

Introduction and Objectives

Tazarotene gel (Tazorac™) is marketed by Allergan for the topical treatment of psoriasis and acne. A GC/MS/MS assay was developed for the metabolite tazarotenic acid using a PFBBr derivative.¹ Adapalene is used as a comparator for acne trials, but the development of a more sensitive assay was required.

The purpose of the present study was to increase the adapalene assay sensitivity by derivatization of the carboxylic acid group of adapalene to add an amine functionality for enhanced detection to achieve the required LLOQ of 2 pg/mL. Method development included extraction optimization and 2-D chromatography to increase specificity.

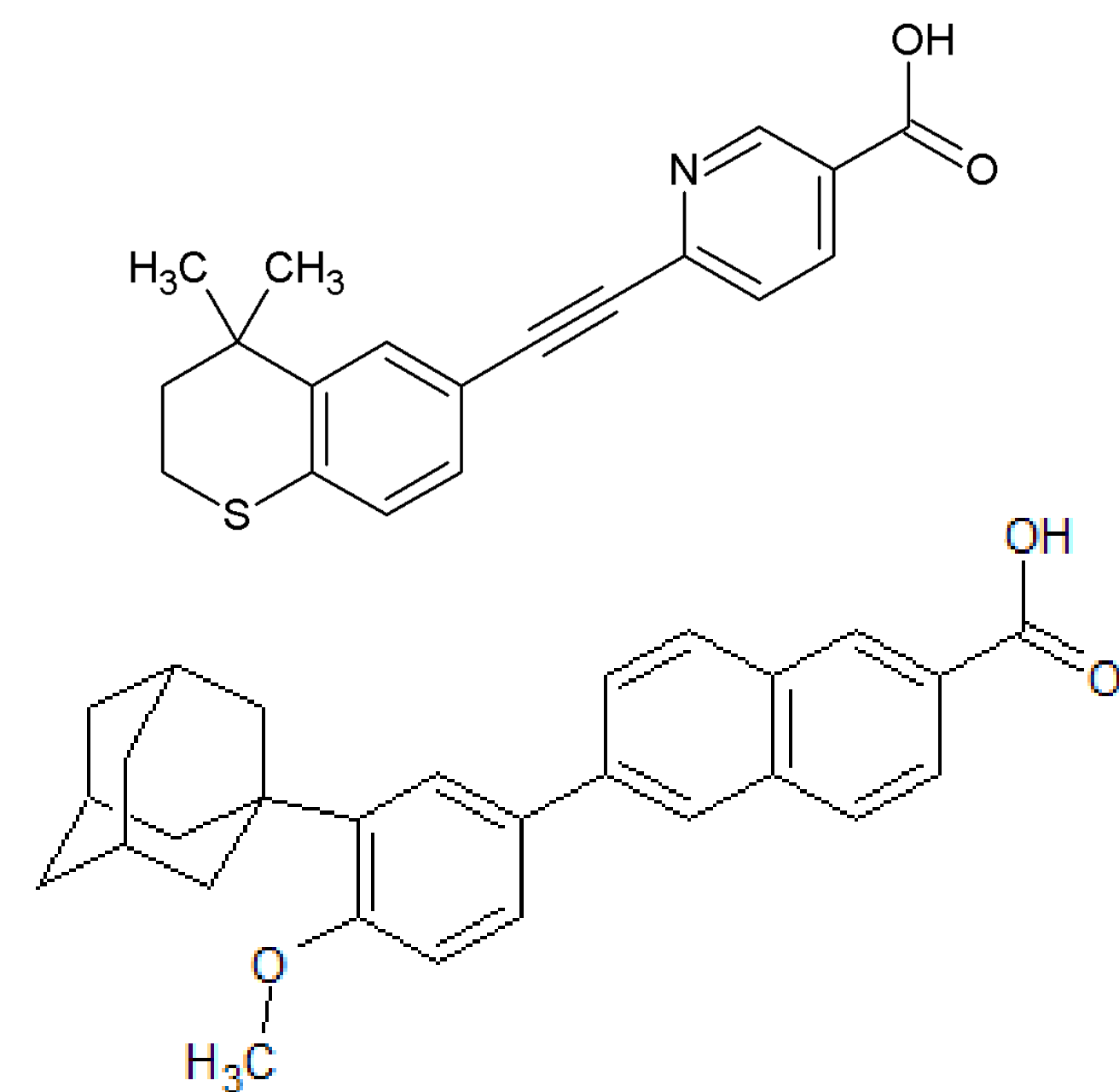


Figure 1: Tazarotenic acid (top) and Adapalene (bottom)

