Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Development and validation of a sensitive liquid chromatography tandem mass spectrometry assay for the simultaneous determination of ten kinase inhibitors in human serum and plasma

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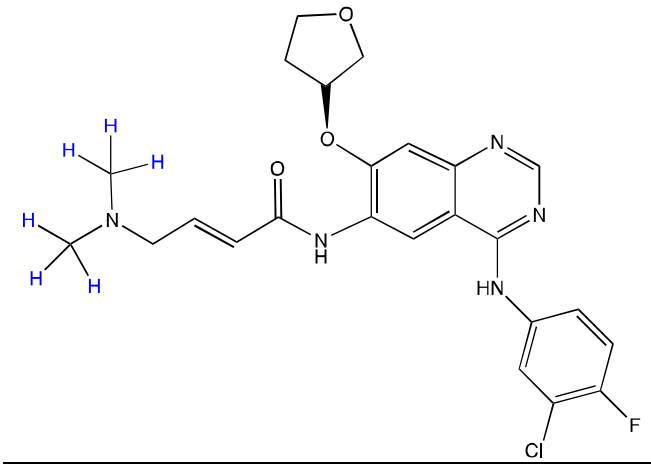
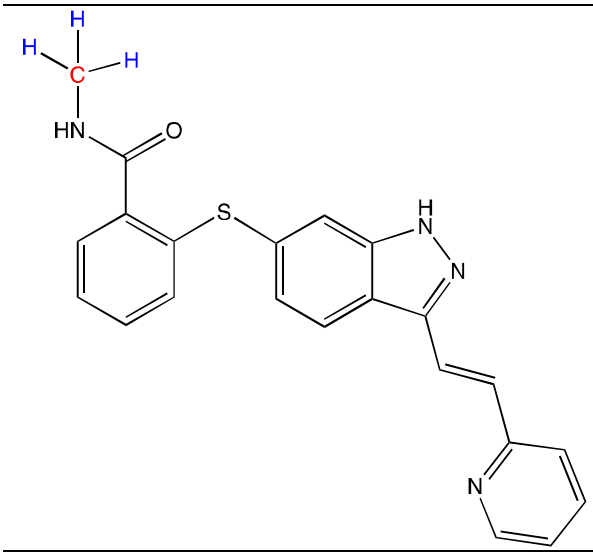
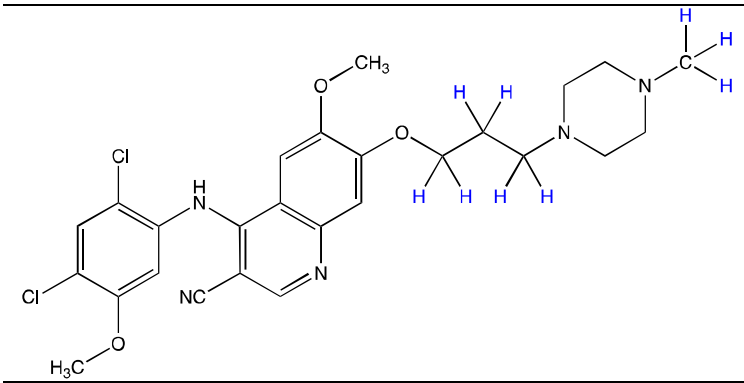
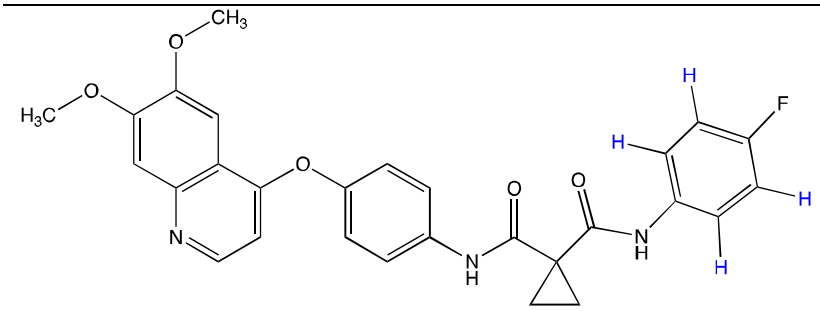
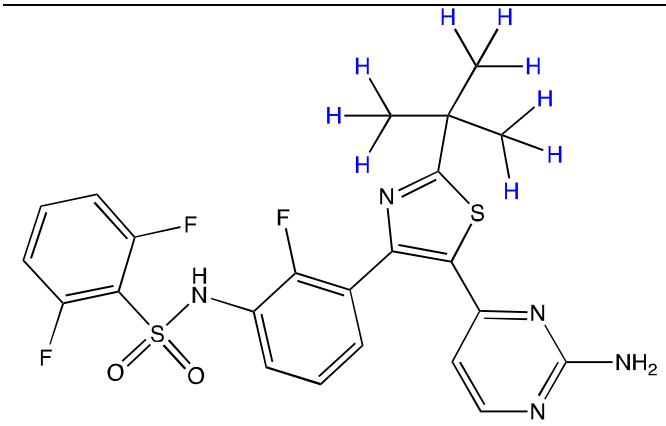
Table S1 Chemical structure of the analytes. Mass transitions of analyte and internal standard were achieved by optimizing the parameters for ionization, fragmentation, and MRM-detection. Colored atoms indicate where protons/carbon-atoms are substituted by $^{2}H/^{13}C$ in the stable isotope labeled internal standards

