

Chiral Separation of Methylphenidate, Ethylphenidate, and Ritalinic Acid from Blood

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INTRODUCTION

Chiral separation of racemic drugs is essential to understand the pharmacokinetic and pharmacodynamic properties of the drug. Methylphenidate (MPH), a drug with two enantiomers, is a medication commonly used to treat attention-deficit/hyperactivity disorder (ADHD). Methylphenidate is used as a cognitive stimulant to combat this disorder by:

- Increasing alertness
- Increasing awareness
- Increasing the ability to pay attention

However, methylphenidate is a chiral compound in which each enantiomer has a differing effect on the body. One enantiomer provides the therapeutic effect while the other proves to be toxic. Therefore, an assay must be utilized that can chirally separate these enantiomers to better understand them. Ethylphenidate (EPH), another chiral stimulant, is a metabolite of methylphenidate in the presence of alcohol. Ritalinic acid (RA) serves as a metabolite of MPH. Due to limited assays, we sought to develop a method that separates the enantiomers of MPH and its metabolites following isolation and extraction from blood samples.

MATERIALS AND METHODS

Sample

- 250 μ L whole blood
- 25 μ L ISTD + standard mixes

Preparation

- 1 mL 100 mM phosphate buffer (pH 6)
- Centrifuge (2000 rpm); 10 min

Condition SPE

- 1 mL methanol
- 1 mL 100 mM phosphate buffer (pH 6)

Load

- UCT Clean Screen DAU Columns (3 mL)

Wash

- 1 mL acetic acid (0.1 M)
- 1 mL methanol

Elute

- 2 mL ammonium hydroxide (2%) in methanol (v/v)

