

Correlation Between Methylphenidate and Ritalinic Acid Concentrations in Oral Fluid and Plasma

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BACKGROUND: We studied the excretion profile of methylphenidate (MPH) and its metabolite ritalinic acid (RA) in oral fluid and plasma, the oral fluid-to-plasma (OF/P) drug ratio, and the variations of oral fluid pH after drug administration.

METHODS: We analyzed oral fluid and plasma samples, obtained from 8 healthy volunteers after ingestion of a single dose of 20 mg fast-release or extended-release MPH, for MPH and RA by LC-MS. We estimated the apparent pharmacokinetic parameters of MPH in plasma and oral fluid and calculated the OF/P ratio for each time interval.

RESULTS: MPH and RA were detected in oral fluid. Whereas parent drug concentrations in oral fluid were an order of magnitude higher than those in plasma, the opposite was observed for RA. Oral fluid concentrations of MPH ranged between 0.5 and 466.7 $\mu\text{g/L}$ and peaked at 0.5 h after administration of the fast-release formulation; they ranged between 0.7 and 89.5 $\mu\text{g/L}$ and peaked at 2 h after administration of the extended-release formulation. Both formulations presented bimodal time-course curves for the OF/P ratio, ranging between 1.8 and 242.1 for the fast-release formulation and between 2.6 and 27.0 for extended-release. Oral fluid pH did not appear to be modified by the administration of the drug, and its influence on OF/P ratio did not affect the correlation of MPH between the 2 body fluids.

CONCLUSIONS: The results obtained support the measurement of MPH in oral fluid as an alternative to plasma if the extended-release formulation is used.

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Monitoring exposure to therapeutic drugs in the pediatric population is more difficult than in adults because of the need to use noninvasive or less invasive methods. Hence, the use of alternative biological matrices should be considered for noninvasive assessment of drug use. Testing of oral fluid has been used successfully as an alternate to blood testing for psychotropic drugs in therapeutic drug monitoring (1), pharmacokinetic studies (2–4), and the detection of drug misuse (5, 6). Specifically, weak basic drugs such as amphetamine derivatives have been reported to concentrate in oral fluid because their pH is slightly acidic compared with that of plasma (3, 7).

Methylphenidate (MPH)⁴ is an amphetamine derivative used in the treatment of attention-deficit hyperactivity disorder (ADHD) in children, adolescents, and adults (8–11). MPH is absorbed rapidly and efficiently after oral administration (12) and is rapidly hydrolyzed at the methyl ester site to its metabolite, ritalinic acid (RA) (13).

Because of a recognized marked individual variability in the dose response to methylphenidate, the drug dose must be titrated for optimal effect and avoidance of toxicity, especially in children (12).

In the last few years, oral fluid has been suggested for monitoring MPH use and identifying recent misuse of MPH in schoolchildren and young adults (13–15). In addition, Pappadopulos et al. (16) recently advocated MPH measurement in oral fluid as an objective assessment of compliance vs the inaccuracy of parental report.

In light of the usefulness of alternative biological matrices for noninvasive assessment of short- and long-term MPH use, we developed LC-MS assays for the determination of MPH and RA in both conventional (blood and urine) and nonconventional (hair, oral fluid, and sweat) biological matrices (17, 18). Us-

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⁴ Nonstandard abbreviations: MPH, methylphenidate; ADHD, attention-deficit hyperactivity disorder; RA, ritalinic acid; AUC, area under the curve; C_{max} , peak concentration; t_{max} , time to peak concentration; $t_{1/2}$, elimination half life; k_e , elimination constant; OF/P, oral fluid-to-plasma; MDMA, methylenedioxymethamphetamine.

ing these LC-MS assays, we performed the study reported here to (1) investigate the presence and time-course concentration of MPH and its main metabolite (RA) in oral fluid after controlled drug administration, (2) assess the correlation between MPH and RA concentrations in oral fluid and plasma, and (3) determine the influence of pH on the MPH and RA oral fluid-to-plasma (OF/P) ratio. We obtained oral fluid and plasma samples from individuals participating in a clinical trial involving the controlled administration of 20 mg fast- and extended-release formulations of MPH.

