Practical Laboratory Medicine 27 (2021) e00258

Contents lists available at ScienceDirect

Practical Laboratory Medicine

journal homepage: www.elsevier.com/locate/plabm

Ritalinic acid in urine: Impact of age and dose

Sheng Feng [a], Erin Strickland [b], Jeffery Enders [c], Michaela Roslawski [d], Timothy McIntire [e], Gregory McIntire [d,*]

[a] Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, 3400 Spruce Street, Philadelphia, PA, 19104, USA
[b] Ameritox, LLC, 486 Gallimore Dairy Rd, Greensboro, NC, 27409, USA
[c] Molecular Education, Technology and Research Innovation Center (METRIC), Department of Biological Sciences, North Carolina State University, Raleigh, NC, 27695, USA
[d] Premier Biotech, 723 Kasota Ave SE, Minneapolis, MN, 55414, USA
[e] Veterans Administration, 1 VA Center, Augusta, ME, 04330, USA

ARTICLE INFO

Keywords:
Methylphenidate
Ritalinic acid
LC/MSMS
Urine drug testing

ABSTRACT

Objectives: The objective of this work was to study the results of urine drug testing for ritalinic acid (RA), the major urinary metabolite of methylphenidate (MP) (e.g., Ritalin®). The impact of age from 4 to 65 years old and older on median levels of RA was investigated as well as potential variations in pH, specific gravity and creatinine content of the patient urine samples.
Design and Methods: Samples from patients who were 1) prescribed MP and found to be positive for RA, 2) prescribed MP but found to be negative for RA and 3) not prescribed MP but tested positive for RA were examined by liquid chromatography – mass spectrometry/mass spectrometry (LC-MS/MS) for RA concentration. The levels of RA were examined for median and average levels and further normalized and transformed to reveal a near gaussian distribution.
Results: Over 20,000 samples from patients who were prescribed MP were examined for this work. Analysis of these data for a subset of patients prescribed MP and testing positive for RA revealed statistically different median values of RA for school age patients of 6 years old through 17 years old from adult patients 18 through 64 years old. Another 6751 samples were positive for RA without a prescription but were not included in the overall assessment of these data.
Conclusions: While not clear as to the reason, these data indicate that school age children under the age of 18 have much higher levels of RA than adult patients. These results can be used to estimate "normal" levels of RA in these chronically dosed populations.

1. Introduction

Methylphenidate (MP) has been used to treat symptoms of Attention Deficit Hyperactivity Disorder (ADHD) for over 50 years [1]. Additionally, it has been used to treat childhood bipolar disorder [2]. Various reports suggest that diagnosis of ADHD and subsequent treatment with stimulant drugs such as MP has grown to include as much as 15% of the population [3]. Inasmuch as MP is a stimulant, it has been - and continues to be - abused [4,5]. The drug is used on college campuses to enhance late night studying [5] and more simply as a semi-legal party drug [5]. Urine drug testing (UDT) is often employed to help assess patient adherence to chronic drug

* Corresponding author.
E-mail address: Gregorymcintire2@gmail.com (G. McIntire).

https://doi.org/10.1016/j.plabm.2021.e00258
Received 1 March 2021; Received in revised form 12 October 2021; Accepted 20 October 2021
Available online 23 October 2021

prescriptions [6]. Since 80% of the oral dose of MP is excreted in urine as ritalinic acid (RA), the primary metabolite of MP, the resulting concentrations of RA can be relatively high making identification of diversion and other abuse pathways difficult in the absence of historical metabolite levels from a normal patient population [7].

The work reported herein was directed at defining "normal" for urine levels of RA. Of the 20,000 plus samples from patients who were prescribed MP, only 11,384 that also tested positive for RA were examined to reach this goal. In addition, over 10,000 additional samples from patients prescribed the medicine were found to be negative for RA. Finally, some 6751 samples were positive for RA without evidence of a prescription for MP. However, in addition to trying to define "normal", the data revealed age dependent concentrations of RA with consistent overall dosing levels for all patients. Further, this was a rare opportunity to evaluate sample validity test results for patients below age 18 down to age 4 and compare with normal adult levels of creatinine, pH, and specific gravity. These data may afford estimates of "normal" to assist in the assessment of UDT results for RA.

2. Materials and methods

Ritalinic acid was part of a larger liquid chromatography – mass spectrometry/mass spectrometry (LC-MS/MS) test panel. Details of the full method and validation can be found in an earlier report by Enders et al. [9]. RA and the corresponding internal standard, ritalinic acid D10, were purchased from Cerilliant Corporation (Round Rock, TX) as 1 mg/mL stock solutions. An enzyme solution was prepared by diluting IMCSzyme® β-glucuronidase solution (IMCS, Irmo, SC) to 10,000 units/mL in 0.02 M sodium phosphate buffer, at pH 7.5. Normal, drug-free urine was purchased from UTAK (Valencia, CA). Samples (30 μL) were diluted six times with 120 μL of enzyme solution and 30 μL of 1000 ng/mL ritalinic acid D10 internal standard. After dilution, samples were incubated at 60 °C for 60 min for hydrolysis and then extracted using a solid-phase extraction method. Ultimately, samples were diluted ten times in 300 μL of 10% methanol:90% water prior to injection and LC-MS/MS analysis. A morphine-3β-D-glucuronide (Cerilliant, Round Rock, TX) standard was used as a hydrolysis control for the method. While conjugation of RA has not been reported, other analytes in this method (e.g., benzodiazepines, opiates, etc.) required hydrolysis for testing. Thus, RA was "hydrolyzed" as part of the overall work flow in this test pathway.

