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Rapid quantitative analysis of methylphenidate and ritalinic acid in oral fluid by liquid chromatography triple quadrupole mass spectrometry (LC-QqQ-MS)

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ABSTRACT

Methylphenidate (MPH), which is metabolized into ritalinic acid (RA), is an amphetamine derivative largely used in the treatment of attention-deficit hyperactivity disorder, a neurological condition commonly diagnosed in early childhood. Ensuring that patients comply with clinical treatment is crucial and compliance is generally monitored in blood or urine specimens which, especially in the case of children, can be challenging to obtain on a repetitive basis. Here we report validation of a specific, non-invasive, and rapid dilute-and-shoot analytical method for the detection and quantitation of MPH and RA in oral fluid (OF). The method is based on liquid chromatography coupled to triple quadrupole MS with electrospray ionization utilizing dynamic MRM mode. Subject OF specimens were collected using a Quantisal™ device, processed, and diluted for analysis with seven-point quadratic calibration curves (weighting of $1/x$) using MPH-d_9 and (±)-threo-RA-d_{10} as internal standards. QC samples and diluted specimens showed intra- and inter-day bias and imprecision values no greater than ± 12%. The LOD and LOQ for MPH were 0.1 and 0.5 ng/mL, respectively, and 0.2 ng/mL and 0.5 ng/mL for RA, respectively, indicating the validity of the method for identification and confirmation at low concentrations. Selectivity was specific for the analytes of interest and matrix effects were minimized through the use of internal standard based quantitation.

1. Introduction

According to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), Attention-Deficit/Hyperactivity Disorder (ADHD) is a disorder generally diagnosed in childhood (around seven years old) that meets five criteria (A–D); traditionally a "*persistent pattern of inattention and/or hyperactivity-impulsivity that is more frequent and severe than is typically observed in individuals at a comparable level of development*" [1]. The 2011 National Survey of Children with Special Health Care Needs reported that 11% of children aged 4–17 were diagnosed with ADHD by a health care provider in the United States, with 6.1% of children in that age group having been prescribed medication for treatment of ADHD [2].

In 1937, psychiatrist Charles Bradley first reported the effects of Benzedrine sulfate, an amphetamine marketed by Smith, Kline & French, on "problem" children while treating them for severe headaches, when he noted that the children showed an improved performance in school, social interactions, and emotional feedback [3]. It is now understood that children with the disorder possess an abnormally low amount of dopamine and norepinephrine in certain brain regions. Amphetamines act as reuptake inhibitors by blocking dopamine (DAT) and norepinephrine (NET) transporters, allowing for a greater neurotransmitter accumulation in the synaptic cleft [4, 5].

Ritalin® is one of several popular orally administered drugs used for the treatment of ADHD in children, adolescents, and adults [6]. Its active ingredient, methylphenidate (MPH), is a phenethylamine and piperidine derivative rapidly absorbed and hydrolyzed by carboxylesterase 1 liver enzymes to the inactive metabolite ritalinic acid (RA), which is excreted primarily in urine [7]. Due to the relatively short half-life of MPH (~2.5 h), it is also advantageous to monitor RA for drug screening or patient compliance purposes. In adults, there is the potential for abuse of MPH; consequently, MPH is a Schedule II drug

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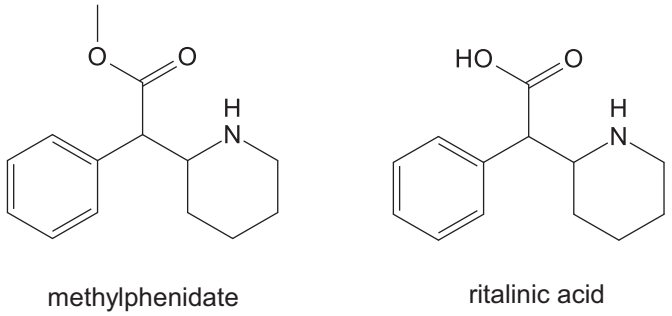

Fig. 1. Chemical structures of methylphenidate (MPH) and its primary metabolite ritalinic acid (RA).

under the U.S. Drug Enforcement Administration and monitoring in drug screens is therefore also of importance [8]. In the case of children, ensuring that parent reports of methylphenidate administration are accurate is crucial for assessing the effectiveness of the clinical treatment regimen and to avoid possible negative effects of MPH treatment [9, 10].

Analytical methods for MPH and RA can be time consuming, and in some instances, sample collection is invasive and tedious, especially when dealing with children. Most methods for the detection and analysis of MPH and RA involve the analysis of sweat, serum, plasma, and urine matrices using liquid-liquid extraction or solid-phase extraction [11, 12]. In contrast, oral fluid (OF) represents a specimen matrix with numerous advantages for MPH/RA monitoring. Collection is non-invasive, relatively rapid, and can be done on-site without the need of same-sex collectors. In addition, since there is very little chance of adulteration, the need for privacy during collection can be eliminated [13]. Furthermore, because MPH (Fig. 1) is lipophilic and expected to readily cross lipid bilayers, there is a good correlation between free drug in blood and that which is excreted by salivary glands into the oral cavity [14, 15].