Materials and Methods

SUBJECTS AND STUDY DESIGN

All subjects provided written informed consent before inclusion and were financially compensated for their participation in the study. The study was conducted in accordance with the Declaration of Helsinki, approved by the local Ethics Committee (Clinical Research Ethical Committee of the Municipal Institute of Health Care), and authorized by the “Agencia Española del Medicamento” (reference AEM 04/0012) of the Spanish Ministry of Health. Eight healthy young men who were not consuming any drugs or pharmaceuticals participated in the study. Each participant underwent a general physical examination, routine laboratory tests, urinalysis, and a 12-lead electrocardiogram. The participants had a mean age of 23.0 years (range 20–26), mean weight of 75.1 kg (range 57.4–87.0), and mean height of 180.3 cm (range 170–187). Two 10-mg oral doses of fast-release tablets (Rubifen®, Laboratorios Rubió) or 1 20-mg oral dose of extended-release capsule (Medikinet®, Medice) were administered with 250 mL water to 5 and 3 volunteers, respectively, at 0900. After collection of oral fluid samples 2 h after drug administration, a light meal was given to the subjects. All volunteers were tested for drugs of abuse consumption before the experimental session, and results were negative (Microgenics® Cedia immunoassay tests for urine) for opiates, cocaine, cannabinoids, and amphetamines.

COLLECTION OF ORAL FLUID AND BLOOD SAMPLES

Samples of oral fluid were collected by having the participants spit in polypropylene tubes without any preservative over a 5-min period at 0, 0.5, 1, 2, 3, 4, 8, 12, and 24 h after drug administration. Approximately 3–5 mL of oral fluid were typically collected. Oral fluid pH was recorded at the time of collection, and the samples were immediately stored at -20°C until analysis. Blood samples were collected in lithium heparin tubes at the same time intervals as the oral fluid. Collected samples

were immediately centrifuged, and the plasma was removed and frozen at -20°C until analysis.

The collection times selected in the present experiment were based on previous data from more extensive pharmacokinetic studies (7).

QUANTIFICATION OF ORAL FLUID AND PLASMA MPH AND RA

We analyzed biological samples (plasma and oral fluid) for the presence of MPH and RA using a previously described liquid chromatography–electrospray ionization–mass spectrometry method (18). The pH of the oral fluid samples was measured at all time-intervals with a pH indicator stick (Riedel-de Haën) with a range of 6.4–8.0 (increments of 0.2 pH units). Two independent observers, who were unaware of the treatment conditions, recorded the results.

PHARMACOKINETICS AND STATISTICAL ANALYSIS

For both oral fluid and plasma concentrations of MPH and RA, we determined the following parameters: peak concentration (C_{max}), time to reach peak concentration (t_{max}), area under the concentration–time curve from 0 to 24 h (AUC_{0-24}), elimination half life ($t_{1/2}$), and elimination constant (k_e).

We calculated AUC by the linear trapezoidal rule and the elimination constants by log-linear regression of the 3 lowest concentrations above the limit of quantification. We assessed correlations between different variables by regression analysis. We used the Wilcoxon test for nonparametric data to assess differences in oral fluid and plasma data between the fast- and extended-release MPH formulations. Differences with P values <0.05 were considered statistically significant.

Results

CONCENTRATION–TIME PROFILES AND PHARMACOKINETICS OF MPH AND RA IN ORAL FLUID AND PLASMA

Fig. 1 shows the time course of MPH and RA concentrations in oral fluid and plasma for each of the 8 volunteers. MPH concentrations peaked in oral fluid (range 26.4–466.7 $\mu\text{g/L}$) at 0.5 h after administration of the fast-release formulation in all subjects. In plasma, MPH peaked at 1 h after administration (21.7 $\mu\text{g/L}$) for 1 subject and at 2 h (range 5.9–9.8 $\mu\text{g/L}$) for the other 7 subjects. With the extended-release formulation, MPH concentrations appeared to be highest both in oral fluid (range 44.4–89.5 $\mu\text{g/L}$) and plasma (range 3.6–7.4 $\mu\text{g/L}$) at 2 h after drug administration. After the absorption phase, MPH concentration declined at 24 h after administration of the fast-release formulation to a median concentration of 2.8 $\mu\text{g/L}$ in oral fluid (range 0.7–6.8 $\mu\text{g/L}$), whereas in plasma the drug was undetected. MPH was undetectable in both oral fluid and plasma 24 h after administration of the

