the value of 60 g of total humic acid per cubic decimetre. This is the "standardised" humic acid solution.

The K-pyrophosphate disclosure of the peat results in a colloidal humic acid solution with 60 g/dm³ concentration, free from any solid accompanying substances, which we apply after a radiation sterilising process, carried out in 10 l PE vessels. On the base of the radiation sterilised humic acid solution, by adding potassium hydrogen phosphate with a concentration of 100 g.K-ion per litre and sterile water, K-humate is prepared for the purpose of producing metallic chelates.

The standardised humic acid solution used during production contains all the components (grey and brown humic acids, fulvic acids, humatormelane acids, humic acid fragments). The molecular mass of the humic acid components in the solution determined by method of cryoscopy is between 3600-4400.

The humic acid is standardised by the mining site of the initial peat and by the potassium pyrophosphate dissolving process.

Steps of the chelate production:

1. The Cu, Zn, Mg and Co stock solutions are being applied simultaneously;
2. the Fe stock solution is introduced into the preparation separately;
3. the V, Mo and Se stock solutions are also added to the preparation separately.

The dipotassium hydrogen phosphate used in the potassium pyrophosphate dissolving process and for the production of potassium humate introduces into the preparation a total quantity of 7.2 g/dm³ of potassium and 2,9 g/dm³ of phosphorus. The sulphur content, introduced together with the initial substances in the form of sulphate makes up 6,6 g/dm³.

4. *Pharmaceutical form (route of administration):*

- suspension syrup

The suspension - being a form of medicine - is a liquid pharmaceutical preparation to be taken orally or used externally, where the solid phase is floating in a liquid dispersion substance evenly distributed, or may be re-dispergated after precipitation (Ph. Hg. VII.).

Composition of the suspension: liquid dispersion medium and solid dispersed material (grain volume: < 200 µm).

Type of the suspension:

According to its application: oral suspension

On the base of the nature of the medium: suspension syrup

Traditional suspension

Requirements towards the suspensions:

- the suspended particle should precipitate slowly; the particles precipitated on the bottom of the bottle should not carburize, might be shaken up by way of slight mechanical shaking and become homogeneous;
- should not be too viscous, nor have any unpleasant taste or scent;
- should be easily poured out of the bottle and measured out;
- should have an appropriate appearance.

4.1 Appearance of the product:

brown suspension with characteristic sourish fruit taste and odour.
Administered as a fruit-flavoured syrup, 30 doses per 300 ml bottle.

5. *Specification of active ingredients*

5.1 The biochemistry of humic acids

(Pal Gömör, MSc., Eötvös Loránd University of Sciences, Budapest, Faculty of Natural Sciences, Dept of General and Inorganic Chemistry)

About ten thousand publications deal with humic acids every year. Of these, the industry related ones amount to about 9500. All decaying vegetable matter is subject to a bacteriological decomposition process. Several centuries have to flow by until the layer commonly referred to as the humus content of soil develops. Where this humus layer is destroyed, the damage lasts for several centuries. To use a commonplace metaphor: the most substantial wealth of Hungary is the upper 20 to 40 cm thick layer that covers the good soil of the country. Hungary is one of the "Great Powers" in this respect, since humus has accumulated in vast masses in the Carpathian Basin during the past 10-30 thousand years. Particularly thick peat marshes could develop in the basins in which ample water had covered the land for several ten thousand years, so for instance in the Transdanubia Region, around Lake Balaton. Owing to this fact, the chemical change proceeded undisturbed with exceptional purity. The region was methodically explored on the initiative of Professor Lóczy, a fact worth noting since the accumulated humus substance was evidenced to be the purest, most uniform deposit of the world. Its depth reaches 8-10 meters in some places. This exceptional purity is why we can safely exploit this substance as the raw material for our preparation.

What is then humus chemically? In attempting to describe it we deal with a substance of unique and incomparable biological importance. This was that 'black earth' which the continental world of vegetable life could take root in as a result of biological processes.

To say some words of the biological relevance of peat: besides its colour, characteristic behaviour and the utilisation of substances extracted from it, peat is well known for farmers and agricultural experts for its effects accelerating germination and improving rhizomic growth. This is attributed to its property of promoting oxygen transport and accelerating respiration.

A wealth of knowledge has accumulated of the ways it affects the growth of algae, intensifies enzyme functions, protein synthesis and processes of fermentation, but also concerning its effects on domestic animals (dogs, rabbits, etc.). Hungarian veterinarians and researchers had a fair share in exploring peat treatment and evidenced its effects on promoting the weight

gain and improving the egg yield of poultry. Owing to its disinfectant effect, peat has even been used as an ingredient of cosmetics. Though human aspects are less often mentioned in the reports, its use in balneo-therapy (mud packing) is well known, and so are the related rheumatological and other practical observations.

The peat used for the manufacture of HUMET_g-R contains calcium humate mixed with shell remnants and calcareous sludge, some sand and minerals. It is of a homogenous fen type (Table 5.1), geologically young and slightly basic (pH 7-8), with an ash content of 28-43% (Table 5.2). Table 5.3 (taking the 105 g/kg amino acid content of the peptides as 100%) shows the particular amino acid composition in this peat that contains several of the essential amino acids as well.

Raw humic acids extracted from peats, lignites and brown coals can be characterised by the Haworth scheme. As with biopolymers in general, it is very difficult to determine the accurate chemical composition of humic acids. According to a view commonly accepted in literature, each and every molecule in a given humic acid fraction is most likely to have a different structure, yet the samples contain functional groups of similar types and in a similar number.

Table 5.1: The composition of the peat vehicle.

Component	% of total weight
Organic substance	55-70
Carbon	20-39
Hydrogen	3-4
Nitrogen	2
Total protein content	10.5

Table 5.2: The ash analysis of the peat vehicle.

