Chiral Separation and Quantitation of Methylphenidate, Ethylphenidate, and Ritalinic Acid Using Supercritical Fluid Chromatography

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Disclosure

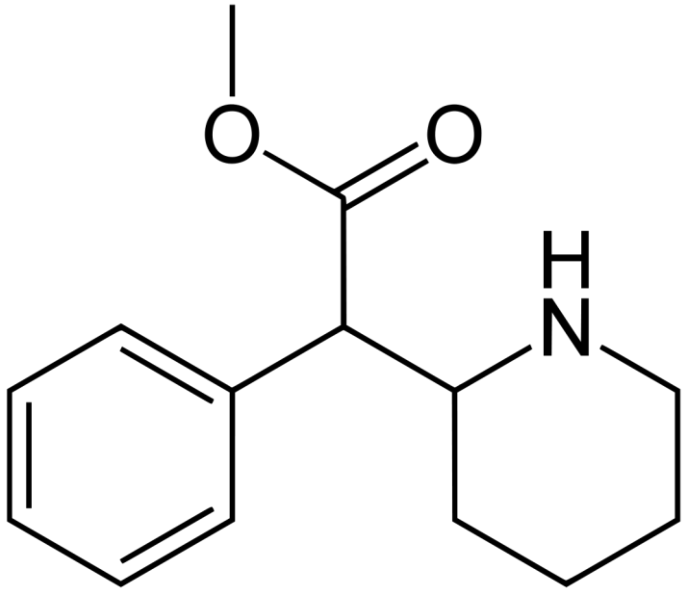
- The authors have nothing to disclose.

Attention-deficit/hyperactivity disorder (ADHD)

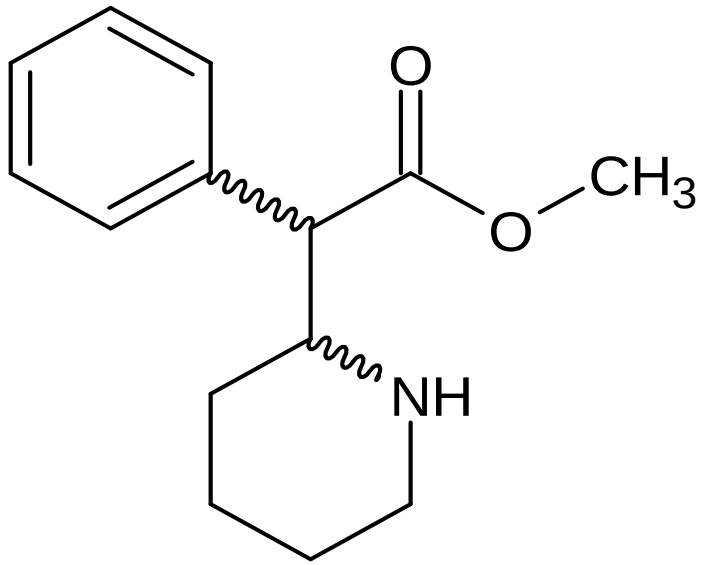
- Neurobehavioral disorder
  - Boredom, difficulty hearing and listening
- Lack of focus
  - Due to: inattention, hyperactivity, impulsivity

Methylphenidate (MPH)

- Most commonly prescribed ADHD medication
- Speeds up brain activity
- 1990s – psychostimulant use, recreational abuse
- Schedule II – medical use, high potential for abuse