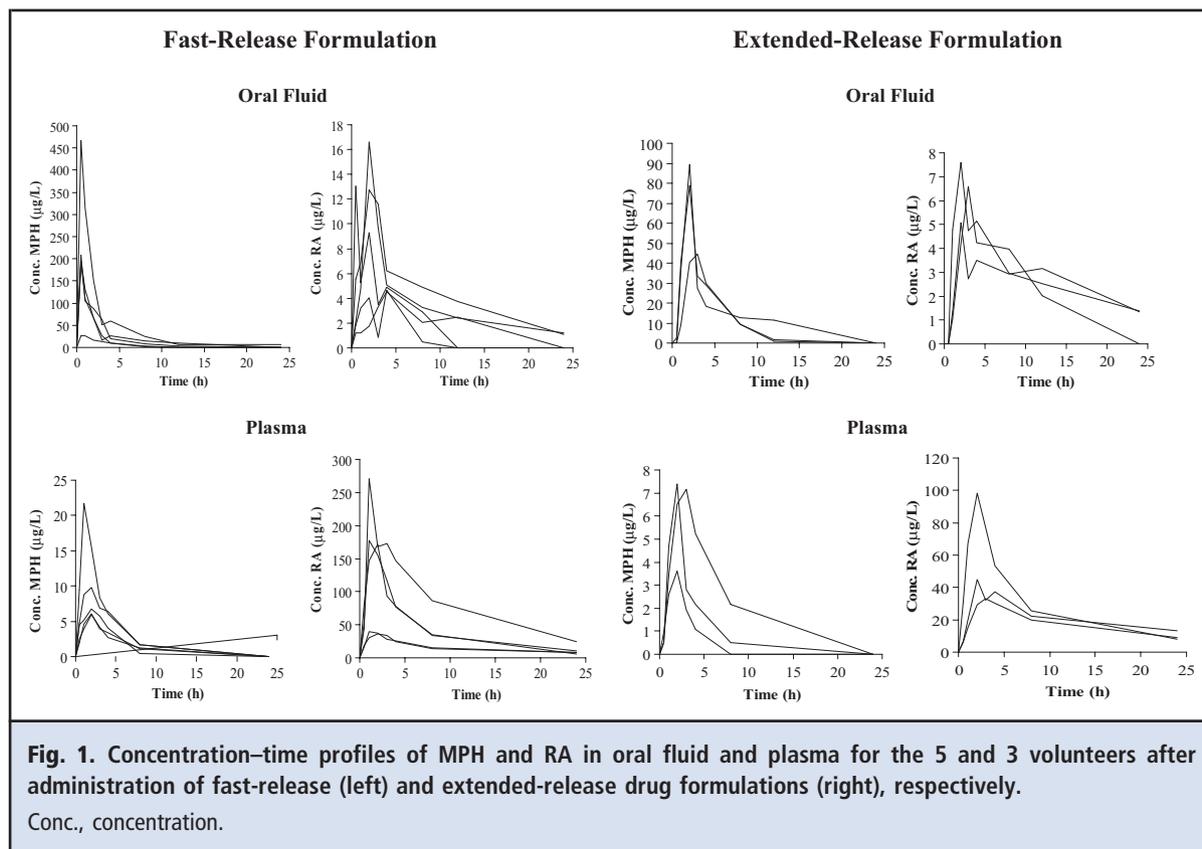


Fig. 1. Concentration–time profiles of MPH and RA in oral fluid and plasma for the 5 and 3 volunteers after administration of fast-release (left) and extended-release drug formulations (right), respectively. Conc., concentration.

extended-release formulation. Mean concentration–time curves for MPH in oral fluid and plasma are shown in Fig. 2. MPH concentrations in oral fluid were always an order of magnitude higher than those observed in plasma, but their time course was well matched, and the shape of the 2 concentration–time curves was almost identical, particularly for the extended-release formulation.

