Altasciences CLINICAL RESEARCH
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Elimination of Endogenous Light Sensitive Compounds in Human Plasma by UV Irradiation for the Determination of Tretinoin and Isotretinoin by LC-MS/MS

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OVERVIEW

NOVEL ASPECT

Preparation of a surrogate matrix by depletion of endogenous light-sensitive compounds in human plasma by UV irradiation.

METHOD

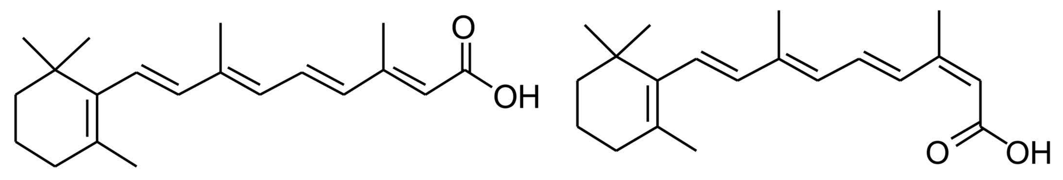
Human plasma samples containing endogenous tretinoin and isotretinoin were subjected, in separate experiments, to normal laboratory light, UV irradiation and light protection at 37 °C for endogenous level depletion. Several aliquots were then sampled from each condition at time-points from $t_{0.5}$ to $t_{7.5}$ hr.

RESULTS

UV irradiation of light-sensitive compounds in plasma successfully reduced their endogenous levels.

INTRODUCTION

The quantitation of endogenous compounds poses bioanalytical challenges since concentrations may be > 20% LOQ. Therefore, a surrogate matrix such as BSA or PBS is often used for the preparation of calibrants and QC samples, even though these differ compositionally from authentic plasma. An alternative approach is the use of charcoal treated (i.e. stripped) plasma. However, the remaining composition is still disparate from untreated plasma, and thus poorly representative of study sample. In the development of an LC-MS/MS assay for the endogenous components tretinoin and isotretinoin, it was determined that surrogate matrix could be depleted of these analytes via UV irradiation, leaving the remaining plasma composition unadulterated and significantly more comparable to study sample composition.



Tretinoin
Chemical Formula: $C_{20}H_{28}O_2$
Exact Mass: 300.21
Molecular Weight: 300.44

Isotretinoin
Chemical Formula: $C_{20}H_{28}O_2$
Exact Mass: 300.21
Molecular Weight: 300.44

Figure 1. Structures of tretinoin and isotretinoin.

METHOD

SAMPLE PROCESSING

- LOQ: 0.50 ng/mL in human plasma
- Matrices: 4 human plasma donors (2 x EDTA, 2 x heparin)
- Each donor was aliquoted into three different containers for each condition evaluated.
- Evaluations:
  - laboratory light
  - UV Irradiation (365 nm) (Figure 2)
  - protection from light at 37 ̊C

Aliquots were sampled at multiple timepoints for each evaluation and extracted by protein precipitation using ACN.