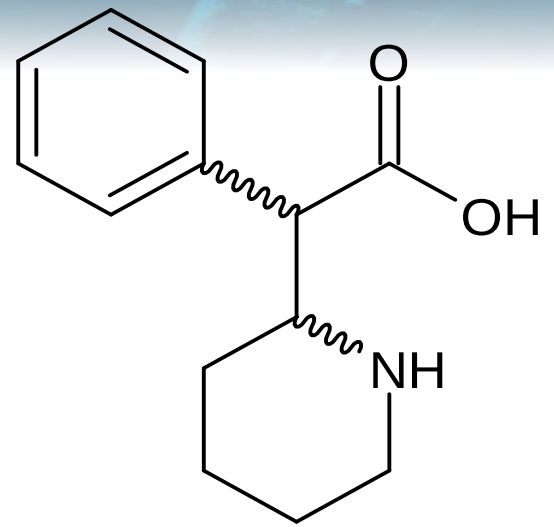
MPH Metabolism



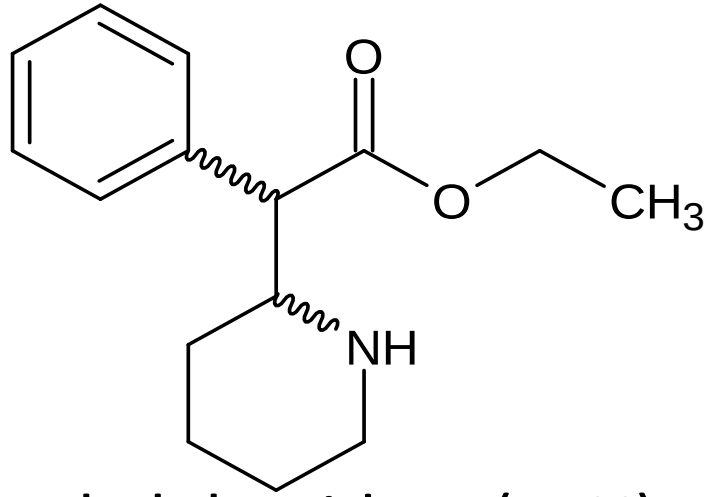
Methylphenidate (MPH)

CES1

+EtOH



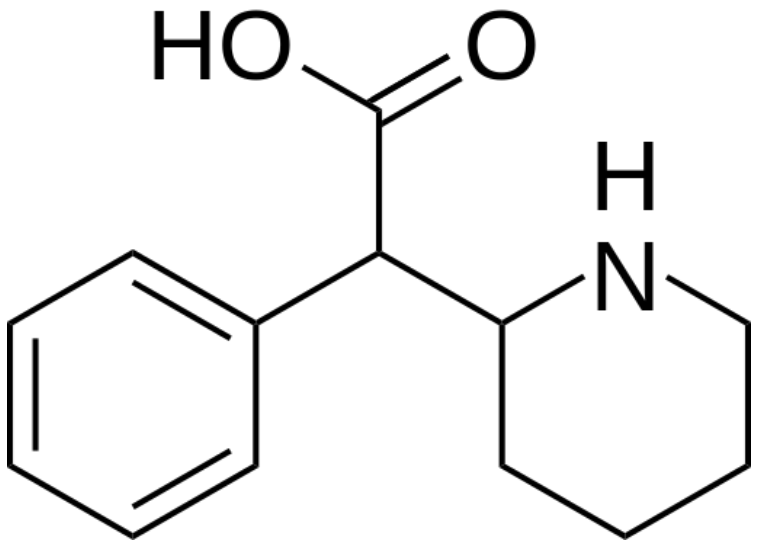
Ritalinic Acid (RA)



Ethylphenidate (EPH)

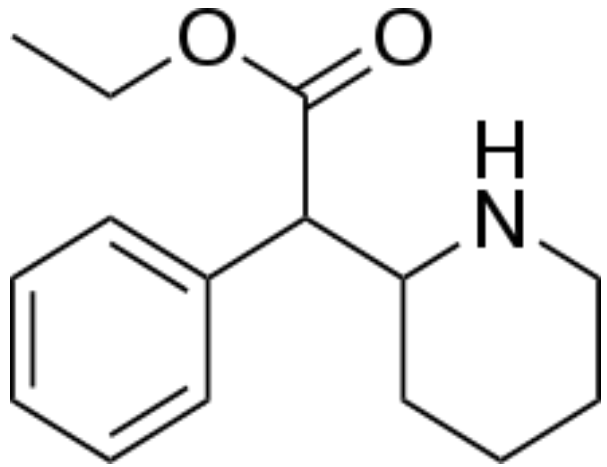
Ritalinic Acid

- Inactive metabolite of MPH
- Structurally different from MPH
  - Carboxylic acid group
  - Poses a challenge for analytical extraction



Ethylphenidate

- MPH and alcohol co-abuse
  - Reported in 92% of cases
- Case studies: 2 fatal overdoses



- More recently: abused alone
  - Purchased over internet

[–] **Borsbakenare** 4 points 8 months ago

I have used ethylphenidate multiple times to study.