Pharmacokinetic parameters for MPH in oral fluid and plasma are presented in Table 1. The mean AUC_{0-24} in oral fluid was 10 times higher than in plasma, for both fast-release (472.2 vs $47.2 \mu\text{g L}^{-1} \text{h}^{-1}$) and extended-release (269.52 vs $28.76 \mu\text{g L}^{-1} \text{h}^{-1}$) formulations. Furthermore, the mean AUC_{0-24} of oral fluid or plasma for fast-release formulation was approximately 2 times higher than for the extended-release formulation, but because of large interindividual variability the differences were not statistically significant between the 2 formulations. Plasma k_e and $t_{1/2}$ were similar for both treatments. The limited number of sampling points in the elimination phase and the bimodal time course in some cases precluded calculation of MPH k_e and $t_{1/2}$ in oral fluid after the administration of the fast- and extended-release MPH formulation.

RA, a major metabolite of MPH, showed an opposite kinetic trend in the 2 biological fluids with respect to that of the parent drug (Figs. 1 and 2). Metabolite concentrations in oral fluid (peak concentration 8.9 and $5.6 \mu\text{g/L}$ at 2 h after administration of fast- and extended-release formulations, respectively) were an order of magnitude lower than those observed in plasma (peak concentration $133.3 \mu\text{g/L}$ at 1 h after administration of fast-release formulation and peak concentration $57.5 \mu\text{g/L}$ at 2 h after administration of extended-release formulation). As in the case of the parent drug, the mean kinetic time course in the 2 biological fluids matched quite well, and the shape of the curves was once again almost identical. Interestingly, for RA, for the fast-release formulation the 2 curves were practically superimposable.

OF/P RATIO OF MPH AND RA CONCENTRATIONS

The time-course curves of OF/P ratio for MPH during the first 8 h (afterward MPH was not measurable in plasma samples and the ratio could not be calculated) and its metabolite RA during the 24 h after drug administration in the 2 formulation groups are presented in Fig. 3. The fast-release formulation shows a first mean maximum value for MPH of 78.2

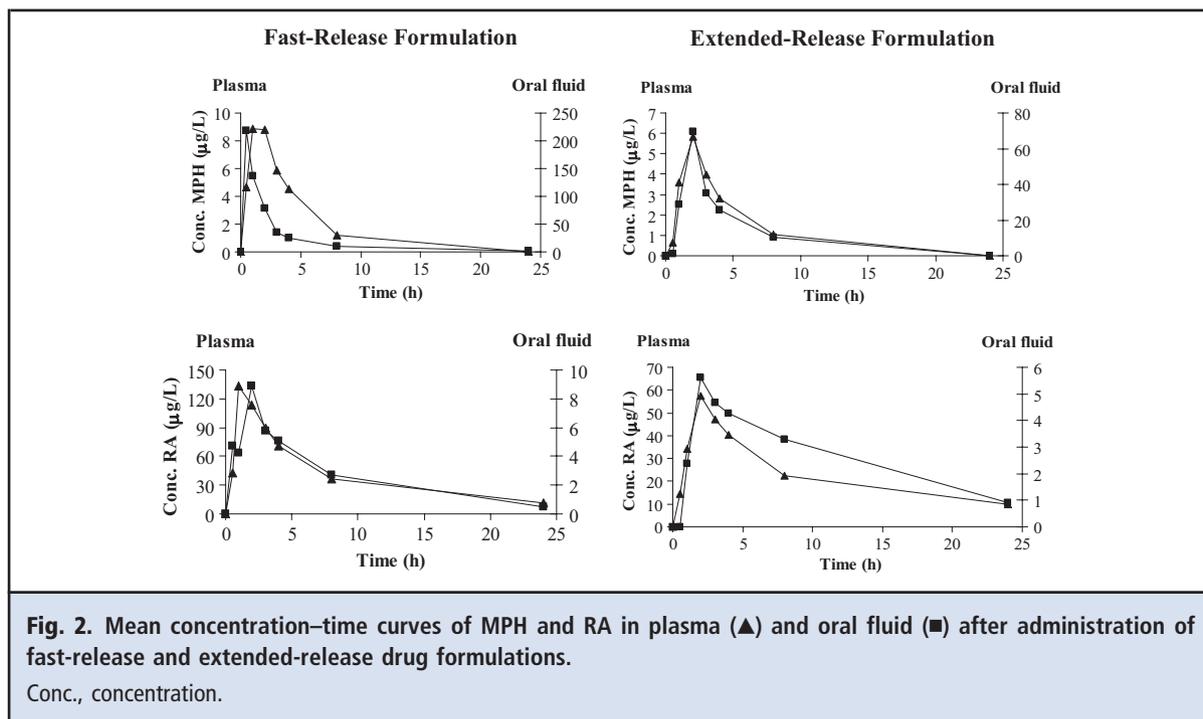


Fig. 2. Mean concentration–time curves of MPH and RA in plasma (▲) and oral fluid (■) after administration of fast-release and extended-release drug formulations.