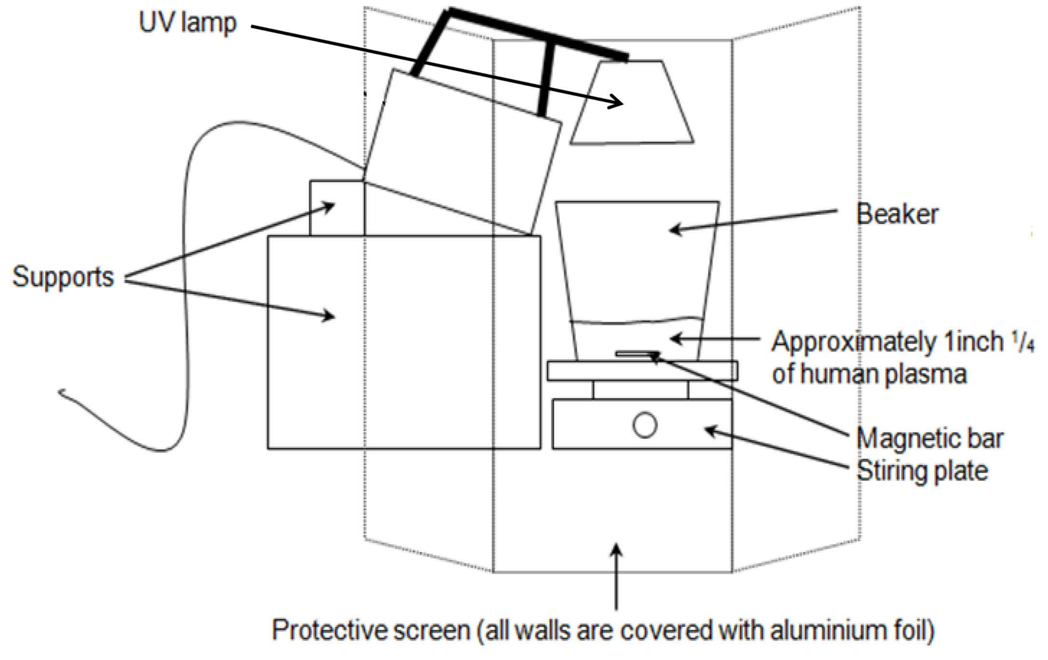


Figure 2. Experimental design for UV irradiation.

CHROMATOGRAPHY

Reversed-phase chromatography with gradient elution.

DETECTION

SCIEX API 5000 in (-)ESI monitoring the MRM transition *m/z* 299 > *m/z* 255 for both tretinoin and Isotretinoin.

RESULTS

Human plasma samples containing endogenous tretinoin and isotretinoin were subjected, in separate experiments, to normal laboratory light, UV irradiation and light protection at 37 °C. Several aliquots were sampled from each condition at the following timepoints: 0.5, 1, 2, 3, 4, 5, 6 and 7.5 hours, then immediately frozen and protected from light. Samples subjected to UV irradiation demonstrated slight discoloration after 3 hours when compared to samples treated with and without laboratory light. Moreover, the temperature of the matrix increased to 39° C after 1 hour and remained stable until the last collection timepoint of 7.5 hours (Figure 3).

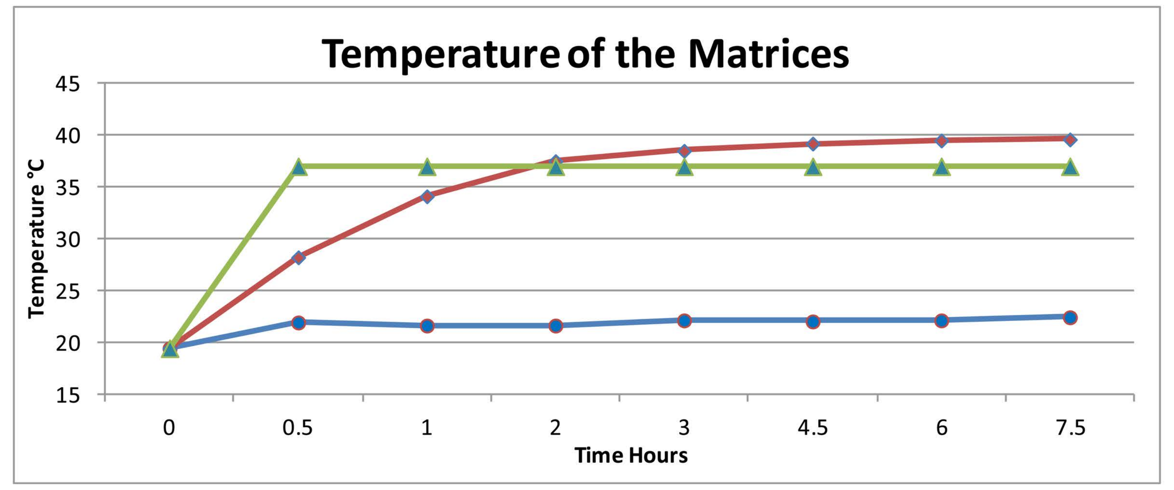


Figure 3. Average temperature of human plasma (EDTA and Heparin) at different processing times.
● human plasma processed under the laboratory light.
♦ human plasma processed under the UV light.
▲ human plasma processed protected from light and at 37 ̊C.

Untreated control plasma at t_0 revealed endogenous levels that were ca. 100 - 300% of the LOQ (0.50 ng/mL) for both tretinoin and isotretinoin. When subjected to UV irradiation, a rapid initial depletion of both compounds was observed.

