

# **The use of Solid Phase Extraction (SPE) for detection Methylphenidate and Ritalinic Acid in small volume plasma samples**

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**This methodology describes an extraction of Methylphenidate (MPH) and Ritalinic Acid (RA), from plasma using solid-phase extraction (SPE), followed by silylation reaction. In addition an ion chromatographic method was developed for the specific GC determination of MPH and RA. Treated plasma samples were passed through SPE cartridge with Hydrophilic-Lipophilic-Balanced (HLB) sorbent to retain and elute target analytes. Using N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) and N-Methyl-bis(trifluoroacetamide) (MBTFA) reagents, eluent was derivatized and the non-polar product was further analyzed using GC-MS. A calibration curve for MPH and RA was constructed in the range 2-250 ug/mL. The SPE resulted in higher extraction recovery (mean x %) with % R.S.D.s similar in both matrix and solvent x%, respectively). (1)**

# 1

## 1.1 Method and materials

Native standards for Ritalinic Acid hydrochloride (1.0 mg/mL in MeOH) and Methylphenidate hydrochloride (1.0 mg/mL in MeOH) were purchased from Sigma Aldrich (St. Louis MO, USA). Both labeled standards for ( $\pm$ )-threo-Methylphenidate- $D_4$  HCl 100ug in MeOH) and ( $\pm$ )-threo-Ritalinic acid- $D_{10}$  HCl (100ug in MeOH) were obtained from Cerilliant (Round Rock, TX). BioChemed Services (Winchester, VA, USA) provided with bovine plasma. HPLC grade formic acid, ammonium hydroxide, methanol and distilled water were purchased from Fisher Chemical (Nazareth, PA). Alongside with solvents, derivatizer MBTFA [N-methyl-bis(trifluoroacetamide)] was also purchased through Fisher Chemical. MSTFA (N-methyl-n-trimethylsilyl-trifluoroacetamide) derivatizer was purchased from RESTEK (Bellefonte, PA). Solid-phase extraction columns (Oasis PRiME HLB 3 cc Vac Cartridge, 60 mg) were acquired from Waters Corp (Milford, MA).

## 1.2 Sample Preparation

## 1.3 Instrumentation

The detection of analytes was performed by GC–MS/MS with XXX . Mass Hunter QQQ software was used for the data acquisition and quantification (2)

## 2 Results and discussion

### 2.1 Linearity

### 2.2 Precision and accuracy

### 2.3 Recoveries

## 3 Discussion and conclusion

### 3.1 Reliability

### 3.2 Practicality

## 4 References

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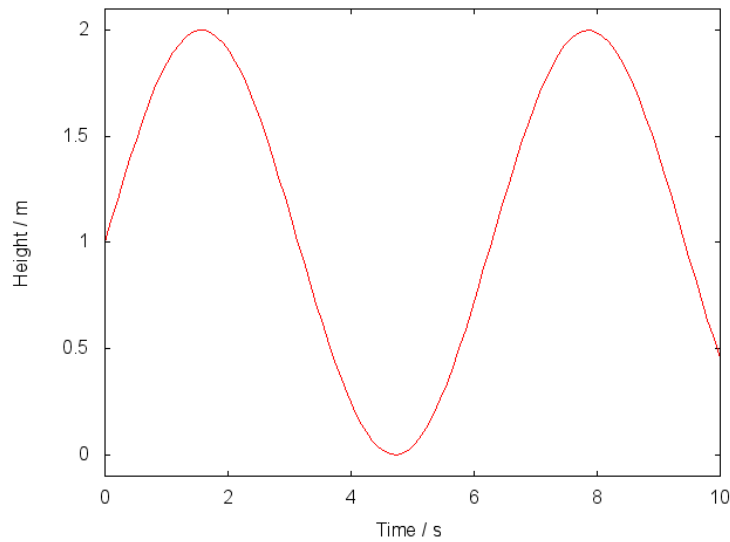
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Sample	<i>A</i>	<i>B</i>	<i>C</i>
	(unit)	(unit)	(unit)
First	1	2	3
Second	4	6	8
Third	5	7	9

## References and Notes

1. R. Thomsen, H. Rasmussen, K. Linnet, A. Pagsberg, Enantioselective Determination of Methylphenidate and Ritalinic Acid in Whole Blood from Forensic Cases Using Automated Solid-Phase Extraction and Liquid ChromatographyTandem Mass Spectrometry. *Journal of analytical toxicology* **36**, 560–568 (2012), doi:10.1093/jat/bks065.
2. Materials and methods are available as supplementary material.
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5. P. One, P. Two, P. Three, An unpublished preprint (2021), arXiv:2101.12345.
6. F. Author, An example dataset, version number, Repository name (2021), doi:10.1000.dataset-DOI.
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8. W. o. Textbooks, *A supplementary reference* (Imaginary publisher) (2021).
9. R. One, R. Two, A talk given at a meeting, in *Proceedings of an Example Conference*, D. Scientist, Ed., vol. 54 of *Series name* (2020), p. 22.

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Materials and Methods

Supplementary Text

Figs. S1 to S3

Tables S1 to S4

References (7-9)

Movie S1

Data S1



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**Methylphenidate and Ritalinic Acid in small volume plasma**  
**samples**

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Figures S1 to S3

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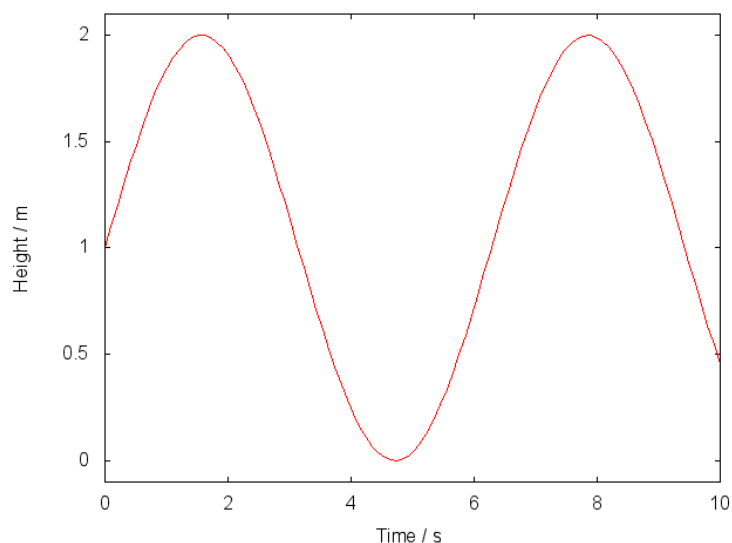
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