European Directorate for the
Quality of Medicines & HealthCare

COUNCIL OF EUROPE CONSEIL DE L'EUROPE

Certification of Substances Division

Certificate of suitability
No. R1-CEP 2001-385-Rev 04

Name of the substance:
SODIUM HYALURONATE
Intrinsic viscosity : not less than 1.6 m^3/kg, by fermentation, for parenteral administration including intra-articular use and intra-ocular use

Name of holder:
HTL S.A.S.
La Boitardière
Z. I. De L' Aumaillerie
France-35133 Javène

Site(s) of production:
HTL S.A.S.
La Boitardière
Z. I. De L' Aumaillerie
France-35133 Javène

THIS CERTIFICATE SUPERSEDES THE PREVIOUS CERTIFICATE
R1-CEP 2001-385-REV 03

After examination of the information provided on the manufacturing method and subsequent processes (including purification) for this substance on the site(s) of production mentioned above, we certify that the quality of the substance is suitably controlled by the current version of the monograph **SODIUM HYALURONATE** no. 1472 of the European Pharmacopoeia, current edition including supplements, only if it is supplemented by the test(s) mentioned below, based on the analytical procedure(s) given in annex.

- Test for residual solvents by gas chromatography (Annex 1)
  Ethanol not more than 5000 ppm

- Test for phosphorous by spectrophotometry (Annex 2)
  Phosphorous not more than 3000 ppm

- Test for aluminium by ICP-AES (Annex 3)
  Aluminium not more than 20 ppm

- Test for calcium and magnesium by AAS (Annex 4)
  Calcium not more than 500 ppm
  Magnesium not more than 100 ppm

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The holder of the certificate has declared the absence of use of material of human or animal origin in the manufacture of the substance.

Compliance with the statements of the Production Section of the monograph is to be considered in the context of a medicinal product containing this substance.

The submitted dossier must be updated after any significant change that may alter the quality, safety or efficacy of the substance.

Manufacture of the substance shall take place in accordance with the Good Manufacturing Practice and in accordance with the dossier submitted.

Failure to comply with these provisions will render this certificate void.

This certificate is renewed from **3 March 2008** according to the provisions of Resolution AP-CSP (93) 5 as amended, and of Directive 2001/83/EC and Directive 2001/82/EC and any subsequent amendment, and the related guidelines.

This certificate has four annexes, the first and the second of 1 page each, the third and the fourth of 2 pages each.
This certificate has:
lines.

On behalf of the
Director of EDQM

Strasbourg, 24 September 2012

DECLARATION OF ACCESS *(to be filled in by the certificate holder under their own responsibility)*

HTL S.A.S., as holder of the certificate of suitability

R1-CEP 2001-385-Rev 04 for SODIUM HYALURONATE

hereby authorises ....URSAPHARM Arzneimittel GmbH.....
(name of the pharmaceutical company)

to use the above-mentioned certificate of suitability in support of their application(s) for the following Marketing Authorisation(s): *(name of product(s) and marketing number(s), if known)*

ANY RELEVANT PRODUCT

The holder also certifies that no significant changes to the operations as described in the CEP dossier have been made since the granting of this version of the certificate.

Date and Signature *(of the CEP holder)*:

Marc DAVID 12/10/2012

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SAS au Capital de 75 000 €uros
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Residual solvents « Standard Operation Procedure »
Current European Pharmacopoeia, 2.2.28. (Gas Chromatography)
Current USP Adapted <467>.
Internal SOP : CI-LAB-032.

