1 Supplementary materials

(Supplementary materials.pdf)

Table S 1: CYP3A inducers listed in the FDA draft guidance on labeling for combined hormonal contraceptives sorted by their CYP3A induction potential

Inducers	Max % ↓ AUC	Objects	Classification
Topiramate	12.0	Ethinylestradiol	
Aprepitant	22.1	Midazolam IV	
Oxcarbazepine	28.1	Flodipine	
Ritonavir	29.2	Ethinylestradiol	
Nevirapine	32.5	Indinavir	**W**eak
Boceptrevir	34.2	Darunavir	
Rufinamide	36.7	Triazolam	
(Fos)amprenavir	43.0	Iopinavir	
Telaprevir	48.4	Darunavir	
Lopinavir	59.7	Amprenavir	
Rifabutin	68.4	Midazolam	
Bosentan	69.0	Sildenafil	**M**oderate
Tipranavir / ritonavir	75.6	Saquinavir	
Efavirenz	76.0	Alfentanil	
Phenobarbital	76.6	Verapamil	
St. John's Wort extract	80.0	Midazolam	
Carbamazepine	86.6	Quetiapine	**S**trong
Phenytoin	89.5	Nisoldipine	
Rifampin	99.7	Budesonide	

The list of CYP3A inducers was taken from the FDA draft guidance on labeling for combined oral contraceptives[5]. Information on the inducers CYP3A induction potential was extracted from the list of "In Vivo Inducers of CYP3A Probes" from the UW Drug Interaction Database (DIDB) Copyright University of Washington, accessed Dec 2019.

Table S 2: Subject characteristics

	LNG N=13	**NET** N=14	**DSG** N=12	**DNG** N=12	**DRSP/EE** N=14	**Total** N=65
Age (years)	58.9 ± 3.66	57.4 ± 5.51	61.2 ± 6.24	60.9 ± 4.34	59.9 ± 6.18	59.6 ± 5.33
Weight (kg)	69.6 ± 11.1	69.7 ± 11.0	71.1 ± 7.70	69.7 ± 6.63	67.1 ± 9.51	69.4 ± 9.25
BMI (kg/m²)	25.3 ± 2.99	26.3 ± 3.27	25.7 ± 1.88	26.4 ± 3.24	24.9 ± 3.00	25.7 ± 2.90
Race = White [a]	13 (100%)	14 (100%)	12 (100%)	12 (100%)	13 (92.9%)	64 (98.5%)
Race = Asian	0	0	0	0	1 (7.1%)	1 (1.5%)

Arithmetic means and standard deviations are presented for age, body weight and body mass index.
[a] not Hispanic or Latino; BMI, body mass index; DNG, dienogest; DRSP, drospirenone; DSG, desogestrel; EE, ethinylestradiol; LNG, levonorgestrel; MDZ, midazolam; NET, norethindrone.

Table S 3: Exposure of midazolam, 4β-hydroxycholesterol, progestins, and EE when the test drug was administered without and with rifampicin

