

Determination of serum retinol using hexane as the serum sample extraction procedure

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1. Reagents and materials

- Methanol (HPLC grade)
- Hexane (HPLC grade)
- Ethanol (HPLC grade/AR grade)
- Normal saline solution (0.9% NaCl)
- Retinol acetate (as internal standard)
- Retinol (as external standard)
- CRM (Certified Reference Material) for serum retinol
- 15 ml centrifuge tube
- Vortex mixer
- Centrifuge (up to 4000 rpm capacity)
- Centrifuge tube rack
- Micropipettes (10-100 μ l and 100-1000 μ l)
- Micropipettes tips for both types
- Stopwatch
- HPLC Vials (1ml)

2. Instrument (HPLC) parameters – we have Shimadzu prominence HPLC.

- Detector - SPD-10A UV/VIS at 325 nm
- Column – SUPELCO SILTMLC-NH₂-NP, 25cm × 4.6mm, 5 μ m
- Mobile phase – HPLC grade methanol
- Elution system – isocratic

3. Sample extraction procedure

- Add 200 μ l serum sample to 15ml plastic test tube.
- Add an equal volume of 50 μ g/dl retinol acetate and ethanol.
- Take 200 μ l of a series of standards (10, 20, 40, 60, 75 μ g/dl) to the 15 ml centrifuge tube and add the same volume of retinol acetate and normal saline solution.
- Mix the standard and sample solutions with a vortex mixer
- Add 1ml of HPLC grade hexane and mix for 45 seconds
- Centrifuge the solution at 4000rpm for 7 minutes and transfer the supernatant to other test tubes
- The solution has to be extract twice by adding 1 ml hexane.

- Dry the hexane using nitrogen gas and reconstitute by 500µl HPLC grade methanol
- It has to be mix for 30 minutes using a vortex mixer and transfer to 1 ml HPLC vials
- Analyze the extracted solutions using reversed-phase HPLC by isocratic elution system, at the flow rate of 1.5 ml/min, and injection volume 30µl

N: B: - Extract and analyse the CRM using the sample extraction procedure.

4. Calculation

- Plot the calibration curve using area ratio of retinol (standard) and retinol acetate (internal standard) vs concentration ratio (retinol: retinol acetate).
- From the linear equation formula, $Y = mx + b$, the concentration of serum retinol can be calculated.

Where Y – is area ratio (retinol: retinol acetate) of sample

M – is slope and b – is Y intercept

X – is the concentration of serum retinol in µg/dl

5. Reference

Quadro, Y.-K. K. and L. (2013). Reverse-Phase High-Performance Liquid Chromatography (HPLC) Analysis of Retinol and Retinyl Esters in Mouse Serum and Tissues. *Methods Mol Biol.* 2010; 652: 263–275. *Doi:10.1007/978-1-60327-325-1_15.*, (12), 1–10. <https://doi.org/10.1007/978-1-60327-325-1>

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