Conc., concentration.

(range 5.8–242.1, median 44.0) at 0.5 h. The OF/P ratio decreased at 2 h postadministration to a mean value of 10.4 (range 2.3–24.3, median 4.1), and at 8 h had a mean value of 8.9 (range 2.3–22.0, median 5.5). The extended-release formulation had a first mean maximum value for MPH of 13.4 (range 6.2–21.9, median 12.1) at 2 h corresponding to MPH t_{max} . At 3 h after administration, the OF/P ratio had a mean value of 11.2 (range 6.2–17.6, median 9.8), and at 8 h, a second mean peak of 16.2 (range 4.5–27.0, median 18.9).

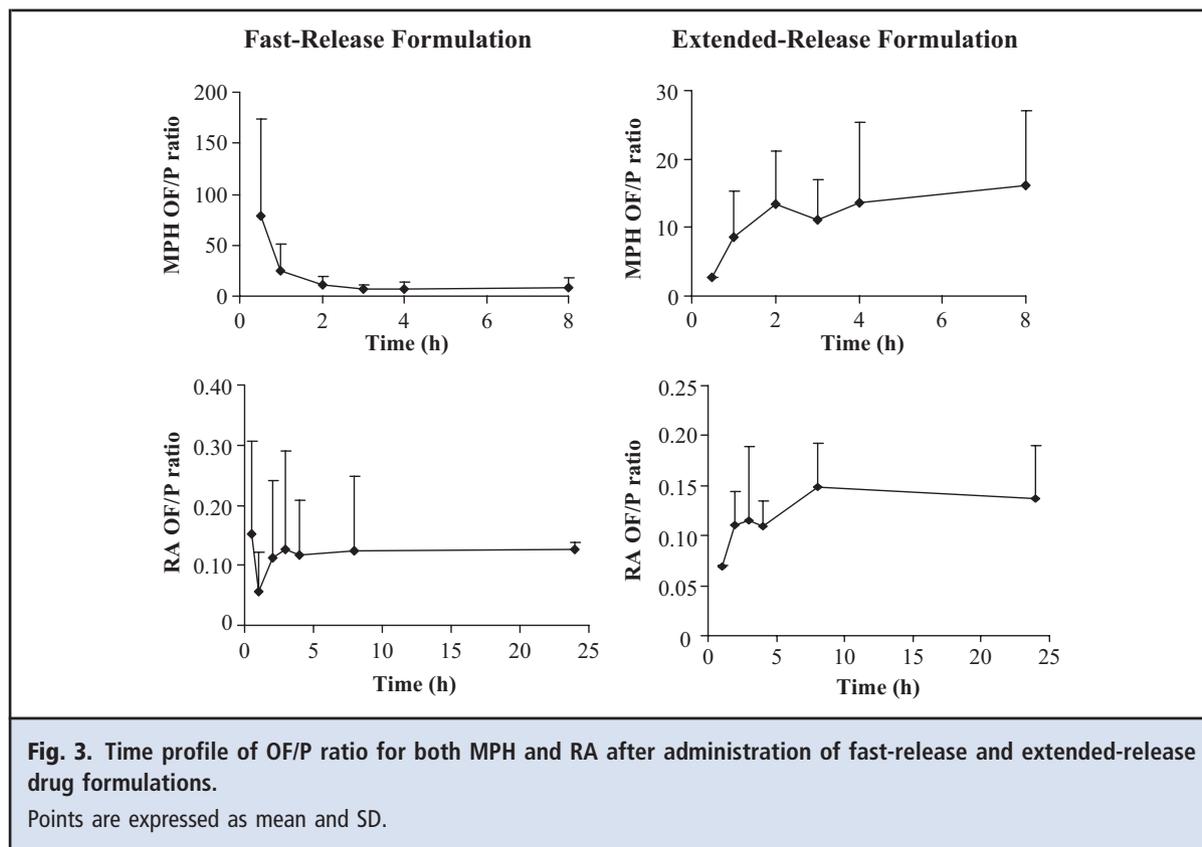
For the subjects receiving the fast-release formulation, the MPH OF/P ratio showed a good correlation with oral fluid drug concentrations ($r = 0.88, P < 0.001$); no correlation was observed with oral fluid pH