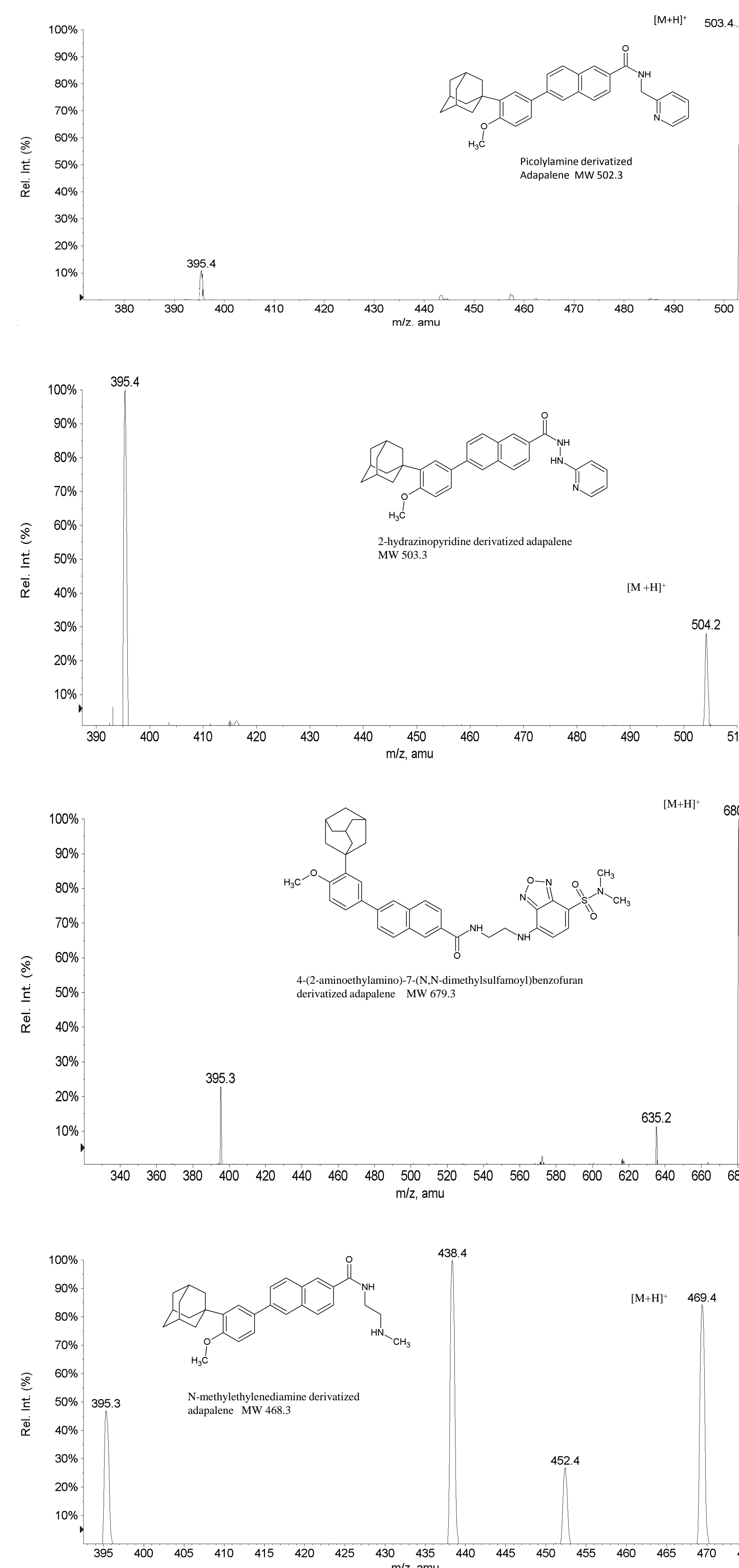


Figure 3. Mass Spectra of Derivatized Adapalene
Mass spectra are product ion scans of the protonated molecular ions using a CE of 20V for the various derivatizing agents.