			Control phase (no RIF)	Weak-induction phase (RIF 10 mg/d)		Strong-induction phase (RIF 600 mg/d)	
Analyte	**Parameter (Unit)**	**N**	**gMean (%CV)**	**gMean (%CV)**	**GMR [90% CI]**	**gMean (%CV)**	**GMR [90% CI]**
MDZ	AUC (µg·h/L)	65	12.7 (36.7)	6.84 (37.3)	0.539 [0.491-0.592]	1.73 (49.3)	0.137 [0.124-0.150]
	C_{max} (µg/L)	65	4.90 (33.0)	3.10 (38.6)	0.633 [0.578-0.695]	0.919 (46.2)	0.188 [0.171-0.206]
1'-OH-MDZ	AUC(0-t_{last}) (µg·h/L) [a]	65	4.05 (38.4)	2.87 (39.4)	0.709 [0.668-0.752]	0.549 (63.1)	0.136 [0.123-0.149]
	C_{max} (µg/L)	65	1.64 (45.5)	1.29 (46.8)	0.784 [0.715-0.859]	0.259 (52.5)	0.157 [0.141-0.175]
	MDZ metabolite ratio for AUC(0-t_{last})	*65*	*0.317 (33.5)*	*0.410 (41.7)*	--	*0.310 (79.2)*	--
4ß-OH	C (µg/L)	65	35.8 (42.3)	39.8 (34.5)	1.14 [1.10-1.19]	108 (24.7)	3.15 [3.03-3.29]
LNG	AUC (µg·h/L)	13	7.95 (36.1)	6.61 (41.4)	0.832 [0.771-0.897]	3.39 (33.1)	0.427 [0.395-0.460]
	C_{max} (µg/L)	13	0.763 (23.9)	0.763 (36.6)	1.00 [0.895-1.12]	0.842 (32.9)	1.10 [0.989-1.23]
$LNG_{unbound}$	AUC (µg·h/L)	13	0.104 (41.8)	0.0887 (39.0)	0.853 [0.744-0.978]	0.0304 (34.7)	0.292 [0.255-0.335]
	C_{max} (µg/L)	13	0.00998 (30.0)	0.0102 (31.0)	1.024 [0.866-1.21]	0.00755 (28.6)	0.757 [0.640-0.894]
NET	AUC (µg·h/L)	14	15.8 (38.1)	13.8 (38.3)	0.875 [0.794-0.965]	8.52 (30.0)	0.539 [0.489-0.595]
	C_{max} (µg/L)	14	3.28 (36.9)	2.94 (33.9)	0.898 [0.805-1.00]	2.66 (30.9)	0.812 [0.728-0.906]
$NET_{unbound}$	AUC (µg·h/L)	14	0.645 (31.6)	0.549 (33.0)	0.850 [0.775-0.932]	0.277 (26.0)	0.430 [0.392-0.471]
	C_{max} (µg/L)	14	0.134 (31.8)	0.117 (32.8)	0.874 [0.794-0.961]	0.0872 (30.0)	0.649 [0.590-0.714]
ENG (DSG)	AUC (µg·h/L)	12	6.68 (35.1)	4.17 (27.5)	0.625 [0.553-0.708]	0.856 (39.3)	0.128 [0.113-0.145]
	C_{max} (µg/L)	12	0.692 (21.0)	0.603 (18.6)	0.871 [0.723-1.05]	0.294 (35.5)	0.425 [0.353-0.512]
$ENG_{unbound}$	AUC (µg·h/L)	12	0.126 (34.1)	0.0793 (31.3)	0.627 [0.547–0.719]	0.0132 (35.4)	0.105 [0.091-0.120]
	C_{max} (µg/L)	12	0.0131 (24.0)	0.0115 (23.3)	0.875 [0.703–1.09]	0.00462 (40.0)	0.353 [0.283-0.439]
DNG	AUC (µg·h/L)	12	560 (25.4)	404 (20.6)	0.722 [0.648-0.804]	72.5 (15.9)	0.130 [0.116-0.144]
	C_{max} (µg/L)	12	44.3 (11.9)	38.9 (20.2)	0.879 [0.792-0.975]	21.8 (19.0)	0.492 [0.444-0.546]
DRSP	AUC (µg·h/L)	14	525 (24.1)	368 (23.3)	0.701 [0.657-0.749]	73.0 (19.0)	0.139 [0.130-0.149]
	C_{max} (µg/L)	14	27.2 (21.4)	25.3 (25.4)	0.931 [0.827-1.05]	16.5 (18.1)	0.605 [0.537-0.680]
EE	AUC (ng·h/L)	14	704 (42.2)	576 (39.0)	0.817 [0.730-0.915]	252 (49.8)	0.358 [0.320-0.400]
	C_{max} (ng/L)	14	57.7 (28.2)	57.5 (37.3)	0.997 [0.895-1.11]	45.1 (39.6)	0.782 [0.702-0.871]

%CV, coefficient of variation [%];1'-OH-MDZ, 1'-hydroxymidazolam; 4ß-OH, 4ß-hydroxycholesterol; AUC, area under the concentration-time curve extrapolated to infinity; AUC(0-t_{last}), AUC from time zero to the last quantifiable concentration; C_{max}, observed maximum concentration; CI, confidence interval; DNG, dienogest; DRSP, drospirenone; DSG, desogestrel; EE, ethinylestradiol; ENG, etonogestrel (active metabolite of DSG); gMean, geometric mean; GMR, geometric mean ratio; LNG, levonorgestrel; MDZ, midazolam; NET, norethindrone.
Weak-induction phase: Administration of the randomly assigned test drug plus midazolam was preceded and followed by once daily administration rifampicin 10 mg for 7 and 4 days, respectively. Strong-induction phase: Administration of the randomly assigned test drug plus midazolam was preceded and followed by once daily administration rifampicin 600 mg for 7 and 4 days, respectively.