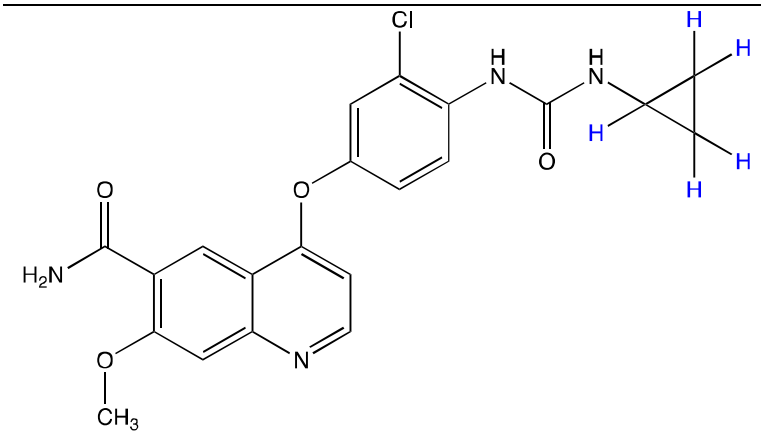
	Afatinib (AFA)
	
Mass transition analyte	486.0 → 371.0
Mass transition isotopically marked internal standard	492.1 → 371.0
Retention time (R_t) (min)	3.04
Declustering potential (volts)	100.0
Enterance potential (volts)	5.0
Collision energy (volts)	34.0
Collision cell exit potential (volts)	11.0

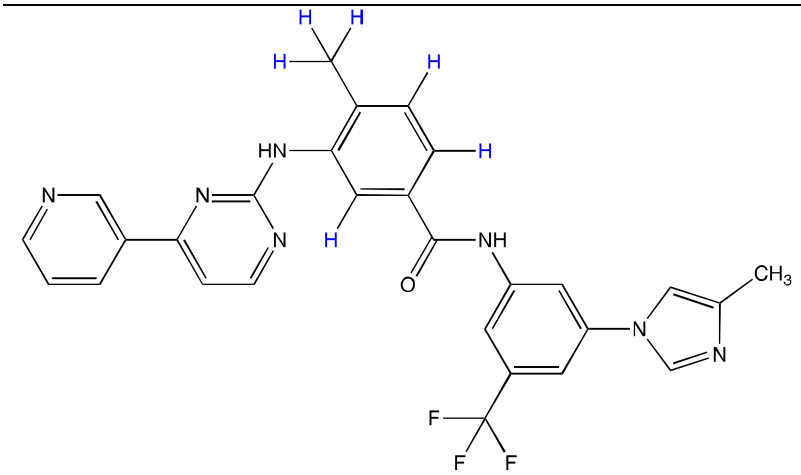
	Axitinib (AXI)
	
Mass transition analyte	387.1 → 356.0
Mass transition isotopically marked internal standard	391.3 → 356.1
Retention time (R_t) (min)	1.84
Declustering potential (volts)	80.0
Enterance potential (volts)	6.0
Collision energy (volts)	27.0
Collision cell exit potential(volts)	16.0