values ($r = 0.15, P > 0.05$) or plasma MPH concentrations ($r = 0.03, P > 0.05$) (see Supplemental Fig. 1, which accompanies the online version of this article at <http://www.clinchem.org/content/vol56/issue4>). Considering all the time course points, oral fluid MPH concentrations showed weak correlation with plasma concentrations ($r = 0.22, P < 0.05$) (Fig. 4); however, if the values for the first time point (0.5 h) were eliminated, the correlation increased to a value of $r = 0.47, P < 0.01$ (see online Supplemental Fig. 2).

In the extended release group, the OF/P ratio showed weak correlation with oral fluid MPH concentrations ($r = 0.46, P < 0.05$), plasma MPH concentrations ($r = -0.41, P < 0.05$), and oral fluid pH values ($r = -0.49, P < 0.05$) (see online Supplemental Fig. 3).

Table 1. Apparent pharmacokinetic parameters for MPH in oral fluid and plasma.

	Fast release formulation (n = 5)				Extended release formulation (n = 3)	
	Oral fluid		Plasma		Oral fluid	Plasma
	Mean (SD)	CV, %	Mean (SD)	CV, %	Mean (range)	Mean (range)
$AUC_{0-24\text{ h}}, \mu\text{g L}^{-1}\text{ h}^{-1}$	472.2 (324.3)	72.5	47.2 (19.6)	41.6	269.5 (209.3–331.5)	28.8 (10.6–51.3)
t_{max}, h	0.5	—	1.8 (0.4)	24.8	2.3 (2.0–3.0)	2.3 (2.0–3.0)
$C_{max}, \mu\text{g/L}$	218.2 (157.9)	72.4	8.8 (7.4)	83.9	69.5 (44.2–89.5)	5.8 (3.6–7.4)
K_e, h^{-1}			0.3 (0.1)	31.6		0.4 (0.2–0.6)
$t_{1/2}, \text{h}$			2.3 (0.6)	25.3		2.1 (1.2–3.1)



Notwithstanding the variation of OF/P ratio during the time course of extended-release administration, oral fluid MPH concentrations were correlated to plasma concentrations ($r = 0.79$, $P < 0.01$) (Fig. 4).