It works like a charm for long hours behind the desk. I got really motivated to get shit done and enjoyed the process of studying.

Compared to methylphenidate it's more euphoric IME.

I stopped using this chemical due to the harsh comedowns, i really hate that

After studying, you feel completely empty and the next day i could feel my heart ache sometimes.

Purpose of this study

- To optimize and validate a method for the chiral separation and quantitation of the *l/d* enantiomers of threo-MPH, EPH and RA in postmortem blood using supercritical fluid chromatography coupled with mass spectrometry

Supercritical Fluid Chromatography

- Supercritical fluid – substance maintained above its critical temperature and pressure
  - Exhibition of chemical properties between liquid and gas
- The “in-between” of GC and LC
- CO_2 is used as the mobile phase
- Analysis of low-moderate molecular weight, temperature labile, separation of chiral compounds

Objective

- To develop and optimize a method to extract MPH, EPH and RA from postmortem blood
- To develop and validate a quantitative method for these analytes utilizing a chiral separation method on SFC-MS
- To compare the quantitation values to those acquired by an achiral LC-MS method

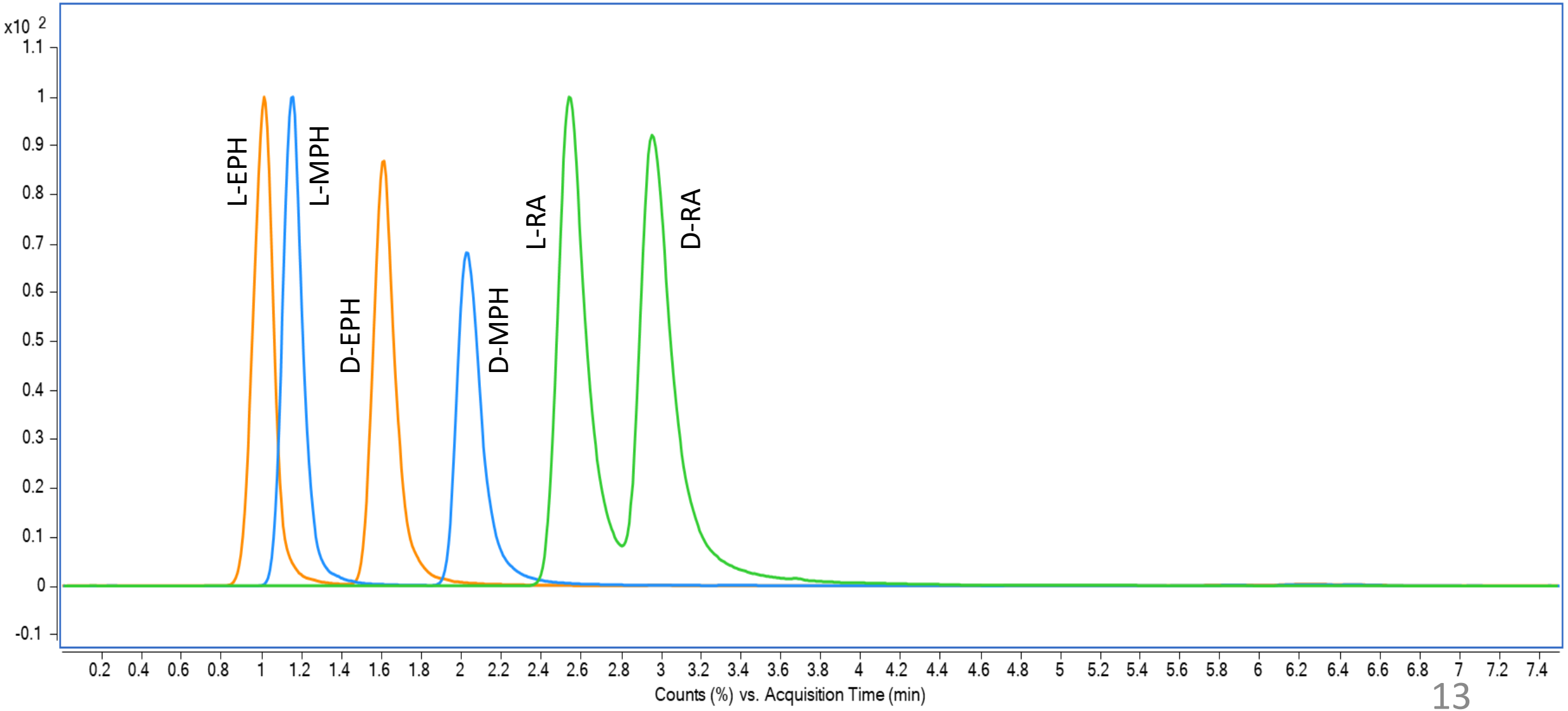
Extraction

- • 250 uL of blood
  • 25 uL of internal standards and drugs
- Add: • 100 mM phosphate buffer (1 mL)
- Wait: • Vortex
  • Let stand for 5 minutes
- Centrifuge: • 2000 rpm, 10 minutes
- Condition: • 1mL Methanol (1 mL)
- Condition: • 100mM phosphate buffer (1 mL)