Reconstitute

- Evaporate under nitrogen
- Reconstitute in 100 μ L of 2:98 mobile phase

RESULTS AND DISCUSSION

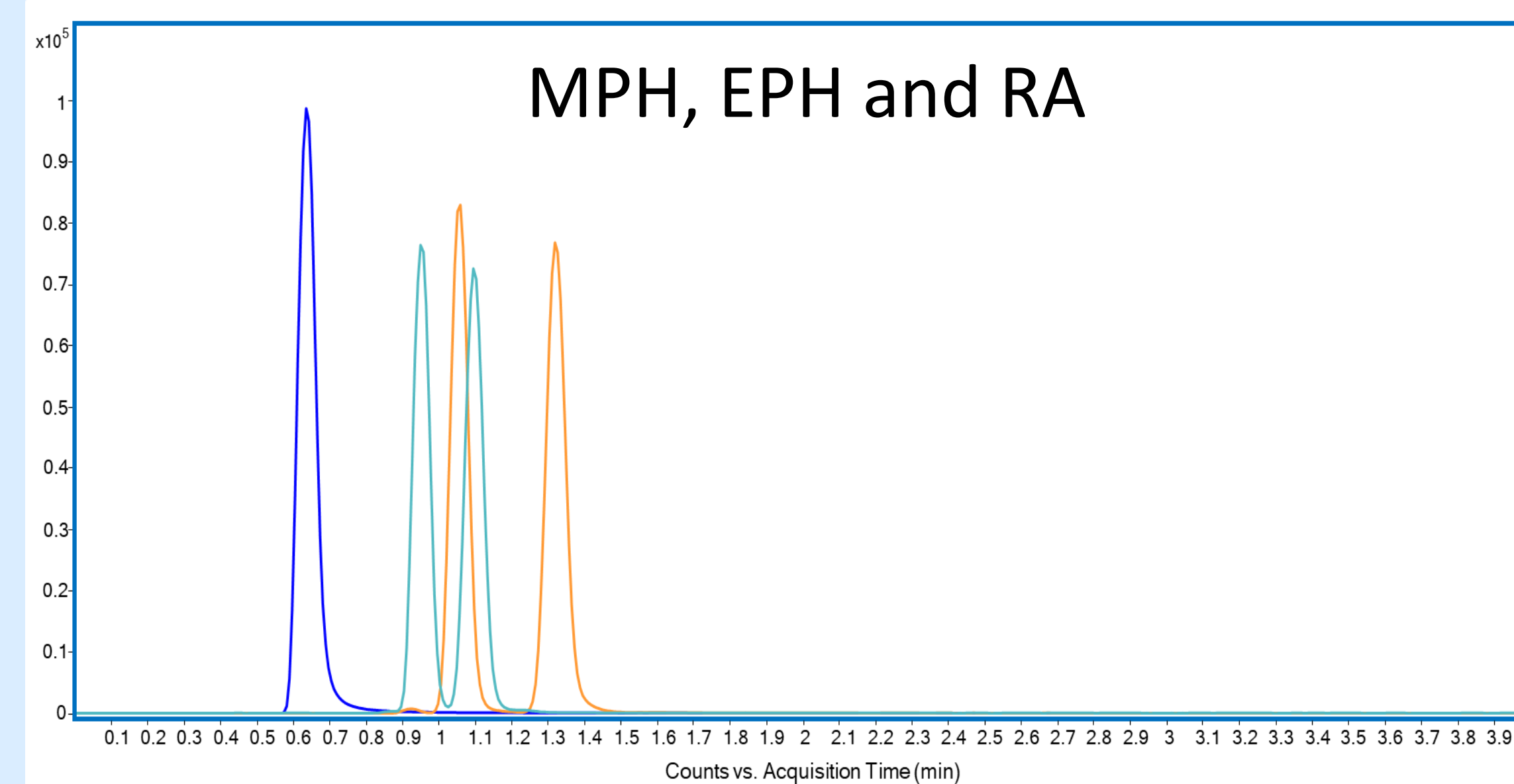


Figure 1. Extracted ion chromatogram for MPH, EPH, and RA from blood (100 ng/mL)

