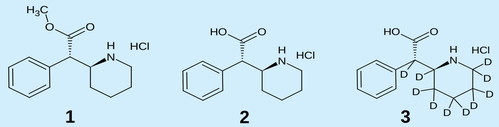
±threo-Ritalinic acid-D10 Hydrochloride an internal standard for quantitation of Ritalinic acid by LCMSMS: Synthesis determination of isotopic distribution by qNMR and LCMS

Authors **Elizabeth B. Marek**, Joshua Cooper, Huahua Jian, Uma Sreenivasan, Isil Dilek

Abstract

As a major metabolite of methylphenidate (**1**, Ritalin), ±-*threo*-Ritalinic acid (**2**) is of clinical relevance. To this end ±-*threo*-ritalinic acid-D_{10} HCl (**3**) was synthesized in seven steps with a purity of 99% and an isotopic purity ratio of D_0/D_{10} = 0% and a significant amount of the D_9-D_7 isomers. Because practical ion monitoring is based on the ratio of D_0/D_{10}, the standard was found to be suitable for use as an internal standard in LC-MS/MS analysis of ritalinic acid and related compounds. The presence of significant amounts of the D_9 isomer prompted extensive structure elucidation work using LC-MS/MS scrambling and 1D, 2D, and qNMR techniques.



1 **2** **3**

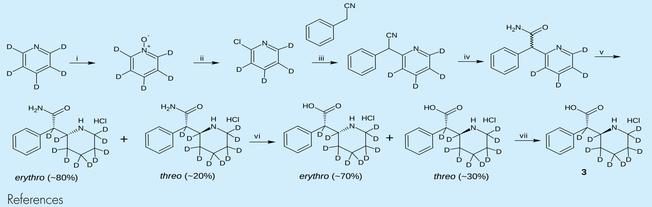
Introduction

Methylphenidate, most commonly known by the Novartis trade name Ritalin®, is a pyschostimulant used to treat attention-deficit hyperactivity disorder, postural orthostatic tachycardia syndrome, and narcolepsy, by increasing alertness and attention and by counteracting fatigue. Methylphenidate was originally sold as a mixture of diastereomers, although it has been shown that the majority of the activity is attributed to the ±-*threo* isomer. More recent products such as Focalin® contain only the active ±-*threo* isomer. While analytical reference standards of the diastereomeric mixture of methylphenidate and its metabolites are available, standards containing only the active isomer are now desirable to reflect the current directive of using only the active isomer in drug products. Therefore it is also desirable to synthesize stable-labeled derivatives of the active isomers of methylphenidate and its metabolites, such as ritalinic acid, for use as internal standards.

Ritalinic acid is a major metabolite of and synthetic precursor to methylphenidate and may be monitored clinically and forensically. The synthesis of deuterated ±-*threo*-ritalinic acid was therefore undertaken to develop an analytical reference standard and as a precursor to deuterated ±-*threo*-methylphenidate.

Synthesis of ±-*threo*-Ritalinic acid-D_{10} HCl

Based on literature precedence [1-3], ±-*threo*-Ritalinic acid-D_{10} HCl was synthesized in seven steps from pyridine-D_5. During the synthesis, the crucial reduction of the pyridine moiety to the fully deuterated piperidine proceeded in good yield but LC/MS-SIM indicated that the product contained a mixture of 55% D_{10}, 34% D_9 and 11% D_8-D_7. The presence of the D_0 isomer was not detected. This deuterium ratio was carried through to the final product.



References
1. J. Heterocyclic Chem. 44; 2007; 1485.
2. US Patent 5936091.
3. J. Med. Chem. 39; 6; 1996; 1201.

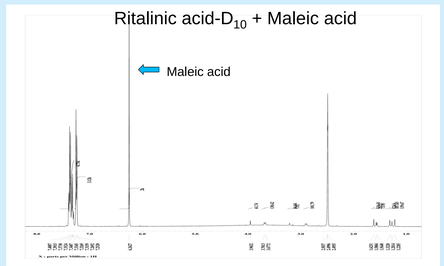
Characterization of ±-*threo*-Ritalinic acid-D_{10} HCl

Isotopic distribution by LC/MS-SIM	
Deuterium Content	Mole Percent (x_i)
D_{10}	55.16%
D_9	33.98%
D_8	9.21%
D_7	1.48%
D_6	0.148%
D_5	0.0128%
D_4-D_0	0.0%

The identity of ±-*threo*-Ritalinic acid-D_{10} HCl was established through NMR and mass spectrometry. The chemical purity was established through HPLC/UV, Karl Fisher, GC/FID Headspace and ROI. LC-MS/MS studies were performed to evaluate isotopic purity, deuterium distribution, fragmentation patterns and suitability for use as an internal standard. HPLC analysis indicated a purity of 99% with isotopic purity ratio D_0/D_{10} = 0% by LC/MS-SIM. Additionally, LC/MS-SIM confirmed the presence of 4.5% D_9-D_7 isomers (see isotopic distribution at left).