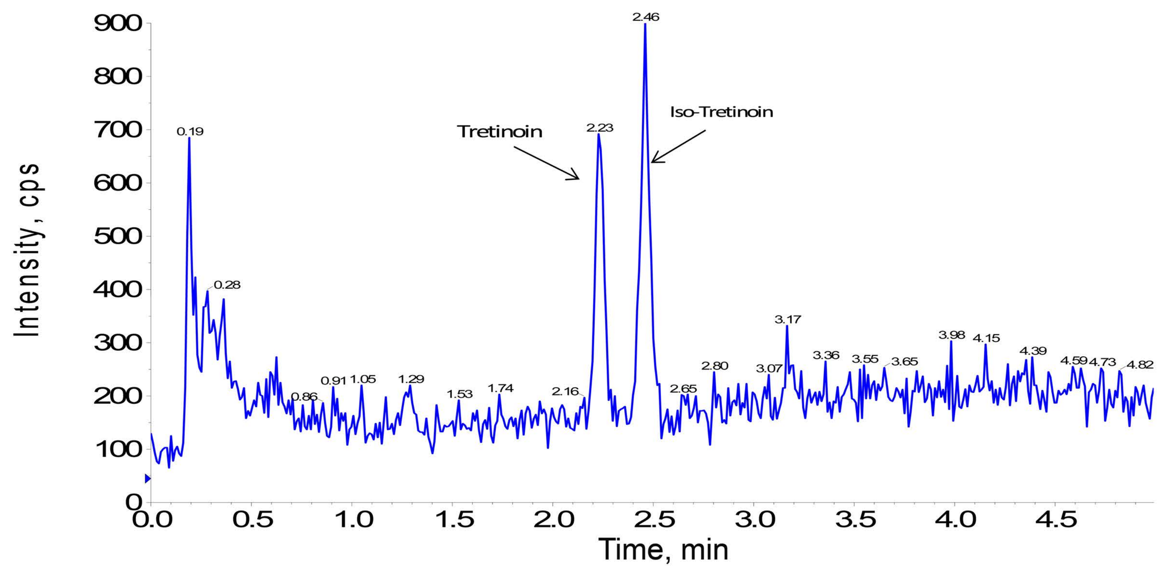


Figure 4, Chromatogram of an extracted LOQ from water (83.3 pg/mL final extract concentration).