2.1. LC-MS/MS method

The original large LC-MS/MS method ran on an Agilent LC-MS/MS system [9]. Some of the data reported in this work were obtained using a Thermo Ultra LC-MS/MS system. The method was revalidated for this system according to CAP and CLIA criteria as laid out in Ref. [9]. A summary of these validation data for RA is given in Table 1. This method used solvents A (5 mM ammonium formate with 0.1% formic acid [aqueous]) and B (5 mM ammonium formate in 75:25 methanol:acetonitrile with 0.1% formic acid) to provide a gradient shown in Table 2. A flow rate of 0.8 mL/min was used throughout. This method was multiplexed so that while the full cycle time was 6.95 min, the net data acquisition window was roughly 6.5 min. A Phenomenex (Torrance, CA) Kinetex 2.6 μm Phenyl-Hexyl 100 Å, 50 × 4.6 mm (00B-4495-E0) LC column was used in this method. The injection volume was 15 μL and column temperature was 30 °C. The RA transitions and MS details for the Thermo Ultra can be found in Table 3. RA produced a quadratic response from 100 ng/mL to 100,000 ng/mL with $1/X^2$ data weighting. A reporting cut-off of 500 ng/mL was used to establish positive results.

2.2. Data analysis

In an attempt to identify an adherent population of patients, the test results for RA from the population of patient samples that tested positive for RA with a prescription were curated as follows:

1. Only samples from patients who were prescribed MP (e.g., Ritalin®, Concerta®, etc.) and tested positive for RA were included.
2. Duplicate patient samples were excluded.

Table 1
Validation summary for ritalinic acid.

	Linearity[a]			Carryover[b]	Precision and Accuracy[c]						Matrix[d]	Interference[e]
	LOQ (ng/mL)	ULOL	r^2	Avg. Conc. (ng/mL) (N = 5)[b]	Avg. % Target (N = 30)			Avg. % CV (N = 30)			% Matrix Effect	
					200 ng/mL	5000 ng/mL	25,000 ng/mL	200 ng/mL	5000 ng/mL	25,000 ng/mL		
RA	100	100,000	0.9925	42.0	90.8%	89.8%	108.4	5.9%	4.1%	3.7%	1.67%	None

[a] The linearity results are compiled for all curve points and points that are between curve points, including 100, 200, 1,000, 5,000, 25,000, 75,000, and 100,000 ng/mL, each run five times. LOD = LOQ; $r^2 = 0.9925$. The reporting cutoff was 500 ng/mL.

[b] Carryover was tested by running a matrix blank immediately following the ULOL.

[c] Precision and accuracy statistics were calculated by data from three separate concentration standards including 200, 5,000, and 25,000 ng/mL, 10 replicates each, prepared and run on 3 separate days.

[d] Matrix data was calculated by dissolving the standards in normal human normal urine compared with a 'neat' preparation in chromatographic starting conditions (10% MeOH in water).

[e] Compounds tested for interference are available in the earlier report [9].

Table 2
The LC gradient parameters for ritalinic acid method[a].

Step	Start (min)	Flow rate (mL/min)	%A	%B
1	0	0.8	95	5
2	1.08	0.8	80	20
3	2.25	0.8	35	65
4	4.12	0.8	2	98
5	6.45	0.8	2	98
6	6.95	0.8	95	5

[a] This gradient is different from the original published method [9].

Table 3
MSMS method acquisition parameters[a].

Analyte	Transition[a]	Collision Energy (V)	Tube Lens Voltage (V)	Retention Time (min)	Time Window (min)
Ritalinic Acid	220.137 → 84.135	35	107	1.75	0.4
	220.137 → 56.199	36	107	1.75	0.4
Ritalinic Acid D10	230.194 → 93.194	21	75	1.75	0.4
	230.194 → 61.132	42	75	1.75	0.4

[a] These parameters differ from the original published method [9].

3. Patients testing positive for any illicit drugs were excluded.
4. Patients who did not test consistent with any other prescription(s) were excluded.
5. Patients who failed sample validity testing (e.g., pH, creatinine, and specific gravity) were excluded.
6. Patient samples without a UDT quantitative result (i.e., >ULOL) were not included.

This filtering process took the original 11,384 data points of the data set "prescribed MP and positive for RA" down to 9674 data points post curating. The data in Fig. 1 are shown as box and whisker plots of the data with 2.5% and 97.5% limits. These data are detailed in Table 4. This was an attempt to remove "outliers" from the curated data set and provide more robust ranges.

