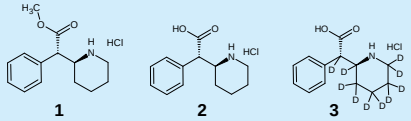
Analytical Reference Standards: Synthesis and Characterization of ±-*threo*-Ritalinic Acid-D_{10} Hydrochloride

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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 1D, 2D, and qNMR techniques.



1 **2** **3**

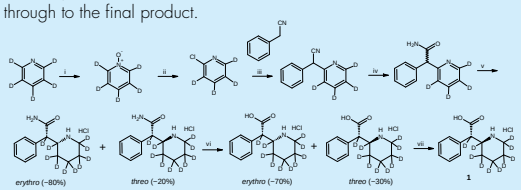
Introduction

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.

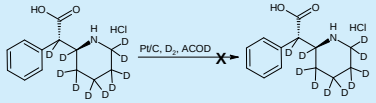
Ritalinic acid is a major metabolite of and synthetic precursor to methylphenidate and may be monitored clinically and forensically. The synthesis of deuterated ±-*threo*-ritalinic acid was therefore undertaken to develop an analytical reference standard and as a precursor to deuterated ±-*threo*-methylphenidate.

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.



Attempt to enrich the deuterium content of ±-*threo*-Ritalinic acid-D_{10} HCl



3, containing 55% D_{10} **3, with enriched D_{10} content**

Further treatment of ±-*threo*-ritalinic acid-D_{10} HCl with Pt/C, D_2, and AcOD did not result in an enrichment of the deuterium content. The isotopic distribution was fully characterized to determine suitability for use.

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

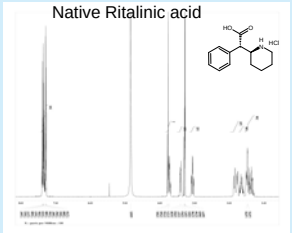
Isotopic distribution by LC/MS-SIM	
Deuterium Content	Percent (x_i)
D_{10}	55.16%
D_9	33.98%
D_8	9.21%
D_7	1.48%
D_6	0.148%
D_5	0.0128%
D_4	0.0010%
D_3	0.0 %
D_2	0.0 %
D_1	0.0 %
D_0	0.0 %

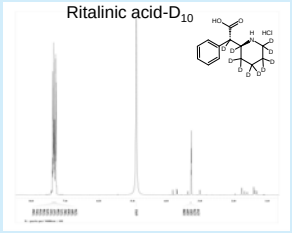
The identity of ±-*threo*-Ritalinic acid-D_{10} HCl was established through NMR and mass spectrometry. The chemical purity was established through HPLC/UV, Karl Fisher, GC/FID Headspace and ROI. LC-MS/MS studies were performed to evaluate isotopic purity, deuterium distribution, fragmentation patterns 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 45% D_9-D_7 isomers (see isotopic distribution at left).

Finally, NMR techniques including ^{1}H, ^{13}C, COSY, HSQC, and qNMR were used to determine the placement of the deuterium on the molecule.

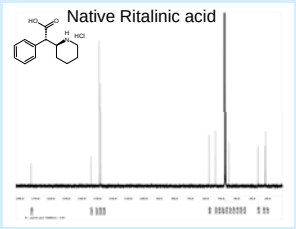
Determination of deuterium placement using NMR techniques

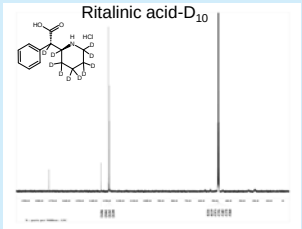
^{1}H NMR





^{13}C NMR



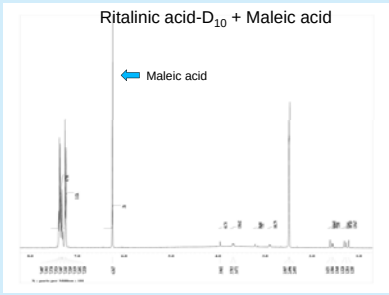


Analysis of LC/MS-SIM, ^{1}H NMR, and ^{13}C NMR

LC/MS-SIM clearly shows incomplete deuterium exchange with only 55% D_{10}. Additionally, the data suggested that some proton to deuterium exchange had also occurred on the aromatic ring. It was therefore desirable to determine the placement of the deuterium labels. ^{1}H NMR clearly shows the presence of a roughly even distribution of protons on the piperidine moiety. Unfortunately, the lack of a reference peak in the ^{1}H NMR made it impossible to determine the extent of the distribution of deuterium on this ring or whether the exchange had also occurred on the aromatic ring. Therefore, Quantitative NMR was used to determine the percent deuterium on each carbon in the molecule.

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.



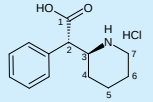
$$H\% = \frac{I_A}{I_{std}} \times \frac{n_{std}}{n_A} \times \frac{m_{std}}{m_a} \times \frac{M_a}{M_{std}} \times \frac{P_{std}}{P_a}$$

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

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

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.



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 some of the aromatic hydrogens deuterium.

CONCLUSIONS

±-*threo*-Ritalinic acid-D_{10} HCl was synthesized in good yield, sufficient ratio of D_0/D_{10}, and 99% purity making it an extremely useful internal standard for the quantitation of ritalinic acid. LC/MS-SIM indicated significant amounts of D_9-D_7 with no D_0. ^{1}H and ^{13}C NMR spectra could not provide definitive information as to where the deuterium were placed. qNMR was, therefore, used to determine exactly what percentage of deuterium is present on each carbon in the molecule. This data indicated that the reduction in step 5 using Pt/C resulted in incomplete deuterium exchange and even caused the exchange of a small percentage of the aromatic protons to deuterium. This study highlights chemical interactions that factor into the design of stable labeled internal standards for LCMS applications.

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

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