

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

Viola Nguyen¹, Blessing Akitunde¹, Dhoone Menezes de Sousa¹,
Shyamalee Dassanayake ¹

¹Environmental Medicine & Climate Science , Icahn School of Medicine NY 10023, USA.

*Corresponding author. Email: viola.nguyen@mssm.edu

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). (I) aaaa

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).

1.1 Sample Preparation

1.2 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 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

but do not attempt a comprehensive review.

General guidance on L^AT_EX: The *Science*-family journals accept papers written in L^AT_EX, but they are a minority of the submissions we receive. Our production department does not handle L^AT_EX directly, instead we use conversion software to automatically process the .tex file into a format they can use. That works well *provided the .tex file is straightforward*. Keep it simple and follow this template. Don't import additional packages or define complex new commands.

Figures and tables: These should be inserted at the end of the main text, as below (not in the middle of the text). Refer to them using e.g. Figure 1 (or Fig. 1) and Table ??.

Citing references: Science uses a numeric citation system. Cite references by number e.g. (?). The template will combine reference numbers automatically (?, ?), including ranges (?, ?, ?). Reference author names and years should be stated in the reference list, not in the text. If you want to add a comment, use the syntax [see (?) for details].

Referring to supplementary material: Whenever more details are given in the Materials and Methods section, cite an entry in the reference list that directs readers there, like this (?). To refer to material in the Supplementary Text section, just write (Supplementary Text). See guidance below for the difference between those two types of supplementary material. Supplementary figures and

tables are referred to in lowercase e.g. figure ?? or table ?. Material in separate files needs to be hand coded e.g. data S1, movie S2.

Mathematics: Simple mathematical expressions can be inserted in the text like $2 \times 3 = 6$. Variables should be italic but textual labels are roman e.g. T_{\max} . Explain the meaning of all variables on their first appearance. More complicated expressions should be entered as numbered equations, such as

Do not indent text immediately after an equation. They can be referred back to as e.g. Equation ?.

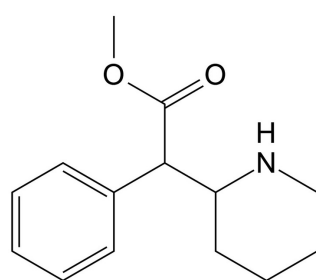
Formatting: Names of software packages should be set in small capitals e.g. NUMPY. Use a non-breaking space between a number and its unit e.g. 7.4 km, and thin spaces between different parts of a unit e.g. 12 m s^{-1} . Use \pm (not parentheses) to indicate uncertainties e.g. $g = 9.8 \pm 0.2 \text{ m s}^{-2}$.

Research Articles and Reviews split the text into sections using headings Use a short (up to 6 words) descriptive phrase, not generic 'Results' or 'Conclusions' Most other formats do not have headings, see the journal instructions to authors for details

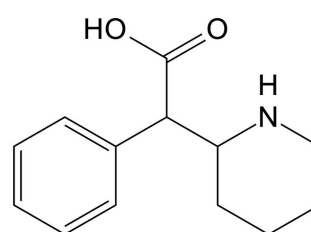
An example heading

Research Articles and Reviews use headings to split the main text into sections; most other formats do not have headings.

Length limits: The *Science*-family journals impose limits on the number of words, figures/tables, and references cited in the main text. The limits vary between the journals and article types. Refer to the instructions to authors on the journal website for the current limits.



methylphenidate



ritalinic acid

Figure 1: Chemical structures of methylphenidate (MPH) and its primary metabolite ritalinic acid (RA).

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.

Acknowledgments

Funding:**Author contributions:****Competing interests:**

Data and materials availability: Specify where the data, software, physical samples, simulation outputs or other materials underlying the paper are archived. They must be publicly accessible when the paper is published (without embargo) and enable readers to reproduce all the results in the paper. Contact the editor if you're unsure what needs to be shared.

Our preference is for digital material to be deposited in a suitable non-profit online data or software repository that issues the material with a DOI. Alternatively, an institutional repository, subject-based archive, commercial repository etc. is acceptable, as are (short) supplementary tables or a machine-readable supplementary data file. 'Available on request' or personal web pages are not allowed.

Cite the relevant DOI (?), URL (?) or reference (?) in this statement. These *do not* count towards the reference limit if they are only cited in the acknowledgements. Be specific and state a unique identifier – such as an accession number, software version number or observation ID – so readers can easily retrieve the exact material used.

Declare any restrictions on sharing or re-use – such as a Materials Transfer Agreement (MTA) or legal restrictions – which must be approved by your editor. Unreasonable restrictions will preclude publication. Consult the journal's editorial policies web page for more details.

Supplementary materials

Materials and Methods

Supplementary Text

Figs. S to S

Tables S to S

References (7-1)

Movie S1

Data S1

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

First Author^{*†}, A. Scientist[†], Someone E. Else

^{*}Corresponding author. Email: example@mail.com

[†]These authors contributed equally to this work.

This PDF file includes:

Materials and Methods

Supplementary Text

Figures S1 to S3

Tables S1 to S4

Captions for Movies S1 to S2

Captions for Data S1 to S2

Other Supplementary Materials for this manuscript:

Movies S1 to S2

Data S1 to S2

Materials and Methods

The Materials and Methods section should contain details of the samples measured, experiments performed, observations taken, simulations run, data analysis, statistical methods etc. Give enough detail for any competent researcher in your field to fully reproduce the results.

To refer to this section from the main text, use the numbered note in the reference list (?). Refer to figures and tables in the same way as in the main text but now all capitalized e.g. Fig. 1, Table ??, Fig. ?? and Table ??. Cite references in the usual way (?), including any that are only cited in the supplement (?, ?).

Example supplement heading

The two main sections of the supplement can be split up using headings.

Supplementary Text

The Supplementary Text section can only be used to directly support statements made in the main text e.g. to present more detailed justifications of assumptions, investigate alternative scenarios, provide extended acknowledgements etc. Material in this section cannot claim results or conclusions that weren't mentioned in the main text. To refer to this section from the main text, just write (Supplementary Text).

Example supplement heading

The two main sections of the supplement can be split up using headings.