Methodology

Extraction:

- 1 ml of human plasma is precipitated with 4 mL of ACN. Internal standard is adapalene-d3.
- Supernatant is partially dried and applied to a 50 mg C8 SPE cartridge. Cartridge is then washed with 1 mL of 40:60 MeOH:H₂O with 2% HOAc and then eluted with 1 mL of ACN and dried.
- Extracts are derivatized with 2-picolyamine² using the catalysts triphenylphosphine² and 2,2'-dithiopyridine², all 100 μL at 20 mM in ACN. This mixture is heated at 60 °C for 40 minutes.
- Water and 0.5M Na₂CO₃ are added and the derivatized extract is extracted with 80:20 hexane:MTBE.
- The organic phase is dried and reconstituted with 225 μL of (40:60 ACN:H₂O) with 0.5% FA.

2D Chromatography: (see Figure 4)

- 4 pumps, two valves, two sets of binary mobile phases (MP), and three columns are used. MP C and MP D are used on the first column and MP A and MP B used for the third analytical column. The second trapping column uses both sets of mobile phase. 85 μL of extract is injected.
- MP C: (95:5 H₂O:MeOH) with 2 mM NH₄OAc and 0.1% FA; MP D: (85:10:5 ACN:H₂O:MeOH) with 2 mM NH₄OAc and 0.1% FA; MPA: 0.1% FA; MPB (50:50 ACN:MeOH) with 0.1% FA. Column 1: Zorbax SB-CN, 2.1 x 50 mm, 5 μm; column 2 (trapping column): HALO phenyl-hexyl 2.1 x 30 mm, 2.7 μm; column 3: ACE 3 C18-AR, 2.1x100 mm, 3 μm.
- The extract is loaded onto column 1 and up to about 1.3 minutes flow is directed to waste. The switching valves change and from about 1.3 to 1.75 minutes, the analyte is heart cut off column 1 with flow directed from column 1 onto the second trapping column. Then the valves switch again and MP A and MPB elute the analyte off the trapping column onto the third column and into the mass spectrometer.

Mass spectrometry:

TIS source on an API-5000 operated in positive ion mode. Transitions used are *m/z* 503.3 → 295.3 for derivatized adapalene and *m/z* 506.4 → 295.3 for derivatized adapalene-d3.

