



Hydromorphone, Tramadol and O-Desmethyltramadol in serum and oral fluid from patients in chronic pain treatment

GERICHTSMEDIZIN
SALZBURG-LINZ

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Introduction

Hydromorphone (H) and Tramadol (T) are opioid agonists prescribed for the relief of moderate to severe pain. O-Desmethyltramadol (DT) is the active metabolite of T formed in the liver by CYP2D6. Interestingly DT, which is no prescription drug, is also abused as an admixed part of powdered Kratom leaves to increase the opioid effects of the pharmacological active component Mitragynine. This substance is marketed under the name "Krypton".
The increasing use and abuse of H, T and other opioids and their involvement in intoxications makes it essential to include these substances into drug screening methods. Drugs of abuse testing traditionally requires urine samples (spl.), while compliance monitoring and TDM normally is based on serum (S) level quantification. Recently oral fluid (OF) is gaining interest as a less intrusive matrix for drugs of abuse and compliance testing. This study aimed at the determination of OF/S-ratios for H, T and DT in paired OF and S spls. from patients (pats.) in steady-state applying a sensitive UPLC-MS/MS method.

Methods

Patients: Pats. in steady-state for H and T were recruited (H: n = 39, 25 male, 14 female, age 28 to 83; T: n = 13, 10 male, 3 female, age 43 - 79) which received their dose once (H: n = 15, dose 2-16 mg/d; T: n = 2, dose 100 mg/d) or twice a day (H: n = 24, dose 2-16 mg/d; T: n = 7, dose 100-300 mg/d), see Tab. 1 and Tab. 2. Spls. were collected nearly simultaneously about 5 h post morning dose and about 0.5 h prior evening dose. The study was approved by the ethics committee at the federal state of Salzburg.

Sample collection: S was prepared from blood collected by venous puncture. OF samples were collected using the liquid based Greiner Bio-One (GBO, Kremsmünster, Austria) SCS pH 4.2 device according to the manufacturer. % OF concentration (conc.) of the OF/SES mixture was quantified on an Olympus AU680 using the GBO saliva quantification kit.

Sample preparation H: 100 µL S or OF/SES, fortified with 20 µL 50 ng/mL H-d₃ in MeOH, was protein precipitated with 180 µL 0.04 M ZnSO₄. After centrifugation 50 µL of the supernatant was diluted with 200 µL 5 mM NH₄FA (pH 3) and 1 µL was injected into the UPLC. SE and OF/SES matrix calibration was performed from 0.05 to 0.4 ng/mL and from 0.5 to 15 ng/mL (n = 8; LoD: 0.05 ng/mL, LoQ: 0.13 ng/mL (according to GTFCh guidelines; Fig. 8)).

Sample preparation T, DT: 100 µL S or OF/SES, fortified with 10 µL 50 ng/mL T-C₁₃-d₃, DT-d₃ in MeOH, was protein precipitated with 200 µL 0.2 M ZnSO₄. After centrifugation 50 µL of the supernatant was diluted with 450 µL (20 mM NH₄FA (pH 3)/ MeOH 0.1% FA (90:10, v/v)) and 10 µL was injected into the UPLC. S and OF/SES matrix calibration was performed from 5 - 1000 ng/mL (n = 10 (Fig. 9); LoD = T: 1.8 ng/mL, DT: 1.1 ng/mL; LoQ = T: 3.2 ng/mL, DT: 2.8 ng/mL (according to GTFCh guidelines)).

UPLC-MS/MS: gradient separation was conducted on a Waters Acquity UPLC connected to a Xevo-TQ-MS with a HSS T3 column (1.8 µm, 2.1x150 mm) kept at 35°C within 9 min. MoPh A was 20 mM NH₄FA (pH 3) and MoPh B was 0.1% FA in MeOH. The instrument was operated in the ESI positive mode. Three transitions were recorded in SRM mode (target ions in bold) for H: **266>185**, 286>157, 286>128; H-d₃: **289>185**, 289>157, 289>128; T: **264>58**, 264>246, 264>121, T-C₁₃-d₃: **268>58**, 268>250, 268>125; DT: **250>58**, 250>77, 250>232; DT-d₃: **256>64**, 256>77, 256>238.