- Apply: • Sample to column – Agilent bond elut (130 mg)
- Wash: • 100mM acetic acid (1 mL)
- Wash: • MeOH (1 mL)
- Dry: • With pressure, 10min
- Elute: • 2% NH4OH in MeOH (2 mL)
- Dry: • Evaporate to dryness under nitrogen
- Recon: • Mobile phase (0.2% TFA in Methanol) (100 uL)

Instrumentation

Agilent Technologies 1260 Infinity SFC Control/1260 Infinity LC	
Column	Agilent Poroshell Chiral-V (2.1 x 100mm x 2.7 µm)
Mobile Phase	0.2% trifluoroacetic acid in supercritical CO_2
Flow Rate	1.8mL/min
Agilent Technologies Ultivo QQQ MS	
Isocratic Pump	0.1% formic acid in MeOH:diH2O (85:15)
Acquisition Mode	MRM
Injection Volume	3µL

EIC


x10 2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
L-EPH
L-MPH
D-EPH
D-MPH
L-RA
D-RA
0.2 0.4 0.6 0.8 1 1.2 1.4 1.6 1.8 2 2.2 2.4 2.6 2.8 3 3.2 3.4 3.6 3.8 4 4.2 4.4 4.6 4.8 5 5.2 5.4 5.6 5.8 6 6.2 6.4 6.6 6.8 7 7.2 7.4
Counts (%) vs. Acquisition Time (min)

Method Validation – Results (blood)

	Linear Range (R^2>0.99)	ME	Bias	Precision (Within Run)	Precision (Between Run)
L-RA	10-1000	-25	-6.8	12.0	9.8
D-RA	10-1000	-27	-4.7	12.5	11.6
L-MPH	0.25-250	-11	-3.7	14.1	10.9
D-MPH	0.25-250	-7.6	-1.8	13.2	10.4
L-EPH	0.25-250	-7.6	-7.4	15.2	11.4
D-EPH	0.25-250	-5.6	-8.6	15.4	12.1

Case samples

- 49 authentic post-mortem samples identified with presence of MPH, EPH and RA by an achiral method
  - Re-analyzed by this SFC chiral method to quantify the enantiomers
- Comparison of the total concentration values (achiral method) to the *l/d* enantiomer concentration values (chiral method)

Analysis of post-mortem samples (n=49)