[a] The AUC for 1'-OH-MDZ could not be calculated reliably because 1'-OH-MDZ concentrations approached the lower limit of quantitation quiet early in some subjects in period 3. Furthermore, in periods 2 and 3, the concentrations increased again 12 hours after MDZ dosing in some subjects – a phenomenon which has also been observed in a prior study with a similar study design. Therefore, AUC(0-t_{last}) was used as describe the exposure and the metabolite ratio.

Table S 4: Metabolic pathways and fraction metabolized via CYP3A4 ($f_{m,CYP3A4}$) of commonly used progestins and ethinylestradiol

Compound	Phase I Metabolism	*In vivo* DDI study with CYP3A inhibitor	$f_{m,CYP3A4}$	Phase II Metabolism
Desogestrel (pro-drug)	Oxidation (CYP2C9, CYP2C19)[a]	-	-	-
Etonogestrel	Oxidation (**CYP3A4**)	Itraconazole	0.44[37]	-
Dienogest	Oxidation (**CYP3A4**)	Ketoconazole	0.67[25]	Glucuronidation & sulfation of phase I metabolites
Drospirenone	Oxidation (**CYP3A4**), reduction, ester hydrolysis	Ketoconazole	0.63[38]	Sulfation of phase I metabolites
Gestodene	Oxidation (**CYP3A4**)	n.a.	n.a.	Glucuronidation of phase I metabolites
Levonorgestrel	Oxidation (**CYP3A4**), reduction	Telithromycin	0.37[2]	Glucuronidation & sulfation of phase I metabolites
Norethindrone	Oxidation (**CYP3A4** [major], CYP2C19 [minor]), reduction	Voriconazole	0.34[2]	Glucuronidation & sulfation of phase I metabolites
Norgestimate (pro-drug)	Ester hydrolysis (deacetylation)	-	-	-
Norelgestromin	Oxidation (**CYP3A4** [major], CYP2B6 & CYP2C9 [minor])	n.a.	0.57[39] / 0.56[40,b]	Glucuronidation (UGT1A1) of phase I metabolites
Norgestrel	Oxidation (**CYP3A4**)	n.a.	0.6[39,b]	Glucuronidation (UGT1A1)
Ethinylestradiol (EE)	Oxidation (**CYP3A4** [major], CYP2C9 [minor])	Ketoconazole	0.20[2] - 0.29[38]	Sulfatation (SULT1E1) [major], Glucuronidation (UGT1A1) [minor]

n.a., not available

[a] not confirmed by Korhonen et al[37]

[b] estimated from *in vitro* studies

The descriptions of the metabolic pathways are based on a paper by Zhang et al[2] and a presentation by Sun and Jargula[41]. $f_{m,CYP3A4}$ data were derived from publications of inhibition studies according to $f_{m,CYP3A4} = CR_{CYP3A4} = 1/IR_{CYP3A4} - 1/AUC_{inhibitor}/AUC_{control} * IR_{CYP3A4}$ (CR = Clearance Ratio, IR = Inhibition Ratio).[42,43]

Methods S 1: Bioanalytical methods

Midazolam (MDZ) and **1'-hydroxymidazolam (1'-OH-MDZ)** were determined in human EDTA K_2 plasma after addition of the internal standards MDZ-d_4 and 1'-OH-MDZ-d_4 and automated liquid-liquid extraction with methyl tert-butyl ether. Separation was achieved by means of a liquid chromatographic system. For the mass spectrometric detection, a triple quadrupole mass spectrometer in positive TurboIonSpray™ ionization mode was applied.