Elements	% of total ash weight
Ca, Al, Si, Fe, Mg	10-18
Na and B	1-10
Ba, Li, Ti, Mn, Cu, Ni, K	0.1-1
Pb, Mo, Be, Zn	0.001-0.0001

The Haworth scheme illustrates that diverse polypeptides, phenol carbonic acids are bound to the polynuclear heteroaromatic nucleus characteristic of humic acids, so their structure is most variable even in pure form. A combination of these structures provides the most important functional groups (-COOH, phenolic -OH, =NH, -NH₂, =C=O). Besides di- and trihydroxy-phenol rings, also a quinonoidal structure is detectable in the nuclear structure. Also carbohydrate type compounds are connected to the nucleus. The average elemental composition is: 54% of C, 37% of O, 4% of N and 5% of H.

Table 5.3: The specific distribution of amino acids.

Amino acid	% of total	Amino acid	% of total
Aspartate	16,9	Isoleucin	5,2
Glutamate	13,1	Lysine	4,5
Glycin	10,4	Prolin	3,9
Alanin	8,4	Arginin	3,3
Valin	7,8	Phenylalanin	2,9
Treonin	7,1	Histidin	2,0
Leucin	6,1	Methionin	1,9
Serin	5,2	Tyrosine	1,3

At several sites and with different strength, all the metals are bound through multiple chelate bonds to the polypeptides and phenol carbonic acids connected to the heteroaromatic nucleus. The important fact is that the metals lose their ionic nature in this kind of bond. The carboxyl group has an obvious role in binding the metals to the amino acids: due to the $-COOH$ functions, the non-chelate links represent one third of the total cation exchanging capacity. This implies then a property of quick metal exchange by ionic dissociation as well.

The peculiar metal binding and metal ion exchanging capacity of humic acids arises from the simultaneous presence of these functional groups. The fact that masking them by methylation and acetylation sharply reduces the extent of metal binding confirms the same.

In evaluating the metal binding and releasing capacity of humic acids it is very important to know that any chelate-bond cation basically modifies the dissociation constant (binding stability) of the other metal linkages. So, for instance, when some alkali metal (K, Na) is bound by the previously empty functional groups, then the chelate bonds of Fe and Al rupture easier than when the humic acid molecule contains some alkali earth metal (e.g. Ca). This is why vegetation suffers from micro-element deficiency in the presence of Ca-humate, a characteristic constituent of basic fen soils, albeit the needed elements abound in the humus.

The dissociation constants for the chelated bivalent metals vary over a very broad range depending on the molecular composition of the humic acids. Certain molecules of a humic acid fraction can bind a given metal, say, Zn^{2+} , very strongly, practically irreversibly, while another would fix or release its zinc content much easier.

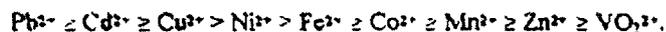
In this way, one finds an infinite number of intermediate values for the dissociation constants with smooth, almost continuous transitions between the two extremes.

If the free metal binding capacity of the humic acids is high, they will, so to say, "extract" the metals from their environment. On the other hand, with a sufficiently high concentration of a metal humate, the humic acid will surely trade off this metal to such (e.g. protein type) molecules of its environment that are able to bind it. This explains why the bioavailability of metallo-humo-

chelates is much better than of the same metals in the ionic forms (high-charge ions are retained by the cell membranes), and why humic acid molecules with a high free capacity of metal binding will remove these elements from the carrier type metallo-proteins.

An important data to the interpretation of metal ion exchange processes in the living organism is that humic acids of a small molecular mass have a metal binding capacity 2 to 6 times higher than the share of the large molecules of the same sample, further that 3 to 5 times more bivalent ions become bound by them than trivalent ones. Compared to bivalent transition metals, the binding capacity is smaller for Ca^{2+} , and still less for Mg^{2+} .

Concerning the cation exchanging capacity for bivalent metals the following sequence was found:



Molecular size of the humic acids is markedly influenced by the pH of the solution. At a lower pH the aggregates are larger, but these will break up into smaller molecules as pH rises. Univalent ions also affect aggregation. So for instance, molecular mass will increase to its 15-fold if 0.2 M NaCl solution is added to a humic acid fraction of approximately 5000 Dalton at pH 4.5.

A summary of some biological effects of humic acids:

- a) Their *antitoxic effect* has been utilised in both medicine and agriculture (Lotosch 1991);
- b) *applied as a bath* in rat experiments, they reduced the number of cheloids caused by surgical interventions (Mesroghĭ et al. 1991);
- c) water containing humic acids drawn from wells in a peat field or the water of peaty fens *normalise trace element deficiencies and have a haematopotetic effect* (Kucera and Ströber 1969);
- d) humic acids *reduce the toxic effect of heavy metals* in unicellular algae (Shanmukhappa and Noelakantan 1990);
- e) physiologically also the human body contains humic acids (Klöcking et al. 1978);
- f) in addition, humic acids *are surface-active and good adsorbents*;
- g) they are present in roasted coffee (Aurich et al. 1967, Klöcking et al. 1967).

5.2 Exorption inducing faculty in radio-caesium contaminated rats
Iános Hideg, MD., PhD., et al., Research Inst. of Public Health and Military Medicine of the Hungarian Army, Department of Toxicological Research

The aim of the experiments was to study the biological effect of the humic acid preparation HUMET_g-R, (HORIZON-MULTIPLAN Ltd., Budapest) in a form that contained no added trace metals.

The experiments were performed in randomised groups of female Wistar rats of 190-200 g body weight. The animals received tap water and either

untreated standard laboratory rat fodder (LATI, Gödöllő) or the same enriched by humic acids.

The humic acid preparation was mixed with ground fodder to produce a dose of 240 mg/animal (about 1200 mg/kg b.w.). The mixture was homogenised and press granulated, then dried at room temperature (20-22 °C). Daily consumption was determined by group measurement (3 to 5 animals/cage).