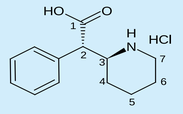
Isotopic distribution by quantitative NMR

Using maleic acid as an internal standard, Quantitative NMR (qNMR) was used to determine the percentage of hydrogen and therefore deuterium on each carbon of ±-*threo*-Ritalinic acid-D_{10} HCl.



$$H\% = \frac{I_A}{I_{std}} \times \frac{n_{std}}{n_A} \times \frac{m_{std}}{m_a} \times \frac{M_a}{M_{std}} \times \frac{P_{std}}{P_a}$$

H% = % hydrogen
I = integral of signal
n = number of protons under the signal of interest
m = mass of compound of interest
M = molecular weight
std = internal standard
A or a = component or analyte whose purity is to be calculated
P = purity



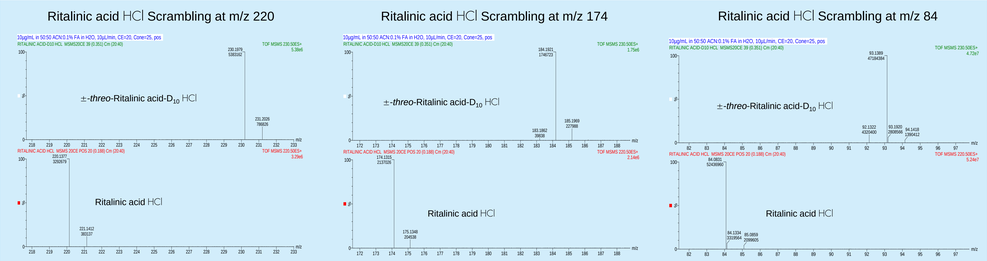
qNMR confirmed the presence of a nearly even distribution of deuterium throughout the piperidine moiety and also revealed that a small percentage of the hydrogens on the aromatic moiety had exchanged for deuterium as well. These results suggest that the Pt/C catalyst used in step 5 of the synthesis also facilitated the exchange of aromatic hydrogens for deuterium.

Product			Internal Standard	
±-*threo*-Ritalinic acid-D_{10}			Maleic acid	
Mass balance	I_{std} =	2000	M_a =	265.223
Purity	P_a =	95.51%	M_{std} =	116.07
	m_{std} =	4.029	m_a =	15.552
	n_{std} =	2	P_{std} =	99.78%
Position	n_A	I_A	*H%*	*D%*
Ortho	2	3122.0571	96.540	3.460
Para&meta	3	4708.465	97.063	2.937
Aromatic	5	7830.522	96.854	3.146
2	1	61.743	3.818	96.182
3	1	130.420	8.066	91.934
4	2	158.628	4.905	95.095
5	2	264.270	8.172	91.828
6	2	185.778	5.745	94.255
7	2	208.357	6.443	93.557

The molar mass of the product is calculated based on isotopic purity results obtained by LC/MS-SIM.

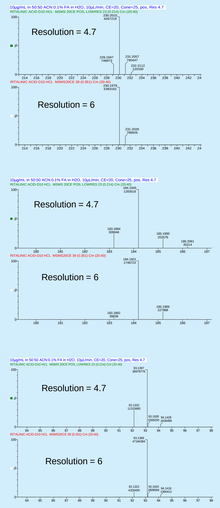
$$\overline{FW} = \sum_{i=0}^{10} x_i (C_{13}H_{18-i}D_iO_2ClN)$$

Investigation of ±-*threo*-Ritalinic acid-D_{10} HCl Scrambling using Waters Xevo G2 Q-Tof



Effect of Resolution on the Scrambling of ±-*threo*-Ritalinic acid-D_{10} HCl

Waters Xevo G2 Q-Tof



Agilent 6410 triple quadrupole

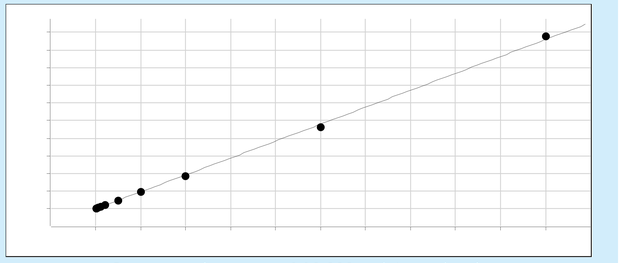
Compound	MS1 Resolution	Collision Energy	Label	Transition(s) d_n	Scrambling % d_{n-1} / d_n
Ritalinic Acid	Unit	20	d_{10}	230.2→93.2	11.19
			native	220.1→84.1	0.45
		10	d_{10}	230.2→230.2	0.39
			native	220.1→220.1	0.3
	Wide	20	d_{10}	230.2→93.2	11.31
			native	220.1→84.1	0.46
		10	d_{10}	230.2→230.2	0.4
			native	220.1→220.1	0.31
	Widest	20	d_{10}	230.2→93.2	51.46
			native	220.1→84.1	0.46
		10	d_{10}	230.2→230.2	47.29
			native	220.1→220.1	0.31

Scrambling was seen on both the Waters Xevo G2 Q-Tof and the Agilent 6410 triple quadrupole, although both instruments indicated the importance of higher resolution. By increasing the resolution, the scrambling was mitigated.

Investigation of the use of ±-*threo*-Ritalinic acid-D_{10} HCl as an Internal Standard

Agilent UHPLC 1290 HPLC-6460 triple quad ESI+
0.4 mL/min
5 µL injection volume
85:15 A:B
A: 0.1% formic acid in water
B: 0.1% formic acid in acetonitrile
Internal Standard: 500 ng/mL in methanol

Compound	Fragmentation (V)	CE (V)	Transition(s) d_n
-*threo*-Ritalinic acid-D_{10} HCl	102	20	230.2→93.1
Ritalinic Acid HCl	102	20	220.14→84.1



Concentration linear range: 1-10,000 ng/mL in methanol

CONCLUSIONS

- ±-*threo*-Ritalinic acid-D_{10} HCl was synthesized in good yield, sufficient ratio of D_0/D_{10}, and 99% purity.
- LC-MS/SIM indicated significant amounts of D_9-D_7 with no D_0.
- The percentage of deuterium present on each carbon was determined by qNMR.
- LC-MS/MS studies indicated that ±-*threo*-Ritalinic acid-D_{10} HCl fragments well and that deuterium scrambling can be minimized.
- Standard curve supports the use of ±-*threo*-Ritalinic acid-D_{10} HCl as an effective internal standard.

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