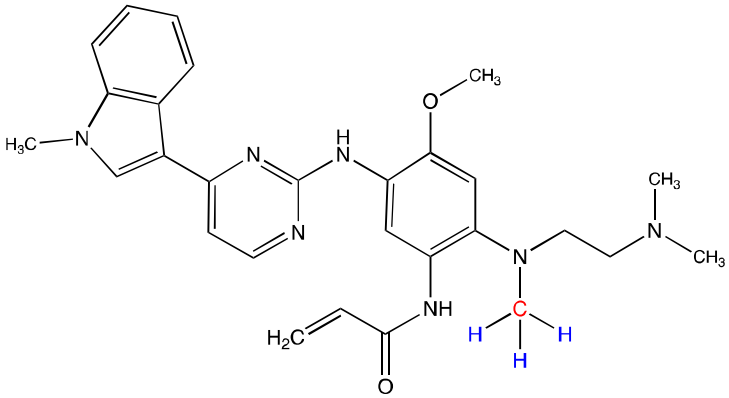
	**Bosutinib (BOS)**
Mass transition analyte	530.4 → 141.3
Mass transition isotopically marked internal standard	539.4 → 150.3
Retention time (R_t) (min)	2.82
Declustering potential (volts)	80.0
Enterance potential(volts)	10.0
Collision energy (volts)	22.0
Collision cell exit potential (volts)	14.0

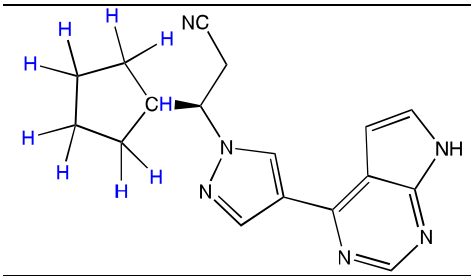
	**Cabozantinib (CAB)**
Mass transition analyte	502.2 → 323.1
Mass transition isotopically marked internal standard	506.4 → 391.4
Retention time (R_t) (min)	3.42
Declustering potential (volts)	50.0
Enterance potential (volts)	10.0
Collision energy (volts)	35.0
Collision cell exit potential (volts)	14.0

	**Dabrafenib (DAB)**
Mass transition analyte	520.1 → 307.1
Mass transition isotopically marked internal standard	529.0 → 316.1
Retention time (R_t) (min)	1.50
Declustering potential (volts)	80.0
Enterance potential (volts)	10.0
Collision energy (volts)	30.0
Collision cell exit potential (volts)	14.0

	**Lenvatinib (LEN)**
Mass transition analyte	428.1 → 371.0
Mass transition isotopically marked internal standard	433.1 → 371.0
Retention time (R_t) (min)	1.55
Declustering potential (volts)	50.0
Enterance potential (volts)	10.0
Collision energy (volts)	43.0
Collision cell exit potential (volts)	14.0

	Nilotinib (NIL)
	
Mass transition analyte	530.0 → 289.1
Mass transition isotopically marked internal standard	536.1 → 295.0
Retention time (R_t) (min)	3.30
Declustering potential (volts)	100.0
Enterance potential (volts)	10.0
Collision energy (volts)	30.0
Collision cell exit potential (volts)	14.0

	Osimertinib (OSI)
	
Mass transition analyte	500.2 → 72.1
Mass transition isotopically marked internal standard	504.2 → 72.0
Retention time (R_t) (min)	3.81
Declustering potential (volts)	50.0
Enterance potential (volts)	10.0
Collision energy (volts)	47.0
Collision cell exit potential (volts)	14.0

	Ruxolitinib (RUX)
	
Mass transition analyte	307.0 → 186.0
Mass transition isotopically marked internal standard	316.0 → 186.0
Retention time (R_t) (min)	1.24
Declustering potential (volts)	80.0
Enterance potential (volts)	10.0
Collision energy (volts)	30.0
Collision cell exit potential (volts)	14.0

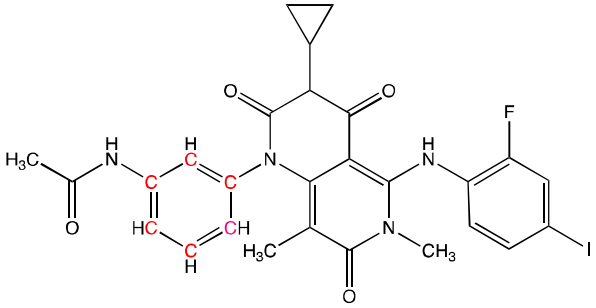
	Trametinib (TRA)
	
