

Supplementary Figures

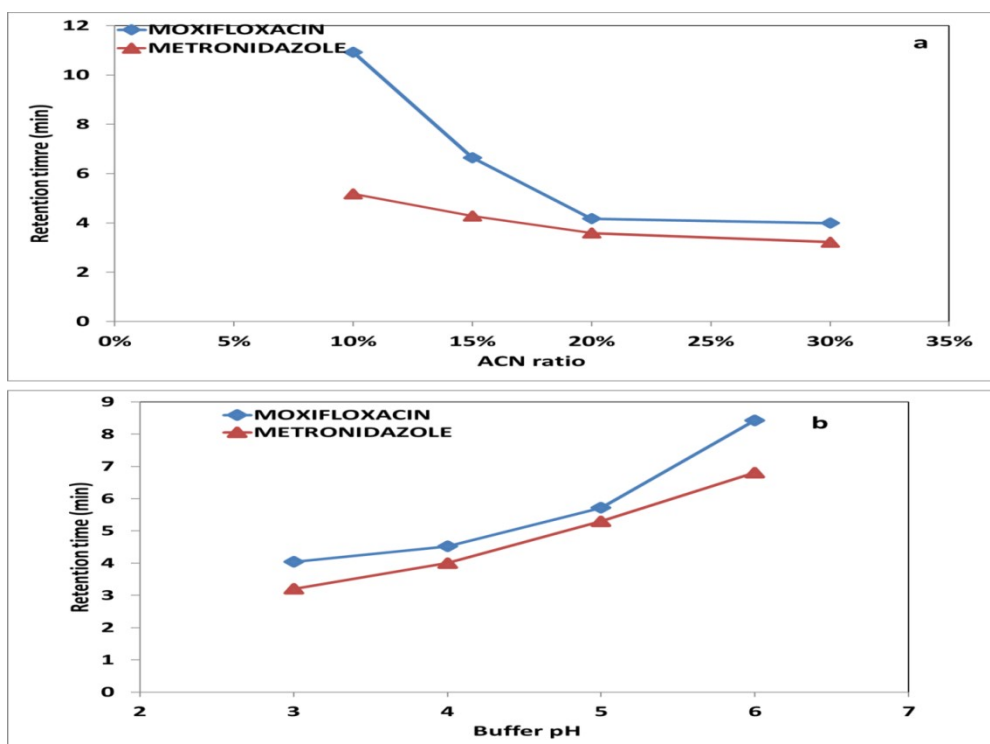


Figure S 1 (Supplementary information): Studying the effect of %ACN (a) and buffer pH (b) on the separation of MX and MT using the proposed RP-HPLC method.

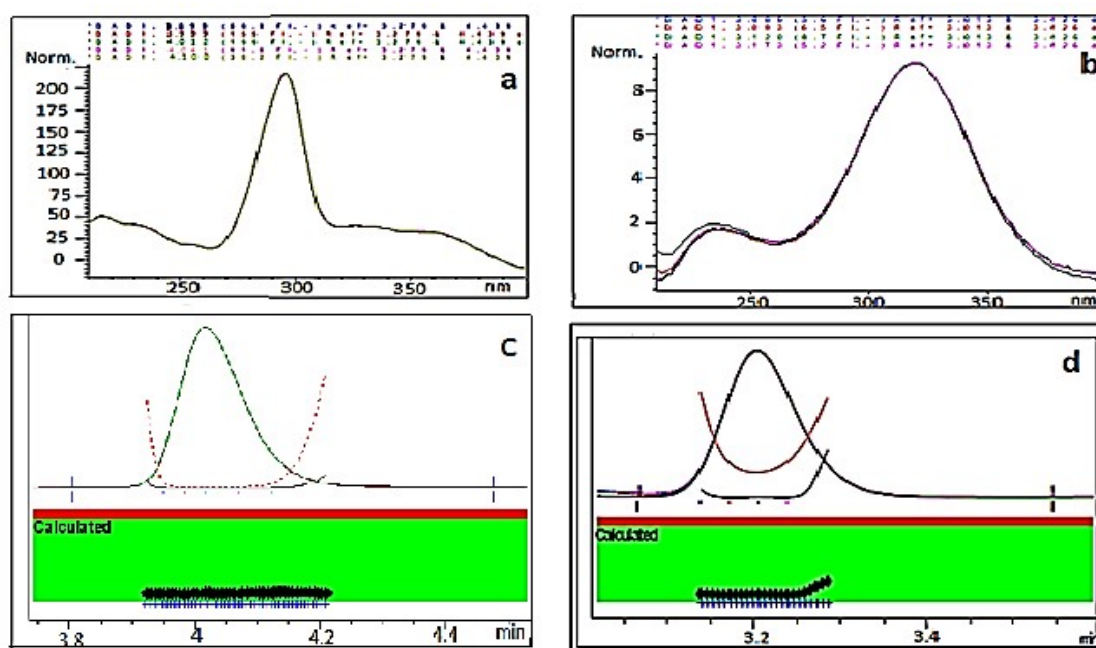


Figure S2 (Supplementary information): Absorption spectra of MX (a) and MT (b) and its peak purity plots (c) and (d) respectively.