Levonorgestrel (LNG) was determined in human EDTA K_2 plasma after addition of the internal standard LNG-d_6 and automated liquid-liquid extraction with a mixture of methyl tert-butyl ether and hexanes. Separation was achieved by means of a liquid chromatographic system. For the mass spectrometric detection, a triple quadrupole mass spectrometer in positive TurboIonSpray™ ionization mode was applied.

Norethindrone (NET) was determined in human EDTA K_2 plasma after addition of the internal standards NET-d_6 and liquid-liquid extraction with l-chlorobutane followed by derivatization. Separation was achieved by means of a liquid chromatographic system. For the mass spectrometric detection, a triple quadrupole mass spectrometer in positive atmospheric pressure chemical ionization mode (Heated Nebulizer) multiple reaction monitoring mode was applied.

Etonogestrel (ENG), the active metabolite of desogestrel (DSG), was determined in human EDTA K_2 plasma after addition of the internal standard LNG-d_6 and liquid-liquid extraction procedure with 1-chlorobutane followed by derivatization. Separation was achieved by means of a liquid chromatographic system. For the mass spectrometric detection, a triple quadrupole mass spectrometer in positive TurboIonSpray™ ionization mode was applied.

Dienogest (DNG) was determined in human EDTA K_2 plasma after addition of the internal standard DNG-$d_4C_{13}N_{15}$ and automated liquid-liquid extraction with methyl tert-butyl ether. Separation was achieved by means of a liquid chromatographic system. For the mass spectrometric detection, a triple quadrupole mass spectrometer in positive TurboIonSpray™ ionization mode was applied. A second calibration range was introduced because many concentrations were above the upper limit of quantitation (ULOQ) applying the initial calibration range from 2 to 16 µg/L. All samples that were above the ULOQ were reanalyzed with the higher range in subsequent sequences.

Drospirenone (DRSP) and **ethinylestradiol (EE)** were determined in human EDTA K_2 plasma after addition of the internal standard DRSP-d_4 and EE-d_4 and liquid-liquid extraction with l-chlorobutane followed by derivatization. Separation was achieved by means of a liquid chromatographic system. For the mass spectrometric detection, a triple quadrupole mass spectrometer in positive APCI ionization mode (Heated Nebulizer) MRM-mode was applied.

Rifampicin (RIF) was determined in human EDTA K_2 plasma after addition of the internal standard RIF-d_3 and automated protein precipitation with methanol and after addition of the internal standard RIF-d_8 and liquid-liquid extraction with ethyl acetate. Separation was achieved by means of a liquid chromatographic system. For the mass spectrometric detection, a triple quadrupole mass spectrometer in positive TurboIonSpray™ ionization mode was applied. Two calibration ranges were applied to cover the concentration range following low and high RIF doses.

4ß-hydroxycholesterol (4ß-HC) was determined in human EDTA K_2 plasma after addition of the internal standard 4ß-HC-d_7, applying liquid-liquid (sodium methoxide, water, hexane) and solid phase (Isolute SPE cartridges) extraction. Separation was achieved by means of a liquid chromatographic system. For the mass spectrometric detection, an API 5500 mass spectrometer in positive APCI mode was applied. Analysis utilized four calibrators within the validated detection range of 4.00 to 100 µg/L [44].

Sex hormone binding globulin (SHBG) was determined serum samples by a target dependent dissociation-enhanced lanthanide fluorescence immunoassay (DELFIA). The method is a solid phase, two-site fluoroimmunometric method based on the direct sandwich technique in which two monoclonal anti-SHBG antibodies are used. The SHBG in standards, quality controls, and samples is bound to streptavidin plates coated with biotinylated capture antibody followed by incubation with a europium-labeled monoclonal antibody. After addition of europium fluorescence intensifier, the time-resolved fluorescence of europium is measured.

All plasma and serum samples were stored at -20 °C until analysis. Stability tests confirmed the stability of the analytes at this temperature.

The possible interference of MDZ, 1'-OH-MDZ and RIF with the LNG, NET, ENG, DNG, DRSP or EE assay was evaluated. No significant interference was observed in all blank samples and zero standards fortified with MDZ, 1'-OH-MDZ and RIF. Furthermore, there was no effect on the quantitation of the progestins or EE.