Achiral method

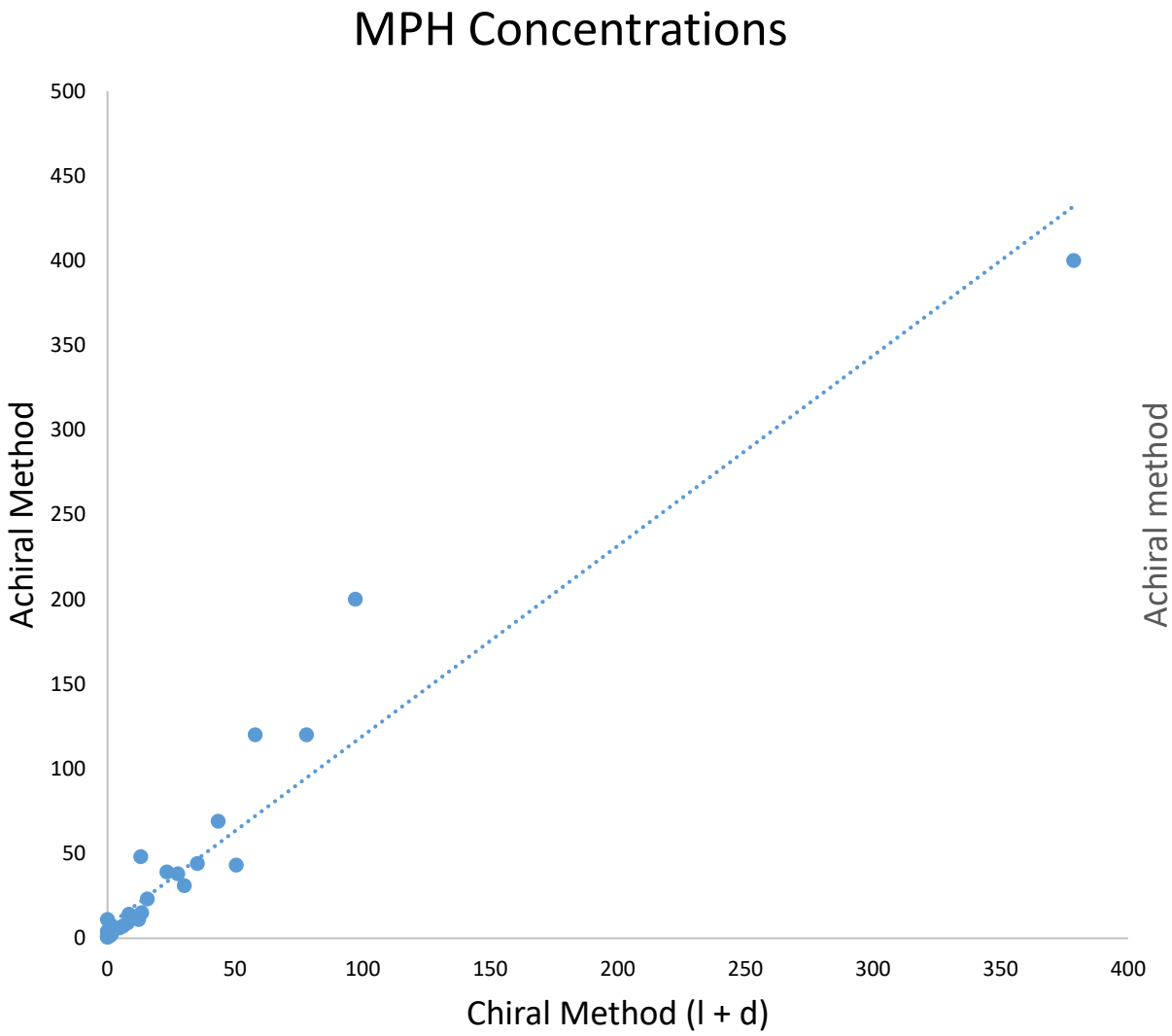
Analyte	Positive Cases	Mean (Range) Concentration (ng/mL)
RA	49	805 (29-5900)
MPH	37	35 (0.6-400)
EPH	7	3 (0.5-10)