Ritalinic acid was further investigated to determine if data modelling could be successful as shown in Fig. 2. The data analysis and model development for RA were conducted using R Project version 3.3, a language and environment for statistical computing and graphing [10]. Data smoothing was conducted by kernel density estimation to smooth continuous data (e.g., histograms) [11]. Model development is detailed in earlier reports [12,13]. The earlier results were simplified for this work resulting in equation (1).

$$NORM_{D_{CONC}} = \ln\left(\frac{RA_{conc}}{CREAT}\right) \quad [1]$$

Where ln is the natural log, RA_{conc} is the concentration of the measured analyte in ng/mL, and CREAT is the sample fluid creatinine concentration in mg/dL. The value of $NORM_{Dconc}$ is then transformed into its corresponding Z_{score} on the standard normal (e.g., Gaussian) distribution using equation (2):

$$Z_{score} = \frac{(NORM_{D_{CONC}} - \mu_A)}{\sigma_A} \quad [2]$$

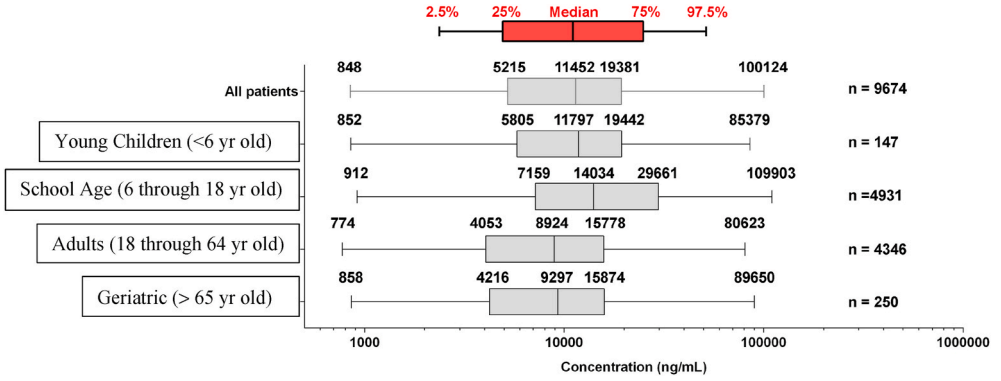

Fig. 1. Box and Whiskers plots for a) all patients, b) young children (<6 yr old), c) school age (6 through 17 years), d) adults (18 through 64 years), e) geriatrics 65 and older.

Table 4
Data from Fig. 1.

Group Names	Age Group	N	2.50%	25%	median	75%	97.50%
Young Children	<6 yr old	147	852	5808	11797	19442	85379
School age	6 through 17	4931	912	7159	14034	29661	109903
Adults	18 through 64	4346	774	4053	8924	15778	80623
Geriatric	65 and over	250	858	4216	9297	15874	89650
	All	9674	848	5215	11452	19381	100124

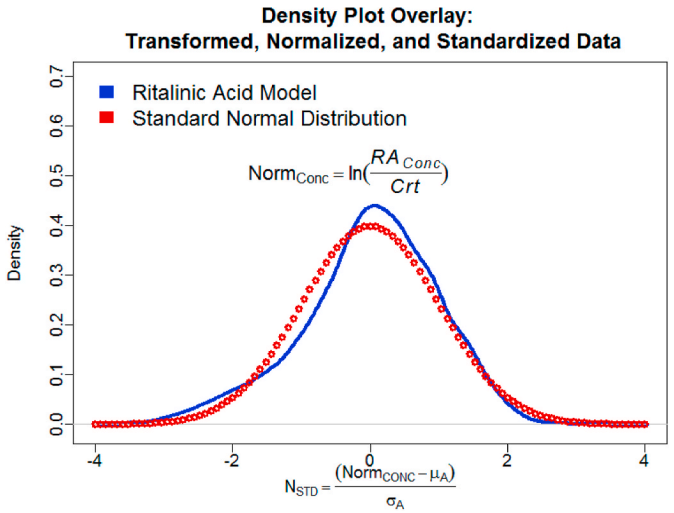

Fig. 2. Kernel density estimation plot derived from the normalized, transformed and standardized raw ritalinic acid data overlaid with the least squares minimized best fit Gaussian distribution curve.

where Z_{score} is the standardized normal value and μ_A (4.695) and σ_A (1.139) are the mean and the standard deviation of the population resulting from the model described in Equation (1). The resulting mean and standard deviation of the standardized normal distribution, Z_{score}, are "0" and "1" respectively.

Patient samples were received and tested over an 8 year period (January 1, 2008 through Dec 31, 2016) at Ameritox, LLC, in Greensboro, NC. All specimens that were used in this analysis were de-identified. Ameritox is accredited by the CAP and abides by CAP, CLIA and Health Insurance Portability and Accountability Act (HIPAA) requirements. The secondary analysis nature of this work and the absence of clinical conclusions, neither the U.S. Food and Drug Administration (FDA) nor other clinical trial review/approval was obtained by Ameritox. Writing this manuscript did not involve human subjects as defined by the U.S. Code of Federal Regulations (45 CFR 46.102); thus, an Institutional Review Board (IRB) approval of these specific research activities was not necessary.

3. Results

Fig. 1 illustrates a box and whiskers plot of the data post curation for the population set described above: "prescribed MP and testing positive for RA". These data are listed in Table 4 for additional clarity. The graph is displayed on a logarithmic scale so that the plots can be displayed together. However, that is strictly a function of the display and has no bearing on the actual data. Nothing in this

Table 5A
Data by Age, Overall Data Set, Less Than 6 Years Old (young children).