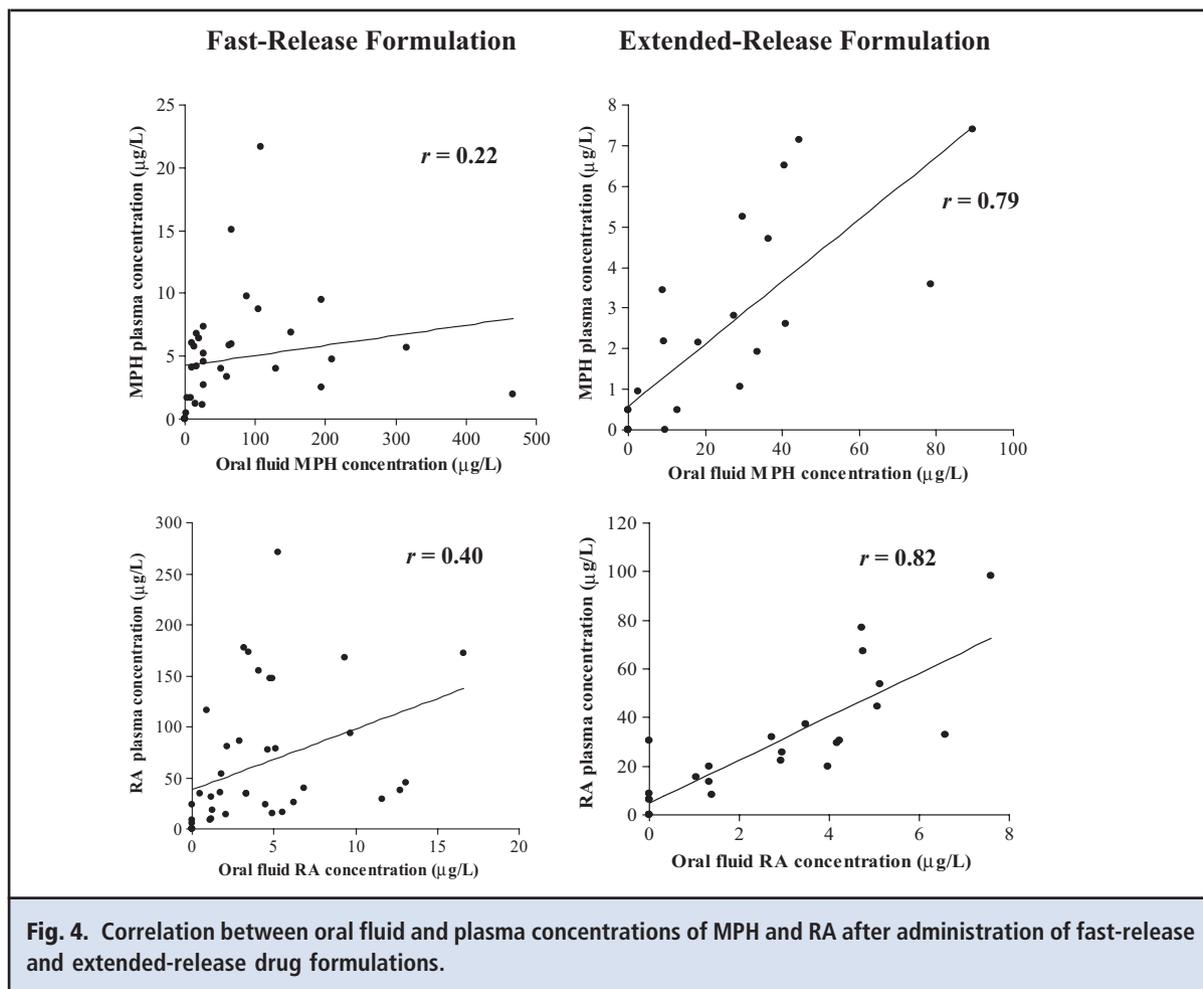
RA showed a bimodal time-course curve of OF/P ratio after the administration of both formulations. With respect to the fast-release formulation, OF/P ratio peaked first at 0.5 h with a mean value of 0.15 (range 0.03–0.34, median 0.07), and then peaked again at 3 h after drug administration, showing a second mean value, lower than the first, of 0.13 (range 0.01–0.41, median 0.10). OF/P ratio for RA achieved a first mean maximum value of 0.12 (range 0.06–0.20, median 0.09) 3.0 h after the administration of the extended-release formulation, and then a second mean maximum of 0.15 (range 0.11–0.20, median 0.13) at 8 h after administration (Fig. 3).

After the fast-release drug administration, no correlation was found between RA OF/P ratios and oral fluid RA concentration ($r = 0.14$, $P > 0.05$) or oral fluid pH ($r = -0.08$, $P > 0.05$), whereas a weak correlation was found with RA plasma concentrations ($r = 0.30$, $P < 0.05$) (see online Supplemental Fig. 1) and between oral fluid and plasma RA concentrations ($r = 0.4$, $P < 0.05$) (Fig. 4).

In the case of the extended-release formulation, RA OF/P ratios did not correlate with oral fluid pH ($r = 0.21$, $P > 0.05$) and showed a minimum correlation with RA plasma concentration ($r = -0.23$, $P < 0.05$) and a weak correlation with RA oral fluid concentration ($r = 0.32$, $P < 0.05$) (see online Supplemental Fig. 3). A good correlation was found between oral fluid and plasma RA concentration ($r = 0.82$, $P < 0.01$) (Fig. 4).

TIME COURSE OF pH IN ORAL FLUID SAMPLES

Oral fluid pH showed a mean value of 7.1 and 6.7 at predose time in the fast- and extended-release groups, respectively. Then, in the first 4 h after drug administration, mean pH values showed variations (both increases and decreases) of 0.2 U for both formulations, which were not statistically significant. After 4 h post-administration, the time course of pH mean values varied between 7.0 and 7.1 in the oral fluid of subjects receiving the fast-release formulation and between 6.7 and 6.8 in those receiving the extended-release formulation. The 24-h profiles of the mean oral fluid pH in both MPH formulation groups are presented in online Supplemental Fig. 4.