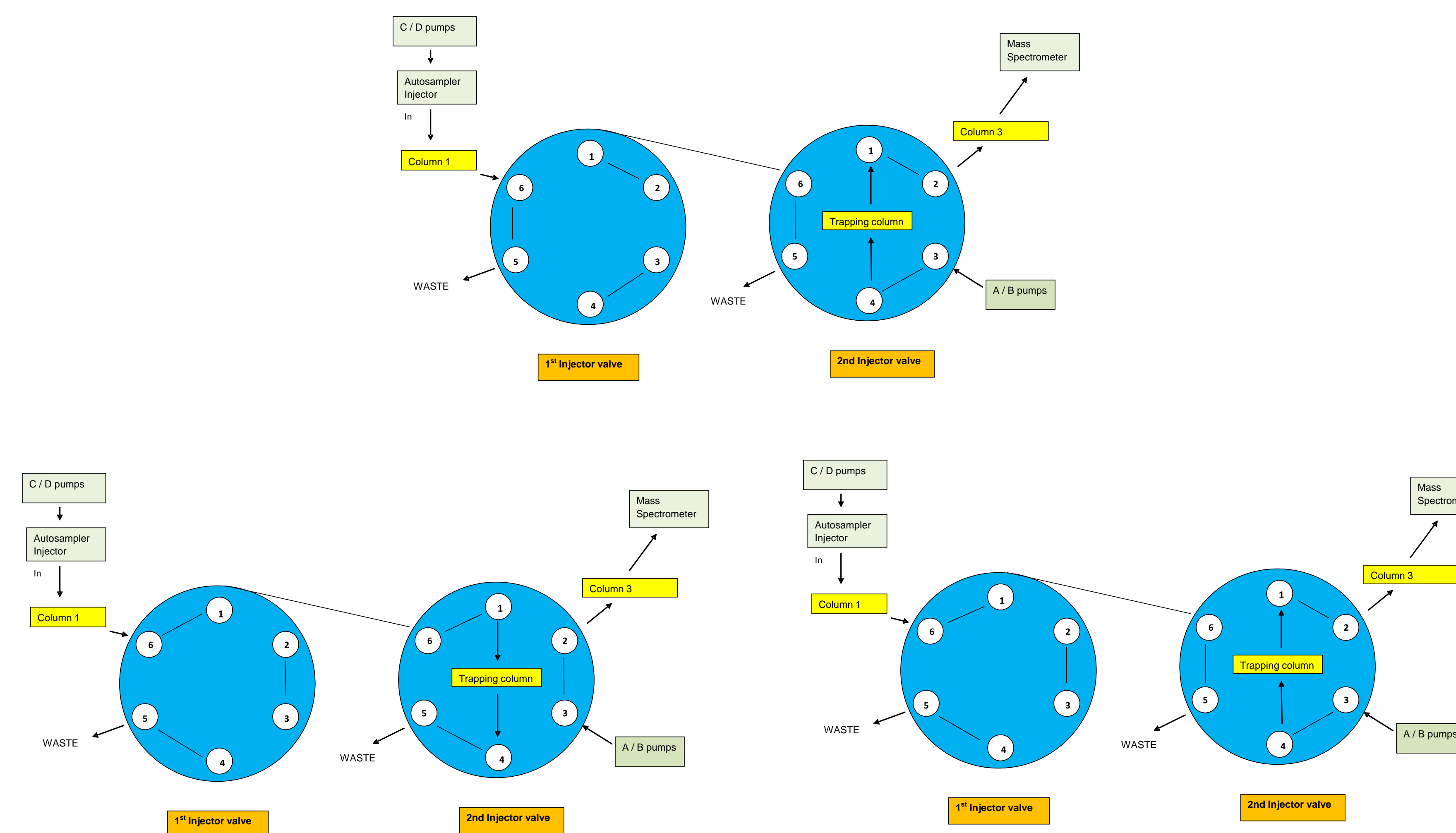


Figure 4

Top: Sample is loaded onto column 1 and flow is directed to waste.

Bottom Left: From about 1.3 to 1.75 minutes the valves switch and the analyte is heart-cut onto the trapping column.

Bottom Right: At t 1.75 min the valves switch again and MP A and MPB elute the analyte off the trapping column and onto the third analytical column and into the mass spectrometer. .