All Ages		Patient Specific Criteria (Average)					Ritalinic Acid (ng/mL)			Dose		
Age	N	wt (lbs)	Ht (in)	pH	Specific Gravity	Creatinine	Avg	std dev	median	median dose	avg dose	Avg Dose/ wt
4–90	**9674**	**144.40**	**62.02**	**6.56**	**1.0128**	**118.12**	**20193.26**	**28800.48**	**11452.00**	**20.0**	**25.37**	**0.14**
<6 years old (young children)												
Age	N	wt (lbs)	Ht (in)	pH	Specific Gravity	Creatinine	Avg	std dev	median	median dose	avg dose	Avg Dose/ wt
4	14	50.33	44.89	6.96	1.0138	89.64	12658.79	20015.22	7793.00	7.5	10.71	0.21
5	133	53.91	45.71	6.88	1.0124	93.94	19270.03	21943.73	12362.00	10.0	11.10	0.21
<6	**147**	**53.64**	**45.65**	**6.89**	**1.0125**	**93.53**	**18640.39**	**21790.28**	**11797.00**	**10.0**	**11.06**	**0.19**

display reflects a "normal" distribution as expected from previous data displays from UDT [14–18] which is why box and whiskers plots were chosen for these data. Notably, the median for school age patients from 6 through 17 years old is much higher than the median for adult patients 18 through 64 and geriatric patients 65 years old and over (Fig. 1). A Kruskal Wallis test indicates there is a significant difference between the median of the RA concentration in school age patients (14,034 ng/mL) and that in adult patients (8924 ng/mL) as well as geriatric patient samples. The other age groups of young children (<6 years old) and geriatric patients are smaller data sets and appear to be consistent with the overall results.

Mathematical normalization and transformation of RA data as per Equations [1] and [2] is shown in Fig. 2. The near Gaussian distribution that results from this process provides a more traditional model for reviewing population data. Note the x-axis is given in standard deviation units where 68% of the population is between ± 1 standard deviation, 95% between ± 2 standard deviations, and 99.7% is between ± 3 standard deviations [19].

Table 5A–D shows a variety of data listed by patient age. The median RA concentrations for school age patients (6 through 17) are significantly higher than those for adult and geriatric patients as shown graphically in Fig. 1 and listed in Table 4. It is not clear that this might be a function of body weight, creatinine or daily dose (also shown in Table 5). While creatinine concentration does appear to increase with age until about age 18, it is neither a big change nor unexpected [20,21]. It is interesting that creatinine concentrations in the young children age group are much lower than adult levels with the exception of the geriatric group which demonstrates nearly the same level of creatinine as do the youngest patient samples. Specific Gravity and pH are consistent across all ages.

Fig. 3 shows the number of patient samples/(year old) for each population as a per cent of that population. These data were converted to percentages of the total population to remove absolute number effects on the resulting profiles. For example, the number of patient samples from patients 6 years old in the group "prescribed MP and testing positive for RA" was divided by the total number of patient samples in that group and the resulting number was multiplied by 100% to create the data point for 6 years old in that group in Fig. 3. These numbers are given in Tables 5A and 5B; e.g., 558 patients 6 years old both positive for and prescribed MP (Table 5B) divided by 9674 total patients in that group (Table 5A).

4. Discussion

Box and whiskers plots of the RA data are shown in Fig. 1. These data were curated as discussed in the methods section in an attempt to define "normal" ranges of RA from MP patients. While the overall range (all data) in Fig. 1a is interesting, the box and whiskers plots representing school age patients (Fig. 1c) and adult patients (Fig. 1d) demonstrate the difference between these unique populations (Fig. 1). It is clear that these two populations have statistically significant different median values with the school age patients exhibiting the highest concentrations. A close look at Table 5 (e.g., Table 5E) does not clearly indicate any correlation between these average/median values and body weight, creatinine, or daily dose. School age patients exhibit a median dose of 27 mg/day while adult patients have a median dose of 20 mg/day, a ratio of 1.35. Yet, the median concentration of RA in school age patients is almost two fold greater than that of the adult patients. Table 5E illustrates that the impact of creatinine normalization does not change the relative order of these age groups.

Normalization and transformation of the RA data from the population of "prescribed MP and testing positive for RA" results in Fig. 2. The fit to a normal Gaussian curve is less than perfect. However, using this approach to define the population affords the determination of patient data points either >2 or <2 standard deviations above or below the mean as outside the range of 95% of this population. This would suggest that these patients are potentially abusing their MP prescription. For example, the actual values of μ_A and σ_A are 4.695 and 1.139 respectively. Using 2 standard deviations from the mean and working backwards through equations (1) and (2) yields an upper value of 115,067 ng/mL (using the median value of creatinine for the entire population of 107.8 mg/dL). This would indicate that any RA value over 115,067 ng/mL would be suspect. Conversely, assessing data points below 2 standard deviations from the mean yields a value of 1208 ng/mL suggesting that patients below this level might not be adherent to their prescription. After curation, only 1.64% of the data points were above 115,067 ng/mL while 4.54% of the data points were below 1208 ng/mL suggesting

Table 5B
Data by age, 6 through 17 years old (school age patients).