Mass transition analyte	616.0 → 490.9
Mass transition isotopically marked internal standard	622.0 → 496.9
Retention time (R_t) (min)	3.89
Declustering potential (volts)	125.0
Enterance potential (volts)	7.0
Collision energy (volts)	46.0
Collision cell exit potential (volts)	12.0

Table S2 Validation results for sensitivity, selectivity, carry-over and dilution integrity (with saline solution)

Analyte	*Sensitivity (n=9)*	*S/N-ratio*[b] *(n=9)*	*Selectivity (n=6) (analyte)*	*Selectivity (n=6) (IS)*	*Carry-over (n = 5) (analyte)*	*Carry-over (n = 5) (IS)*	*Dilution integrity (n = 6)*	
	Acceptance Factor[a]	-[b]	(%)	(%)	*(%)*	*(%)*	*Accuracy (%)*	*CV%*
AFA	0.25 ± 0.20 (0.05-0.84)	20.7 ± 5.7	1.75	0.19	6.83	0.05	102.0 ± 1.33	1.39
AXI	0.13 ± 0.07 (0.01-0.31)	77.0 ± 32.6	0.15	0.01	1.84	0.01	88.4 ± 4.02	3.70
BOS	0.35 ± 0.24 (0.09-0.91)	11.9 ± 4.4	3.52	0.05	3.36	0.02	95.0 ± 2.77	2.82
CAB	0.14 ± 0.10 (0.02-0.34)	51.6 ± 18.7	2.79	0.11	4.06	0.08	89.9 ± 2.21	2.20
DAB	0.06 ± 0.03 (0.02-0.14)	34.9 ± 12.6	0.65	0.08	0.48	0.01	94.1 ± 2.64	2.80
LEN	0.02 ± 0.02 (0.01-0.08)	46.5 ±14.0	0.23	0.03	0.84	0.02	100.0 ± 2.15	2.39
NIL	0.20 ± 0.18 (0.02-0.86)	47.5 ± 19.1	2.60	0.23	7.33	0.05	98.0 ± 3.35	3.53
OSI	0.03 ± 0.01 (0.01-0.06)	25.2 ± 10.3	1.35	0.26	12.3	0.16	109.0 ± 4.31	4.87
RUX	0.03 ± 0.02 (0.01-0.11)	79.4 ± 33.5	0.28	0.30	0.71	0.36	109.0 ± 2.36	2.32
TRA	0.04 ± 0.04 (0.02-0.16)	47.0 ± 18.9	0.31	0.004	0.94	0.01	95.4 ± 1.91	1.75

[a] mean ± SD (range)

[b] S/N-ratios calculated for the lowest calibration level expressed as mean ± SD

Table S3 Accuracy and precision of quality control (QC) samples prepared in hemolytic, icteric and lipemic serum, quantified against a calibration curve prepared in plasma (n = 2)

Analyte	**Sample**	**Accuracy (%)**	**CV (%)**	**Accuracy (%)**	**CV (%)**	**Accuracy (%)**	**CV (%)**
		Hemolytic		Icteric		Lipemic	
AFA	QC high	96.1 ± 4.90	5.10	102.3 ± 7.36	7.19	101.2 ± 0.95	0.94
	QC low	99.4 ± 0.84	0.84	92.8 ± 3.35	3.61	92.3 ± 6.78	7.35
AXI	QC high	88.1 ± 0.44	0.50	96.3 ± 0.59	0.61	93.7 ± 0.15	0.16
	QC low	96.5 ± 1.71	1.77	96.6 ± 1.00	1.04	92.1± 8.12	8.81
BOS	QC high	95.9 ± 4.17	4.35	99.6 ± 1.34	1.35	99.8 ± 0.45	0.45
	QC low	89.4± 6.5	7.28	90.5 ± 5.08	5.61	88.6 ± 7.62	8.60
CAB	QC high	90.2 ± 2.42	2.67	96 ± 2.91	3.03	92.6 ± 2.90	3.14
	QC low	96.6 ± 0.99	1.03	91.4 ± 0.99	1.09	92.1 ± 2.65	2.87
DAB	QC high	100.2 ± 1.49	1.49	103 ± 0.49	0.48	104 ± 2.98	2.87
	QC low	105.2 ± 6.44	6.12	100.2 ± 2.03	2.03	102.1± 2.71	2.66
LEN	QC high	103.9 ± 8.89	8.56	110 ± 3.95	3.59	110.5 ± 1.27	1.15
	QC low	104.8 ± 5.77	5.51	96 ± 5.87	6.11	94.6 ± 0.96	1.02
NIL	QC high	95.6 ± 0.99	1.03	103 ± 2.47	2.40	102.2 ± 0.49	0.48
	QC low	96.3 ± 1.35	1.41	94.8 ± 2.71	2.86	94.8 ± 2.03	2.14
OSI	QC high	99.2 ± 0.00	0.00	97.5 ± 1.18	1.21	109.6 ± 5.30	4.84
	QC low	100.6 ± 0.82	0.82	101.5 ± 1.23	1.22	96.5 ± 1.64	1.70
RUX	QC high	92.2 ± 3.72	4.03	91.6 ± 3.12	3.41	93.8 ± 2.08	2.22
	QC low	99.1± 3.24	3.27	95 ± 7.90	8.32	96.8 ± 2.74	2.82
TRA	QC high	95.1± 1.30	1.36	100 ± 0.14	0.14	98.2 ± 1.30	1.32
	QC low	94.2 ± 3.05	3.24	89.3 ± 1.57	1.76	88.4 ± 1.67	1.89

