

Supplemental Information

for

Quantification of oligonucleotides by isobaric internal
standards using tandem mass spectrometry

by

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1. DoE parameter optimization.....	3
Table S1: Runs performed for intensity optimisation with respective results for ion at m/z = 690.6	3
Figure S1: Contour plot of DOE results.....	5
2. MSMS Quantification of non-thiolated Oligonucleotides	6
Figure S2: Calibration curve of isobaric pair 2 via intensity.....	6
Table S2: Raw data for creation of calibration curve in Figure S1 via signal intensity (An = analyte; IS = internal standard).....	6
Figure S3: Calibration curve of isobaric pair 2 via MRM TIC area.	7
Table S3: Raw data for creation of calibration curve in Figure S2 via MRM TIC area (An = analyte; IS = internal standard).....	7
3. Method Qualification – Linearity	8
Figure S4: Calibration curve of isobaric pair 1 via intensity.....	8
Table S4: Raw data for creation of calibration curve in Figure S3 signal intensity (An = analyte; IS = internal standard).....	8
Figure S5: Calibration curve of isobaric pair 1 via MRM TIC area. (see Figure 4).....	9
Table S5: Raw data for creation of calibration curve in Figure S4 via MRM TIC area (An = analyte; IS = internal standard).....	9
4. Method Qualification – Sensitivity.....	10
Figure S6: Sensitivity experiments of isobaric pair 1 analyte via MRM TIC area on a logarithmic scale. With LLOQ of 1 ng/ml.	10
Table S6: Raw data for sensitivity experiment in Figure S5 via MRM TIC area (An = analyte; IS = internal standard).....	10
Table S6: Raw data for precision determination of LLOQ in Figure S5 via MRM TIC area (An = analyte; IS = internal standard).....	10
5. Method Qualification – Accuracy & Precision.....	11
Figure S7: Calibration curve of isobaric pair 1 via MRM TIC area; non-extracted. (see Figure 5)	11
Figure S8: Calibration curve of isobaric pair 1 via MRM TIC area; extracted from bovine plasma. (see Figure 5)	11
Table S8: Raw data for non-extracted calibration curve in Figure S6 via MRM TIC area (An = analyte; IS = internal standard).....	12
Table S9: Raw data for calibration curve extracted from bovine plasma in Figure S7 via MRM TIC area (An = analyte; IS = internal standard).	12
Table S10: Raw data for sample testing extracted from water in Figure S6 & S7 via MRM TIC area (An = analyte; IS = internal standard).	12
Table S11: Raw data for sample testing extracted from bovine plasma in Figure S6 & S7 via MRM TIC area (An = analyte; IS = internal standard).	12
Table S12: Raw data for precision calculation by triplicate replica from analyte sample testing.....	13
6. Method Qualification – Short term biofluid stability study.....	13
Table S13: Raw data for biofluid stability study in bovine plasma via MRM TIC area; set 1 (An = analyte; IS = internal standard).....	13
Table S14: Raw data for biofluid stability study in bovine plasma via MRM TIC area; set 2 (An = analyte; IS = internal standard).....	14

Table S15: Raw data for biofluid stability study in bovine plasma via MRM TIC area; set 3 (An = analyte; IS = internal standard)..... 14

1. DoE parameter optimization

For optimal MS-parameters, we assessed the critical parameters influencing the intensity of one of the charge states of our molecule. For this purpose, we were using the software MODDE Version 13. We used the screening objective with full factorial design with interactions. The runs used to build the model are shown in supplementary table 1. Figure S1 shows the Contour plot of the DoE experiments calculated by MODDE software. Best results were considered for final method settings. See Material and methods. The optimizations were all performed with Precursor 690 m/z ($z = 7$). The optimizations were all performed with Precursor 690 m/z ($z = 7$). However, the use of other charge states is also possible and results in equivalent fragment spectra. As an example, a comparison of the MSMS spectra of precursor 690 m/z and 805 m/z was compared in Figure S1. Here, the fragments 465 m/z & 485 m/z relevant for quantification are clearly visible.

Table S1: Runs performed for intensity optimisation with respective results for ion at m/z = 690.6