Despite the potential advantages of using OF for routine methylphenidate monitoring, there are only a limited number of validated methods reported in the literature [12, 16]. In addition, there are currently no reports of a validated dilute-and-shoot method for MPH or RA specifically in OF. Here we confirm the suitability of oral fluid as a matrix for routine monitoring of MPH and RA in children enrolled in an ADHD treatment program and report on validation of a rapid, sensitive, and specific LC-QqQ-MS method for screening and quantitation of both MPH and RA in this matrix. Application of the method to 149 authentic OF specimens confirmed the ease of collection and sample preparation and the high sample throughput associated with this approach.

2. Materials and methods

2.1. Chemicals and reagents

Methylphenidate (MPH) HCl (1.0 mg/mL, free base), ritalinic acid (RA) HCl (1.0 mg/mL, free base), (±)-threo-ritalinic acid-D_{10} (RA-d_{10}) (100 µg/mL, free base), and methylphenidate-D_9 (MPH-d_9) HCl (100 µg/mL, free base) were purchased from Cerilliant (Round Rock, TX). Ammonium formate (99%) and formic acid Optima grade (99%) were obtained from Thermo Fisher Scientific (Waltham, MA). OraFlx synthetic oral fluid was from Dyna-Tek (Lenexa, KS) and extraction buffer containing phosphate-buffered saline (PBS) with protein stabilizers was from Immunalysis (Pomona, CA). Acetonitrile (ACN) and MeOH solvents were HPLC grade from Thermo Fisher Scientific and deionized water was obtained using a Barnstead Nanopure™ purification system with UV/UF capability.

2.2. Instrumentation

Liquid chromatography (LC) triple quadrupole mass spectrometry (QqQ-MS) was performed on an Agilent 1290 Infinity autosampler and binary LC (Santa Clara, CA) coupled to an Agilent 6460 triple quadrupole MS equipped with electrospray ionization (ESI) and Agilent Jet Stream (AJS) Technology. Agilent MassHunter Workstation software (Version B.06.00) was used for data acquisition and analysis.

2.3. LC-QqQ-MS conditions

Gradient elution was performed using 5 mM ammonium formate + 0.1% formic acid as phase modifier (solvent A) and MeOH + 0.1% formic acid (solvent B) for organic phase. Solvent composition was set to flow at 0.3 mL/min varying between 95% A (0 min), 30% A (3 min), and 95% A (4 min). Total run time was 5 min. A Poroshell 120 EC-C18 (2.1 × 100 mm, 2.7 µm) column (Agilent, Santa Clara, CA) maintained at 40 °C and connected to an Agilent 1290 Infinity in-line filter (0.3 µm) was used for chromatography. A needle wash consisting of ACN:MeOH:DI-H_2O (25:25:50, v/v/v) was used to minimize carryover effects between injections. Sample injection volume was 1 µL. The mass spectrometer was operated in positive ESI mode using dynamic multiple reaction monitoring (dMRM), with a cycle time of 500 ms and three MRM repeats for each optimized transition (Table 1). The drying and sheath gas were set to 325 °C (6 L/min) and 350 °C (11 L/min), respectively, while the nebulizer operated at 40 psi. The capillary voltage was fixed at + 4000 V.

2.4. Analytical procedures

2.4.1. Standards and calibration

A working stock solution of MPH (1.0 mg/mL) was prepared by diluting 1 mL of standard in 10 mL of MeOH for a final concentration of 100 µg/mL. The standard for RA (1.0 mg/mL) was prepared similarly. Working stock solutions for internal standards MPH-d_9 (100 µg/mL) and RA-d_{10} (100 µg/mL) were prepared by diluting 1 mL of each standard in 10 mL of MeOH for a final concentration of 10 µg/mL. Working standards were prepared on a daily basis and all solutions were stored at − 20 °C. Calibration (CAL) standards were prepared in MeOH as a mix of MPH and RA from a set of two primary dilution standards (PDS)

Table 1
Optimized QqQ-MS transitions for MPH, RA, and internal standards.

Analyte	Formula	Precursor ion (*m/z*)	Product ion (*m/z*)[a]	Fragmentor (V)	CE (V)	CAV (V)	Ret. time (min)
MPH	$C_{14}H_{19}NO_2$	234.2	**84.1**	75	20	2	3.32
			56.1	75	56	2	
MPH-d_9	$C_{14}H_{10}D_9NO_2$	243.2	**93.2**	75	24	2	3.32
			61.1	75	56	2	
RA	$C_{13}H_{17}NO_2$	220.1	**84.1**	75	16	2	3.12
			56.1	75	56	2	
RA-d_{10}	$C_{13}H_7D_{10}NO_2$	230.2	**93.1**	80	24	4	3.12
			61.1	80	56	4	

[a] Product ion used for quantitation in bold.