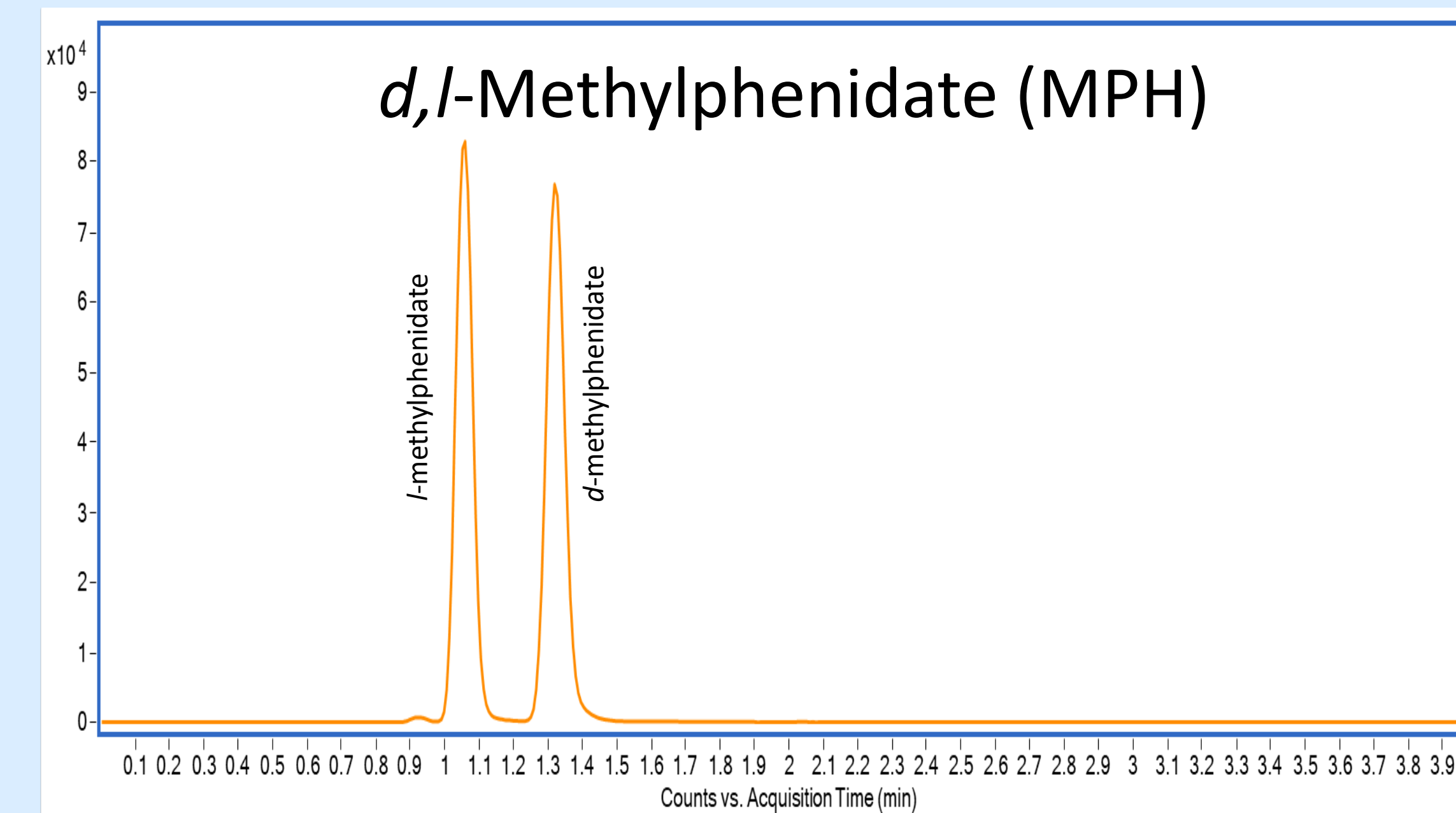


Figure 3. Extracted ion chromatogram for *d,l*-methylphenidate from blood (100 ng/mL)

Table 1. Matrix effects, recovery, and limits of detection (LOD) for MPH, EPH, RA and internal standards in blood

Analyte	Matrix Effects (%) (n=3, 10ng/mL)	Recovery (%) (n=3, 10ng/mL)	LOD (ng/mL)
<i>l</i> -Methylphenidate	1.2	73	0.1
<i>d</i> -Methylphenidate	-10.7	73	0.1
<i>l</i> -Ethylphenidate	-3.9	72	0.1
<i>d</i> -Ethylphenidate	1.4	72	0.1
Ritalinic Acid	-77.4	90	0.5
<i>l</i> -d10-Methylphenidate	-0.8	72	-
<i>d</i> -d10-Methylphenidate	-12.2	70	-
d10-Ritalinic Acid	-76.6	89	-

CONCLUSIONS

- Unique approach to chiral separation and extraction of cognitive-stimulant drugs from blood
- First comprehensive method for chromatographic separation of methylphenidate and ethylphenidate enantiomers, in addition to ritalinic acid
- Method provides simple SPE to isolate the drug and metabolites from a single sample of blood (0.25 mL)