Table S4 Accuracy and precision of quality control (QC) samples prepared in serum, quantified against a calibration curve prepared in plasma (n = 3)

Analyte	Sample	Accuracy (%)	CV (%)
AFA	QC high	100.4 ± 2.33	2.32
	QC low	99.3 ± 0.76	0.77
AXI	QC high	96.5 ± 2.18	2.26
	QC low	97.7 ± 1.29	1.32
BOS	QC high	104.7 ± 3.06	2.92
	QC low	97.9 ± 3.91	3.99
CAB	QC high	96.2 ± 2.75	2.86
	QC low	99.8 ± 1.95	1.95
DAB	QC high	101.9 ± 3.70	3.63
	QC low	100.4 ± 4.37	4.35
LEN	QC high	98.4 ± 2.21	2.25
	QC low	92.3 ± 0.61	0.66
NIL	QC high	101.9 ± 3.10	3.04
	QC low	98.2 ± 5.10	5.20
OSI	QC high	103.0 ± 3.00	2.91
	QC low	101.0 ± 0.45	0.45
RUX	QC high	98.8 ± 2.02	2.04
	QC low	97.0 ± 6.02	6.21
TRA	QC high	103.2 ± 4.31	4.18
	QC low	97.33 ± 2.93	3.01

Table S5 Validation results for freeze-thaw stability cycles and long-term stability (n = 3)