Performance data for the above assays are summarized in the following table:

Accuracy calculated as percent of nominal and precision (CV%) of the bioanalytical methods

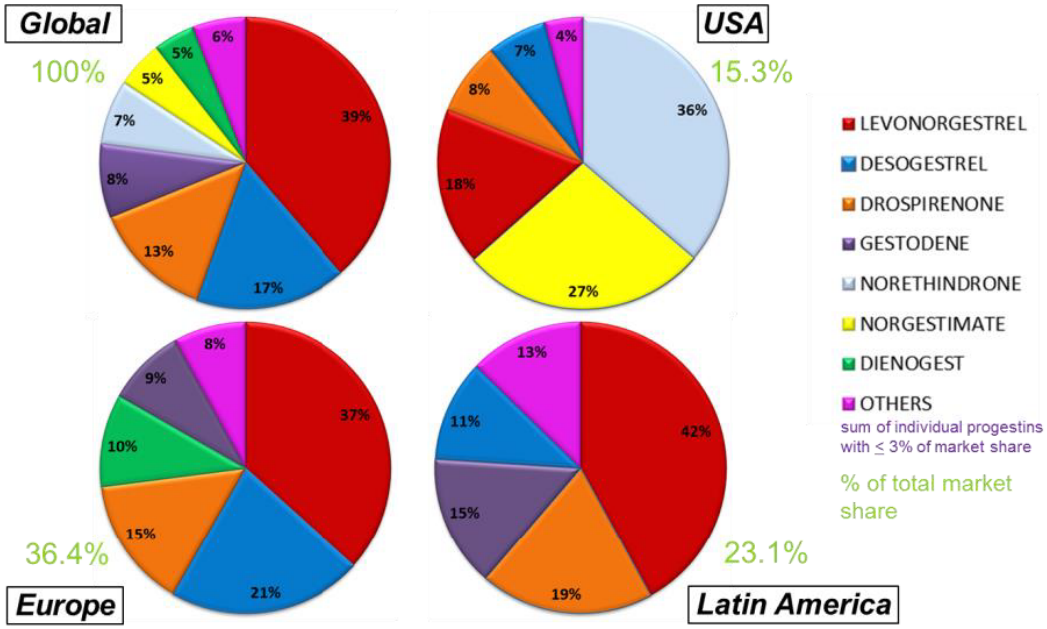
	Calibration standards					Quality control samples		
	Calibration range [µg/L]	Accuracy [a, b] [%]	Precision [b] [%]	Accuracy at LLOQ [a] [%]	Precision at LLOQ [%]	Concentration range [µg/L]	Accuracy [a] [%]	Precision [%]
MDZ	0.002–2	99.5–101	≤4.59	100	6.45	0.006–1.5	98.8–100	3.35–6.46
1'-OH-MDZ	0.005–5	99.4–101	≤5.79	100	6.00	0.015–3.75	100–101	3.80–6.78
LNG	0.01–10	97.4-102	≤2.69	98.9	4.48	0.03–7.5	96.9–99.3	2.72–3.86
NET	0.05–10	96.6–105	≤6.23	97.8	1.97	0.15–7.5	90.1–100	3.55–7.52
ENG	0.01–2	98.6–101	≤4.03	100	6.37	0.03–1.5	98.0–103	3.30–7.48
DNG low	0.2–16	93.3–112	≤5.34	100	2.50	0.6–12	88.3–93.2	0.70–3.20
DNG high	0.2–100	96.2–105	≤2.09	99.0	3.22	0.6–75	96.7–99.8	0.93–1.28
DRSP	0.1–40	95.8–104	≤6.34	98.7	3.37	0.3–30	93.3–98.7	3.36–4.14
EE	0.001–0.4	97.0–104	≤6.18	99.0	4.56	0.003–0.3	94.3–101	3.76–4.40
RIF low	0.1–100	97.0–102	≤6.91	101	7.52	0.3–75	96.3–97.2	3.60–6.26
RIF high	5–5000	99.0–102	≤6.06	100	7.58	15–3800	90.5–92.4	4.37–5.78
4ß-HC	4–100	97.8-101	≤4.4	100	1.3	10-80	98-104	3.30-5.70
SHBG	10–512 nmol/L	98.8–101	≤3.39	99.4	1.53	20–384 nmol/L	98.3–109	3.29–5.43

[a] mean inter-assay accuracy of back-calculated concentration; [b] except LLOQ

%CV, coefficient of variation [%]; 1'-OH-MDZ, 1'-hydroxymidazolam; 4ß-HC; 4ß-hydroxycholesterol; DNG, dienogest; DRSP, drospirenone; EE, ethinylestradiol; ENG, etonogestrel; LLOQ, lower limit of quantitation; LNG, levonorgestrel; MDZ, midazolam; NET, norethindrone; RIF, rifampicin; SHBG, sex hormone binding globulin.