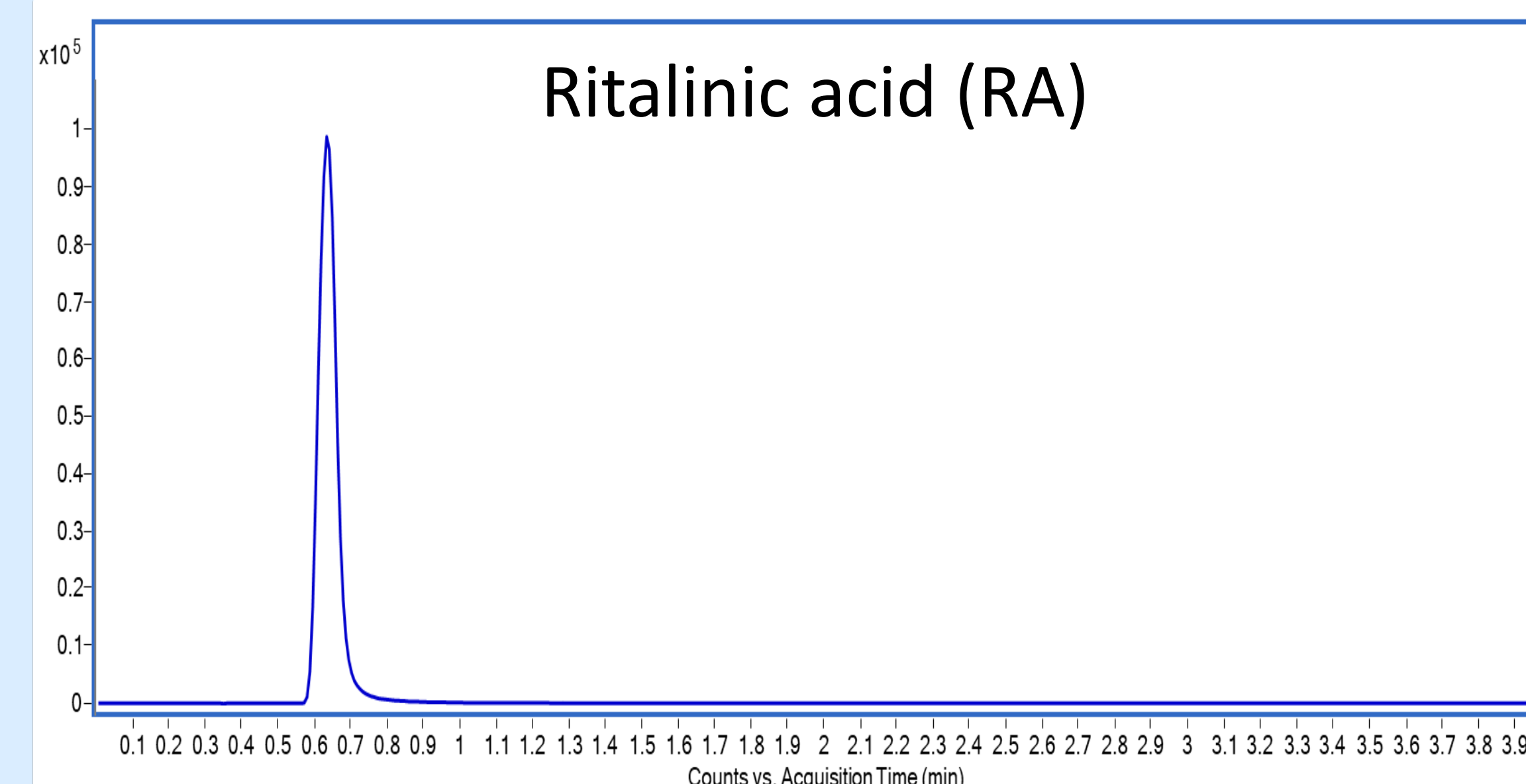


Figure 2. Extracted ion chromatogram for ritalinic acid from blood (100 ng/mL)