Analyte	QC level	*Freeze-thaw cycle 1*		*Freeze-thaw cycle 2*		*Freeze-thaw cycle 3*		*Long-term stability –20 °C (3 months)*		*Long-term stability –80 °C (3 months)*	
		Accuracy (%)	CV%	Accuracy (%)	CV%	Accuracy (%)	CV%	Accuracy (%)	CV%	Accuracy (%)	CV%
AFA	QC-H	106.6 ± 3.98	3.73	106.2 ± 3.28	3.09	105.9 ± 2.26	2.14	86.2 ± 4.66	5.40	102.1 ± 1.83	1.79
	QC-L	107.9 ± 6.57	6.09	108.5 ± 6.11	5.63	111.7 ± 3.32	2.97	103.4 ± 0.00	0.00	107.8 ± 4.57	4.24
AXI	QC-H	95.6 ± 1.94	2.02	95.9 ± 1.47	1.53	102.6 ± 1.68	1.64	91.3 ± 4.68	5.13	99.4 ± 0.70	0.71
	QC-L	97.4 ± 1.45	0.98	102.3 ± 1.45	1.41	97.6 ± 1.12	1.15	104.8 ± 0.13	0.12	105.0 ± 5.72	5.45
BOS	QC-H	105.4 ± 1.73	1.64	103.9 ± 3.22	3.10	106.3 ± 0.79	0.75	96.3 ± 8.26	8.58	104.7 ± 3.94	3.77
	QC-L	101.2 ± 2.04	2.01	99.4 ± 0.74	0.74	101.2 ± 5.04	4.98	101.8 ± 2.34	2.30	105.0 ± 6.21	5.92
CAB	QC-H	98.2 ± 4.50	4.58	98.5 ± 2.05	2.09	99.6 ± 2.16	2.17	89.7 ± 4.57	5.10	97.9 ± 2.02	2.07
	QC-L	103.5 ± 1.48	1.43	102.8 ± 2.89	2.81	105.6 ± 1.96	1.86	103.7 ± 1.27	1.22	102.0 ± 3.70	3.63
DAB	QC-H	98.1 ± 2.47	2.52	98.4 ± 1.26	1.28	100.3 ± 3.33	3.32	92.4 ± 8.79	9.51	100.1 ± 0.95	0.95
	QC-L	98.5 ± 6.74	6.84	101.5 ± 2.70	2.66	102.8 ± 0.37	0.36	99.0 ± 3.03	3.06	104.3 ± 2.97	2.85
LEN	QC-H	105.6 ± 0.63	0.59	106.0 ± 2.55	2.41	107.5 ± 3.02	2.81	92.5 ± 3.99	4.32	100.5 ± 2.44	2.43
	QC-L	103.8 ± 4.83	4.65	109.0 ± 6.64	6.09	105.3 ± 2.77	2.64	98.4 ± 1.48	1.50	97.8 ± 0.80	0.82
NIL	QC-H	97.0 ± 7.29	7.52	100.9 ± 2.94	2.91	100.6 ± 4.21	4.18	92.3 ± 5.27	5.71	98.9 ± 1.65	1.67
	QC-L	97.5 ± 6.31	6.48	98.3 ± 4.01	4.08	102.3 ± 5.93	5.79	99.4 ± 4.76	4.79	98.6 ± 2.45	2.49
OSI[a]	QC-H	93.5 ±1.47	1.57	92.8 ± 2.97	3.20	89.5 ± 1.02	1.14	89.8 ±1.47	1.63	89.5 ± 0.49	0.55
	QC-L	91.8 ±1.49	1.63	90.5 ± 2.36	2.61	81.5 ± 2.46	3.02	92.4 ± 3.31	3.58	91.9 ± 4.82	5.25
RUX	QC-H	98.6 ± 2.02	2.05	96.3 ± 7.12	7.40	98.4 ± 2.35	2.39	92.3 ± 6.13	6.64	96.1 ± 0.50	0.52
	QC-L	98.6 ± 4.65	4.72	102.5 ± 1.76	1.72	103.3 ± 2.38	2.31	95.8 ± 1.04	1.08	101.8 ± 1.40	1.38
TRA	QC-H	100.4 ± 0.50	0.50	103.7 ± 0.28	0.89	100.9 ± 1.33	1.32	93.6 ± 9.69	10.3	100.5 ± 1.00	0.99
	QC-L	100.8 ± 3.70	3.67	96.9 ± 1.47	5.72	101.5 ± 0.82	0.81	102.6 ± 7.16	6.98	101.1 ± 4.98	4.93

[a] long term stability was only assessed for four weeks in the case of OSI: - 20 °C and -80 °C

Table S6 Validation results for stock solution (SL) in DMSO and working solution in methanol (WSL) stability

Analyte	*WSL in methanol for 4 months*		*SL in DMSO for 4 months*	
	Accuracy [%]	CV% (n=3)	Accuracy [%]	CV% (n=3)
AFA	87.6 ± 0.51	0.58	82.8 ± 1.23	1.49
BOS	102.2 ± 3.04	2.97	99.2 ± 1.25	1.26
CAB	95.1 ± 3.78	3.97	89.2 ± 2.89	3.24
DAB	92.0 ± 2.89	3.14	88.2 ± 4.33	4.91
LEN	108.1 ± 2.93	2.71	109.0 ± 3.47	3.18
NIL	96.8 ± 5.09	5.26	99.6 ± 2.40	2.41
OSI	109.4 ± 2.45	2.24	105.3 ± 5.05	4.80
RUX	93.8 ± 5.03	5.36	95.0 ± 3.82	4.02
TRA	100.0 ± 2.71	2.71	103.2 ± 0.85	0.82

Table S7 Co-medication and additional condition of the patients taking OSI and AFA