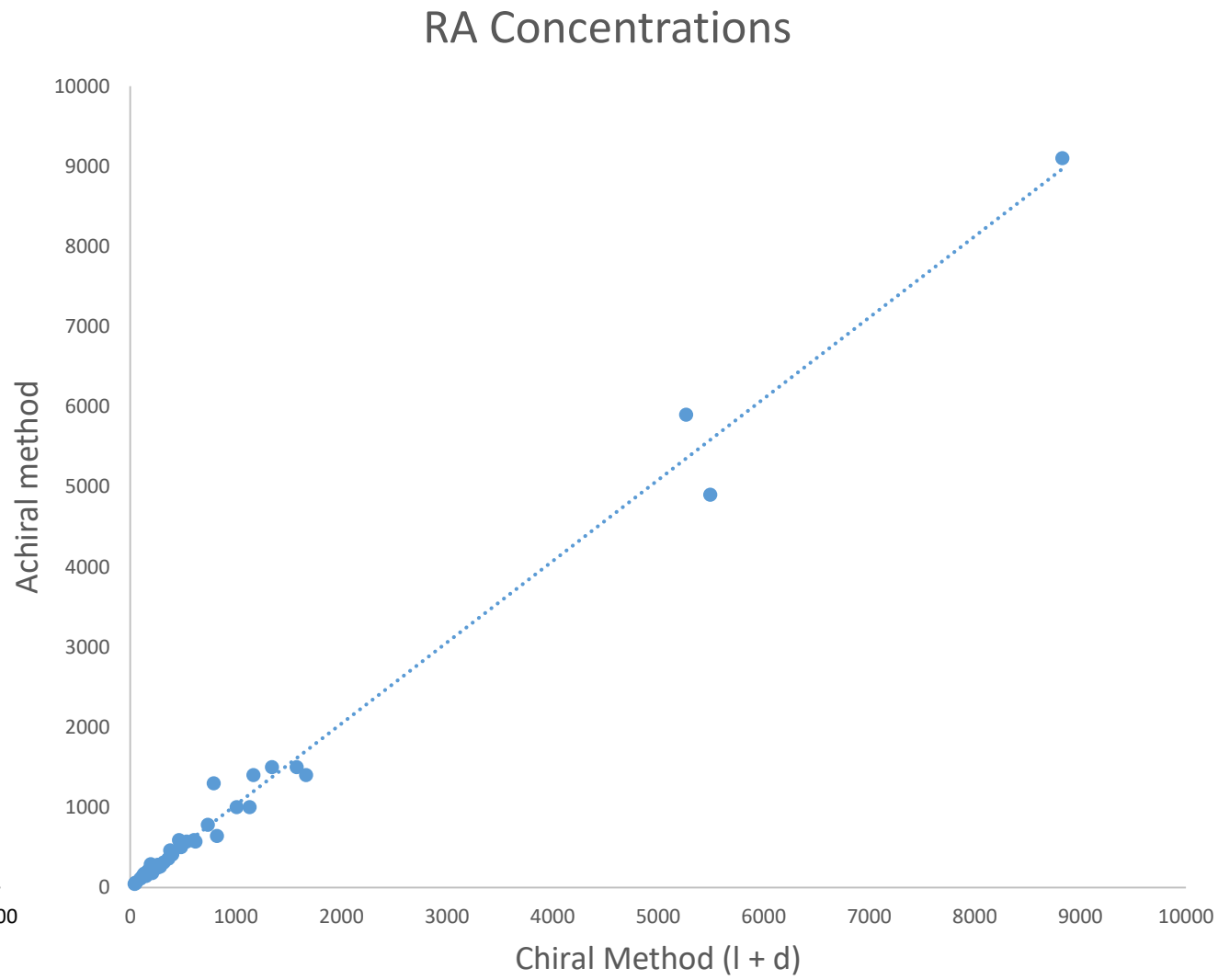
Chiral method

Analyte	Positive Cases	Mean (Range) Concentration (ng/mL)
l-RA	49	343 (<LOQ–3419)
d-RA	49	436 (17–5410)
l-MPH	15	6 (<LOQ–20.5)
d-MPH	30	28.5 (<LOQ–79.4)
l-EPH	5	2.5 (0.62–8.2)
d-EPH	1	<LOQ