Through a gastric tube, the animals were given a single dose of ¹³⁷cesium chloride (Amersham, England) of 74-111 kBq (2-3 µCi) activity dissolved in 0.5 ml distilled water. The radioactivity of the cesium administered was determined by an NK-370 model energy selective spectrometer. Measurements were always compared to a standard of an activity identical with the isotope administered. Whole-body measurements were performed daily during the first week, then two to three times a week. Time changes in the whole-body radioactivity of the rats were followed for 24 days. Retention values were determined separately for each test animal, then the data so obtained were averaged for each group. Biological half time of radio-caesium was estimated by a double exponential plot of best fit to the measurements.

Test animals consumed humic acid-added fodder as a pre-treatment for 7 days, then for another 25 days after the administration of radio-caesium, i.e. for 32 days altogether. Colloidal Prussian blue was used as the reference compound for the exsorption inducing faculty.

The haematological (erythrocyte, white blood cell and platelet counts, haemoglobin, haematocrit, mean corpuscular volume, haemoglobin content and haemoglobin concentration) and clinical laboratory parameters (blood glucose and urea nitrogen concentrations, activities of glutamic oxaloacetate and pyruvate transaminases, hydroxy-butyrate dehydrogenase, lactate dehydrogenase, creatine kinase, gamma-glutamyl transpeptidase, alkaline phosphatase and amylase, serum total protein content, albumin, bilirubin, creatinine, ionised calcium, phosphorus, cholesterol, triglycerides and uric acid levels) were compared both at the start and end of the study.

In the course of the several weeks long experiment, the general physical condition (hair, weight gain, food intake, agility, etc.) of the animals treated with 1200 mg/kg b.w. (i.e. 600 times the human dose of 2 mg/kg b.w.) of the humic acid preparation showed no difference from the controls. The comparison of the internal organs of animals sacrificed at the end of the experiment evidenced that neither of the treatments did cause any change in liver, kidney and spleen weights or in the macroscopic appearance of these organs. No pathological alterations were seen in any of the organs. White blood cell and platelet counts were unaffected by the prolonged (32 day) treatment with the humic acid preparation. *On Days 1-5 after the isotope administration, radio-caesium retention of the animals subjected to humic acid pre-treatment was by 20-25% less than in the controls.*

- 5.3 The effect of humic acids on the rat subjected to whole-body irradiation
János Hideg, MD, PhD, et al, Research Inst. of Public Health and Military Medicine of the Hungarian Army, Department of Toxicological Research

The aim of the experiment was to study the radio-protective effect of the metal carrier humic acid preparation (serving as an active substance of

HUMET₂-R, (HORIZON-MULTIPLAN Ltd., Budapest) in a form that contained no added trace metals. The experiment was performed in randomised groups of female Wistar rats of 250 g mean weight. The animals were given tap water and standard rat fodder (IATL, Gödöllő).

Humic acid preparation was administered in a dose of 240 mg/animal (about 960 mg/kg b.w.) through a gastric tube (2 ml/animal).

By using plastic irradiation stocks (4 animals/stocks), whole-body irradiation (employing a dose of 7.0 Gy and a dose rate of 0.653 Gy/min.) was carried out in the Siemens-Gammatron-3 model gamma irradiator of the OSSKI (National Research Institute of Radiation Biology and Radiation Medicine, Budapest).

At the start and end of the study, haematological data (white blood cell, erythrocyte and platelet counts, total iron binding capacity) were determined from blood sampled from the abdominal aorta under ether anaesthesia.

The results of the pilot study (the mean of 3 rats used for each measurement time) showed that platelet count, which had markedly decreased after irradiation, began to normalise one week earlier than in the untreated controls, even when only a single dose of the humic acid preparation was used.

II.B. Control of the finished product

Quality description of the product

1. Properties

- 1.1 Characteristics: A brown suspension with typical sourish fruity odour and taste. The suspension can be mixed with water in every proportion, its colour remains dark, brownish even after a tenfold dilution.

2. Drug test

- 2.1. Filling volume: 300 ml \pm 3%
Test: according to Ph.Hg. VII.1.425.o. K/g. 3.2.1., on the base of which in this case I.419./K/g. 1.2.1. is applicable.

For the volume control calibrated jars or cylinders with ml graduation are used.

Acceptable limits: 300 ml \pm 3% i.e. from 291 to 309 ml.

2.2. Mechanical impurities

The suspension should not contain any macroscopically detectable extraneous particles. Test: according to Ph.Hg. VII.1.425.o. K/g. 3.2.2.

"Approx. 10 ml of the well shaken-up and homogeneous suspension is to be tested in a test tube, at appropriate illumination, against a black- white background, in incident and transmitted light. For the detection of extraneous particles in case of necessity a manual eyeglass with fourfold magnification may be used as well"

- 2.3 Grain size
None of the linear dimensions of the suspended grains in the suspension should exceed 200 micrometer.
Test: according to Ph.Hg. VIII.425.o. K/g. 3.2.3.
"A 0.10 g sample of the well shaken-up suspension is to be smeared into a thin, even layer on an object slide, by means of another slide. In four different sections of the layer we measure the largest linear dimension of 25 neighbouring particles of the dispersed substance under a microscope."
During the test we show the largest, smallest and the average size, as well as the occurrence in %.
- 2.4 Homogeneity, dispergating capacity
The particles sedimented during storage should be homogeneously re-dispergated by way of shaking
Test: according to Ph.Hg. VII.L425.o. K/g. 3.2.4.
From the suspension, after shaking it vigorously 8-10 times, we pour 10 ml into a test tube with grinded glass-stopper. The content of the test tube is to be shaken up repeatedly, three times. The suspension liquid should seem homogenous during 60 seconds, when being tested macroscopically.
- 2.5 pH: 3.0-3.5
Test: with litmus paper or with Radelkis pH/ION ANALYSER (OP- 274)