Supplementary Tables

Table S1 (Supplementary information): System suitability parameters for simultaneous determination of MX and MT using the proposed RP-HPLC method.

Parameter	MX (DAD)	MX (FLD)	MT
$t_R \pm SD$ (min) ^a		3.99 ± 0.03	3.22 ± 0.02
Capacity factor (k')		2.99	2.22
Selectivity (α)		1.41	1.14
Resolution (R_s)		5.43	3.19
Theoretical plates (N)	4799	3612	10264
Tailing factor (T)	1.92	1.79	1.40

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- ^a Average of five determinations.
 - System suitability recommendations: $k' > 2$, $R_s > 2$, $N > 2000$, and $T \leq 2$.

Table S2 (Supplementary information): Regression and statistical parameters for simultaneous determination of MX and MT in biological fluids using the proposed RP-HPLC method.

Parameter	Saliva			Human plasma			Rat plasma		
	MX (DAD)	MX (FLD)	MT	MX (DAD)	MX (FLD)	MT	MX (DAD)	MX (FLD)	MT
Linearity range	1-20	0.2-20	0.5-20	1-20	0.2-20	0.5-10	2-20	2-10	1-20
LLOQ ($\mu\text{g/mL}$)	1.0	0.2	0.5	1.0	0.2	0.5	2.0	2.0	1.0
Intercept (a)	7.36×10^{-2}	1.90×10^{-2}	-3.16×10^{-2}	9.72×10^{-3}	3.05×10^{-3}	6.13×10^{-2}	6.45×10^{-2}	1.03×10^{-4}	-5.40×10^{-1}
Slope (b)	1.80×10^{-1}	4.23×10^{-2}	2.08	5.28×10^{-2}	1.15×10^{-2}	3.90×10^{-1}	3.74×10^{-2}	3.38×10^{-3}	6.77×10^{-1}
Correlation	0.9992	0.9992	0.9998	0.9991	0.9991	0.9995	0.9992	0.9994	0.9991
S_a	2.99×10^{-2}	4.68×10^{-3}	1.54×10^{-1}	9.62×10^{-3}	7.27×10^{-3}	3.16×10^{-4}	7.27×10^{-3}	3.16×10^{-4}	1.21×10^{-1}
S_b	2.90×10^{-3}	5.38×10^{-4}	1.64×10^{-2}	9.35×10^{-4}	5.95×10^{-4}	4.76×10^{-5}	5.95×10^{-4}	4.76×10^{-5}	1.19×10^{-2}
S_b^2	8.42×10^{-6}	2.89×10^{-7}	2.68×10^{-4}	8.74×10^{-7}	3.54×10^{-7}	2.26×10^{-9}	3.54×10^{-7}	2.26×10^{-9}	1.41×10^{-4}
$S_{y/x}$	4.51×10^{-2}	9.57×10^{-3}	2.76×10^{-1}	1.45×10^{-2}	8.92×10^{-3}	3.01×10^{-4}	8.92×10^{-3}	3.01×10^{-4}	1.87×10^{-1}
F	3832.97	6174.51	16132.39	3188.84	3956.34	5062.04	3956.34	5062.04	3260.52
Significance F	9.28×10^{-6}	6.32×10^{-9}	2.30×10^{-8}	1.22×10^{-5}	8.85×10^{-6}	6.12×10^{-6}	8.85×10^{-6}	6.12×10^{-6}	1.18×10^{-5}

Table S3 (Supplementary information): Intra-day and inter-day precision and accuracy for simultaneous determination of MX and MT in standard solutions using the proposed RP-HPLC method.

Conc. ($\mu\text{g/mL}$)	Average % recovery \pm SD			%RSD			%E _r		
	MX (DAD)	MX (FLD)	MT	MX (DAD)	MX (FLD)	MT	MX (DAD)	MX (FLD)	MT
Intra-day precision									
0.2:20	99.15 \pm 0.16	100.07 \pm 1.71	101.52 \pm 0.30	0.16	1.70	0.29	-0.85	0.07	1.52
40:0.2	99.48 \pm 1.40	101.21 \pm 0.97	100.26 \pm 0.67	1.41	0.96	0.67	-0.52	1.21	0.26
60:80	98.45 \pm 0.48	99.70 \pm 1.20	99.73 \pm 0.46	0.49	1.20	0.46	-1.55	-0.30	-0.27
100:40	99.61 \pm 0.19	99.72 \pm 0.06	99.94 \pm 0.43	0.19	0.06	0.43	-0.39	-0.28	-0.06
Inter-day precision									
0.2:20	100.76 \pm 1.32	100.96 \pm 0.55	101.18 \pm 0.69	1.31	0.54	0.68	0.76	0.96	1.18
40:0.2	100.79 \pm 0.80	100.82 \pm 1.29	100.70 \pm 1.55	0.80	1.28	1.54	0.79	0.82	0.70
60:80	98.83 \pm 0.26	100.05 \pm 0.34	100.71 \pm 0.42	0.27	0.34	0.41	-1.17	0.05	0.71
100:40	99.99 \pm 0.06	101.12 \pm 0.91	100.68 \pm 1.06	0.06	0.90	1.05	-0.01	1.12	0.68

Table S4 (Supplementary information): Robustness evaluation for the simultaneous determination of MX and MT using the proposed RP-HPLC method.