Conclusion

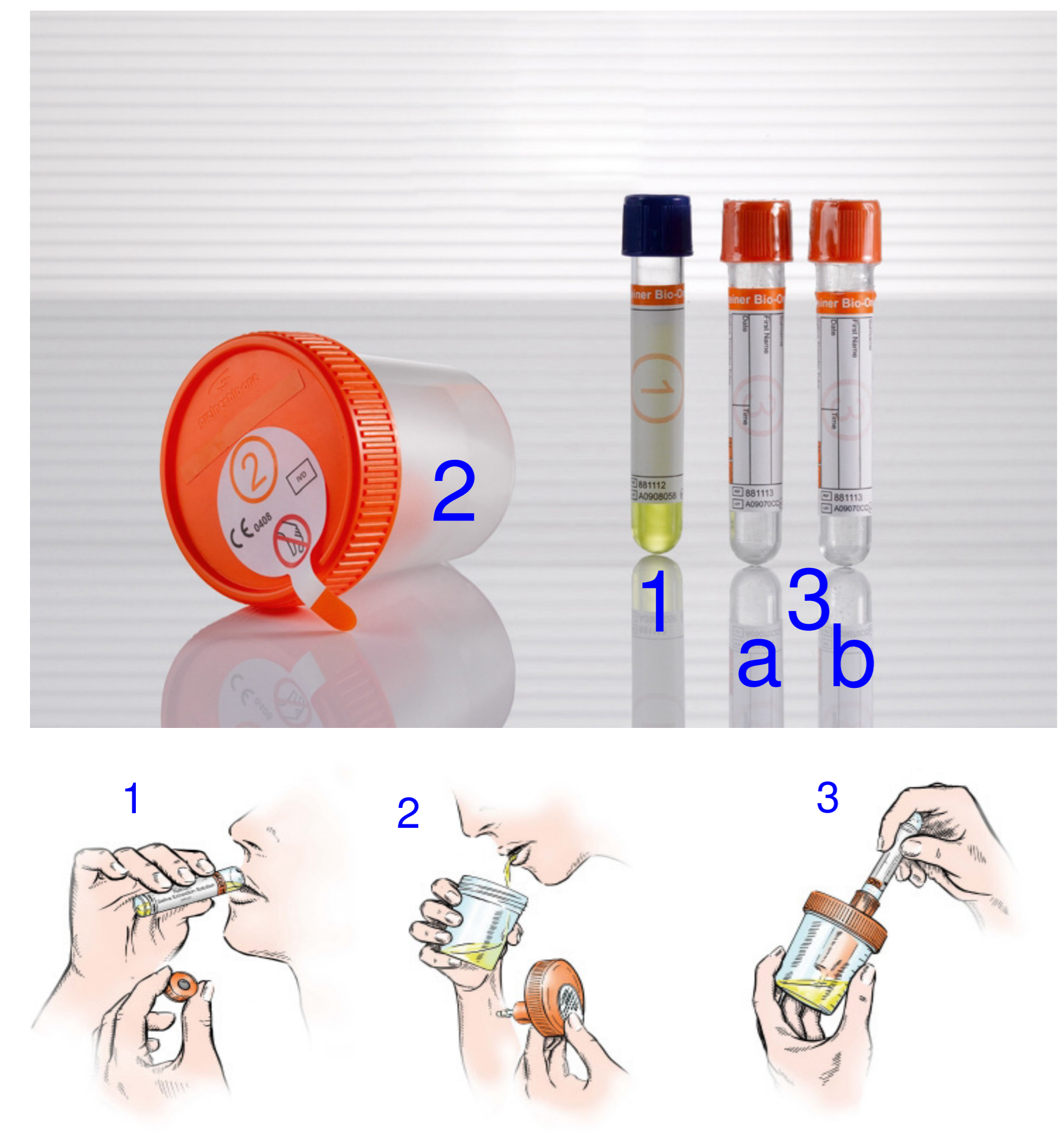
-- The mean OF/S ratio of **H** was 0.9 (median: 1.0; see Tab. 4). This is somewhat lower than expected from pKa (8.2) and plasma protein binding (~10%).

-- The mean OF/S ratio of **T** was 5.7 (median: 4.7; see Tab. 5) This could be expected from pKa (9.4) and plasma protein binding (~20%).

-- The mean OF/S ratio of **DT** was 2.1 (median: 1.2; see Tab. 6). Even though the parent drug is the target analyte when spotting T consumption, parallel detection of DT is a helpful plausibility control. In addition DT is a drug on its own ("Krypton") and should therefore be detectable in the absence of T.

-- Oral fluid is a promising alternative matrix for drugs of abuse and compliance testing for H, T and DT.

Fig. 1 Greiner Bio-One Saliva collection system pH 4.2



Saliva collection

- (1) rinse oral cavity with Saliva Extraction Solution (SES) for minimum 2 minutes
- (2) spit OF/SES into beaker
- (3) transfer OF/SES into evacuated tubes containing bactericides and send to lab
- (4) after centrifugation Amylase and OF concentration are determined on an Olympus AU680