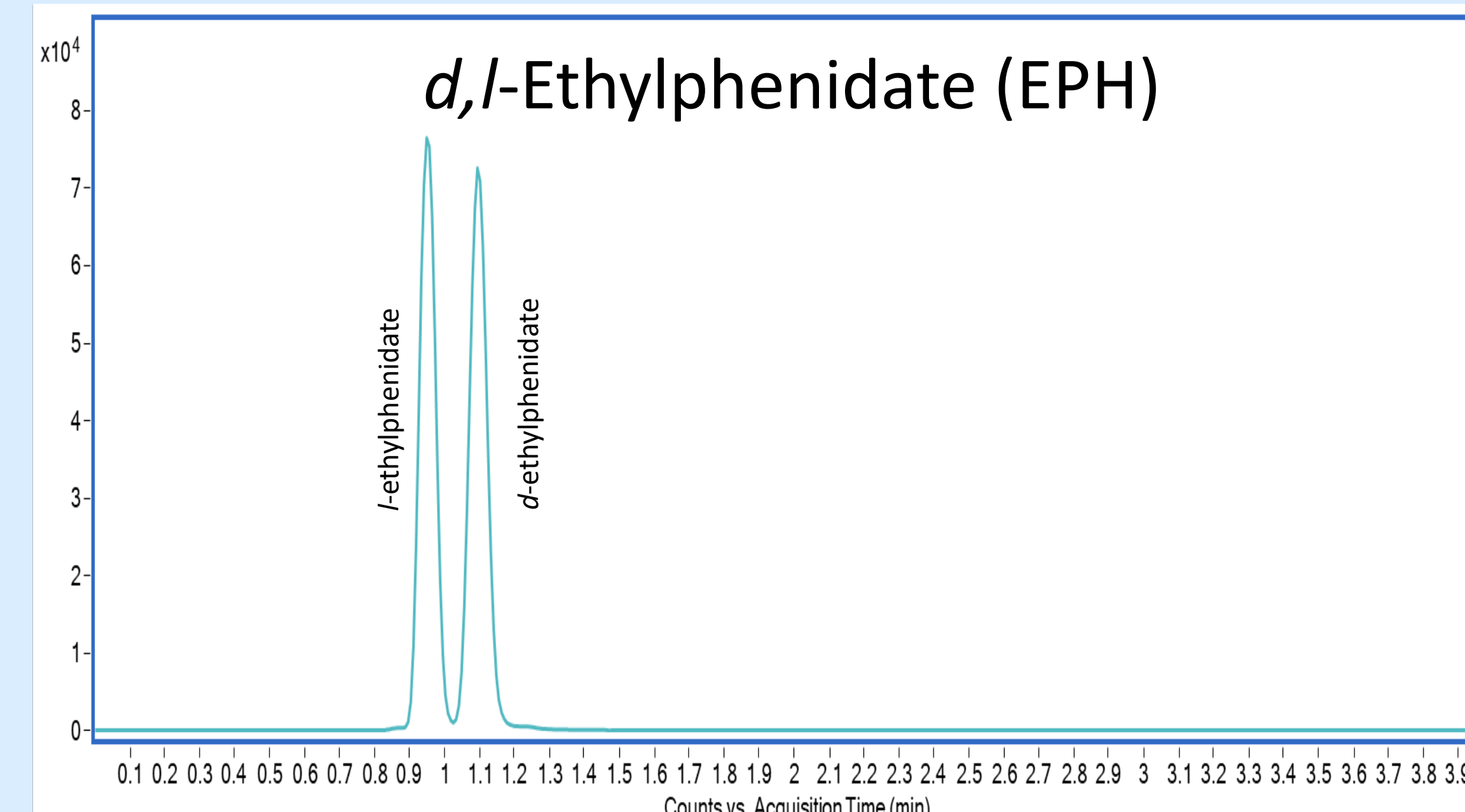


Figure 4. Extracted ion chromatogram for *d,l*-ethylphenidate from blood (100 ng/mL)

MATERIALS AND METHODS

Instrumentation:

- Agilent 1290 Infinity II Liquid Chromatograph
- Agilent 6470 Triple Quadrupole MS

Column:

- Agilent Poroshell Chiral-V (2.1 x 100 mm, 2.7 μ m)

Mobile phase

- A: diH₂O
- B: 0.025% Ammonium acetate (w/v) + 0.0125% Trifluoroacetic acid (v/v) in methanol

Flow rate:

- 0.6 mL/min (isocratic, 2% A: 98% B)