Figure S 1: Market shares of different progestins and number of CYP3A induction studies with progestins

(A) Market shares of different progestins based on the number of oral contraceptive units sold in 2016

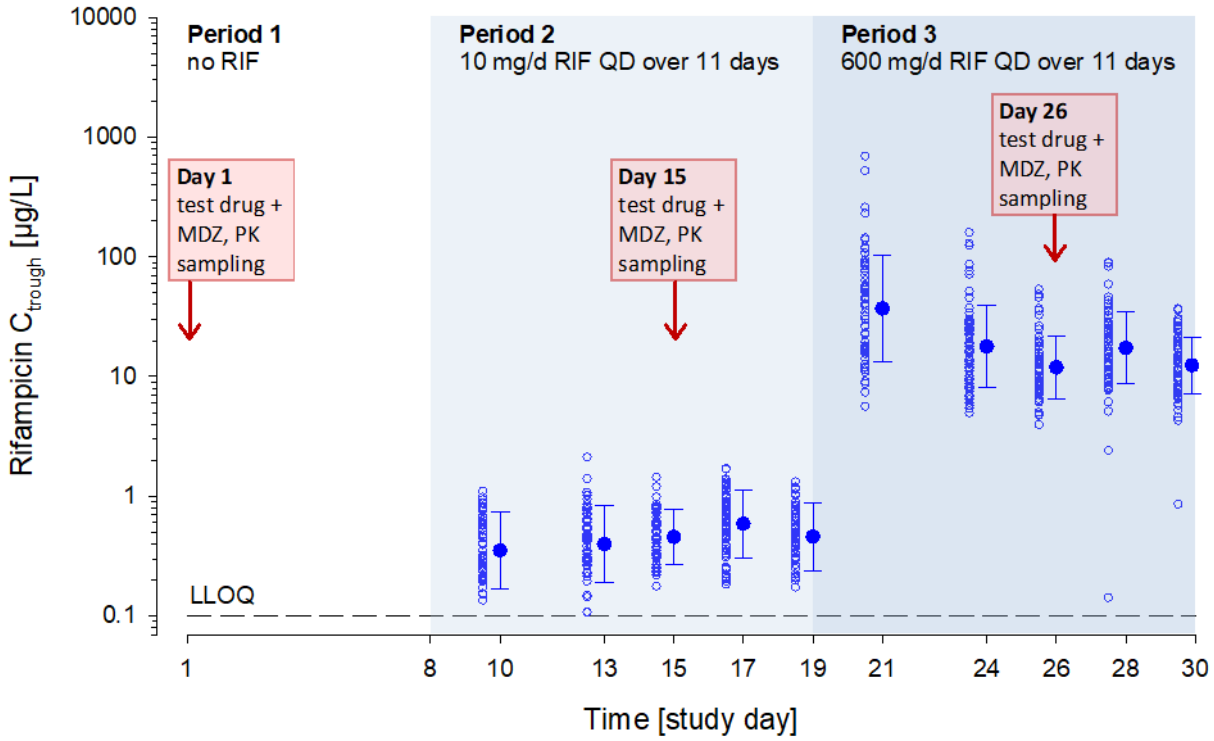


(B) Clinical induction studies with progestins

Victim drug (progestin)	Number of published studies
Norethindrone	27
Levonorgestrel	25
Desogestrel + Etonogestrel	5
Norgestimate / Norelgestromin / Norgestrel	3 / 1 / 1
Dienogest	1
Gestodene	1
Medroxyprogesterone	1