Results

Fig. 2 Hydromorphone conc. range: S and OF

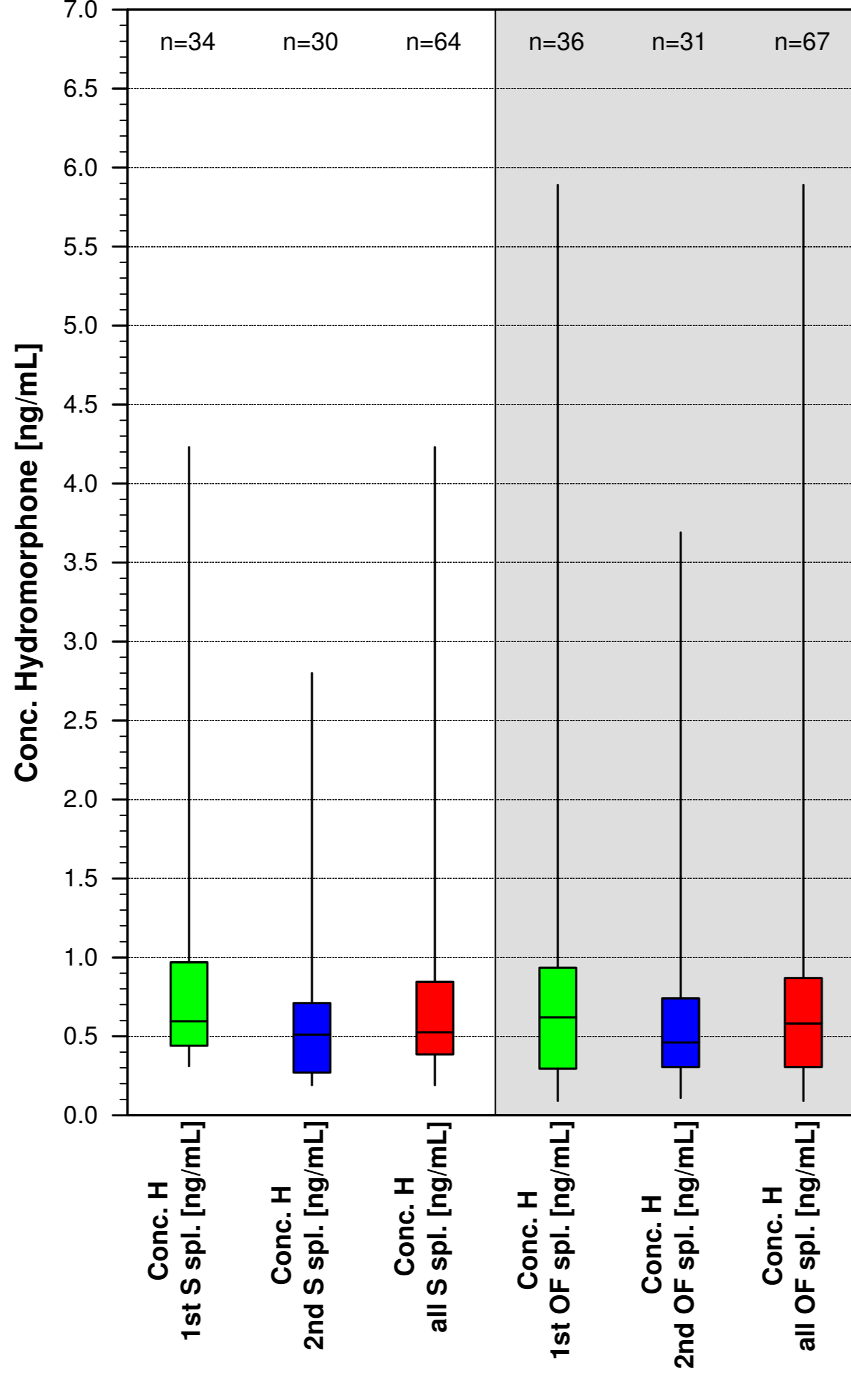


