H.R. BIOANALYSIS srl

BIOCHEMICAL LABORATORY

HYALURONIC ACID HR ASSAY (50 assays)
HYALURONIC ACID [0.001 ÷ 0.01 %]

Intented use

HYALURONIC ACID HR ASSAY is a spectrophotometric assay for the quantitative determination of hyaluronic acid (HA) in pharmaceuticals, cosmetics and medical devices which contain linear or cross-linked HA.
The assay is not suitable for blood and urine analysis.

Principle of the method

Hyaluronic acid react rapidly in weak acidic medium with bisphenylnaphthylmethane dye to form a complex which causes the change of absorption spectra. The increase of absorbance values near 680 nm are proportional to the concentration of HA, Beer's law is obeyed in the range between 0.001~0.01 %.

Reagents and standard provided

- DYE REAGENT — 1x200 ml bottles
- HA STANDARD, $[\mathbf{HA}, \%] = 0.005$ — 1x10,0 ml vial

Materials required but not supplied

- Spectrophotometer with 1.0 cm glass cuvettes
- Pipettes (200 µl, 1000 µl)
- 100 ml Volumetric flask
- Syringe filter
- 5 ml test tubes

Storage

Store at room temperature (15-25 °C).
Stability: 12 months.

Detection range

[0.001 ÷ 0.01 %]

Assay procedure

PREPARATION LIQUID OR HYDROGEL TEST SAMPLE
Place 1.0 ml (g) sample in 100 ml volumetric flask and made up volume with deionized water or other dilutent (i.e.: sodium lauril sulfate solution). Incubate for 15 min at room temperature (15–25 °C) with constant shaking to ensure complete dissolution of the sample (dilution factor = 100). Sample's solution turbid should be filtered using a syringe filter.

Mesurement of HA

1. Mix by gentle rotation of the Dye Reagent and pipet 4,0 ml into the 5 ml test tubes.
2. Transfer 150 µl aliquots of HA Reference sample / Reagent Blank / Test Samples to the 5 ml test tubes containing the Dye Reagent.
3. Mix by gentle rotation of the test tubes and incubate for 15 min at room temperature (15–25 °C).
4. Mix again and using a spectrophotometer read the Absorbance values at 680 nm, therefore convert data obtained.

Calculation of results

The results are given in % by the formula:

$$[\mathbf{HA}, \%] = \mathbf{0.005} \times \mathbf{ABS_2} \times \mathbf{F}/\mathbf{ABS_1}$$

ABS_1 = Absorbance value at 680 nm of HA Reference sample
ABS_2 = Absorbance value at 680 nm of Test sample
F = Diluition factor made in sample preparation

RECOMMENDATION

- **If absorbance values of test samples are: $ABS_2 > ABS_1 + 0{,}05$, dilute further the samples.**
- **If absorbance values of test samples are: $ABS_2 < ABS_1 - 0{,}05$, reduce the diluition factor.**

Therefore repeat steps 1-2-3-4.

References:
[1] L. H. Chen, Y. Jian, H. Q. Luo et al. *Chinese Chemical Letters* 18 **(2007)** 1099–1102
[2] L. H. Chen, S. Liu , H. Q. Luo and X. Hu. *Journal of Chemical and Pharmaceutical Research*, **2014**, 6(6):1695-1698

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