McLaughlin, G, Morris, N, Kavanagh, PV, Power, JD, Dowling, G, Twamley, B, O'Brien, J, Hessman, G, Murphy, B, Walther, D, Partilla, JS, Baumann, MH and Brandt, SD

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Analytical characterization and pharmacological evaluation of the new psychoactive substance 4-fluoromethylphenidate (4F-MPH) and differentiation between (±)-threo- and (±)-erythro- diastereomers.

Gavin McLaughlin, a,b* Noreen Morris, a Pierce V. Kavanagh, b John D. Power, b,c Geraldine Dowling, b,d Brendan Twamley, e John O’Brien, e Gary Hessman, e Brian Murphy, d Donna Walther, f John S. Partilla, f Michael H. Baumann, f and Simon D. Brandt g

a Department of Life and Physical Sciences, School of Science, Athlone Institute of Technology, Dublin Road, Westmeath, Ireland

b Department of Pharmacology and Therapeutics, School of Medicine, Trinity Centre for Health Sciences, St. James’s Hospital, Dublin 8, Ireland

c Forensic Science Ireland, Garda HQ, Phoenix Park, Dublin 8, Ireland

d School of Chemical and Pharmaceutical Sciences, Dublin Institute of Technology, Dublin 2, Ireland

e School of Chemistry, Trinity College Dublin, Dublin 2, Ireland

f Designer Drug Research Unit of the Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD 21224, USA

g School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, UK

*Correspondence to: Gavin McLaughlin, Department of Pharmacology & Therapeutics, School of Medicine, Trinity Centre for Health Sciences, St. James’s Hospital, Dublin 8, Ireland. E-Mail: gmclaug@tcd.ie or gavinmclaughlin@research.ait.ie
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1. Examples of powdered 4F-MPH and tablet products
2. TLC analysis of vendor sample containing both (±)-threo- and (±)-erythro-racemates of 4F-MPH, followed by LC-MS analysis of the isolated bands.
3. Preparation of 4F-MPH tablets for GC-MS/LC-MS analysis

For analysis of the 4F-MPH tablets by gas chromatography mass spectrometry (GC-MS), the tablet was crushed using a mortar and pestle and 10 mg was added to 1 mL of methanol. This solution was added to a Corning® Costar® Spin-X® centrifuge tube filter (cellulose acetate membrane, 0.45 µm) (Corning Inc, United States) and centrifuged at 2500 rpm for 3 minutes. Furthermore, 100 µL of this filtered solution was added to 900 µL methanol in a GC vial.

For analysis of the 4F-MPH tablets by liquid chromatography mass spectrometry (LC-MS), the tablet was crushed using a mortar and pestle and 10 mg was added to 1 mL of acetonitrile/water (1:1) with 0.1% formic acid. This solution was added to a Corning® Costar® Spin-X® centrifuge tube filter (cellulose acetate membrane, 0.45 µm) (Corning Inc, United States) and centrifuged at 2500 rpm for 3 minutes. Furthermore, 20 µL of this filtered solution was added to 980 µL acetonitrile/water (1:1) with 0.1% formic acid in a LC vial.
4. GC-induced thermal degradation of 4-fluoromethylphenidate isomers (4F-MPH) to methyl 4-fluorophenyl acetate (7.26 min)

![Graph showing GC-MS analysis of 4F-MPH samples]

- **4F-MPH sample 1 (Powder)**: (±)-threo-4F-MPH (18.00 min), (±)-erythro-4F-MPH (18.13 min)
- **4F-MPH sample 2 (Powder)**: (±)-threo-4F-MPH (18.01 min), (±)-erythro-4F-MPH (18.13 min)
- **4F-MPH sample 3 (Tablet)**: (±)-threo-4F-MPH (18.13 min)

**Chemical Structures:**
- **Enol tautomer**
- **Methyl 4-fluorophenyl acetate**
5. EI-MS data obtained for vendor samples 1-3

Vendor 1: both (±)-threo- and (±)-erythro racemates of 4F-MPH (Powder)

Vendor 2: (±)-threo-4F-MPH only (Powder)
Vendor 3: (±)-threo-4F-MPH only (Tablet)

Vendor 1: both (±)-threo- and (±)-erythro racemates of 4F-MPH (Powder)
Vendor 2: (±)-threo-racemate of 4F-MPH (Powder)
Vendor 3: (±)-threo-racemate of 4F-MPH (Tablet)

7. $^{13}$C NMR spectra obtained for the isolated (±)-erythro- and (±)-threo-racemates of 4F-MPH (HCl salt in DMSO- $d_6$).
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(±)-threo-4F-MPH

(±)-threo-4F-MPH
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11. Two-dimensional NMR experiments

- Heteronuclear single quantum coherence spectroscopy (HSQC)

(±)-*threo*-4F-MPH racemate (HCl salt in DMSO)

(±)-*erythro*-4F-MPH racemate (HCl salt in DMSO)
4F-MPH vendor product identified as (±)-threo- and (±)-erythro-4F-MPH racemates (HCl salt in DMSO)

4F-MPH vendor product identified as (±)-threo-4F-MPH racemate (HCl salt in DMSO)
Heteronuclear Multiple Bond Correlation (HMBC)

(±)-*threo*-4F-MPH racemate (HCl salt in DMSO)

(±)-*erythro*-4F-MPH racemate (HCl salt in DMSO)
4F-MPH vendor product identified as (±)-threo and (±)-erythro-4F-MPH racemates (HCl salt in DMSO)

4F-MPH vendor product identified as (±)-threo-4F-MPH racemate (HCl salt in DMSO)
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Powdered product containing mixture of (±)-erythro and (±)-threo-4FMPH racemate
Tablet containing (±)-threo-4F-MPH racemate
13. X-ray crystallography supporting information

Left: *erythro* salt hydrogen bonded dimer with hydrogen atoms omitted for clarity. The cell is shown. Only atoms involved in H-bonding interactions are labelled. Right: *threo* salt hydrogen bonding network extending parallel to the b-axis
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14. High resolution mass spectrometry data obtained for isolated (±)-threo-racemate, isolated (±)-erythro-racemate, and vendor samples

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SmartFormula Settings

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Supervisor
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Low value of mSigma indicates good isotopic pattern match
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Acquisition Date  06/07/2016 15:14:03
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SmartFormula Settings
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Trinity College Dublin
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Analysis Name
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Station
Supervisor
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Low value of mSigma indicates good isotopic pattern match
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