Fig. 3 Tramadol conc. range: S and OF

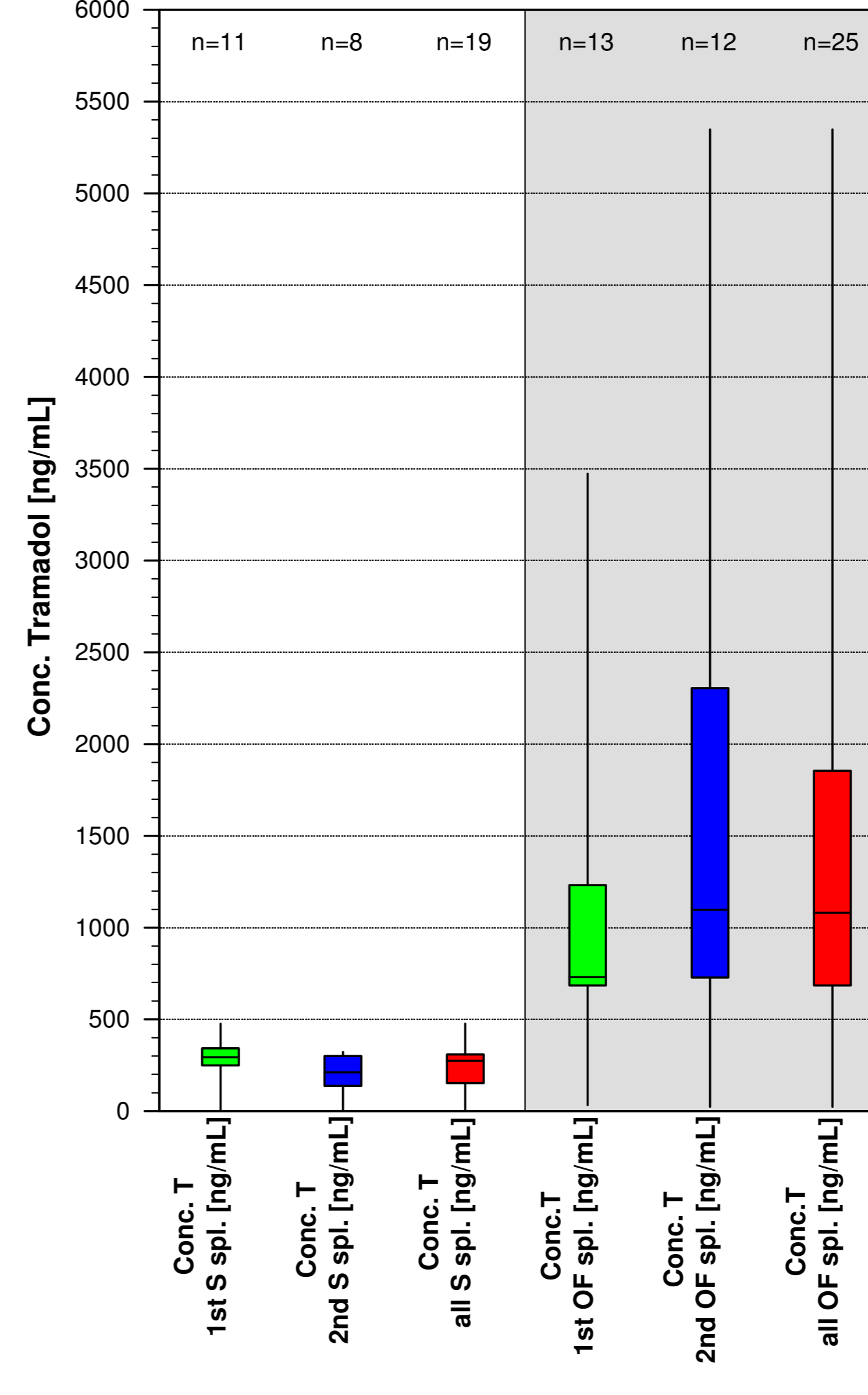
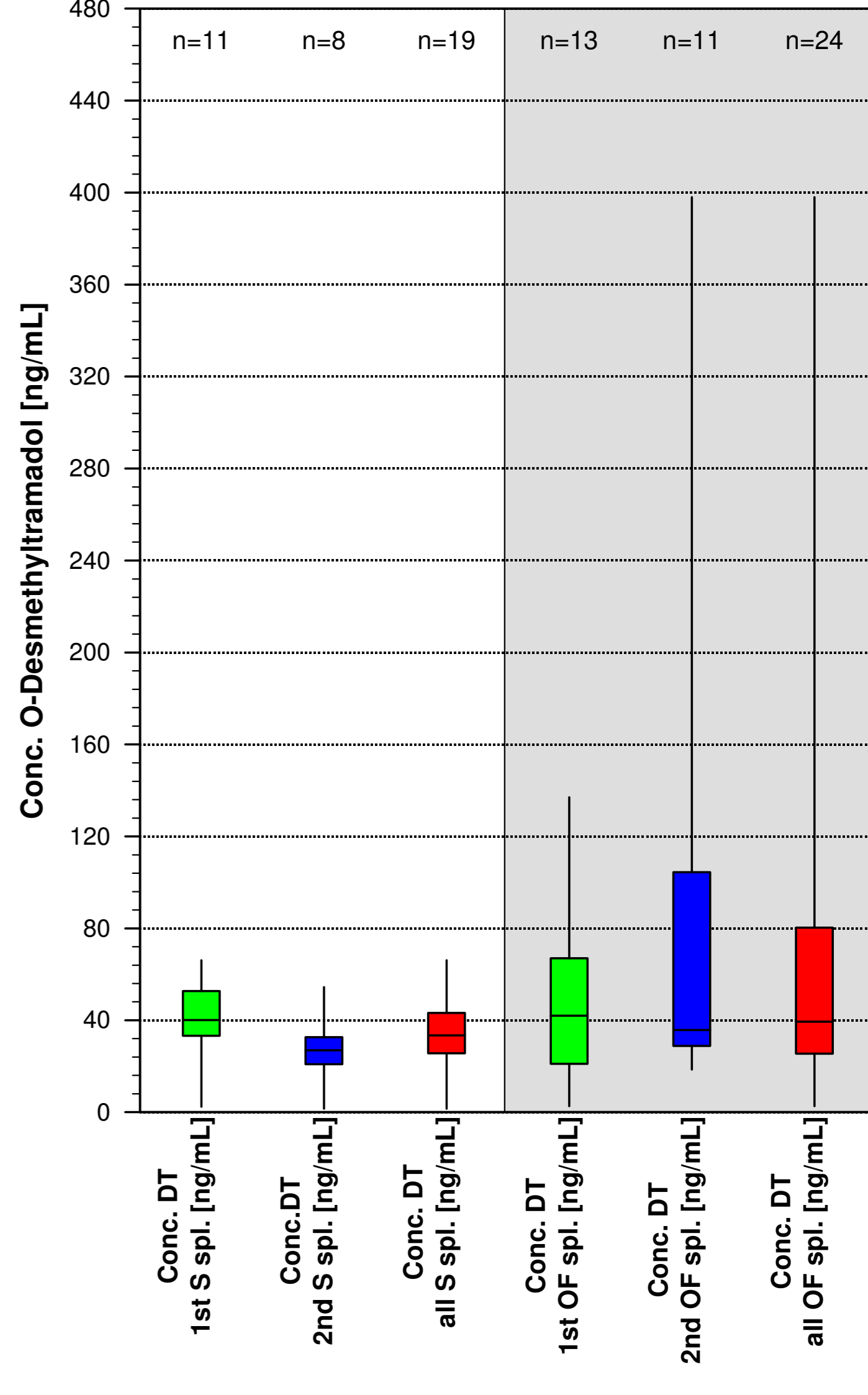