OSI	**Patient 1**	Candesartan 24 mg QD, Hydrochlothiazide 12.5 mg QD Crizotinib 250 mg BID (8/14)* Zolendronat every 8 weeks	Arterial hypertension
	Patient 2	Zolendronate every 6 weeks Crizotinib 250 mg BID (3/11)* Zolendronate every 6 weeks Enoxaparin sodium (50.000 IE/5 mL) 0.4 mL BID	No additional conditions
AFA		Levothyroxine 100 µg Doxycycline 50 mg QD (short-time therapy) no further medication documented	Condition after thyroidectomy after papillary carcinoma of the thyroid, arterial hypertension and mitral regurgitation (Grade II classified by Carpentier)

* ratio of samples (x/total) taken during the combination of the two kinase-inhibitors (osimertinib and crizotinib)

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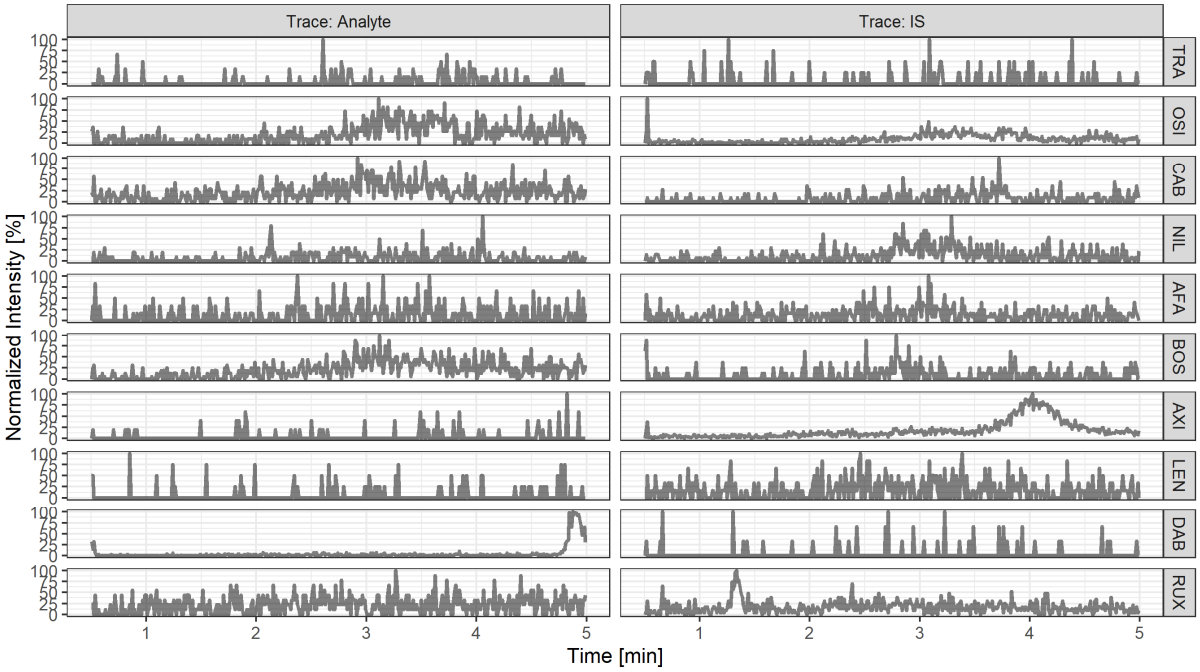

Fig. S1 Chromatographic traces of all monitored MRM transitions in a blank sample

