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Objective

In the therapy of attention deficit hyperactivity disorder (ADHD) methylphenidate represents nowadays first line treatment in combination with psychotherapy. It is one of the best-studied pediatric psychopharmacological drugs with a long clinical experience time. Since methylphenidate has addiction potential and shows different metabolic characteristics in children, therapeutic drug monitoring is advised, but not being applied in daily clinical practice [1]. Similar conditions concerning metabolic properties can be found in patients with hepatic or renal insufficiency, these would benefit as well from a less invasive and painful sampling. To avoid burdening invasive blood collection, oral fluid may be a non-invasive, simple and cost-effective alternative laboratory matrix. However, in pediatric drug therapy, there are only few reliable studies about the inter- and intraindividual pharmacodynamics of methylphenidate in oral fluid. In this study we quantified methylphenidate and its metabolite ritalinic acid from saliva with mass spectrometry.

Methods

From 40 ADHD patients (27 children, 2 adolescents and 11 adults) taking methylphenidate, serum and oral fluid were obtained. Methylphenidate and its major metabolite ritalinic acid were quantified using LC-MS/MS measurements. Sample preparation was performed using the Chromsystems MassTox TDM Parameter Set Antidepressants 2/ Psychostimulants-kit. The blood and saliva samples were taken as possible two to three hours after drug intake, when highest concentrations can be expected both in serum and oral fluid. [2] From 15 of these patients, 10 oral fluid samples were obtained about 2 hours after intake on different days to analyze intraindividual variance. The study participants took predominantly long-acting sustained-release formulations like Medikinet retard® or Ritalin LA®. The daily intake ranged between 10 and 60 mg MPH, corresponding to a dosage of 0.1 to 1.4 mg per kilogram of body weight.

Literature

- [1] van den Anker JN, Schwab M, Kearns GL. (2011) Developmental pharmacokinetics. Handbook of experimental pharmacology **205**: 51-75.
- [2] Marchei E, Farré M, Pellegrini M, Rossi S, García-Algaró, Vall O, Pacifici R, Pichini S. (2010) Pharmacokinetics of methylphenidate in oral fluid and sweat of a pediatric subject. *Forensic Science International* **196**(1-3): 59-63.
- [3] Novartis Pharma. (2013) Fachinformation Ritalin® 10mg Filmtabletten
- [4] Marchei E, Farré M, García-Algaró O, Pardo R, Pellegrini M. (2010) Correlation between methylphenidate and ritalinic acid concentrations in oral fluid and plasma. *Clinical Chemistry* **56**(4): 585-92.
- [5] Stegmann B, Dorfeldt A, Haen, E. (2015): Quantification of Methylphenidate, Dexamphetamine and Atomoxetine in Human Serum and Oral Fluid by HPLC with Fluorescence Detection. In: *Therapeutic drug monitoring*, **38**(1):98-107.

Results

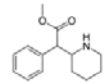
Study populations

Patients	Interindividual		Intraindividual	
	n	mean ± SD (min; max)	n	mean ± SD (min; max)
Kids (8-12 years)	9	18	18	18
Adolescent (13-18 years)	1	1	1	1
Adults (>18 years)	9	2	2	2
Age (years)				
mean ± SD	19.4 ± 13	12.5 ± 10.8		
(min; max)	(8; 48)	(7; 54)		
Female	6	3		
Male	13	18		
Height (cm)				
mean ± SD	155.6 ± 19.3	140.2 ± 14.6		
(min; max)	(133; 193)	(122; 175)		
Weight (kg)				
mean ± SD	55.54 ± 25.28	35.6 ± 14.1		
(min; max)	(26.2; 105)	(20.9; 71.7)		
BMI (kg/m ²)				
mean ± SD	21.7 ± 6	17.5 ± 3.4		
(min; max)	(14.2; 36.3)	(13.4; 25.6)		

Dose rate and serum/saliva concentration

Dose rate per day (mg, mean ± SD (min; max))	Interindividual		Intraindividual	
	n	mean ± SD (min; max)	n	mean ± SD (min; max)
30.4 ± 12.1 (10; 60)				
Dose rate/ kg BW (mg/kg BW)				
mean ± SD	0.6 ± 0.4	1.1 ± 0.4		
(min; max)	(0.1; 1.4)	(0.3; 1.8)		
Serum concentration MPH (ng/ml)				
mean ± SD	10.9 ± 9.8	16.4 ± 7.6		
(min; max)	(0.6; 35.3)	(9.1; 31)		
Oral fluid concentration MPH (ng/ml)				
mean ± SD	41.8 ± 42.6	80.3 ± 18		
(min; max)	(1.2; 167.2)	(1.7; 216.5)		
Oral fluid/serum ratio MPH				
mean ± SD	5.1 ± 4.9	4.6 ± 2.4		
(min; max)	(1.4; 20.6)	(1.8; 9.3)		

