

## Problem

Estradiol is measured by a highly sensitive LC-MS/MS assay and utilized by clinicians to investigate different processes and disorders in women including menstrual cycle regulation, reproductive function and infertility as well as amenorrhea and oligomenorrhea.

It is used to evaluate pregnancy maintenance, precocious puberty, menopause and estrogen deficiency in men and women.

There is an interfering peak in the LC-MS/MS results that requires further evaluation and separation. It is unclear if this peak is due to underlying patient medications or disease characteristics. The interference peak only occurs in 1% of sample runs in a select group of 7 patients in multiple samples collected over the last 7 months.

## Method Information

Estradiol is measured using ultra-fast liquid chromatography (UFLC) coupled with tandem mass spectrometry (MS/MS). Estradiol is extracted from serum using a mixture of hexane and ethyl acetate, dried under nitrogen and derivatized using dansyl chloride.

The sample is injected into the LC-MS/MS system where it is eluted onto the analytical column with a gradient of water and organic solvent (methanol and acetonitrile).

Addition of an internal standard (carbon 13 labeled estradiol) allows for quantification of estradiol compared with a six-point standard curve and three quality control samples are run in each batch.

Instruments: Sciex 5500 QTrap mass spectrometer and Shimadzu Prominence UFLC

Column: Phenomenex Kinetex Phenyl-Hexyl, 2.6 micron, 100x3mm

Mobile Phase A: 0.1% formic acid in water

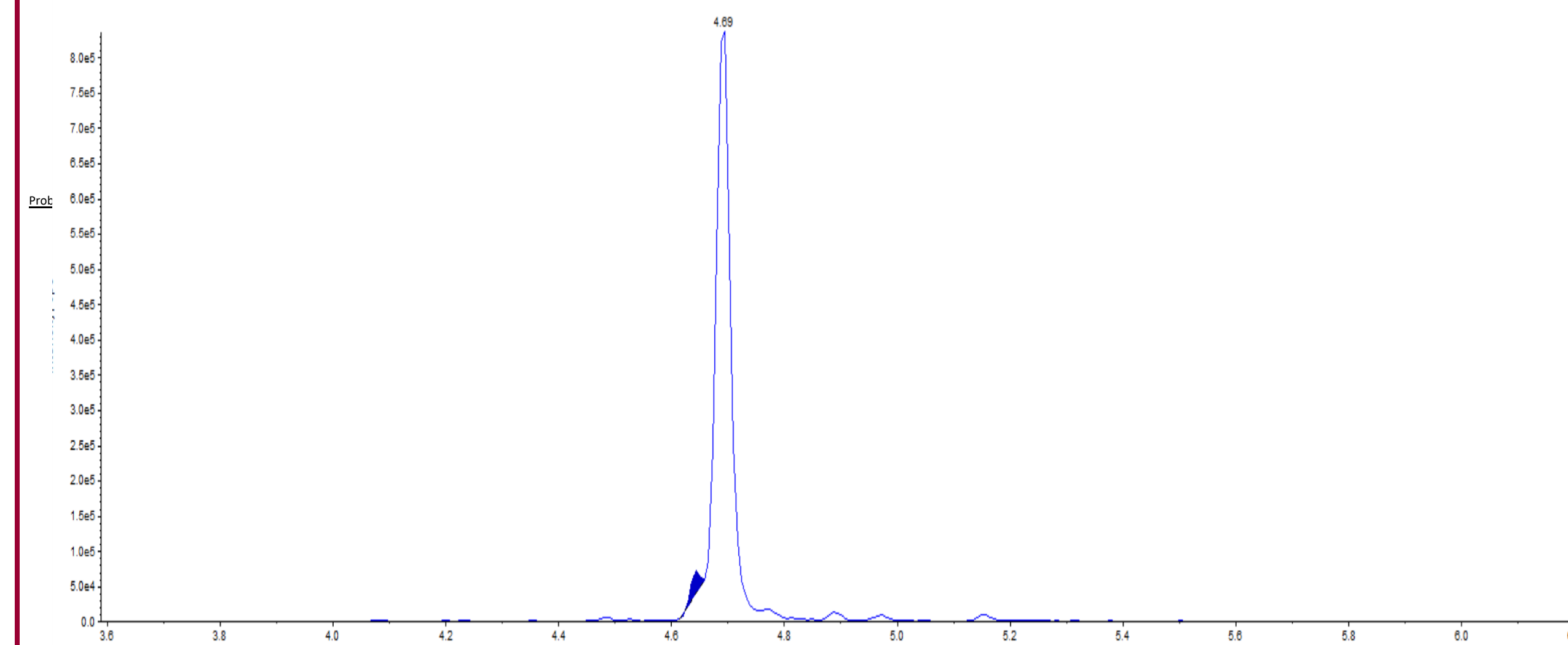
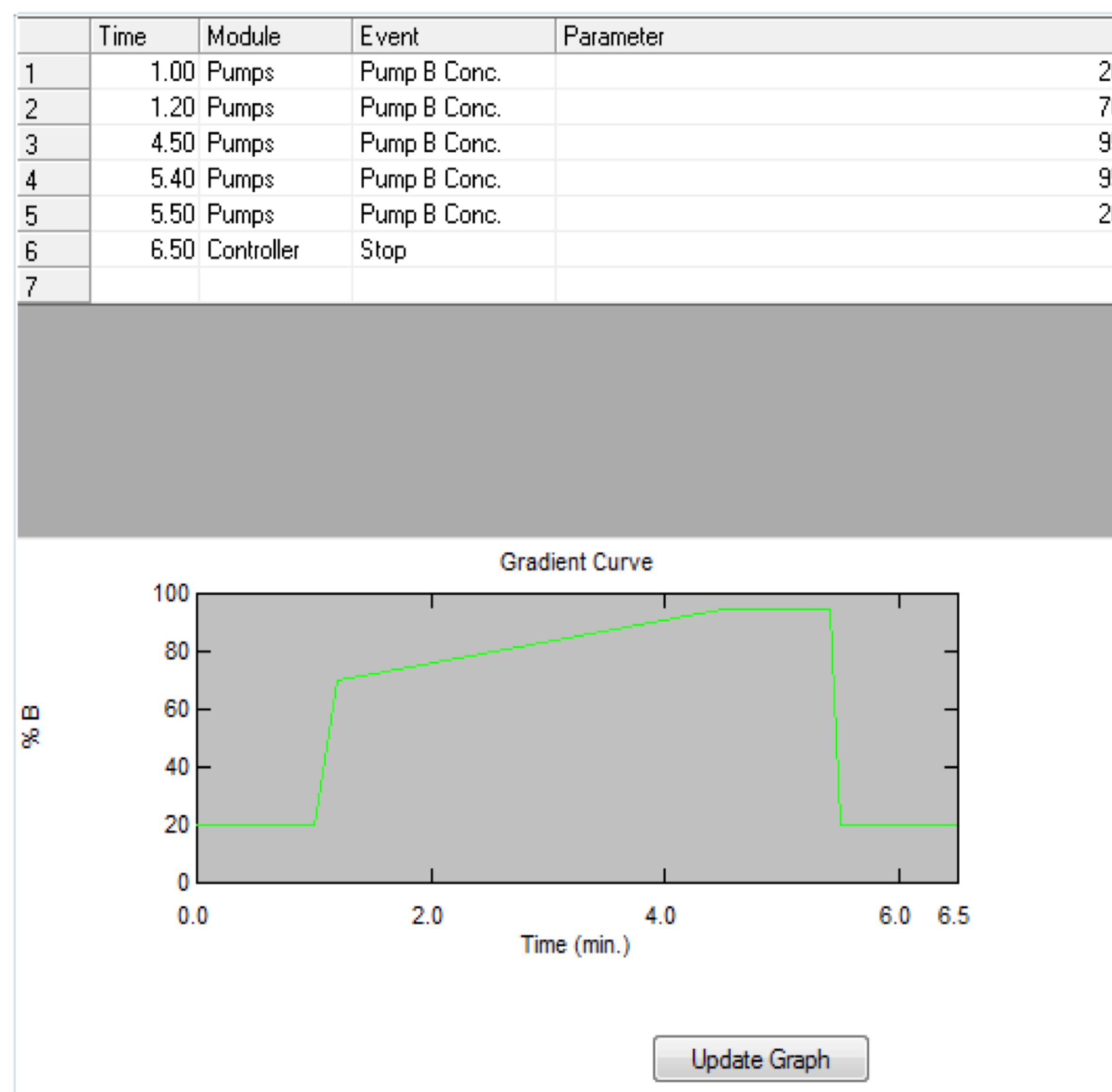
Mobile Phase B: 0.1% formic acid in 70% methanol and 30% acetonitrile