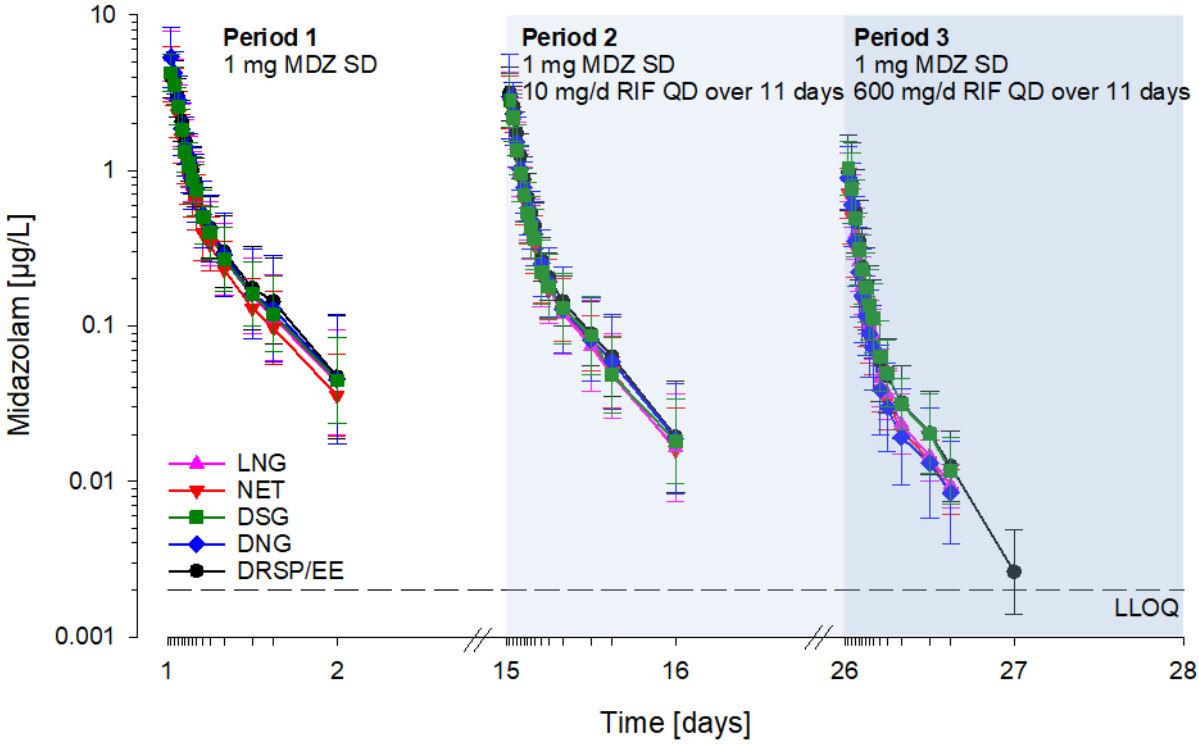
Sources of market shares data: IQVIA MIDAS Q4/2016, as base for cycle calculation in database Womenshealth; Farminform (Netherlands) Q4 2016; GERS (France) Q4/2016.
Source of CYP3A induction study data: UW Drug Interaction Database (DIDB) Copyright University of Washington, accessed Dec 2019.

Figure S 2: Rifampicin trough concentrations in plasma during repeated oral administration of rifampicin