3. Tests

- 3.1. Composition test
- 3.1.1. Detection of carbohydrate content
After carefully heating 2-3 ml of sample in a metal spoon or a porcelain skillet, a caramel formation melt with the odour of burnt sugar will appear, which after further heating will carbonise.
- 3.1.2. Detection of humic acid - metal chelate content
Approx. 10 ml of homogenised sample we dilute with approx. 30 ml of distilled water, then mix it with 10 ml 2N HCl solution and boil. The appearing fall-out is, after filtering and rinsing, humic acid metal chelate, free from accompanying substances. The fall-out is soluble in 2N NaOH.
- 3.2. Purity test
- 3.2.1. Microbiological purity
To the product apply the prescriptions concerning the preparations which belong to Ph.Hg.VII III. purity class.
Test: according to Ph.Hg.VII. I.307.0.F.2.
Table F/1, purity class III.
Requirement: Max.: 1000 micro-organisms/ml;
among them: max. 100 fungi/ml
-

Not allowed: Enterobacteriaceae, Pseudomonas aeruginosa
 Staphylococcus aureus

(The requirements apply to the preparation, basic and auxiliary materials and intermediates).

Microbiological purity test of not sterile drugs (Ph.Hg.VII. I.F.4.)

The preparation should not contain pathogens detrimental to health, nor other non-pathogenic, so-called indicator micro-organisms indicating hygienic insufficiencies during production, storage etc.

(Appendix 4.: Commission contract to carry out microbiological tests;
Appendix 5.: Microbiological protocol)

Preparation of the tests, taking of samples:

For the tests should be taken a quantity corresponding to 3-4 g of sample. In order to avoid external contamination, the sample should be taken according to the general rules of aseptic procedure, with sterilised tools, and be put in sterilised container. The preparation of the samples and the tests should be carried out under aseptic conditions, also with sterilised tools. It is necessary to be convinced of the sterility of the culture-media as well.

Control of the inhibitory effect:

For the evaluation of the results of the microbiological purity tests it is necessary to control previously, whether the substance to be tested has any anti-microbial effect, and if so, to which extent, and in regard of which micro-organism species. To the test of the inhibitory effect the following tribes should be used:

Escherichia coli
Staphylococcus aureus
Pseudomonas aeruginosa
Bacillus subtilis
Candida albicans
Aspergillus niger

For the control test we measure in at least 3 parallel series into sterile Petri dishes 1 ml of test sample diluted in 1:10 and 1:100 proportion, adding 1.00 ml micro-organism suspension, while using fresh 24-hour bacterium culture and 72-hour fungi culture. Into the same quantity of sterile Petri dishes we measure for each control 1.00 ml diluting solution No.1. (Ph.Hg.VII. I.F.4.7.1.) and 1.00 ml micro-organism suspension. Then, by using the corresponding culture-medium (culture-medium 2. and 3., Ph Hg VII. I.322./F.4.7.) we pour out a plate (L. 319./F.4.5.1.), and incubate the plates for 5 days at the prescribed temperature. There is no inhibitory effect, if the observed quantity of micro-organisms in the substance to be tested and in the culture-media does not differ substantially (by more than 25%). In case of an inhibitory effect adequate inactivating agents should be used.

Determination of the total number of micro-organisms

F.4.5.1. Pouring of plates

From the properly prepared sample we measure 1.00 ml substance and, adding to it thinning solution No. 1 or if needed, also emulsifying agent, we prepare a 10-scale dilution sequence, if necessary, even up to a 1:10000 proportion.

From the dilutions we measure into 3 Petri dishes 1.00 ml each, and from the culture-media No. 2. and 3., melted and cooled down to 45 °C, we pour plates. We place the hardened plates for 5 days in a thermostat with a temperature range of +0 -32 °C. The culture media for the testing of fungi we place for 5 days in a thermostat with a temperature range of 22-25 °C. After the incubation we count the appearing colonies. In each dilution sequence it is necessary to prepare also control samples which do not contain the substance to be tested, in which no growth should be observed after the incubation.

F.4.5.2. Membrane filtering

1.00 ml of the properly prepared sample is diluted in adequate proportion with sterile thinning solution No. 1., then the solution will be filtered through 2 filtering plates with 0.45 µm pore size. After filtering, the filtering plates are washed at least 3 times with 100 ml sterile thinning solution, then the filtering plates are placed on adequate culture-medium and incubated during 5 days, one at a temperature of 30-32 °C, the other at 22-25 °C. After the incubation we count the colonies grown on the filtering plates.

F.4.5.3. Evaluation

We take the arithmetical mean of the number of colonies on the parallel plates which may be evaluated, and the results are shown related to 1.00 ml substance. The total number of micro-organisms equals to the sum of all aerobic bacteria and fungi (Appendix 2.)

Special test for the detection of pathogens

For the test we measure 1.00 ml sample into thickeners prescribed for the given test, directly, (or, in case of inhibitory effect in the necessary dilution), then, after incubation in the thickener, we smear it on an elective culture-medium. The volume of the thickener usually exceeds 100 times that of the sample.

F.4.6. Special test for the detection of pathogens

For the tests we measure usually 1.00 g or 1.00 ml properly prepared average sample [I.318./F.4.3.] into thickeners prescribed for the given test, directly, (or, in case of inhibitory effect in the necessary dilution), then, after incubation in the thickener, we smear it on an elective culture-medium. The volume of the thickener usually exceeds 100 times that of the sample.

F.4.6.1 Test for the detection of the members of the Enterobacteriaceae family

1.00 ml of the properly prepared sample [I.318./F.4.3.] is measured in 100 ml thickener No. 4. (lactose thickener) and incubated for 24 hours at a temperature of 37 °C. After incubation we smear 0.10 ml of the lactose thickener containing the sample on the surface of the culture-medium No. 5.

(lactose-indicator agar), and place for 24 hours in a 37 °C thermostat. During incubation no colonies should appear, which are characteristic to the members of the Enterobacteriaceae family.

