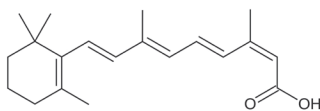
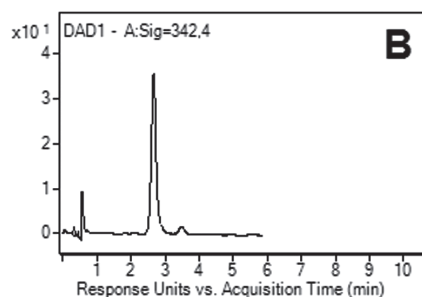
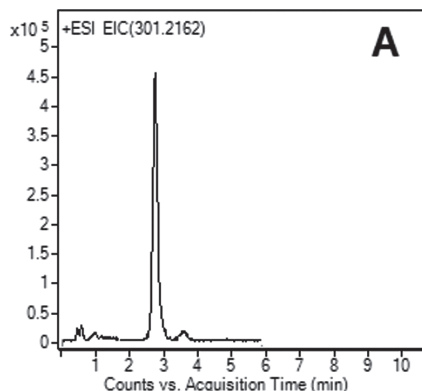


# Isotretinoin in Human Plasma

Drug used for treatments of cystic acne, cancer, and keratinization



Isotretinoin

**Note:** Isotretinoin was originally synthesized with intent as a potential treatment for skin cancer. Investigation revealed it was ineffective in that application but also showed notable efficacy for acne treatment.

## Method Conditions

**Column:** Cogent Bidentate C18™, 4µm, 100Å

**Catalog No.:** 40018-05P-2

**Dimensions:** 2.1 x 50 mm

**Mobile Phase:** 70% A: DI H<sub>2</sub>O / 0.1% formic acid (v/v)  
30% B: Acetonitrile / 0.1% formic acid (v/v)

**Injection vol.:** 1µL

**Flow rate:** 0.4 mL/min

**Detection:** Fig. A: ESI - POS - Agilent 6210 MSD TOF mass spectrometer

Fig. B: Agilent DAD detector

**Sample:** Fig. A: A 0.5 mL aliquot of the collected plasma sample was pipetted into a 10 mL glass centrifuge tube. 4.0 mL of acetonitrile: dichloromethane (1:1, v/v) was added. The mixture was vortexed for 3 min. After centrifugation, the upper organic layer was transferred to another 10 mL centrifuge tube and evaporated to dryness under nitrogen gas in a water bath at 37°C. The residues were then redissolved in 100µL of acetonitrile and dichloromethane (1:1) mixture. Fig. B: Isotretinoin reference standard used for method development. Stock solution was 500µg/mL in a diluent of ethanol/ dichloromethane. The sample for injection was diluted 1:100.

**Peak:** Fig. A: Isotretinoin 301.2162 m/z [M+H]<sup>+</sup>

Fig. B: UV 342 nm

t<sub>0</sub>: 0.4 min

## Discussion

Method development for isotretinoin was first performed with a standard on an HPLC-UV system. The final isocratic method was then transferred to an LC-MS system for analysis of isotretinoin in human plasma. The potential matrix effect on the ionization of the isotretinoin was evaluated by comparing the peak area of the analyte dissolved in the supernatant of the processed blank plasma to that of standard solutions at the same concentration. Three different concentration levels of isotretinoin (20, 500 and 1200 ng/mL) were evaluated by analyzing three samples at each concentration level. A matrix effect was observed, leading to an 18–25% reduction of the peak area in plasma compared to the standard solution. The ion suppression effect was relatively high but reproducible.