Fig. 4 O-DM-Tramadol conc. range: S and OF



Tab. 1 Patients and dosing: Hydromorphone

patients	total	n	age	dosage [mg]
	total	39	28-83	
	male	25	32-74	
	female	14	28-83	
dosing range	daily			2-16
	morning			2-16
	evening			2-8
doses per day	2 [morning and evening]	24		
	1 [morning]	14		
	1 [evening]	1		

Tab. 2. Patients and dosing: Tramadol

patients	total	n	age	dosage [mg]
	total	13	43-79	
	male	10	43-79	
	female	3	54-79	
dosing range	daily			100 - 300
	morning			100
doses per day	2 [morning and evening]	7		
	1 [morning]	2		
	not specified	4		

Tab. 3 Pharmacokinetic properties

Pharmacokinetic properties*	Tramadol	Hydromorphone
pKa	9.4	8.2
oral bioavailability	~75%	36.4% (Hydal® retard)
elimination half life	Tramadol ~ 6h O-Monodesmethyltramadol ~ 9h	~ 2.5 h
plasma protein binding	20 %	< 10%
distribution volume	~3 L/kg	~1.2 L/kg
main metabolite	O-Monodesmethyltramadol	Hydromorphone-3-Glucuronide
main excretion	renal	renal

* from the literature
-- The pats. received Hydal® retard as standard medication.
-- Immediate release Hydal® was given if needed.

Tab. 4 Hydromorphone OF/S ratio

statistics	OF/S ratio 1st spls.	OF/S ratio 2nd spls.	OF/S ratio all spls.
range	0.2 - 2.9	0.4 - 2.2	0.2 - 2.9
mean	0.9	1.0	0.9
median	0.8	1.2	1.0
SD	0.5	0.7	0.6
CV	55%	59%	58%
25% percentile	0.6	0.6	0.6
75% percentile	1.2	1.5	1.2
5% percentile	0.2	0.4	0.4
95% percentile	2.3	2.9	2.4
n	34	27	61

Tab. 5 Tramadol OF/S ratio

statistics	OF/S ratio 1st spls.	OF/S ratio 2nd spls.	OF/S ratio all spls.
range	2.3 - 8.9	1.8 - 16.2	1.8 - 16.2
mean	4.8	6.8	5.7
median	4.2	6.2	4.7
SD	2.3	4.3	3.3
CV	48%	63%	58%
25% percentile	2.6	4.5	3.0
75% percentile	7.3	7.7	7.3
n	11	8	19

Tab. 6 O-Desmethyltramadol OF/S ratio

statistics	OF/S ratio 1st spls.	OF/S ratio 2nd spls.	OF/S ratio all spls.
range	0.6 - 4.1	0.55 - 9.0	0.55 - 9.0
mean	1.5	3.1	2.1
median	1.1	1.3	1.2
SD	1.0	3.5	2.4
CV	67%	113%	114%
25% percentile	0.6	0.7	0.7
75% percentile	1.9	7.3	2.1
n	11	7	18