6–17 years		Patient Specific Criteria (Average)					Ritalinic Acid (ng/mL)			Dose		
Age	N	wt (lbs)	Ht (in)	pH	Specific Gravity	Creatinine	avg	std dev	median	median dose	avg dose	Avg Dose/wt
6	297	66.07	47.50	6.88	1.0133	91.80	23227.52	29176.54	13251.00	10.0	15.35	0.23
7	403	60.64	49.93	6.81	1.0132	95.76	24841.15	31819.84	13780.00	18.0	20.66	0.34
8	485	67.46	51.46	6.72	1.0133	98.79	24014.21	28559.98	13982.00	18.0	21.54	0.32
9	544	75.61	53.74	6.79	1.0133	105.99	25877.47	30051.32	14074.00	20.0	25.57	0.34
10	558	86.02	55.05	6.62	1.0136	116.51	27699.36	30989.85	15616.50	22.0	26.62	0.31
11	558	97.66	57.32	6.58	1.0136	118.07	26514.37	31425.09	15041.50	27.0	28.18	0.29
12	493	110.67	59.68	6.50	1.0142	130.47	25689.89	30599.23	13552.00	30.0	30.32	0.27
13	400	124.59	62.13	6.62	1.0142	141.72	23638.34	26656.62	14308.00	30.0	30.74	0.25
14	371	140.73	64.17	6.53	1.0141	146.10	25393.62	35058.70	13646.00	36.0	33.40	0.24
15	336	155.17	65.97	6.59	1.0139	147.71	26973.88	57041.26	13265.00	36.0	33.93	0.22
16	261	164.23	66.87	6.47	1.0136	154.03	20109.14	23453.55	13022.00	30.0	32.20	0.20
17	225	165.82	66.60	6.72	1.0131	157.18	21583.00	23702.06	14271.00	36.0	33.35	0.20
6–17	**4931**	**104.99**	**57.73**	**6.65**	**1.0136**	**122.23**	**25064.78**	**32493.79**	**14034.00**	**27.0**	**27.32**	**0.26**

Table 5C
Data by age, 18 through 64 Years old (adult).

18–40 years		Patient Specific Criteria (Average)					Ritalinic Acid (ng/mL)			Dose		
Age	N	wt (lbs)	Ht (in)	pH	Specific Gravity	Creatinine	avg	std dev	median	median dose	avg dose	Avg Dose/wt
18	171	168.70	66.91	6.75	1.0128	144.10	17513.62	20111.02	13054.00	36.0	35.53	0.21
19	105	181.14	67.57	6.62	1.0133	157.50	19022.91	22737.54	10982.00	30.0	31.86	0.18
20	96	169.77	67.44	6.64	1.0130	141.50	11291.54	10785.52	8084.50	20.0	27.28	0.16
21	88	171.26	66.50	6.70	1.0125	139.81	20281.01	23346.16	12325.00	36.0	34.85	0.20
22	80	174.39	67.55	6.64	1.0123	125.27	11511.95	12151.80	7809.00	20.0	26.30	0.15
23	89	184.45	67.64	6.52	1.0125	132.93	15498.43	28617.35	7379.00	20.0	26.48	0.14
24	92	179.03	67.47	6.62	1.0118	133.73	16042.42	22163.64	8813.00	20.0	25.94	0.14
25	84	187.52	67.88	6.54	1.0118	120.46	11997.62	13427.58	8808.00	20.0	28.31	0.15
26	84	190.16	67.62	6.64	1.0118	117.16	12743.88	18423.59	8285.50	20.0	23.15	0.12
27	151	181.92	65.83	6.57	1.0124	112.51	14617.54	23159.17	7410.00	20.0	27.46	0.15
28	145	176.73	65.87	6.54	1.0113	121.03	15732.34	24542.25	9126.00	20.0	25.26	0.14
29	122	185.71	66.75	6.56	1.0134	130.70	15292.34	22281.95	8480.50	20.0	24.56	0.13
30	121	181.01	66.83	6.59	1.0115	117.94	15881.90	23706.73	8970.00	20.0	26.03	0.14
31	110	185.13	67.14	6.49	1.0133	128.76	12973.70	16786.80	8389.50	20.0	22.05	0.12
32	104	183.07	65.72	6.48	1.0123	121.86	14216.89	18883.01	9384.50	20.0	21.05	0.11
33	97	182.75	65.74	6.47	1.0103	113.20	12862.08	15742.56	9080.00	20.0	21.78	0.12
34	129	178.87	66.35	6.59	1.0114	118.27	14987.79	22453.19	9275.00	20.0	23.48	0.13
35	103	181.09	66.97	6.48	1.0118	109.81	13526.04	18543.44	8893.00	20.0	23.03	0.13
36	95	188.96	67.47	6.43	1.0114	107.25	12728.93	14763.54	8331.00	20.0	21.73	0.12
37	81	204.32	67.36	6.35	1.0122	126.34	14442.63	20407.98	9201.00	20.0	23.38	0.11
38	107	184.55	66.37	6.57	1.0115	112.24	14766.12	23400.91	8652.00	20.0	22.05	0.12
39	89	192.62	67.35	6.43	1.0128	120.69	18155.92	28971.85	11453.00	20.0	29.40	0.15
40	110	186.08	66.35	6.57	1.0102	103.84	10718.43	9239.49	8183.50	20.0	23.21	0.12
Age 41–64		Patient Specific Criteria (Average)					Ritalinic Acid (ng/mL)			Dose		
Age	N	wt (lbs)	Ht (in)	pH	Specific Gravity	Creatinine	Avg	std dev	median	median dose	avg dose	Avg Dose/wt
41	104	190.63	66.48	6.42	1.0106	102.12	13318.44	19798.78	8130.50	20.0	25.94	0.14
42	89	179.82	66.29	6.41	1.0118	113.41	22678.62	48659.88	12053.00	20.0	23.32	0.13
43	92	194.43	67.65	6.44	1.0126	119.17	12305.41	13799.03	7687.00	20.0	22.99	0.12
44	104	190.16	66.50	6.42	1.0124	111.63	16149.48	25965.50	8730.50	20.0	23.47	0.12
45	95	192.62	66.90	6.31	1.0112	113.74	13518.52	20585.17	8405.00	20.0	23.73	0.12
46	98	192.52	66.60	6.37	1.0116	107.12	11854.41	14634.88	8859.50	20.0	20.96	0.11
47	111	187.55	65.86	6.41	1.0124	107.91	16801.92	30044.70	8364.00	20.0	22.12	0.12
48	101	183.48	65.55	6.39	1.0128	104.20	14124.92	19174.94	7543.00	20.0	23.62	0.13
49	59	196.45	67.44	6.31	1.0120	98.44	8610.71	6313.33	6578.00	20.0	19.17	0.10
50	74	179.07	65.95	6.56	1.0120	101.80	22016.05	53915.33	10399.50	20.0	20.39	0.11
51	80	180.11	65.83	6.47	1.0104	89.48	13664.83	14364.65	8160.50	20.0	25.12	0.14
52	69	189.77	66.22	6.41	1.0101	90.36	8767.81	8849.55	6638.00	20.0	18.64	0.10
53	92	180.06	66.28	6.21	1.0118	90.96	12755.15	18489.42	7141.00	20.0	20.64	0.11
54	108	189.98	66.16	6.24	1.0121	106.13	19027.30	31109.55	8593.00	20.0	19.27	0.10
55	81	177.78	66.27	6.30	1.0118	98.02	13109.53	21962.11	7648.00	10.0	18.15	0.10
56	77	181.66	65.81	6.26	1.0111	100.51	22331.22	43228.73	10375.00	20.0	22.11	0.12
57	84	186.14	67.05	6.22	1.0111	90.26	14032.94	15571.95	9782.50	20.0	19.79	0.11
58	67	187.28	67.72	6.36	1.0128	121.44	15747.82	26783.16	9646.00	20.0	21.77	0.12
59	69	194.94	67.84	6.25	1.0116	100.96	14900.22	25286.99	6036.00	20.0	18.40	0.09
60	64	191.75	67.31	6.20	1.0119	105.93	16653.53	22263.58	9851.00	20.0	20.14	0.11
61	44	189.33	64.29	6.30	1.0116	100.87	20553.30	32106.85	8732.00	20.0	17.03	0.09
62	47	185.63	66.22	6.09	1.0121	105.73	13800.15	16247.68	9379.00	10.0	15.61	0.08
63	42	197.06	66.61	5.72	1.0139	110.15	17262.69	28292.69	6810.00	20.0	21.15	0.11
64	42	179.31	64.06	6.18	1.0128	104.40	14144.07	15091.64	8918.00	20.0	18.85	0.11
18–64	**4346**	**184.28**	**66.64**	**6.46**	**1.01**	**115.54**	**15007.58**	**23485.76**	**8924.00**	**20.0**	**24.00**	**0.13**