1.1 - Equipment and experimental conditions
. Gas chromatograph GC 3900 (Varian)
. Column: CP-SELECT 624 CB DF = 1.8 WCOT FUSED SILICA 30 m x 0.32 mm
. Vector Gas: Helium (Alphagaz 2)
. Flow rate: 1.7 mL/min (35 cm/s), Division ratio: 1/5
. Oven temperature : isotherm 40°C for 12 min, then 40 to 240°C (10°C/min), then isotherm 240°C for 10 min.
. Injector temperature: 280 °C, Detector temperature: 280 °C
. Detector FID (Hydrogen type Alphagaz 2 – Air type Alphagaz 1)
. Injection: 100 µl syringe for gas

1.2 - Solution preparation
- Standard solution
Start Solution
Ethanol: 3.0 g of Ethanol R in 100 ml of water R: solution A
"Internal standard" Solution
Put 1 ml of THF (tetra hydrofurane) in 100 ml of water R: solution EI.
Standard bottle
In a glass bottle of 50 ml (lyophilization type), put 50 µl of solution A and add, 10 µl of solution EI. Close the bottle with appropriate stopper and crimp with aluminum cap.
Standard bottle contains 1500 µg of ethanol.
Test bottle
In a glass bottle of 50 ml (lyophilization type), put 300 mg of substance to be examined and add, 10 µl of solution EI. Close the bottle with appropriate stopper and crimp with aluminum cap.
Prepare the test bottle in duplicate.

1.3 - Standard operation procedure and results
Put "test" and "standard" bottles in an oven at 110 °C for 15 minutes (maximum 20 min). Take 100 µl of gas in each bottle and inject through septum of injector with a syringe for gas (syringe must be heating at 110 °C for 2 or 3 minutes before sampling).
Inject twice 100 µl of gas from each bottle "standard" and "test".
Standard chromatogram has 1 peak (ethanol) with retention times of about 4.1 minutes. Retention time for THF (internal standard) is close to 10.3 minutes.
Peak observed on the "test" chromatogram does not to have been higher than peak observed on the "standard" chromatogram. Calculate for ethanol the ratio R [Area (peak ethanol)/Area (peak THF)].
R ratios calculated for "test" chromatogram must be lower than R ratios calculated for "standard" chromatogram.

Limit test at 5000 ppm of ethanol.

Phosphorous content « Standard Operation Procedure »
Internal SOP : CI-LAB-027.

1.1 - Reagents
Reagent A : Aqueous solution of 0.2 % (w/v) of ammonium métavanadate (R).
Reagent B : Aqueous solution of 5 % (w/v) of ammonium molybdate (R).

1.2 - Solution preparation
Test solution:
In a Kjeldhal matrass, add an HA Na sample that is accurately weighed (Pe) (close to 250 mg) to 25 ml of nitric acid (R) and 5 ml of sulfuric acid (R).
Heat to a light boil until discoloration. If necessary add some drops of perchloric acid. Cool down. Transfer the mineralisate into a 50-ml volumetric glass. Rinse the matrass with water R and complete with water R to 50 ml (volumetric glass).
Standard solution
Aqueous solution of 0.02 % (w/v) of phosphorous (prepared from potassium dihydrogenophosphate R: 879.76 mg/liter).

1.3 - Quantitative analysis
Use seven 100-ml volumetric glasses.

	T1	T2	T3	T4	T5	Assay	Blank
Standard solution (ml)	0.5	1.0	2.5	5.0	10.0	-	-
Test solution (ml)	-	-	-	-	-	40	-
Neutralize the test solution to pH 7 with sodium hydroxide and bring all glass volumes to 50 ml							
Nitric acid (ml)	5	5	5	5	5	5	5
Reagent A (ml)	10	10	10	10	10	10	10
Reagent B (ml)	10	10	10	10	10	10	10

Leave in contact for one hour and complete with water to 100 ml.
The standard concentrations are 0.1 - 0.2 - 0.5 - 1.0 and 2.0 mg of phosphorous/100 ml.
Read the Optical Density at 440 nm for each solution against the blank solution, by using a Spectrophotometer. Plot the standard curve O.D. = f(c)
The phosphorous content in the test product is given in percent by:

$$T\ \% = \frac{C \cdot 10 \cdot 100 \cdot 100}{Pe \cdot (100 - \text{Loss On Drying})}$$

With C = phosphorous concentration of the test solution in mg/100 ml

The phosphorous content (on dried product) is not more than 0.3 % (w/w).