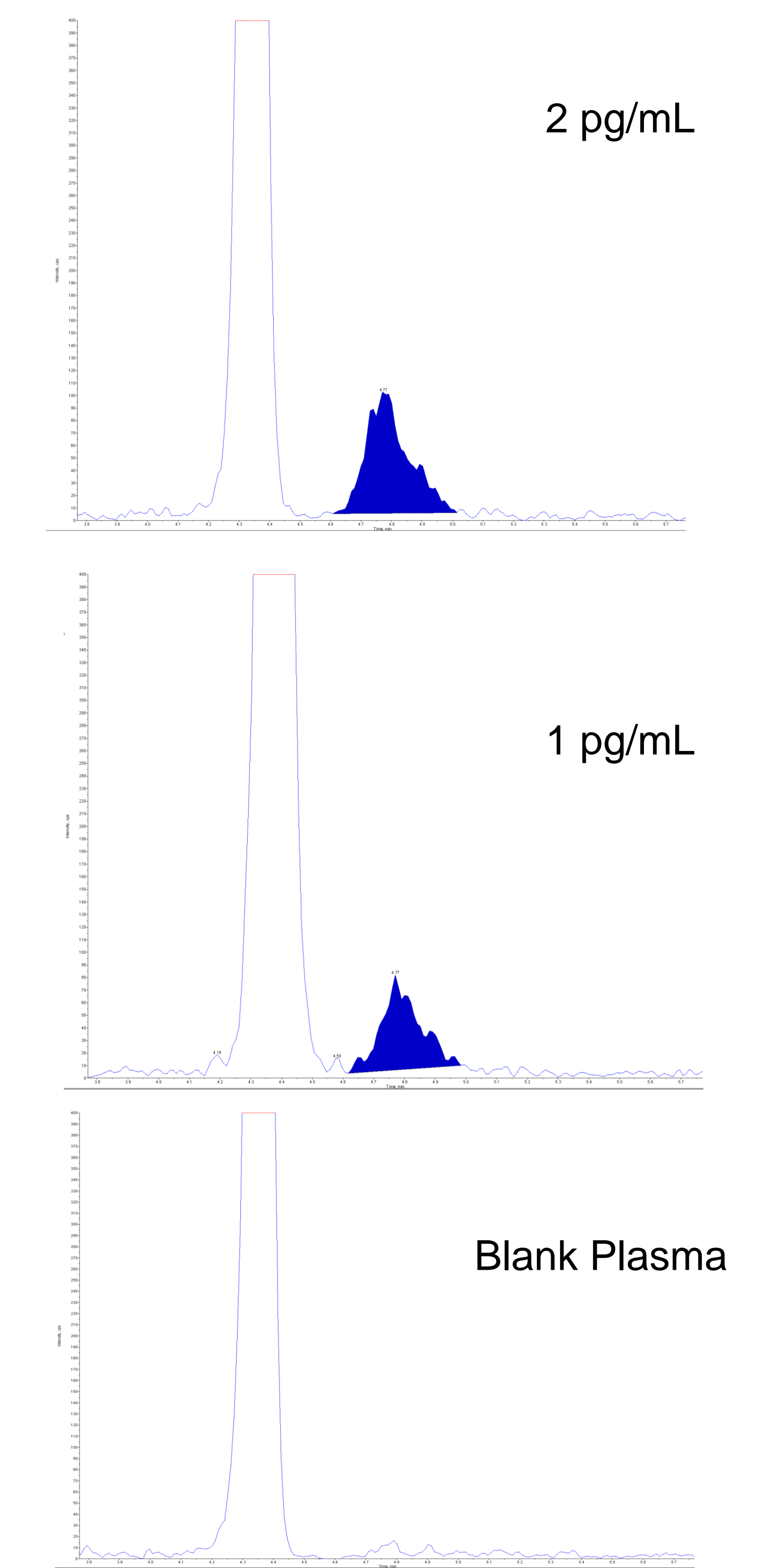


Figure 5: Blank and LLOQ chromatograms of derivatized adapalene.

Results and Conclusions

Figures 1 and 2 display the structures of tazarotenic acid, adapalene and the derivatizing reagents used in the study, respectively.

Figure 3 shows product ion spectra (CE 20) of the protonated molecular ions for adapalene using the various derivatizing agents.

Figure 4 shows the 2D chromatography valve configuration.

Figure 5 shows potential LLOQ and Blank chromatograms of derivatized adapalene.

Several derivatizing reagents were used, having in common a primary amine which condenses with carboxylic acids to form an amide linkage. These derivatizing reagents (Figure 2) contain, in addition, either a secondary or tertiary amine for efficient mass spectrometric ionization in the positive ion mode.

In plasma, the derivatives of 2-picolyamine and 2-hydrazinopyridine yielded the highest sensitivity with several ion transitions available. Overall, the 2-picolyamine derivative yielded the greatest signal to noise at the targeted LLOQ with the highest selectivity in plasma and was used in the further development and validation of the bioanalytical assay. Two-dimensional chromatography was required to achieve a 2 pg/mL LLOQ with a ≥ 20:1 S/N ratio.

The bioanalytical method was sufficiently sensitive to achieve the desired LLOQ and subsequently was successfully validated.

References

1. Matsumoto, R.M. et al., Pharm Res. 14, S259-260, 1997.
2. Higashi T., et al, J. Pharm. Biomed. Anal., 52, 2010, 809-818.
3. Prados P., et al. Anal. Chim. Acta 344, 1997, 227-232.

Figure 2: Derivatizing reagents

From top to bottom: 2-picolyamine²; 2-hydrazinopyridine²; 4-(2-aminoethylamino)-7-(N,N-dimethylsulfamoyl)benzofuran³; N-methylethylenediamine