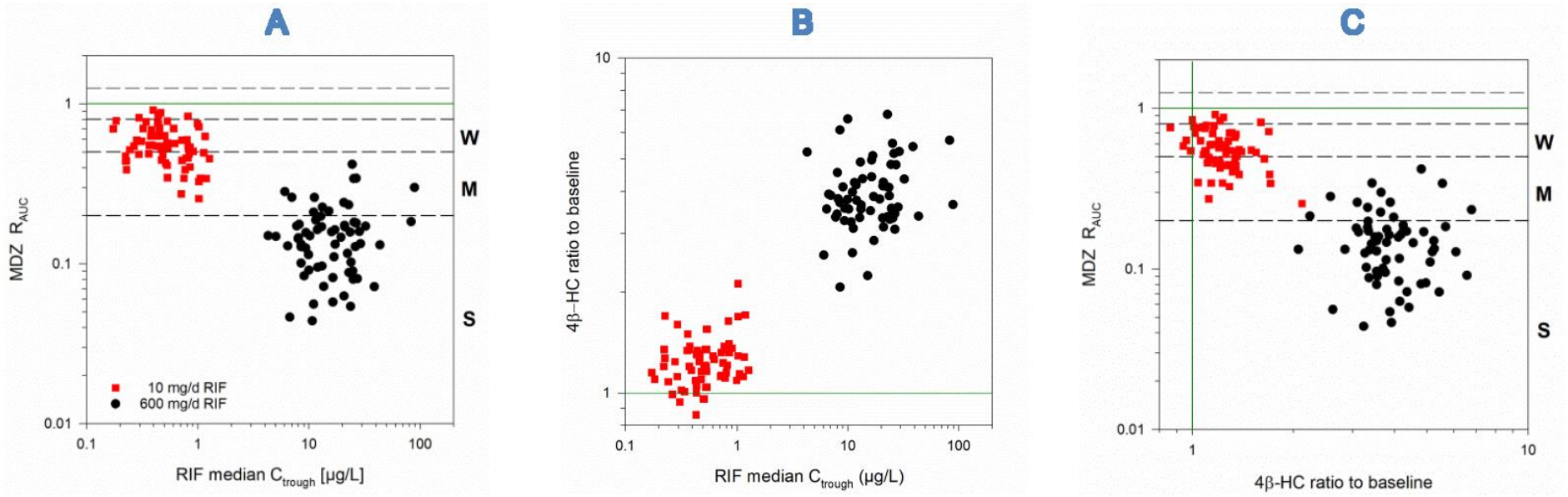
Geometric means with geometric standard deviations; N=65.
C_{trough}, concentration of the drug before dosing; LLOQ, lower limit of quantitation; MDZ, midazolam; PK, pharmacokinetic; QD, once daily; RIF, rifampicin.
Blood samples for the determination of RIF were generally taken in the morning prior to intake of RIF. On Days 15 and 26, RIF was given 12 hours after the test drug; ie, on these days the blood samples for the determination of RIF were taken 36 h after the last dose of RIF. RIF C_{trough} increased more than dose proportional, likely due to extensive and saturable first-pass metabolism [45]). The decrease in RIF concentrations observed in period 3, ie, after the first doses of RIF 600 mg, probably reflects the known ability of RIF at high doses to induce its own metabolism [46,47]. Trough concentrations of RIF were comparable between treatment groups after 10 mg/d and 600 mg/d RIF administration. Two low RIF trough concentrations were observed in period 3 on Study Days 28 and 30. Both samples belong to one subject in the DNG group. Since both MDZ and DNG were eliminated within 24 h when high-dose RIF was coadministered (In-Text-Figure 2), this potential non-compliance of tablet intake had no impact on the PK assessment and, therefore, the subject was not excluded from PK analyses.

Figure S 3: Midazolam plasma concentration-time curves obtained after single administration of midazolam 1 mg without and with coadministration of rifampicin 10 mg or 600 mg



Geometric means with geometric standard deviations.
DNG, dienogest group; DRSP/EE, drospirenone/ethinylestradiol group; DSG, desogestrel group; LNG, levonorgestrel; MDZ, midazolam; NET, norethindrone group; SD, single dose.

Figure S 4: Scatterplots of individual data



A: Trough concentration of RIF in plasma (median of days 13, 17, and 19 and days 24, 28 and 30) vs AUC ratio (with RIF/no RIF) for MDZ. B: Trough concentration of RIF in plasma (median of days 13, 17, and 19 and days 24, 28 and 30) vs concentration ratio for 4ß-HC (day 19 or 30/day 8). C: concentration ratio for 4ß-HC (day 19 or 30/day 8) vs AUC ratio (with RIF/no RIF) for MDZ.

4ß- HC, 4ß-hydroxycholesterol; AUC, area under the concentration-time curve extrapolated to infinity; C_{trough}, concentration of the drug before dosing; MDZ, midazolam; RIF, rifampicin; R_{AUC}, AUC ratio with RIF/no RIF; RIF, rifampicin.
The magnitude of the induction effect is classified into 3 categories: weak induction (W, R_{AUC} >0.5 and ≤0.8), moderate induction (M, R_{AUC} >0.2 and ≤0.5) and strong induction (S, R_{AUC} ≤0.2).