Injection volume: 30 microl

Column Temperature: 40 degrees Celcius

Autosampler temperature: 15 degrees Celcius

FIGURE 1. LC Gradient with Interference Peak



## Variations in Troubleshooting

Change the Gradient (timing in the program) – try different variations as follows

Change the timing of the gradient – Change to a longer or more complex gradient.

Change the organic composition of the mobile phase

Mass spec option: Collect product ion spectra of estradiol and of the interfering peak using the QTrap with a collision energy spread.

This might tell us if there are any significant fragments that differ between the two peaks.

Change the columns from phenylhexyl to C18

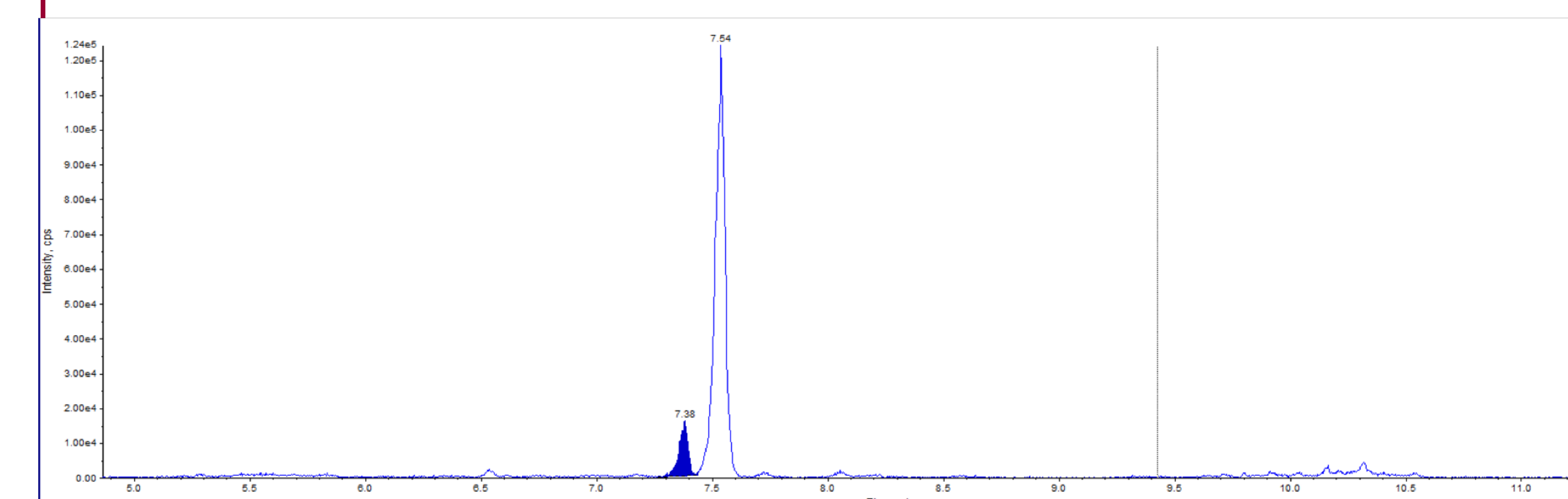
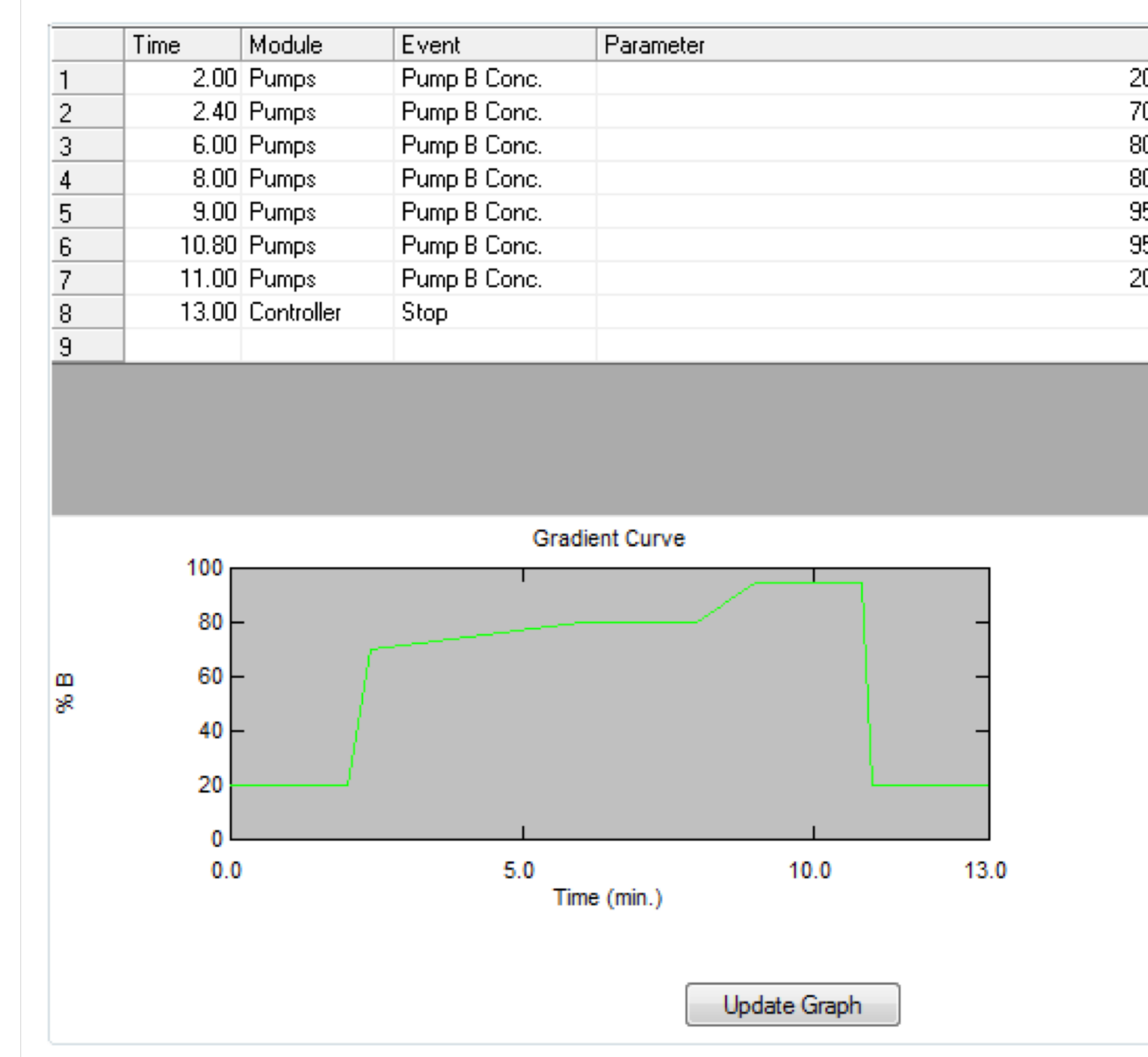
## Troubleshooting steps taken

➤ LC gradient time increased from 6.5 to 13 min total

➤ Gradient steps modified to the following parameters:

1. Longer start and stop period with 2.0 mins at 20% B
2. Increase %B more gradually to 80% and hold for 3.0 mins followed by 90% hold for 2.0 mins allowed peaks to separate

FIGURE 1. LC Gradient with Interference Peak Corrected



## Outcome

➤ Interference peak separated from the Estradiol peak with increased LC gradient time and %B

➤ Overall would ideally still like to see a shorter total retention time for peak separation to maintain efficiency of testing clinically.