SUPPLEMENTARY MATERIAL

Use of a monolithic column for the development and validation of a HPLC method for the determination of famotidine, cimetidine and nizatidine in biological fluids

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Table S1. Extraction efficiency of H_2RAs from standard solutions and spiked samples.

H_2RA	Absolute recovery[a] (%)	Relative recovery[b] (%)	
		Urine	Serum
FAM	86.4	93.4	77.1
CIM	91.4	81.4	80.0
NIZ	96.6	90.8	76.7

[a] Peak area of extracted H_2RAs from a standard mixture (10 μg mL^{-1}) versus peak area of respective H_2RAs in a non-extracted standard mixture of identical concentration.
[b] Peak area of extracted H_2RAs from spiked sample (10 μg mL^{-1}) versus peak area of respective H_2RAs extracted from a standard mixture of identical concentration.

Table S2. The stability of FAM, CIM and NIZ in human serum under tested conditions[a] (n = 3).

	Mean ± SD in serum					
	FAM		CIM		NIZ	
	2 mg L^{-1}	20 mg L^{-1}	2 mg L^{-1}	20 mg L^{-1}	2 mg L^{-1}	20 mg L^{-1}
Short-term stability	96.8±0.50	102.1±0.35	97.5±0.76	99.8±0.81	95.8±0.63	98.3±0.77
Long-term stability	95.1±0.77	97.3±0.91	90.1±1.1	95.3±0.91	88.7±0.73	90.5±1.2
Post-preparative stability	97.7±0.57	93.4±0.64	89.8±0.82	97.5±0.41	89.4±0.91	103.4±0.75
Freeze- and thaw-stability	93.3±0.33	97.7±0.73	89.9±0.88	96.0±0.33	90.5±78	93.9±0.90

[a]Stability was expressed as mean percentage of the analyte concentration determined at certain time point relative to that at time zero (nominal concentration).

Table S3. The stability of FAM, CIM and NIZ in human urine under tested conditions[a] (n = 3).

	Mean ± SD in urine					
	FAM		CIM		NIZ	
	2 mg L^{-1}	20 mg L^{-1}	2 mg L^{-1}	20 mg L^{-1}	2 mg L^{-1}	20 mg L^{-1}
Short-term stability	100.9±0.78	102.1±1.3	95.5±0.88	101.1±0.31	92.0±2.3	99.8±1.2
Long-term stability	95.2±0.75	89.3± 2.1	88.7±3.2	100.4±0.77	92.0±0.77	101.0±1.1
Post-preparative stability	89.2±0.33	97.0±0.76	90.5±0.51	99.4± 0.88	89.9±0.67	100.3±0.91
Freeze- and thaw-stability	90.4± 0.88	99.7±0.75	96.0± 2.1	93.0±0.51	88.8± 1.6	100.5±0.52

[a]Stability was expressed as mean percentage of the analyte concentration determined at certain time point relative to that at time zero (nominal concentration).

Table S4. Effects of the analytical parameters in resolution and retention factor of the chromatographic method according to Youden's test.

H_2RAs	$D_{A/a}$	$D_{B/b}$	$D_{C/c}$	$D_{D/d}$	$D_{E/e}$	$D_{F/f}$	$D_{G/g}$
	Effect in resolution (R_s)						
FAM-CIM	1.33	0.54	-0.52	-0.13	-0.32	-0.35	-0.47
CIM-NIZ	0.46	0.30	-0.1	0.12	0.08	-0.08	-0.32
	Effect in retention time (t_R)						
FAM	0.19	0.83	0.01	0.04	-0.04	-0.75	0.15
CIM	1.41	2.07	-0.15	0.33	-0.08	-1.20	0.04
NIZ	2.56	3.68	-0.24	0.75	0.01	-1.59	-0.09