Parameter	Average %recovery \pm %RSD		
	MX (DAD)	MX (FLD)	MT
Buffer pH (3 \pm 0.1)	100.57 \pm 0.35	98.33 \pm 0.65	99.35 \pm 0.69
Buffer concentration (5mM \pm 0.1)	101.13 \pm 1.22	100.97 \pm 0.98	100.77 \pm 1.05
Detection wavelength \pm 2nm	99.21 \pm 0.47	99.61 \pm 0.66	101.11 \pm 0.42
Flow rate (1 mL/min \pm 0.1)	100.12 \pm 1.67	101.28 \pm 1.11	98.47 \pm 0.85

Table S5 (Supplementary information): Stability studies for MX and MT spiked in human saliva using the proposed RP-HPLC method.

Stability	Storage conditions	Conc.	Average %recovery \pm % RSD ^a		
			MX (DAD)	MX (FLD)	MT
Freeze and thaw	After 3 cycles at -20°C for 1h	LQC ^b	110.05 \pm 2.59	97.65 \pm 10.25	95.37 \pm 7.23
		HQC ^c	107.34 \pm 3.17	89.12 \pm 3.78	110.01 \pm 5.06
Short term	At 25°C for 8 h	LQC ^b	112.11 \pm 1.97	113.23 \pm 8.66	87.90 \pm 5.99
		HQC ^c	90.15 \pm 4.31	96.98 \pm 2.47	114.67 \pm 3.31
Post-preparative	at 4°C for 24 h	LQC ^b	88.75 \pm 3.70	109.78 \pm 11.31	108.67 \pm 5.40
		HQC ^c	94.09 \pm 7.99	96.77 \pm 12.53	106.55 \pm 3.25
Long term	at-20°C for 10 days	LQC ^b	113.28 \pm 4.77	94.47 \pm 7.25	114.67 \pm 10.16
		HQC ^c	87.66 \pm 10.23	104.29 \pm 5.13	92.17 \pm 8.01

^a Percentage of recoveries average \pm relative standard determination of three replicates (n=3).

^b LQC is 2 μ g/mL for MX and MT.

^c HQC is 20 μ g/mL for MX and MT.

Table S6 (Supplementary information): Stability studies for MX and MT in spiked human and rat plasma using the proposed RP-HPLC method.

Stability	Storage conditions	Conc.	Average %recovery ± %RSD ^a					
			Human plasma			Rat plasma		
			MX (DAD)	MX (FLD)	MT (DAD)	MX (DAD)	MX (FLD)	MT
Freeze and thaw	3 cycles at -20°C for 1h	LQC	110.05±2.59	97.65±10.25	95.37± 7.23	105.12± 6.78	103.22± 8.87	92.12± 6.65
		HQC	107.34±3.17	89.12± 3.78	110.01± 5.06	91.17± 5.47	94.37± 5.09	89.12± 5.06
Short term	At 25°C for 8 h	LQC	112.11±1.97	113.23± 8.66	87.90± 5.99	111.78± 3.45	114.38± 6.13	104.12± 2.16
		HQC	90.15± 4.31	96.98± 2.47	114.67± 3.31	87.91± 6.12	109.12± 9.18	93.67 ± 4.22
Post-preparative	at 4°C for 24 h	LQC	88.75±3.70	109.78± 9.31	108.67± 5.40	107.45± 4.47	87.99± 11.30	104.67± 9.45
		HQC	94.09±7.99	96.77±12.53	106.55± 3.25	114.33± 8.39	90.90±10.53	93.77± 12.40
Long term	at-20°C for 10 days	LQC	113.28±4.77	94.47± 7.25	114.67± 9.16	86.65± 9.49	91.67± 12.25	110.45 ± 7.05
		HQC	87.66±10.23	104.29±5.13	92.17± 8.01	113.10± 5.23	109.47±11.62	109.97± 8.04

^a Percentage of recoveries average ± relative standard determination of three replicates (n=3).

* In human plasma - LQC is 2 µg/mL for MX at DAD and FLD but LQC is 1 µg/mL for MT.

- HQC is 20 µg/mL for MX at DAD and FLD and 10 µg/mL for MT.

* In rat plasma - LQC is 4 µg/mL for MX at DAD and FLD but LQC is 2 µg/mL for MT.

-HQC is 20 µg/mL for MX and MT but it is 10 µg/mL for MX at FLD.