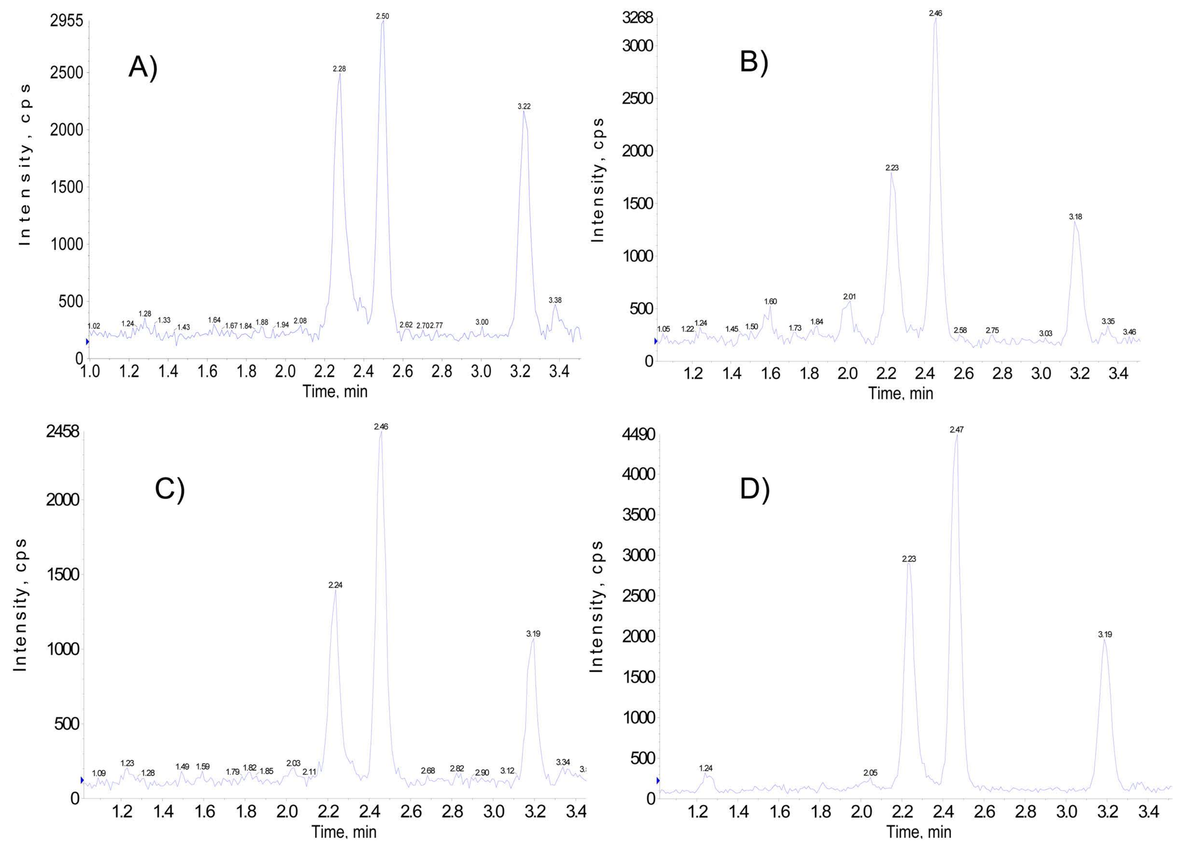


Figure 5. Extracted blank chromatograms from two different donors from human EDTA plasma (A, B) and heparinized plasma (C, D).

After 4.5 hours of UV irradiation, both tretinoin and isotretinoin were entirely depleted in all donors. In contrast, there was no depletion of endogenous levels when samples were subjected to either normal laboratory light or light protection (Figures 6 to 9). Therefore, human plasma treated with UV irradiation was successfully implemented as a surrogate matrix for the preparation of calibration curves and quality control samples, with the advantage of maintaining the integrity of plasma constituents.

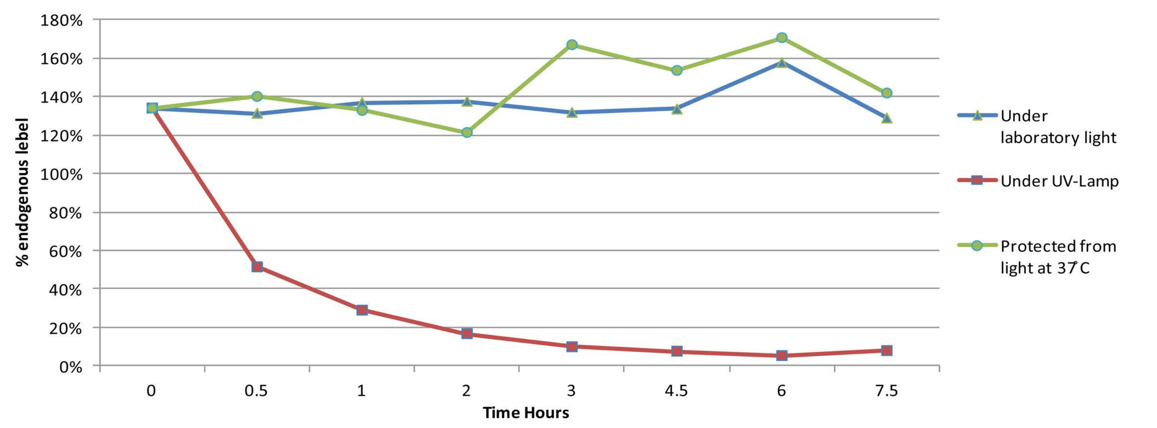


Figure 6. Depletion of tretinoin in human plasma (Donor 1, EDTA).

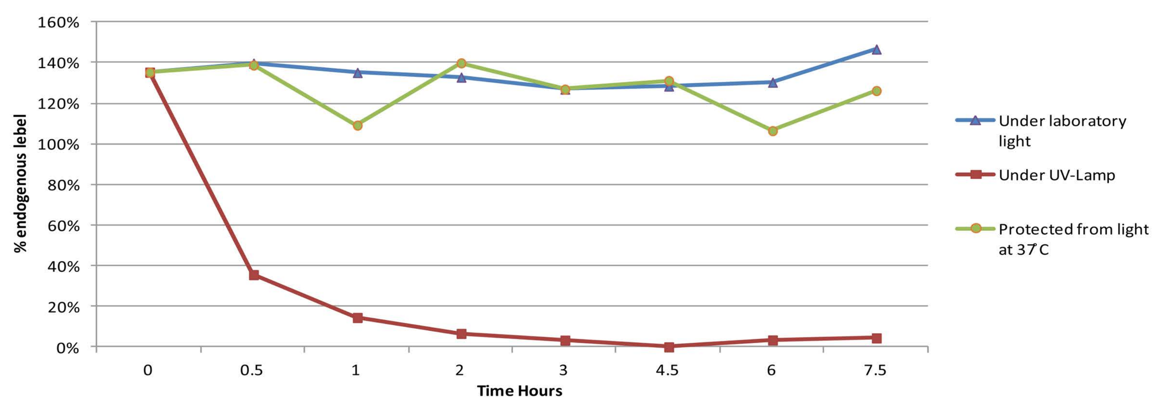


Figure 7. Depletion of tretinoin in human plasma (Donor 2, EDTA).