In case of suspicious colonies we inoculate parts of the individual colonies on culture-medium No. 6. (eosin-methylene blue agar) or No 7. (Endo-agar). After incubation (24h at 37 °C) there should be on eosin-methylene blue or Endo-agar colonies consisting of characteristic Gram-negative bacteria which, after being placed on culture-medium No. 19. (iron(II)-triple-sugar-agar) and incubated once more (24h at 37 °C), might show no gas or gas-and-acid formation.

F.4.6.2. Test for the detection of *Pseudomonas aeruginosa*

1 ml of the properly prepared sample [L318./F.4.3.] is measured in 100 ml culture-medium No. 8. (glucose thickener) and incubated for 24 hours at a temperature of 37 °C. This glucose thickener is used also for test [4 6.3.].

After incubation we smear 0.10 ml of the thickener containing the sample on the surface of culture-medium No. 9. (cetrimide agar) and No 11. (TTC-agar), and place this for 24 hours in a 37 °C thermostat. If no colony formation, or only slowly growing colonies are observed, the plates are to be incubated for further 24 hours. On the cetrimide culture-medium there should not be any greenish colonies producing fluorescent pigments, and on the TTC-agar there should be no red colonies. If such colonies develop on any of the plates, they have to be placed, in order of further verification, on culture-medium No. 10 (blood-agar) and incubated for 24 hours at 37 °C, then we shall carry out the so-called oxidase test.

Oxidase test. We drop reagent No. 20. on a stripe of filtering paper placed on an object slide and smear onto it the colony grown on blood-agar culture-medium with the edge of a slide. The reaction is positive if in 20 seconds red coloration is observed. For the test may also be used indicator papers specially produced for this purpose, available in the shops. They can be evaluated according to the enclosed instructions.

On the base of the performed test series, the tested drug samples should not contain oxidase-positive rods with colony morphological or eventual pigment formation characteristics as described above.

Test for the detection of *Staphylococcus aureus*

After incubation we smear 0.10 ml glucose thickener [L320./F.4.6.2.] containing the properly prepared sample on the surface of culture-medium No. 12. (mannitol-salt agar), and place the plate for 24 hours in a 37 °C thermostat.

On the culture-medium no colonies should develop which dye the medium yellowish. In case of appearance of such colonies they have to be inoculated onto culture medium No. 10. (blood-agar) and No. 13. (Baird-Parker) or No. 14 (Vogel-Johnson) and the plates have to be incubated for 24 hours at 37 °C. On the elective culture media no bright black colonies should develop, nor should any haemolizing colonies appear on the blood-agar.

In case of suspicion coagulase test and Gram staining are also to be carried out

Coagulase test. Into the plasma (reagent No. 21.) dropped on the object slide we mix a tendril of colony from one of the elective culture-media (N. 13. or 14.). Neither the plasma nor the colony should be cooler than room temperature. The reaction is positive if the colony cannot be evenly mixed, while a sudden coagulation takes place. During the test a positive control is to be carried out as well. In the substance to be tested there should be no coagulase-positive or Gram-positive cocci.

Culture-media and reagents

The solutions, culture-media and reagents necessary for the tests may only be made from "analytically purest" materials or of pharmacopoeia quality, or from those produced for bacteriological purposes.

The solid substances necessary for the various culture-media are usually dissolved either in distilled water or meat extract of prescribed quantity, by heating. The solutions are brought to the boil but are not boiled. It is advisable to swell the prescribed agar-agar previously in a small quantity of distilled water or meat extract. The pH value of the solutions is to be set, by using 0.1 M sodium hydroxide solution or 0.1 M hydrochloric acid solution, in a way that the required pH value after sterilisation should remain between the given prescribed limits. The still warm culture-media have to be always filtered; the agar-agar through gelatine containing cotton-wool and the other ones through filtering-paper. The indicator paints are usually introduced in the culture-medium after solving in a small quantity of the already prepared solution. The way of preparation of culture-media requiring special procedure is shown in the instructions concerning such culture-media.

For the purpose of the tests we can also use controlled, standard powdered culture-media with composition required by the corresponding prescriptions and with electivity appropriate to the purpose of the test.

F.4.7.1. Thinning solution No.1. (attenuating phosphate solution)

The pH value of the solution after sterilisation: pH = ± 0.2

Composition:

potassium dihydrogen phosphate	0.58 g
disodium hydrogen phosphate (2H ₂ O)	1.02 g
sodium chloride	9.00 g
distilled water	ad 1000.0 ml

Sterilising: at 121 °C, for 20 minutes.

F.4.7.2. Culture-medium No. 2. (for the determination of total number of all aerobic bacteria).

The pH value of culture-medium after sterilisation: pH = 7.2±0.2

Composition:

casein digested by trypsin	10.00 g
sodium chloride	3.00 g
glucose (counted as dehydrated)	1.00 g
dipotassium hydrogen phosphate	2.00 g
agar-agar (powdered)	15.00 g
meat extract [L312./F.3.7.1.]	ad 1000.0 ml

Sterilising: at 121 °C, for 15 minutes.

F.4.7.3. Culture-medium No. 3. (modified Sabouraud medium for the determination of number of fungi).

The pH value of culture-medium after sterilisation: pH = 5.7±0.2

Composition:

glucose (counted as dehydrated)	40.00 g
casein digested by trypsin	10.00 g
amphenicol chloride	40.00 mg
agar-agar (powdered)	15.00 g
distilled water	ad 1000.0 ml

Sterilising: at 121 °C, for 15 minutes

F.4.7.4. Thickener No. 4. (for testing of Enterobacteriaceae tribes).

The pH value of culture-medium after sterilisation: pH = 7.3±0.2

Composition:

casein digested by trypsin	10.00 g
lactose	5.00 g
disodium hydrogen phosphate (2H ₂ O)	8.00 g
potassium dihydrogen phosphate	2.00 g
ox bile extract	20.00 g
brilliant green	15.0 mg
distilled water	ad 1000.0 ml

Sterilising: for 30 minutes in streaming vapour or in autoclave at 121 °C, for 10 minutes.