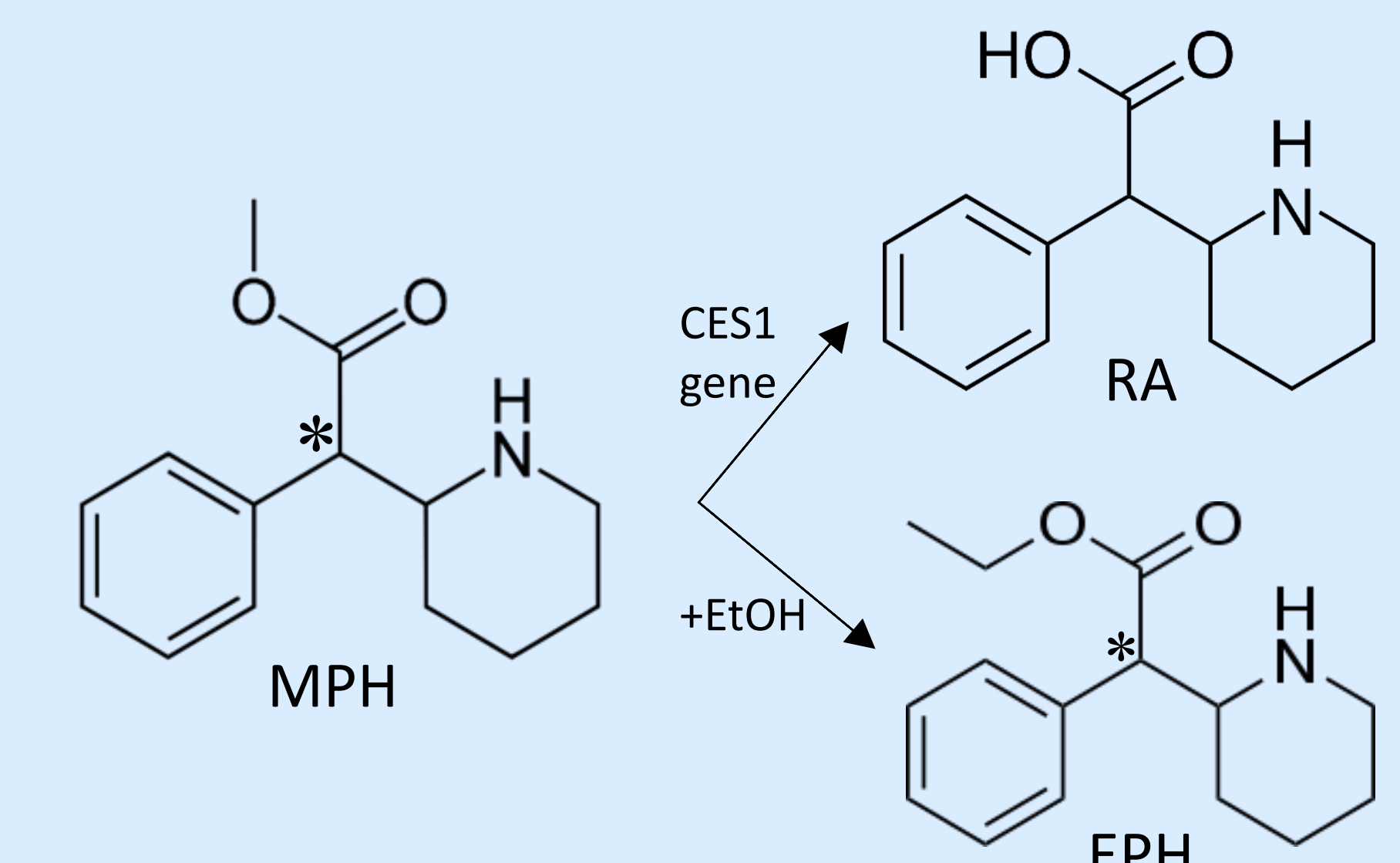


Figure 5. Metabolic pathway of methylphenidate

Table 2. MRM data for MPH, EPH, RA and internal standards

Analyte	Precursor Ion	Product Ion	CE (V)	Frag (V)
MPH	234.1	84.1	21	102
		56.0	49	
EPH	248.2	84.1	21	107
		56.0	57	
RA	220.1	84.0	21	92
		56.1	49	
d10-MPH	244.2	93.1	25	97
		61.1	61	
d10-RA	230.2	93.1	21	107
		61.1	53	

REFERENCES

- Zhu H-J, Patrick KS, Markowitz JS. *Journal of Chromatography B*. 2011;879:783-8
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