Chemical structures

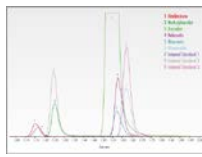


Methylphenidate (MPH)



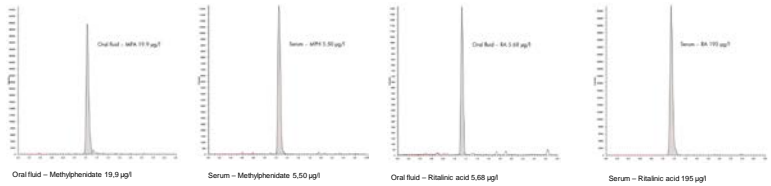
Ritalinic acid (RA)

Example chromatogram LC-MS/MS measurement



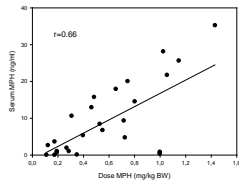
Example chromatogram of an LC-MS/MS measurement. The eluates were obtained with the MassTox TDM Series A: Antidepressants 2/ Psychostimulants Kit in serum/plasma. MPH eluted at a retention time of approximately 1.25 min and RA of about 1.15 min.

Serum and oral fluid chromatograms of a patient taking methylphenidate

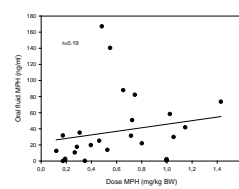


These figures show the chromatograms of a patient who has taken Medikinet adult® with a dosage of 30 mg per day. The results of the serum levels show a value of 5.5 ng/ml for MPH and 195 ng/ml for its metabolite RA. As described from other working groups before, the values for MPH in saliva were significantly higher - here about factor 4, whereas significantly lower values for RA were determined, which could be explained by the fact that oral fluid is more acidic than blood. [2,4]

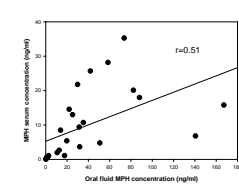
MPH dose/serum correlation



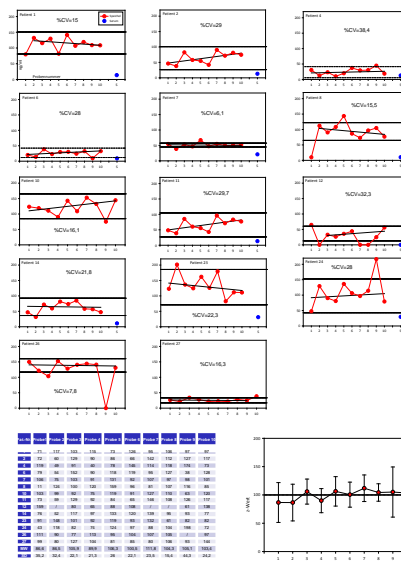
MPH dose/oral fluid correlation



MPH oral fluid/serum correlation

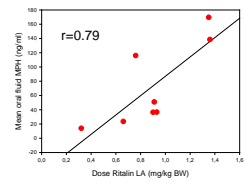


Intraindividual MPH concentrations in oral fluid and serum

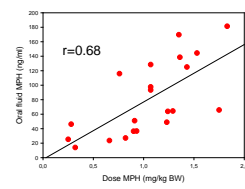


Intraindividual z-Transformation of oral fluid MPH-concentrations in children (means ± SD).

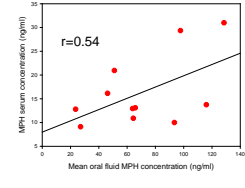
Ritalin LA dose/oral fluid correlation



MPH dose/oral fluid correlation



MPH oral fluid/serum correlation



Conclusion

- The LC-MS/MS kit "MassTox TDM Parameter Set Antidepressants 2/ Psychostimulants" is suitable and reliable for the determination of methylphenidate and ritalinic acid in oral fluid.
- The measured serum concentration values for methylphenidate correspond to the previously published data. [3]
- Oral fluid methylphenidate concentrations were about 5 times higher than in serum, while the concentrations of ritalinic acid were significantly lower in oral fluid.
- Correlations between serum and saliva concentrations for MPH described by Marchei et al. range between $r=0.22$ (fast-release formulation) and $r=0.79$ (extended-release formulation). Our result with $r=0.51$ for MPH is precisely in between, for Ritalin LA comparable. Probably fast-releasing pharmaceutical formulations cause buccal contamination and falses the oral fluid concentration. [4]
- Taken together oral fluid seems to be a valid matrix for TDM of MPH in children with ADHD.