F.4.7.5. Culture-medium No. 5. (for the detection of Enterobacteriaceae tribes)
The pH value of culture-medium after sterilisation: pH = 7.4±0.1

Composition:

yeast extract (powdered)	3.00 g
casein digested by trypsin	7.00 g
sodium chloride	5.00 g
ox bile extract	5.00 g
lactose	10.00 g
agar-agar (powdered)	15.00 g
neutral red	30.0 g
crystal violet	2.0 mg
distilled water	ad 1000.0 ml

Sterilising: at 121 °C for 15 minutes

F.4.7.6. Culture medium No. 6. (eosin-methylene blue-agar for the detection of Enterobacteriaceae tribes)
The pH value of culture-medium after sterilisation: pH = 7.0±0.1

Composition:

peptone	10.00 g
lactose	10.00 g
dipotassium hydrogen phosphate	3.00 g
eosin	0.40 g
agar-agar (powdered)	15.0 g
methylene blue	65.0 mg
distilled water	ad 1000.0 ml

Sterilising: at 121 °C for 15 minutes.

F.4.7.7. Culture-medium No. 7. (for the detection of Endo-agar Enterobacteriaceae tribes)
The pH value of culture-medium after sterilisation: pH = 7.5±0.1

Composition:

peptone	10.00 g
lactose	10.00 g
dipotassium hydrogen phosphate	3.50 g
sodium sulphite (7H ₂ O)	2.50 g
fuchsin	0.40 g
agar-agar (powdered)	15.00 g
distilled water	ad 1000.0 ml

Sterilising: at 121 °C for 15 minutes.

The prepared culture-medium is to be stored protected from light!

F.4.7.8. Thickener No. 8. (liquid thickener for the detection of *Pseudomonas* and *Staphylococcus tribes. pre-thickener for that of Salmonella tribes*)
The pH value of culture-medium after sterilisation: pH = 7.2±0.2

Composition:

casein digested by trypsin	10.00 g
sodium chloride	3.00 g
dipotassium hydrogen phosphate	2.00 g
glucose (counted as dehydrated)	1.00 g
meat extract [L312./F.3.7.1.]	ad 1000.0 ml

Sterilising: at 121 °C for 15 minutes.

F.4.7.9. Culture-medium No. 9. (for the detection of Centrimid-agar *Pseudomonas*)
The pH value of culture-medium after sterilisation: pH = 7.2±0.1

Composition:

peptone	20.00 g
mercuric(II) chloride	1.40 g
potassium sulphate	10.00 g
N-cetyl-N,N,N-trimethyl-ammonium-bromide (Cetrimide)	0.30 g
agar-agar (powdered)	15.00 g
glycerine	10.00 ml
distilled water	ad 1000.0 ml

Sterilising: at 121 °C for 15 minutes.

F.4.7.10. Culture medium No. 10. (blood-agar)
The pH value of culture-medium after sterilisation: pH = 7.3±0.1

Composition:

(basic culture-medium)	7.00 g
powdered meat	5.00 g
peptone	5.00 g
sodium chloride	5.00 g
agar-agar (powdered)	15.00 g
meat extract [L312./F.3.7.1.]	ad 1000.0 ml

We sterilise the basic culture-medium for 20 minutes at a temperature of 121 °C, than we mix into the basic culture-medium, cooled down to 45-50 °C, 7% of defibrinated horse or cattle blood. The poured plates have to be stored for 24 hours in a thermostat, and then have to be tested.

F.4.7.11 Culture-medium No. 11. (for the detection of TTC-agar *Pseudomonas*)
The pH value of culture-medium after sterilisation: pH = 7.4±0.1

Composition:

protease peptone	10.00 g
sodium chloride	3.00 g
dipotassium hydrogen phosphate	2.00 g
agar-agar (powdered)	15.00 g
meat extract [L312./F.3.7.1.]	ad 1000.0 ml

Sterilising: at 121 °C for 20 minutes.

Into the culture-medium cooled down to 45-50 °C we mix, in aseptic conditions, 100 ml of freshly prepared, sterile filtered 1% 2,3,5-triphenyl-tetrazolium-chloride solution.

F.4.7.12. Culture-medium No. 12. (for the detection of mannitol-salty-agar *Staphylococcus*)
The pH value of culture-medium after sterilisation: pH = 7.5±0.1

Composition:

casein digested by trypsin	10.00 g
D-mannitol	10.00 g
sodium chloride	75.00 g
agar-agar (powdered)	15.00 g
phenol red	25.00 mg
meat extract [L312./F.3.7.1.]	ad 1000.0 ml

Sterilising: at 121 °C for 15 minutes.

F.4.7.13. Culture medium No. 13. (Baird-Parker-agar for the detection of *Staphylococcus*)
The pH value of culture-medium after sterilisation: pH = 7.1±0.1

Composition:

casein digested by trypsin	10.00 g
powdered meat	2.00 g
yeast extract (powdered)	1.00 g
lithium chloride	5.00 g
agar-agar (powdered)	15.00 g
glycerine	12.00 g
sodium piruvate	10.00 g
meat extract [L312./F.3.7.1.]	ad 1000.0 g

Sterilising: at 121 °C for 15 minutes.

To the sterilised and cooled basic culture-medium it is necessary to add, under aseptic circumstances, 50 ml yolk emulsion and 10 ml sterile filtered 1% potassium telluride solution, then to mix this carefully and pour plates.

F.4.7.14. Culture-medium No. 14. (Vogel-Johnson-agar for the detection of Staphylococcus)

The pH value of culture-medium after sterilisation: pH = 7.2±0.2

Composition:

casein digested by trypsin	10.00 g
yeast extract (powdered)	5.00 g
D-mannitol	10.00 g
dipotassium hydrogen phosphate	5.00 g
glycerine	10.00 g
lithium chloride	5.00 g
agar-agar (powdered)	13.00 g
phenol red	25.0 mg
distilled water	ad 1000.0 ml

The solution is to be boiled for one minute and sterilised for 15 minutes at 121 °C, then cooled down to 45-50 °C, and 20.00 ml sterile filtered 1% potassium telluride solution will be added to it under aseptic circumstances.