Table 5D
Data by age, 65 Years and older (geriatric).

≥65 Years		Patient Specific Criteria (Average)					Ritalinic Acid (ng/mL)			Dose		
Age	N	wt (lbs)	Ht (in)	pH	Specific Gravity	Creatinine	Avg	std dev	Median	median dose	avg dose	Avg Dose/ wt
65	31	193.10	66.30	6.15	1.0123	126.99	21455.80	27932.70	11268.00	25.0	34.35	0.18
66	26	178.88	66.29	6.53	1.0091	81.52	14837.04	19787.68	8258.50	20.0	23.96	0.13
67	24	179.09	66.48	6.32	1.0129	96.27	9910.25	8349.86	8904.50	10.0	20.05	0.11
68	23	197.05	67.39	6.10	1.0115	89.88	16208.87	17870.40	9703.00	20.0	19.68	0.10
69	21	196.24	65.82	5.98	1.0129	98.64	9123.76	6927.26	7161.00	10.0	13.85	0.07
70	28	167.56	65.46	6.35	1.0101	92.78	22313.89	35874.28	9351.00	20.0	23.96	0.14
71	15	178.67	65.79	6.09	1.0121	97.45	12278.00	10768.44	10236.00	10.0	14.00	0.08
72	18	164.43	65.29	6.53	1.0115	93.58	18230.72	18283.55	13232.00	15.0	17.83	0.11
73	17	174.87	65.93	6.14	1.0124	88.33	19858.71	34620.05	8652.00	10.0	15.76	0.09
74	9	190.11	66.00	5.99	1.0120	104.72	11312.78	8417.87	12991.00	10.0	17.89	0.09
>75	40	162.81	65.63	6.38	1.0116	94.69	11609.30	13468.66	6958.00	15.0	17.63	0.11
>65	**250**	**178.81**	**66.03**	**6.27**	**1.0116**	**96.12**	**15168.33**	**21172.03**	**9297.00**	**20.0**	**19.08**	**0.11**