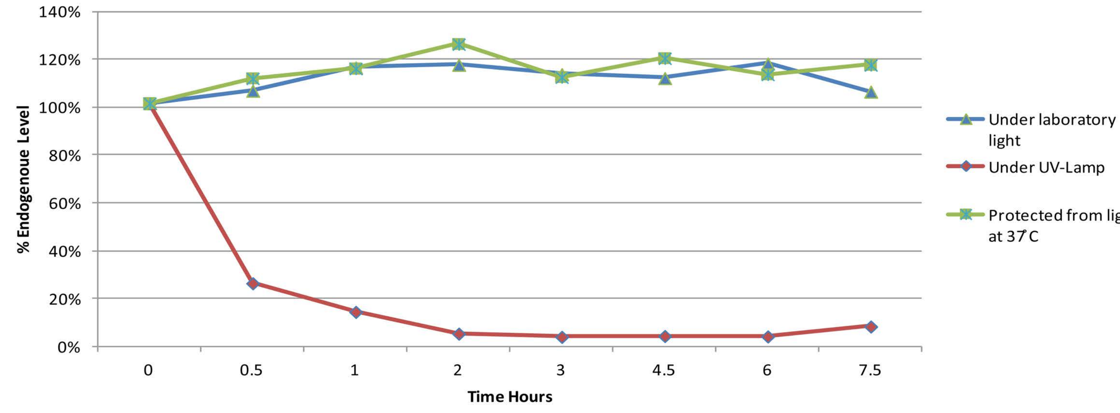


Figure 8. Depletion of tretinoin in human plasma (Donor 1, heparin).

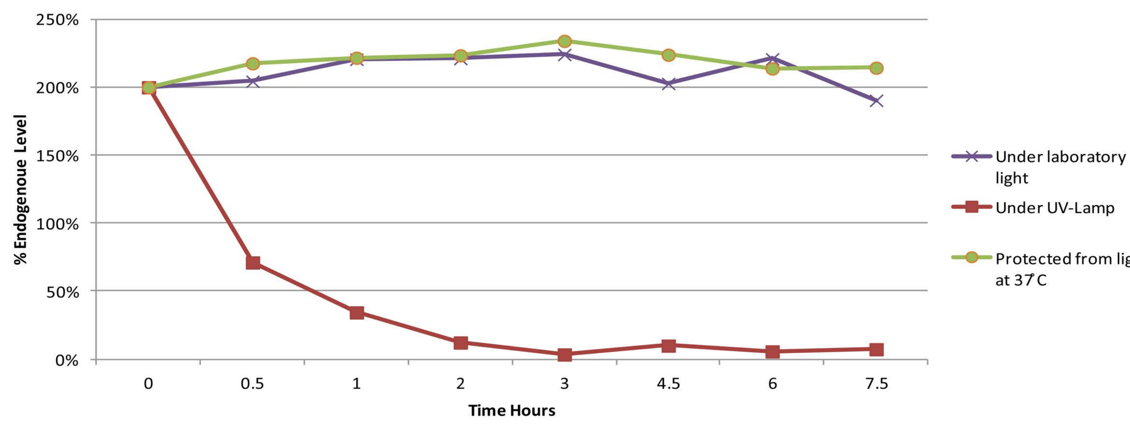


Figure 9. Depletion of tretinoin in human plasma (Donor 2, heparin).

Depletion profiles similar to those of tretinoin were observed for isotretinoin (data not shown), and thus surrogate matrix generated by UV irradiation furnished endogenous concentrations of tretinoin and isotretinoin that were ca. 10% LOQ, suitable for calibrant and QC sample preparation.

CONCLUSION

While there are several options when a surrogate matrix is required, the current investigation demonstrates the implementation of UV irradiation as a viable option for reducing endogenous levels of light sensitive compounds, whilst preserving plasma integrity. Notably, in optimizing the depletion process, it was necessary to optimize plasma volume against irradiation time, temperature and mixing procedure.

ACKNOWLEGMENTS

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