Aluminum content « Standard Operation Procedure »
Current European Pharmacopoeia, 2.2.22, Atomic Emission Spectrophotometry.
Current USP, Adapted <730>, ICP-AES.
Internal SOP: CI-LAB-116

1.1 Principle:
Aluminum content is controlled using atomic emission spectrophotometry (Inductively Coupled Plasma Atomic Emission Spectroscopy or ICP-AES) according to the standard monograph test described in the current European Pharmacopoeia - 2.2.22, Method I.
The technique of the standard curve uses five standard solutions of known concentrations, of which two will bracket the presumed concentration of the unknown test product.

1.2 Equipment and experimental conditions
- Inductively Coupled Plasma Atomic Emission Spectroscopy or ICP-AES: Vista MPX (Varian Company), long torch equipped.
- Working wavelength: 396.152 nm

1.3 Reagents
- Standard multi elements solution (including Aluminum, 100 mg/liter): Ref. TECHLAB N° QCS-O2-R1-1,
- Nitric acid 65 % (w/w) free from heavy metals: NORMATOM, ultra pure, Ref. VWR International N° 29675.291,
- Highly purified water,
- Hydrochloric acid R (free from heavy metals): Ref. VWR International N° 100318.

1.4 Preparation of solutions
Standard Solutions
Prepare a set of standard solutions in highly purified water 0.05, 0.1, 0.3, 0.6 and 0.8 mg/liter.
Prepare the standard solutions from 1 mg of aluminum /liter (1) according to the following table:
(1) Dilute the Standard multi elements solution to 1/100 with highly purified water.

	Al 0 mg/l	**Al 0.05 mg/l**	**Al 0.1 mg/l**	**Al 0.3 mg/l**	**Al 0.6 mg/l**	**Al 0.8 mg/l**
1 mg/liter of aluminum standard solution	0 ml	1.25 ml	2.5 ml	7.5 ml	15 ml	20 ml
Nitric acid 65 % (w/mw) free from heavy metals	1.25 ml	1.25 ml	1.25 ml	1.25 ml	1.25 ml	1.25 ml
Highly purified water	To 25 ml					

Preparation of the sample solutions
Prepare the total ashes (according to EP, 2.4.16) on about 1 g of substance to be examined.
Make a crucible of silica red-hot for 30 minutes. Then cool in a dissector and weigh.
Accurately weigh approximately 1.0 g of the substance to be examined in the crucible of silica.
Make the crucible of silica red-hot for 30 minutes and then ignite in a muffle furnace, at 600 ± 25°C until the carbon is completely burned off. Cool in a dissector.
Add 2 ml of 30 % (w/v) hydrochloric acid R (free from heavy metals) on the total ashes residue.
Evaporate until dry.

Add 5 ml 2 N hydrochloric acid R (free from heavy metals) and digest 15 min in a boiling water bath. Slowly evaporate until dry. Add a 10 ml of hot highly purified water and digest for 2 min in a boiling water bath. Decant in a 25 ml volumetric glass and rinse the crucible with highly purified water. Filter if necessary and complete at 25 ml with highly purified water: sample solution S1.
Dilute the solution S1 to 1/2 with highly purified water: sample solution S2.

Preparation of the blank solution

Make the crucible of silica red-hot for 30 minutes (without product) and then ignite in a muffle furnace, at 600 ± 25°C. Cool in a dissector.
Add 2 ml of 30 % (w/v) hydrochloric acid R (free from heavy metals) on the total ashes residue. Evaporate until dry.
Add 5 ml 2 N hydrochloric acid R (free from heavy metals) and digest 15 min in a boiling water bath. Slowly evaporate until dry. Add a 10 ml of hot highly purified water and digest for 2 min in a boiling water bath. Decant in a 25 ml volumetric glass and rinse the crucible with highly purified water. Filter if necessary and complete at 25 ml with highly purified water: blank solution B1.
Dilute the solution B1 to 1/2 with highly purified water: blank solution B2.