Fig. 5 Hydromorphone conc.: OF vs. S

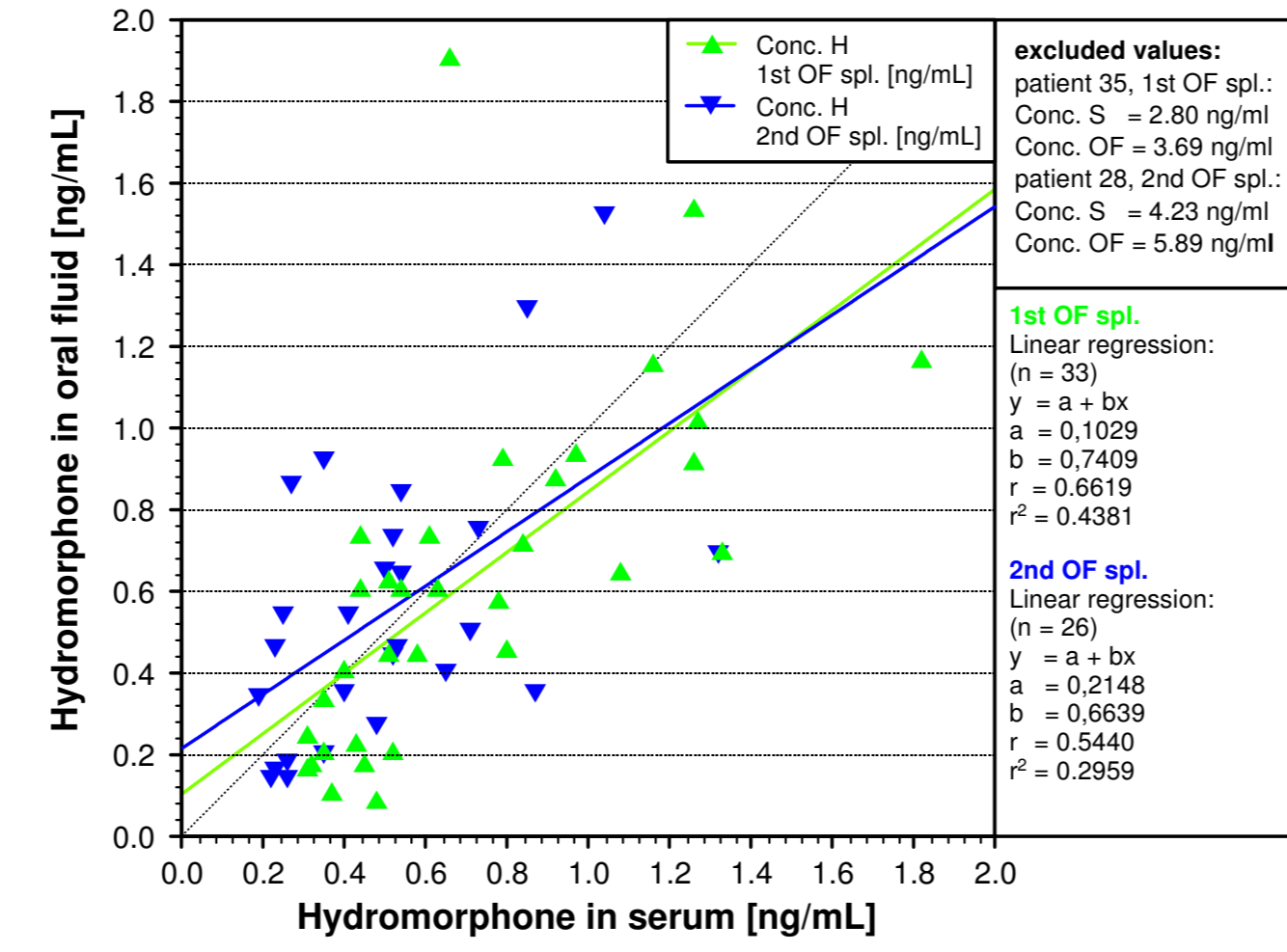


Fig. 6 Tramadol