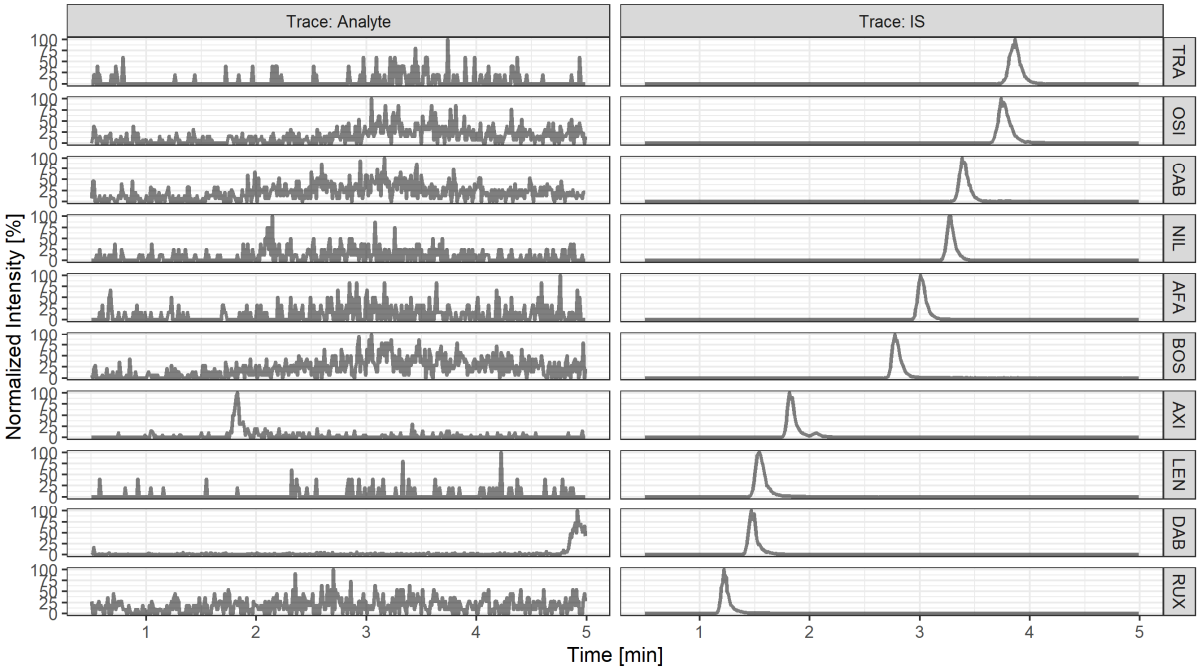

Fig. S2 Chromatographic traces of all monitored MRM transitions in a blank sample containing internal standards

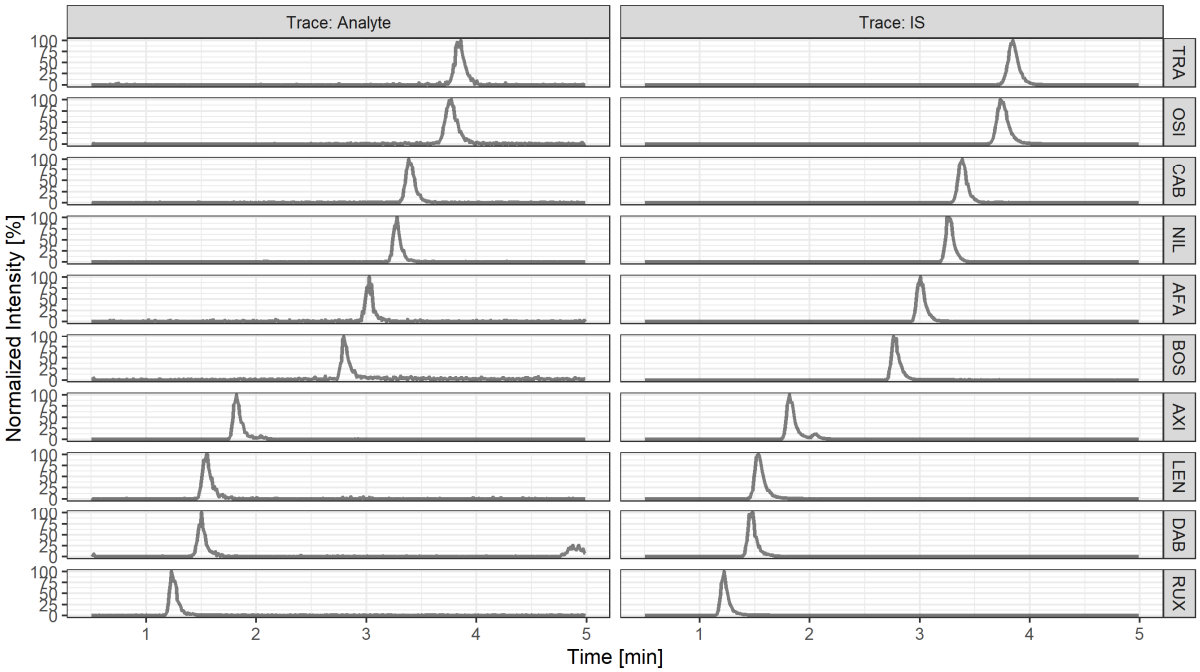

Fig. S3 Chromatographic traces of all monitored MRM transitions in an LLOQ sample