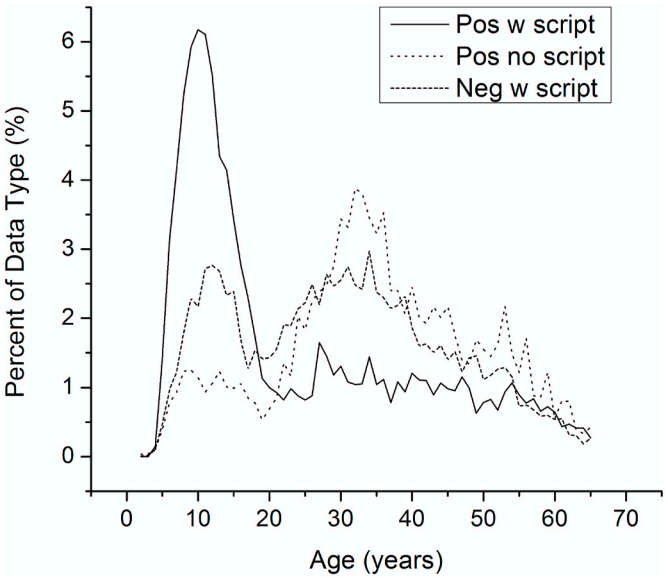

Fig. 3. Population percentages.

the Gaussian fit is less than perfect. Thus, while interesting, the approach of describing the population via normalization and transformation requires some data and mathematical skills from the practitioner.

Of course, patients prescribed MP but diverting their pills would test negative for RA even if they attempted to add MP directly to their urine sample since the MP would only be minimally 'metabolized' in the urine sample if at all via hydrolysis (i.e., "pill scrapers"). Hence, these values would be less than the reporting cutoff of 500 ng/mL (Table 1).

Abuse of MP is acknowledged especially on college campuses where it is used to enable all night studying and is used recreationally via intranasal administration [5]. In the same time frame where 11,384 patients with prescriptions tested positive, 10,421 (47.8%) patients with listed prescriptions tested negative indicating that they might not take their drug and/or divert it to other users as discussed above. Another group of 6751 patients over the same time limits were positive for RA without a prescription for MP listed. After curating this group as above without the requirement for a prescription, the median concentration of RA was 6227 ng/mL (5787 patient samples), much lower than any of the age groups or overall shown in Fig. 1. While knowledge of the presence of a prescription is totally dependent upon information provided by the testing physician on the sample requisition, it would appear that non-prescription use of MP results in lower levels of RA in urine consistent with less frequent dosing than those on chronic prescriptions.

As shown in Fig. 3, the profile of each group with respect to the number of patients by age is different. The "normal" group of patients prescribed MP and testing positive for RA exhibits a peak among younger patients between 4 and 20 with a maximum at approximately 10 or 11 years old. Beyond age 20, the numbers for this group are nearly flat at lower percentages. Those with a prescription for MP but testing negative for RA parallel the "normal" population below age 20 but reflect those who are positive for RA without a prescription above age 20. Finally, those without a prescription but testing positive for RA show a peak in numbers between

Table 5E
Data summaries.

age group	median RA	median creatinine	median RA/ median creatinine	Avg RA	avg creatinine	Avg RA/ avg creatinine	median dose/wt	ratio median dose/wt to adult dose/wt value	ratio median RA to median adult RA	avg dose	ratio avg dose to avg adult dose	median dose	ratio median dose to adult median dose
<6 yr old	11797	90.7	130.1	18640	93.5	199.4	0.19	1.5	1.32	11.1	1.5	10	0.5
6 through 17	14034	112.4	124.9	25064	122.2	205.1	0.26	2	1.57	27.3	2.0	27	1.4
18 through 64	8924	105	85.0	15007	115.5	129.9	0.13	1	1	24	1.0	20	1.0
≥65 Years	9297	91.95	101.1	15268	96.1	158.9	0.11	0.85	1.04	19.1	0.8	20	1.0

the ages of 20 and 45. These profiles suggest that these are individual populations and thus, combining all these data or even data from patients with a prescription either negative or positive would be ill advised. Interestingly, of the prescribed population (e.g., positive with a prescription + negative with a prescription), 41.2% are female and 58.6% male while for those positive without a prescription, 56.7% are female and 43.3% male. Further, the number of patient samples under age 18 for the "prescribed MP and testing positive" population is 54.7% while the same number for the positive without prescription group was 13.9%. Again, while interesting and perhaps not surprising, it is difficult to make conclusions based on these data.

Part of the focus for this paper is to aid in determining patient adherence. To be successful, RA outliers should be readily identified from a comparison with Fig. 1 or Fig. 2. Necessarily, 5% of the patient data used in making Fig. 1 (Table 4) is outside the limits of this box and whiskers plot. This correlates with the amount of a population between ± 2 standard deviations around the mean (95%) of a population. The difference between Figs. 1 and 2 is that the excluded points will not be symmetric around the mean in Fig. 1 whereas the near Gaussian distribution of Fig. 2 makes that more likely. Making decisions from population based data displays is difficult for those patients who fall above, but near the upper limit of the "normal range" and should be made in conjunction with other clinical observations of the individual patient.

The ability to quickly compare UDT results without further mathematical manipulation to results from a large test population should help determine patient adherence from their UDT data. While various normalizations and transformations have been reported [12–17], they all require additional mathematical manipulations often using demographic data that may or may not be available. There is value in defining the population and in being able to use Gaussian statistics to predict consistency with that population as shown in Fig. 2. However, in the absence of such comparisons by the testing company on the report, direct comparison with raw data (albeit curated for inconsistent results) may be the easiest and most impactful way to help assess patient adherence.