- Results were comparable for RA (7.3%) to that of validated LC-MS method but time and concentration played a factor in instability of MPH (55%) and EPH (36%)
- Stability studies suggest MPH breakdown to RA at a faster rate
  - RA could also be more abundant due to abuse

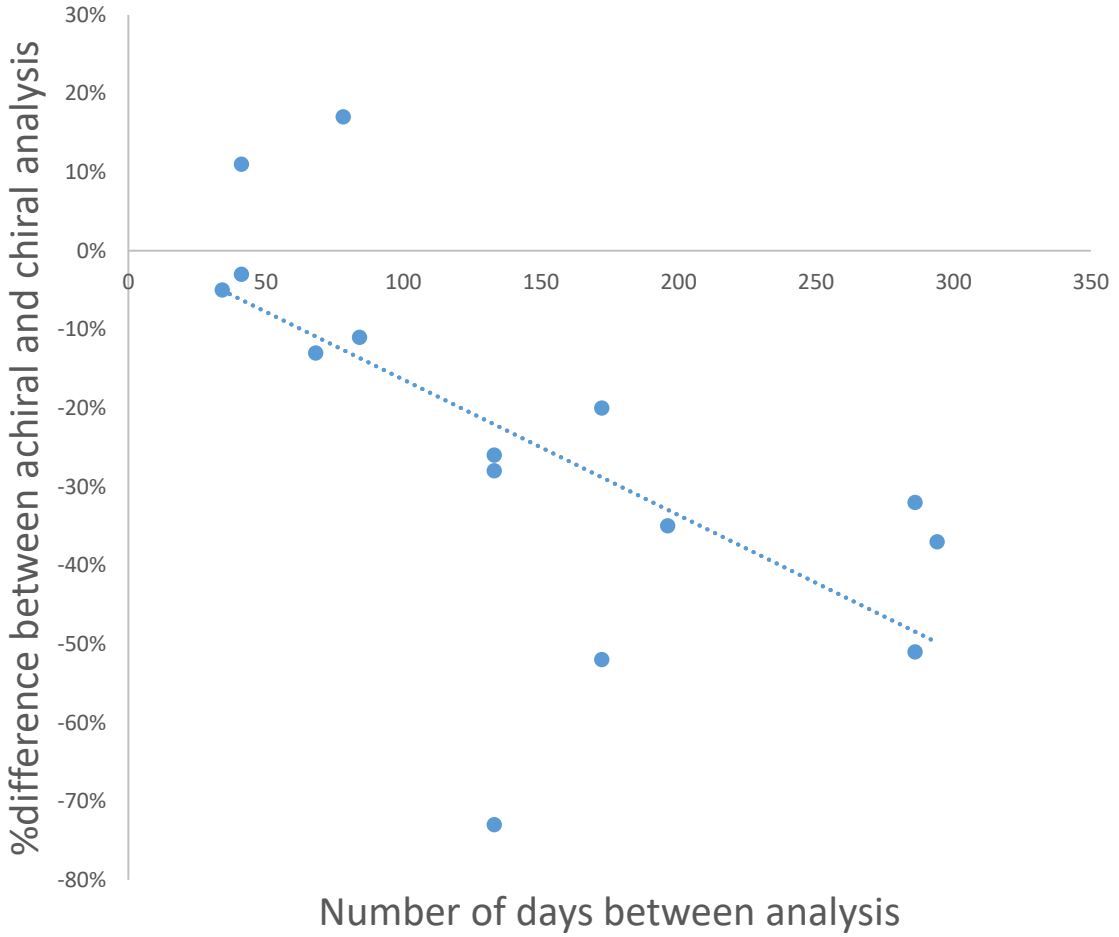
Analysis of post-mortem samples



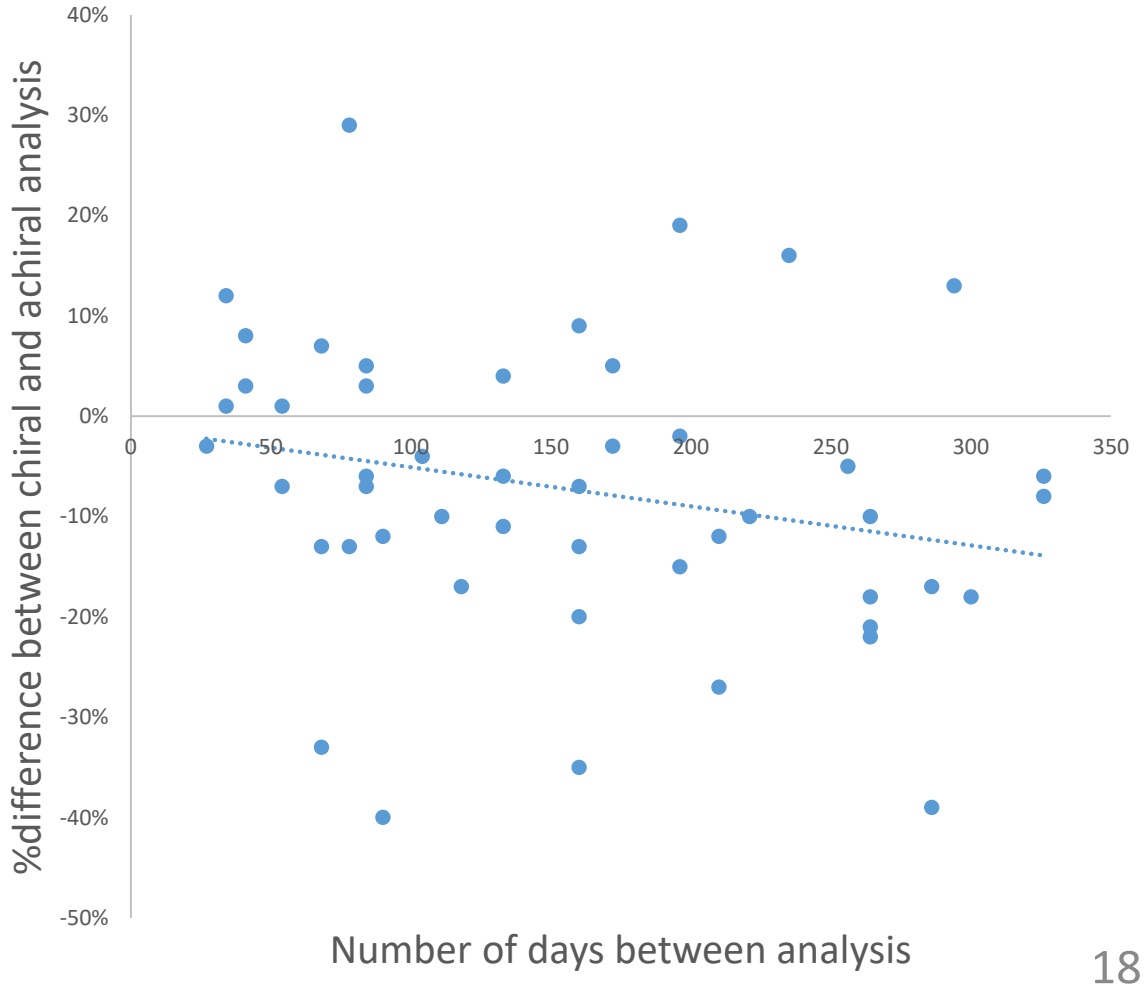


Stability of samples

MPH



Ritalinic Acid



Analysis of post-mortem samples

Analyte	Number of cases	*l/d* ratio Mean (Range)
l-RA	49	0.68 (0.07-1.84)
d-RA		
l-MPH	15	0.14 (<LOQ-0.38)
d-MPH		
l-EPH	1	<LOQ
d-EPH		

- MPH is usually taken as a racemic mixture
- l/d ratio for RA and MPH
  - *d* configuration is more abundant
- Time of intake may play a factor in the difference of l/d ratios

Conclusion

- A SFC-MS method was developed and validated for the chiral separation of threo-MPH, EPH and RA
- This method was applied to 49 postmortem samples
  - These values were compared to an achiral method utilizing LC-MS
- The SFC-MS method gave insight into metabolism and stability issues of these compounds

Thank you!

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