1.5 Quantitative analysis of aluminum

Analyze the following standard solutions: A_1, A_2, A_3, A_4 and A_5 for their intensity readings. Plot intensity versus concentration and obtain the standard curve by drawing a straight line going through the points.
Analyze the sample and blank solutions: A_S and A_B for their intensity readings. Plot (A_S - A_B) on the standard line and deduct the aluminum concentration of the sample solution. Then, calculate the aluminum content of the product to be examined.

The aluminum content (on dried substance) is not more than 20 ppm.

Calcium and magnesium « Standard Operation Procedure »
Current European Pharmacopoeia, 2.2.22., Atomic Absorption Spectrophotometry.
Current USP, <851>
Internal SOP : CI-LAB-034 and CI-LAB-026.

1.1 Principle:
Calcium and magnesium are measured using Atomic Absorption Spectroscopy according to the method described in the current European Pharmacopoeia - 2.2.23.
The technique of standards is based on five standard solutions of known concentrations, of which two will bracket the presumed concentration of test product (Method I).

1.2 Equipment
. Atomic absorption spectroscopy

1.3 Preparation of solutions
Standard solutions
. Aqueous standard solution: 1.0, 2.0, 3.0, 4.0 and 5.0 ppm of calcium with 10.0 % (v/v) of the following lanthane oxide solution. Dissolve slowly 11.6 g of lanthane oxide (La_2O_3) in 20 ml of chlorhydric acid R and complete to 200 ml with water R.
. Aqueous standard solutions: 0.2, 0.3, 0.4, 0.6 and 0.8 ppm of magnesium with 10.0 % (v/v) of the following lanthane oxide solution. . Dissolve slowly 11.6 g of lanthane oxide (La_2O_3) in 20 ml of chlorhydric acid R and complete to 200 ml with water R.

Test solution
Perform total ashes (2.4.16.) on 1.00 g of the substance to be examined.
Accurately weigh approximately 1.0 g of the substance to be examined in crucible of silica.
Dry at 100 - 105 °C during 1 hour and then ignite, in a muffle furnace, at 600 ± 25°C until the carbon is completely burned off (until constant mass).
Add 2 ml of hydrochloric acid R (concentrated) on the total ashes residue.
Evaporate until dry
Add 5 ml 2N hydrochloric acid and digest 15 min in a boiling water bath. Slowly evaporate until dry. Add 10 ml of hot water R and digest for 2 min. Decant in a glass and rinse the crucible with water R. Filter if necessary and put the solution in 50 ml volumetric glass. Complete with water R at 50 ml: **solution S**.
For calcium test, dilute the solution S to 1/2 with water R and 10 % (v/v) of lanthane oxide solution.
For magnesium test, dilute the solution S to 1/2 with water R and 10 % (v/v) of lanthane oxide solution.

1.4 Analysis - Experimental settings
Calcium
- Calcium lamp (working wavelength: 422.67 nm)
- Flow: - Air: 10.0 L/min
  - Acetylene: 2.7 L/min
- Opening diameter (width/height): 2.7 mm/0.6 mm

Magnesium

- Magnesium lamp (working wavelength: 285.21 nm)
- Flow: - Air: 10.0 L/min
  - Acetylene: 2.5 L/min
- Opening diameter (width/height): 2.7 mm/1.05 mm

Quantitation analysis and results

Examine the standard solutions of calcium and magnesium according to their respective experimental settings.
Simultaneously analyze the corresponding sample solutions.
Calculate the calcium and magnesium content in the tested product.

On dried substance, calcium content is not more than 500 ppm [0.05 % (w/w)] and magnesium content is not more than 100 ppm [0.01 % (w/w)].