Discussion

Our results demonstrate the presence of MPH and its metabolite in oral fluid after the administration of fast- and extended-release formulations. Notwithstanding some interindividual variations, the overall patterns of oral fluid and plasma MPH and RA concentration–time profiles agreed well in different subjects.

MPH appeared in oral fluid in concentrations remarkably greater than those in plasma (Figs. 1 and 2). This is not surprising, since this drug is an amphetamine-like compound that shows the characteristics of a weak base ($\text{pK}_a = 8.9$), low plasma protein binding (approximately 15%), and low molecular weight (233 Da). Thus, in agreement with what was reported for amphetamine type substances (3) and specifically for methylenedioxymethamphetamine (MDMA) (7), and with the fact that oral fluid is more acidic than blood, this substance is incorporated in oral fluid by passive diffusion of the free fraction of the drug in its ionized form, which cannot diffuse back into plasma.

The theoretical OF/P ratio should be around 3.1 as calculated using the Henderson–Hasselbach equation (19). In our study, however, OF/P ratio mean values ranged between 78.2 at peak MPH oral fluid concentrations and 8.9 at 8 h after administration of the fast-release formulation and between 13.4 at peak MPH concentrations and 16.2 at 8 h after administration of the extended-release formulation. It must be noted that, for the fast-release drug formulation, plasma drug concentrations were quite homogeneous for the 5 subjects, with a 4-fold variation at peak time, whereas oral fluid concentrations presented enormous fluctuations (more than 15-fold) at peak MPH concentrations (Fig. 1). As a possible explanation, buccal contamination must be considered, since in this case the drug was administered as tablets. In the case of the extended-release formulation, MPH concentrations were more homogeneous in both plasma and oral fluid, probably because the drug was administered in capsules. In any case, considering buccal contamination in the first hours after drug administration and excluding the enormous OF/P ratios obtained in the first hours after

fast-release formulation administration, it appears that accumulation of MPH in oral fluid occurs to a greater extent than expected. In accordance with our previous study on MDMA (7), we can postulate a sympathomimetic effect of MPH consisting in a reduction of oral fluid production, with higher fluid concentration, lowered buffering capacity, and thus a lower pH at the site of oral fluid secretion. Hence, as already demonstrated for MDMA, a concentration gradient takes place that produces OF/P ratios higher than that calculated by the Henderson–Hasselbach equation. Conversely, in the case of RA, OF/P ratios under the unity can be easily explained by the acidic nature of this compound, which prevents its excretion in oral fluid. For all these reasons, MPH is the target compound which can be and has to be measured in oral fluid, as has been already shown in a pediatric case (20).

Furthermore, measurement of MPH in oral fluid appears to be a suitable alternative to plasma analysis. Despite the changes in OF/P ratio at the single time points, the eventual buccal contamination when consuming tablets (reflected in the enormous concentrations found at 0.5 h in the case of fast-release formulation and resulting in a lower correlation between the 2 fluids), the correlation between MPH concentration in the 2 biological fluids indicates that oral concentrations of this drug may be a predictor of plasma concentrations if the extended formulation is used.

Unlike MDMA (7), in the case of MPH only weak nonsignificant pH variations were observed, which could also be due to physiological changes (time, food) as already demonstrated for the placebo group in the abovementioned study (7).

A limitation of this study is that data were obtained from a small cohort in controlled conditions where eventual drug–drug interaction potential was not taken into consideration. Although it does not reflect the “real-life” situation of chronic dosing in a patient population, we consider the data of this first study of MPH kinetics in oral fluid as promising. Furthermore, these results are supported by the data obtained when

developing the analytical assay for oral fluid drug detection (18), where the compliance of individuals in treatment with the drug could be verified by oral fluid testing and by the data presented for a pediatric case (20). In this latter case, oral fluid measurement of MPH and RA was successfully applied to verify the 4-week compliance of a 12-year-old boy treated with the extended-release drug formulation. Nonetheless, these data have to be confirmed in a larger number of individuals. The application of MPH oral fluid monitoring in clinical practice and clinical toxicology may help to evaluate compliance with the treatment and monitor nonprescribed use of the drug with the possibility of on-site sample collection, using a less invasive method than plasma concentration monitoring.

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