5. Conclusions

While an increasing number of children and young adults continue to be dosed with MP as well as other stimulants for the treatment of ADHD, little has been written about testing concentrations of RA in urine and what is "normal" vs. what is diversion/abuse [3,8]. The data presented herein (Table 4) provide an estimate of "normal" such that physicians can or they may be abusing/diverting their prescription. Interestingly, the "normal" concentration of RA in urine is different for school aged patients from 6 through 17 years old than for adults (18 through 64 years) and geriatric patients. It is clear that on average, the school age patients are dosed at higher levels than adults or geriatrics. These higher dose levels coupled with lower body weight in this age group might account for a portion of the observed differences. A more interesting question concerns the elevated dose levels for children vs adults. The observed differences between these groups cannot easily be attributed to a single factor or even a small collection of factors. Finally, if a UDT concentration is outside "normal" ranges, other clinical information/observations should guide decision making.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author statement

We thank the reviewer for their time and expertise in the review of our paper.

Declaration of competing interest

The authors do not have any conflict of interest in the publication of this work.

References

[1] R.L. Findling, Evolution of the treatment of attention-deficit/hyperactivity disorder in children: a review, Clin. Therapeut. 30 (5) (2008) 942–957.
[2] R.A. Kowatch, M. Fristad, B. Birmaher, K.D. Wagner, R.L. Findling, M. Hellander, Treatment guidelines for children and adolescents with bipolar disorder: child psychiatric workgroup on bipolar disorder, J. Am. Acad. Child Adolesc. Psychiatry 44 (3) (2005) 213–235.

[3] New York Times, The selling of attention deficit disorder. https://www.nytimes.com/2013/12/15/health/the-selling-of-attention-deficit-disorder.html.
[4] W.A. Morton, G.G. Stockton, Methylphenidate abuse and psychiatric side effects, primary care companion, J. Clin. Psychiatr. 2 (5) (2000) 159–164.
[5] Q. Babcock, T. Byrne, Student perceptions of methylphenidate abuse at a public liberal arts college, J. Am. Coll. Health 49 (2000) 143–145.
[6] P.J. Christo, L. Manchikanti, X. Ruan, M. Bottros, H. Hansen, D.R. Solanki, A.E. Jordan, J. Colson, Urine drug testing in chronic pain, Pain Physician 14 (2) (2011) 123–143.
[7] R.C. Baselt, Disposition of Toxic Drugs and Chemicals in Man, eleventh ed., Biomedical Publications, Seal Beach, CA, 2011.
[8] D'Agostino, The drugging of the American boy. https://www.esquire.com/news-politics/a32858/drugging-of-the-american-boy-0414.
[9] J.R. Enders, J.P. Smith, S. Feng, E.C. Strickland, G.L. McIntire, Analytical considerations when developing an LC-MS/MS method for over 30 analytes, Journal of Applied Laboratory Medicine 2 (4) (2018) 543–554.
[10] R. R Core Team, A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2014. http://www.R-project.org.
[11] E. Parzen, On estimation of a probability density function and mode, Ann. Math. Stat. 33 (3) (1994) 1065–1076.
[12] G.L. McIntire, A.A. Morris, O.T. Cummings, Methods of normalizing measured drug concentrations in urine using patient specific data and testing for potential non-compliance with a chronic drug treatment regimen, U.S. Patent Application No. (October 13, 2016), 20160300038.
[13] O.T. Cummings, J.R. Enders, G.L. McIntire, Fentanyl–norfentanyl concentrations during transdermal patch application: LC–MS-MS urine analysis, J. Anal. Toxicol. 40 (8) (2016) 595–600.
[14] O.T. Cummings, A.A. Morris, J.R. Enders, G.L. McIntire, Normalizing oral fluid hydrocodone data using calculated blood volume, J. Anal. Toxicol. 40 (7) (2016) 486–491.
[15] S.A. Tse, R.S. Atayee, B.M. Best, A.J. Pesce, Evaluating the relationship between carisoprodol concentrations and meprobamate formation and inter-subject and intra-subject variability in urinary excretion data of pain patients, J. Anal. Toxicol. 36 (4) (2012) 221–231.
[16] M.M. Hughes, R.S. Atayee, B.M. Best, A.J. Pesce, Observations on the metabolism of morphine to hydromorphone in pain patients, J. Anal. Toxicol. 36 (4) (2012) 250–256.
[17] A. Pesce, C. West, K. Egan City, J. Strickland, Interpretation of urine drug testing in pain patients, Pain Med. 13 (7) (2012) 868–885.
[18] S. Feng, L. Ward, B. Parish, E.C. Strickland, G.L. McIntire, Urine drug testing concentration ranges for select benzodiazepines, J. Anal. & Pharm Res. 8 (1) (2019) 8–13.
[19] NIST/SEMATECH e-handbook of statistical methods, 6.5.1, http://www.itl.nist.gov/div898/handbook/pmc/section5/pmc51, April 2012.
[20] E.J. Cone, Y.H. Caplan, F. Moser, T. Robert, M.K. Shelby, D.L. Black, Normalization of urinary drug concentrations with specific gravity and creatinine, J. Anal. Toxicol. 33 (1) (2009) 1–7.
[21] G.K.S. Singh, B.W.R. Balzer, R. Desai, M. Jimenez, K.S. Steinbeck, D.J. Handelsman, Requirement for specific gravity and creatinine adjustments for urinary steroids and luteinizing hormone concentrations in adolescents, Ann. Clin. Biochem. 52 (6) (2015) 665–671.