conc.: OF vs. S

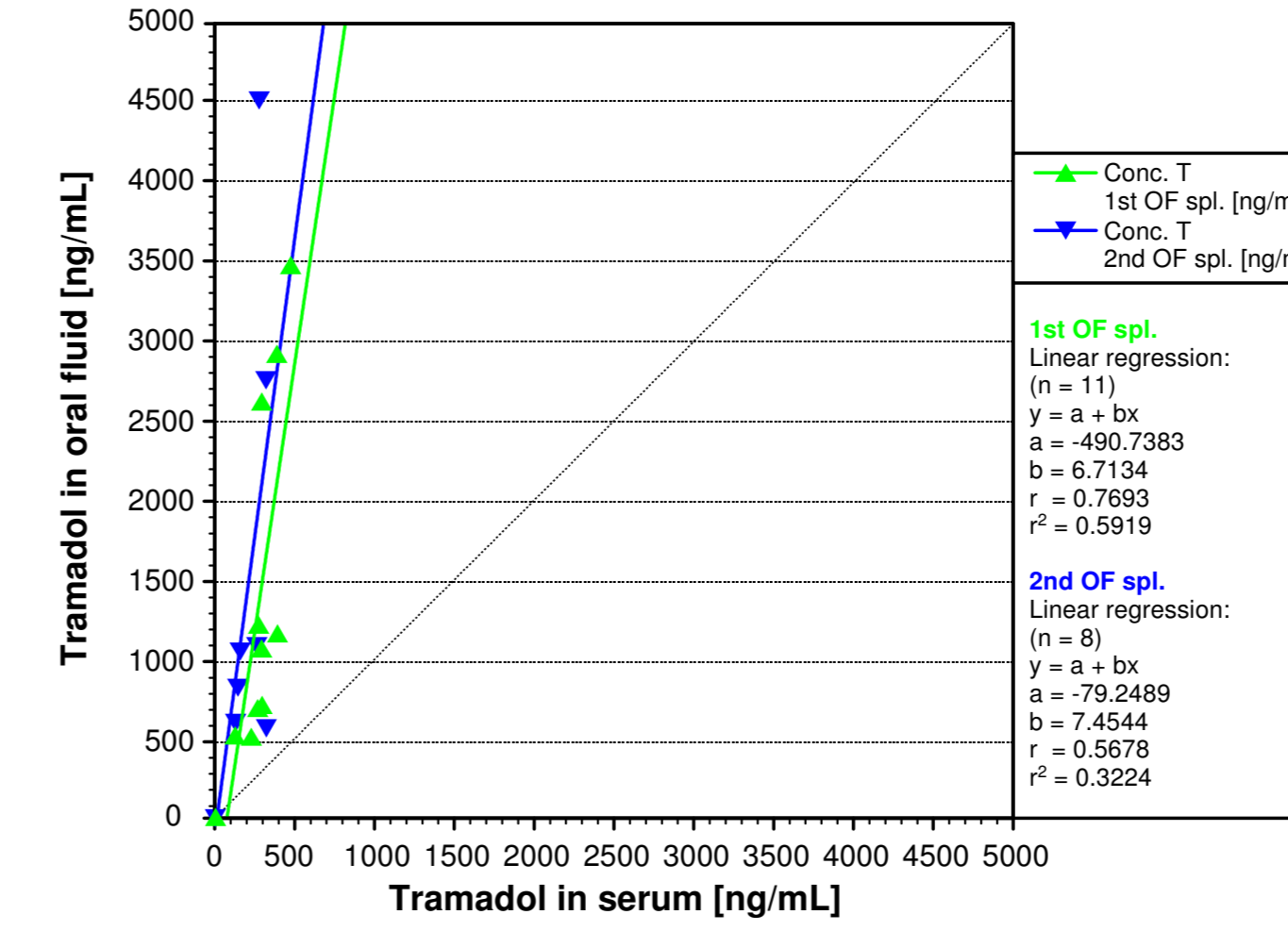
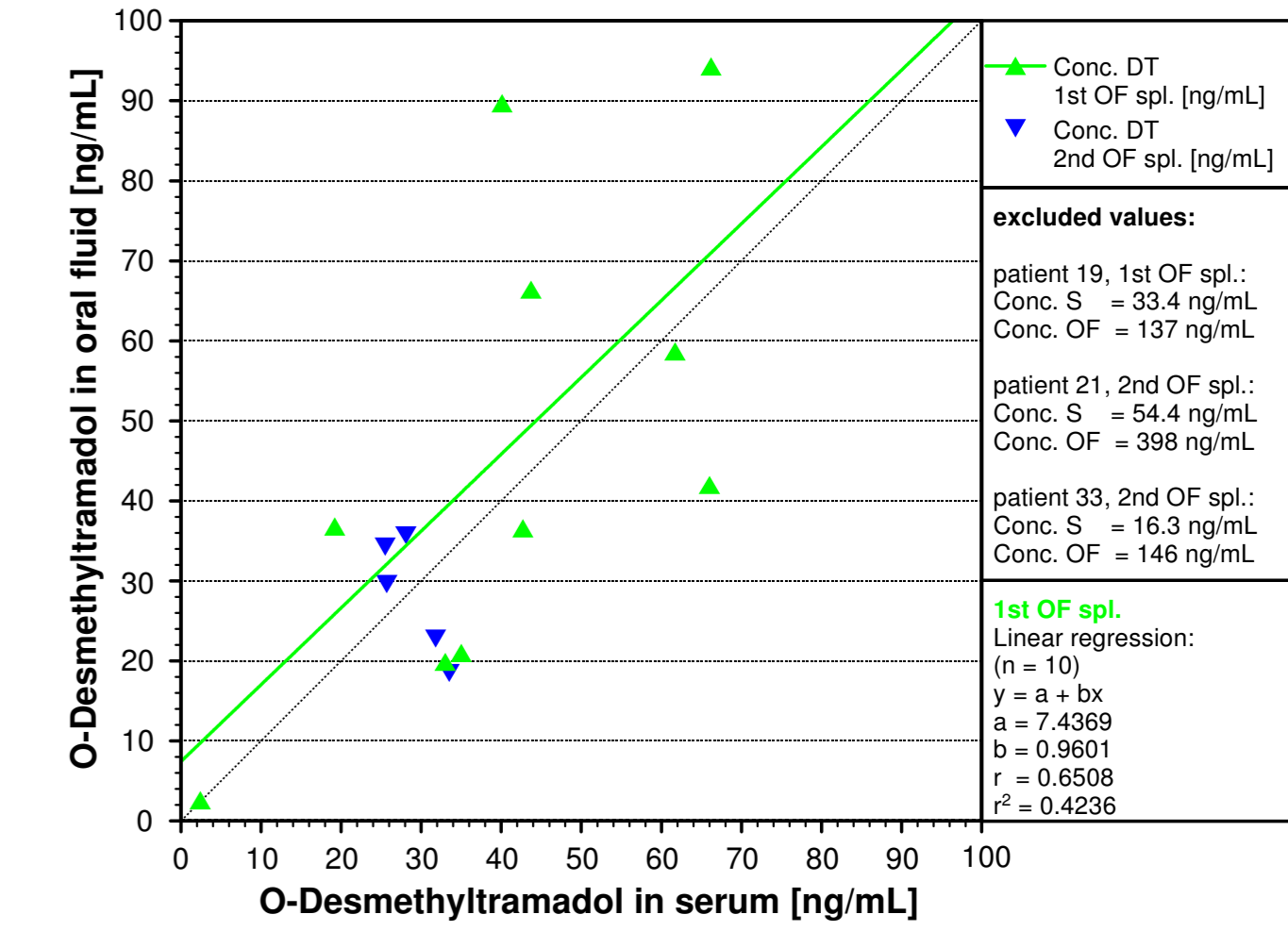