F.4.7.20. Oxidase reagent No. 20. (for the verification of Pseudomonas tribes)

The reagent is a 1% aqueous solution of N,N-dimethyl-1,4-phenylene-diammonium-dichloride ($C_6H_{12}N_2 \cdot 2HCl \cdot M_r: 209.1$). Being kept in a refrigerator, it can be used upto 3 days.

F.4.7.21. Coagulant reagent No. 21. (for the test of Staphylococcus tribes)

To 1 volume part of sterile 3.8% trimetallic sodium citrate ($2H_2O$) solution we measure 4 volume parts of human blood, centrifuge this, then we add to 19.0 ml plasma 1.0 ml of 0.2% phenyl mercury (II) borate or 0.2% thiomersale solution. Instead of human blood rabbit plasma can be used as well, diluted in 1:2 or 1:3 proportion with isotonic sodium chloride solution and preserved as above.

3.2.2. Lead content: max.: 5 ppm

Test: according to Ph.Hg.VII. L189.o.C.7.1.1.

Previous destruction is necessary on the base of Ph.Hg.VII. I.182/C.1. or according to I.183/C.1.4.

We heat 5 ml sample (HUMET-R syrup) on water bath in an Erlenmeyer flask mixed with 5 ml cc HNO_3 , until the foaming stops. The sample is destructed in the mixture of 10 ml cc $P \cdot H_2SO_4$ and 20 ml R-50% HNO_3 in a destruction flask by heating. The sample is dosed slowly by 1 ml, in order to avoid the fall-out of carbon particles. Then we add 6 ml H_2O_2 and 6 ml R-30% HNO_3 , by turns, by 1 ml.

The superfluous sulphuric acid will be expelled by Bunsen flame heating, on small flame, taking care that the flame could not reach the glass above the surface of the liquid (sulphuric acid vapours will appear vigorously, then the dark brown mixture will fade into a yellowish-white colour).

The solution will be evaporated to 0.5 ml, then after cooling down diluted with 2.0 ml of water and neutralised to pH 7 with 5 M NaOH solution. Then

we wash it with equal quantity of bidistilled water in a test tube so that the volume of the solution would reach 15.0 ml. The solution prepared in such a way will be tested as to lead content.

Then we measure into one tube 1.00 ml of this solution and into the other one 1.00 ml of the lead measuring solution.

Both solutions will be acidated with 1 drop of 2M HCl, mixed with 1.0 ml freshly prepared 1% ascorbic acid solution and heated to the boil. The cooled down solutions will be filled up with water upto 12.0 ml and mixed with 1 ml M KCN solution, then with 2.0 ml 2M NaOH solution. The solutions will be shaken up, then we add to both solutions 5 drops of R-Na-sulphide solution and shake up the content of the test tubes again.

After 5 minutes we record the changes.

Result:

The observed change in the solution to be tested should be at most of such an extent, as it is in the lead measuring reference flask. Preparation of lead measuring solutions: Ph.Hg VII L597/R.4.1.

3.3. Determination of content

3.3.1. Metal content test

Prescription:

Zn	300 mg/300 ml	+0/-20%
Cu	60 mg/300 ml	+0/-20%
Fe	420 mg/300 ml	+0/-20%
Co	6 mg/300 ml	+0/-20%
Mn	90 mg/300 ml	+0/-20%

Test: Atom absorption analysis following wet destruction

Preparation of the sample

After complete homogenisation of HUMET-R containing syrup (after thorough shaking-up) we measure 50 cm³ sample (3 parallel samples) into a tall 50 cm³ heating glass.

We add 5 ml cc. HNO₃. We start heating carefully, because the reaction begins vigorously, and after stabilisation of the dissolving process we add 5 ml more cc HNO₃ and heat this covered with watch-glass for approx. 3-4 hours at 130-150 °C until the sugar and the organic substances get destructed. Then we carefully add 3.0 ml cc HClO₄ and evaporate the solution dry. The remaining perchlorine acid will be evaporated from the surface of the heating glass by small Bunsen flame. We add 20.0 ml 0.4 M Selektion-B2 solution with 9 pH and let this stand for 12 hours.

Finally we pour it together with Selektion B-2 solution into a 25 ml measuring flask upto the mark.

Preparation of Selektion B-2 solution:

148.8 g Selektion-B2 ad 1000 ml
To stabilise with NaOH at 9 pH.

This solution is called *stock solution "A"*.

Preparation of standards

We prepare 5 standards which contain:

- the metals to be measured,
- humic acid, K, Ca and syrup contained in the sample.

Composition of the standards:

Into 5 heating glasses we measure by pipette the following standard solutions:

Co standard

1.	2 ppm Co=	1 ml	from 100 ppm Co solution
2.	4 ppm Co=	2 "	"
3.	6 ppm Co=	3 "	"
4.	8 ppm Co=	4 "	"
5.	10 ppm Co=	5 "	"

Mn standard

1.	20 ppm Mn=	1 ml	from 1000 ppm Mn solution
2.	40 ppm Mn=	2 "	"
3.	60 ppm Mn=	3 "	"
4.	80 ppm Mn=	4 "	"
5.	100 ppm Mn=	5 "	"

Cu standard

1.	20 ppm Cu=	1 ml	from 1000 ppm Cu solution
2.	40 ppm Cu=	2 "	"
3.	60 ppm Cu=	3 "	"
4.	80 ppm Cu=	4 "	"
5.	100 ppm Cu=	5 "	"

Fe standard

1.	100 ppm Fe=	1 ml	from 5000 ppm Fe solution
2.	200 ppm Fe=	2 "	"
3.	300 ppm Fe=	3 "	"
4.	400 ppm Fe=	4 "	"
5.	500 ppm Fe=	5 "	"

Zn standard

1.	100 ppm Zn=	1 ml	from 5000 ppm Zn solution
2.	200 ppm Zn=	2 "	"
3.	300 ppm Zn=	3 "	"
4.	400 ppm Zn=	4 "	"
5.	500 ppm Zn=	5 "	"

To the standards we add:

- + 750 ppm K = 3.75 ml 10,000 ppm K
- + 300 ppm Ca = 1.5 ml 10,000 ppm Ca

The 5 composed standard solutions will be evaporated to 2-3 ml, then we add:

- + 1.25 ml 60 g/l humic acid
- + 5 ml syrup

The composed standards will be destructed under the same conditions as the samples and diluted in a twofold quantity of acids, then we dilute this in a

twofold quantity of Selektion-B2 and pour into a 50 ml measuring flask upto the mark.