Run order	Exp Name	Source temperature [°C]	Capillary voltage [kV]	Cone voltage [V]	Desolvation temperature [°C]	Desolvation gas flow [L/h]	Intensity of 690.6 m/z
1	N9	80	2.8	40	500	725	19825
2	N11	80	3.2	40	500	725	29093
3	N27	80	3.2	40	500	775	17335
4	N21	80	2.8	60	450	775	18026
5	N35	100	3.0	50	475	750	12513
6	N17	80	2.8	40	450	775	10839
7	N15	80	3.2	60	500	725	19488
8	N8	120	3.2	60	450	725	
9	N18	120	2.8	40	450	775	14946
10	N33	100	3.0	50	475	750	13748
11	N24	120	3.2	60	450	775	15600
12	N10	120	2.8	40	500	725	20146
13	N23	80	3.2	60	450	775	16407
14	N32	120	3.2	60	500	775	16391
15	N25	80	2.8	40	500	775	22271
16	N12	120	3.2	40	500	725	24479
17	N29	80	2.8	60	500	775	13354
18	N2	120	2.8	40	450	725	26737
19	N7	80	3.2	60	450	725	24079
20	N19	80	3.2	40	450	775	15007
21	N31	80	3.2	60	500	775	18303
22	N14	120	2.8	60	500	725	19481
23	N3	80	3.2	40	450	725	30323
24	N30	120	2.8	60	500	775	14311
25	N1	80	2.8	40	450	725	26263
26	N20	120	3.2	40	450	775	18005
27	N13	80	2.8	60	500	725	17320
28	N22	120	2.8	60	450	775	18053
29	N26	120	2.8	40	500	775	19403
30	N5	80	2.8	60	450	725	18336
31	N28	120	3.2	40	500	775	17444
32	N4	120	3.2	40	450	725	24337
33	N34	100	3.0	50	475	750	13365
34	N6	120	2.8	60	450	725	16409
35	N16	120	3.2	60	500	725	19488

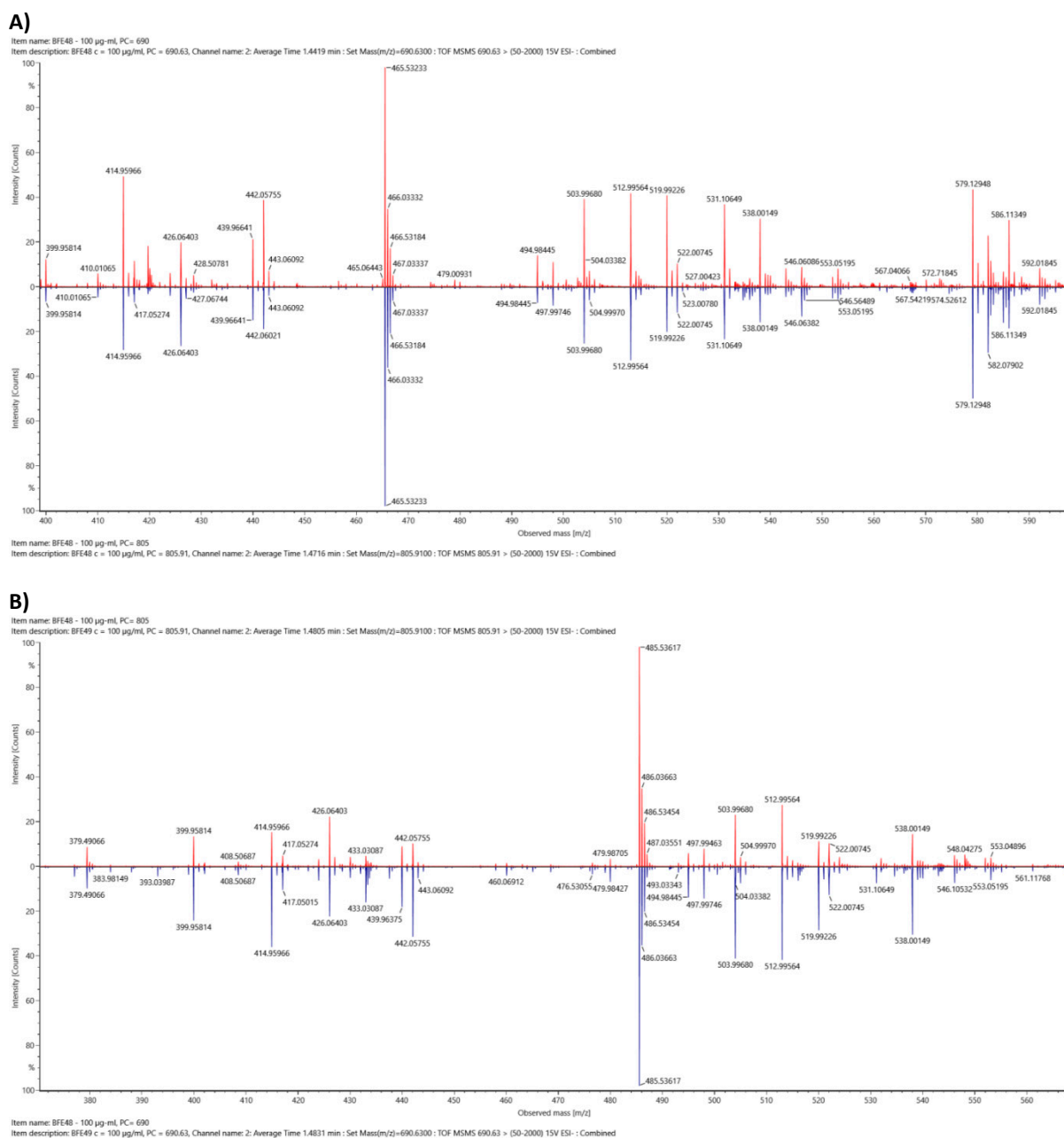


Figure S1: Comparison of the fragment spectra of the 690 m/z and 805 m/z precursors. **A:** Mass range with the fragment 465 m/z from precursor 805 m/z (top) and 690 m/z (bottom), which is relevant for quantification. **B:** Mass range with the fragment 485 m/z from Precursor 805 m/z (top) and 690 m/z (bottom) that is relevant for quantification.