Fig. 7 O-Desmethyltramadol conc.: OF vs. S



UPLC-MS/MS method

Fig. 8 Hydromorphone: serum calibration LoD, LoQ

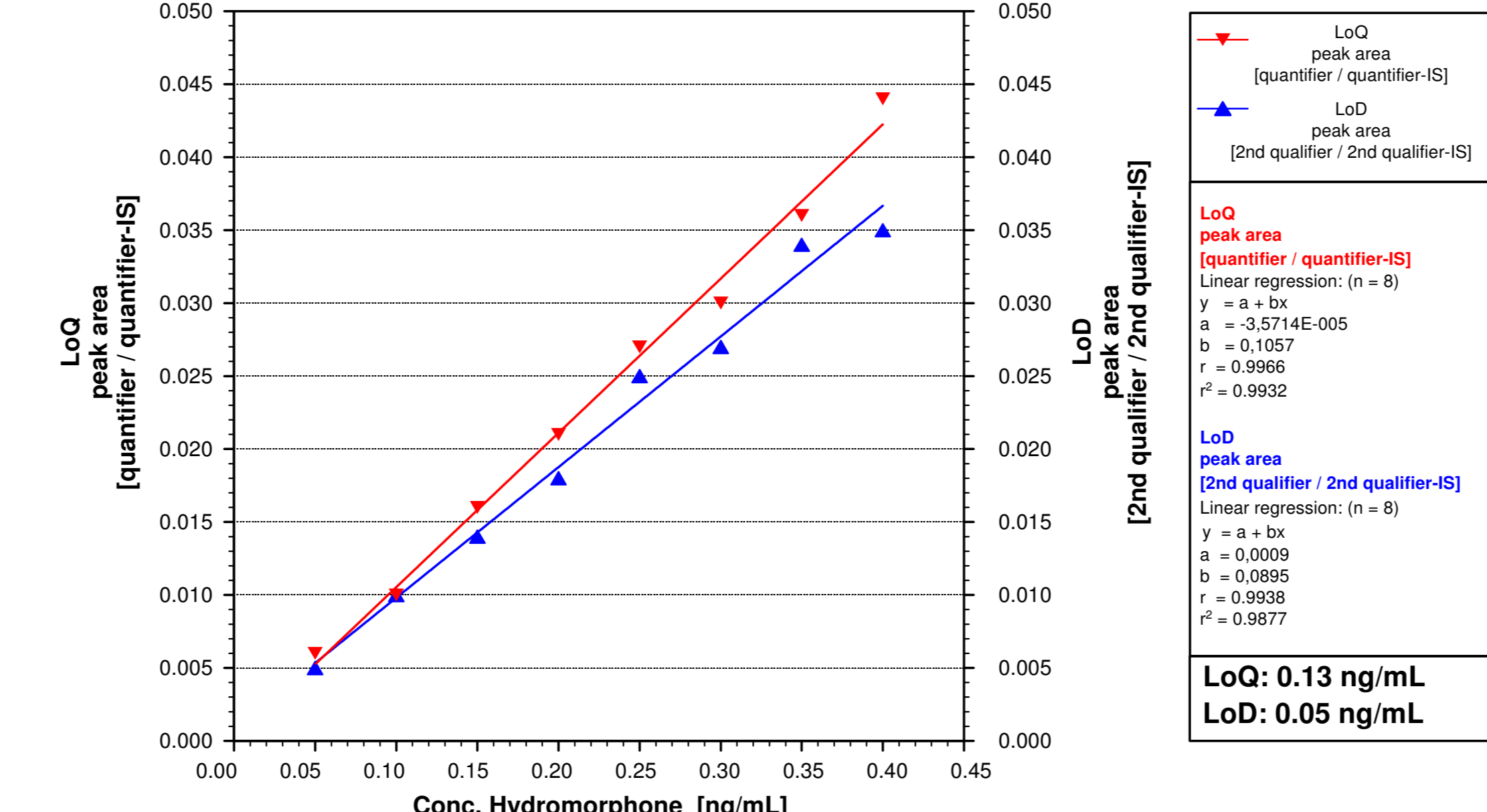


Fig. 9 Tramadol and DT: serum working calibration