The device to be applied

Two-ray atom absorption device of VARIAN SPECRA A-20 type.

Determination of Co:

The measuring is being carried out from stock solution "A" with 5-fold dilution, using the basic standard line, at 240.7 nm wave length and 0.2 nm slit width, in air-and-acetylene flame.

Determination of Cu:

We dilute the stock solution "A" and the basic standard line 20-fold with distilled water. The measuring is being carried out at 324.8 nm wave length and 0.5 nm slit width, in an air-and-acetylene flame.

Determination of Fe:

We dilute the stock solution "A" and the basic standard line 20-fold with distilled water. The measuring is being carried out at 248.3 nm wave length and 0.2 nm slit width, in an air-and-acetylene flame.

Determination of Mn:

We dilute the stock solution "A" and the basic standard line 20-fold with distilled water. The measuring is being carried out at 279.5 nm wave length and 0.2 nm slit width, in an air-and-acetylene flame.

Determination of Zn:

We dilute the stock solution "A" and the basic standard line 500-fold with distilled water. The measuring is being carried out at 213.9 nm wave length and 1.0 nm slit width, in an air-and-acetylene flame.

Final dilutions:

Co - 50 x
Cu - 100 x
Fe - 100 x
Mn - 100 x
Zn - 2500 x

Calculating of the result:

Metal ion concentration=

resulting pptn x dilution rate x original volume (300 ml)

1000

II.C. Stability

1. Packing unit:

300 ml/bottle/pack

2. Packing:

exterior: cardboard

interior: brown bottle, white plastic cap with guarantee seal

3. Storage conditions:

The product is to be kept at room temperature (15-20 °C), protected from light. After opening, the bottle should be kept well closed, in a dark, cool place (refrigerator).

In one cardboard box we place 20 bottles (packs) of HUMET preparation.

The collecting boxes are being kept in a dry, cool place, on standard loading platforms with joint straps.

4. Keeping time:

12 months counted from the date of production,
1 month from the opening.

Annex 1.



ORSZÁGOS GYÓGYSZERÉSZETI INTÉZET
1032 Budapest V., Zrínyi u. 3
Levél cím: 1372 Postafiók 450.
Telefon: 117-1488
Telefax: 118-1187

Budapest, 19 95. szeptember 20.
Szám: 7419/51/95
Előadó: Szoke Krisztina
Melléklet: -
Tárgy: Nyilvántartási adatok
módosítása

A 10/1987.(VIII.19.) Eü. Min. rendeletben kapott felhatalmazás alapján
a következő határozatot hozom.

Az alábbi, gyógyszernek nem minősülő gyógyhatású készítmény (gyógytermék) eredetileg
8575/50/93 számon kiadott és 6245/51/94, 10008/51/94 számokon módosított
"nyilvántartásba vételi igazolásának" I. sz. mellékletét az alábbiak szerint
módosítom.

Gyógytermék neve: H U M E T - R S Z I R U P

Nyilvántartási száma: O G Y I - 4 3 0 / 1 9 9 3

Forgalomba hozatali engedély száma: 6 8 0 8 / 5 1 / 9 4

A gyógyszerék módosított nyilvántartási adatai a készítmény
lejáratii idejére vonatkoznak.

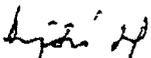
A módosított adatokat lásd alább:

ad 8575/50/93 sz. határozat:

Lejáratii ido: 12 hónap,
(felbontás után 1 hónap)

Indoklás: Az Intézethez beérkezett dokumentációk alapján a fenti változás
szakmailag megalapozott.

A jelen határozat ellen kézhez vételtől számított 15 napon belül,
a Népjóléti Minisztériumnak címezve, de hozzánk benyújtva fellebbezéssel élhet.


Dr. Pál Tamás
főigazgató

NATIONAL INSTITUTE OF PHARMACY Budapest, September 20, 1995
1051 Budapest, V., Zrínyi u 3. No.: 7419/51/95
Mailing address: 1372 P.O Box 450 Our Clerk: Krisztina Szöke
Phone: 117-1488 Annexes: -
Fax: 118-1167 Re.: Modification of registration data

Authorized by Order No. 10/1987 (VIII. 19)EÜ. Min. issued by the
Minister of Health, herewith I make the following **d e c i s i o n** :

'Decision on Registration' of the paramedicinal product below, originally
issued under No. 8575/50/93, and amended under Nos. 6245/51/94,
10008/51/94, is modified as follows:

Name of paramedicinal product: HUMET-R Syrup

Registration No.: OGYI-430/1993

No. of Distribution Licence: 6808/51/94

The **a m e n d e d** registration data of the paramedicinal product apply
to the **s h e l f l i f e** of the product.

For amended data, see below:

to Decision No. 8575/93:

Shelf life: 12 months
(1 month after the product is opened up)

Reasoning: on the base of documentation submitted to the Institute, the
above modification is professionally well-grounded.

Appeals against the above decision may be executed within 15 days as
from the delivery, as addressed to the Ministry of Public Welfare, but
submitted to us.

Stamp, Signature
Tamás Paál, M.D.
General Director