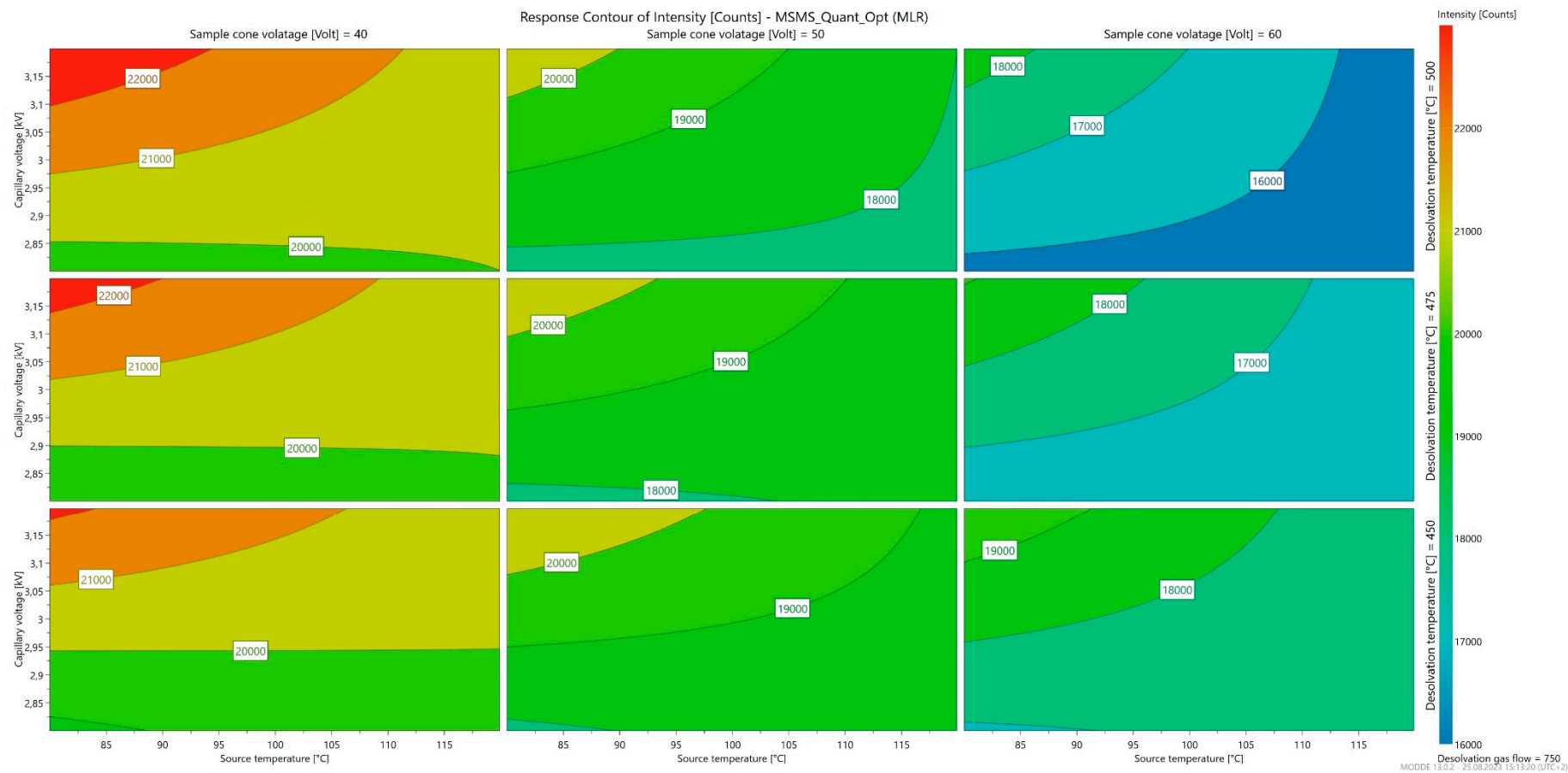


Figure S2: Contour plot of DOE results.

2. MSMS Quantification of non-thiolated Oligonucleotides

In addition to the isobaric pair used in the publication, proof of principle was also performed with a non-thiolated oligonucleotide pair (Chapter 4.1, Table 2). In this case, the MRM TIC area was calibrated via the signal intensity and the MRM TIC area. It was found that a linear trend line cannot be applied to the data points when calibrating via the intensity. Instead, the curve can be described via a power equation. This can also be seen in the case of the thiolated oligonucleotide pair, which is why calibration via MRM TIC areas also appears to make more sense in this case.

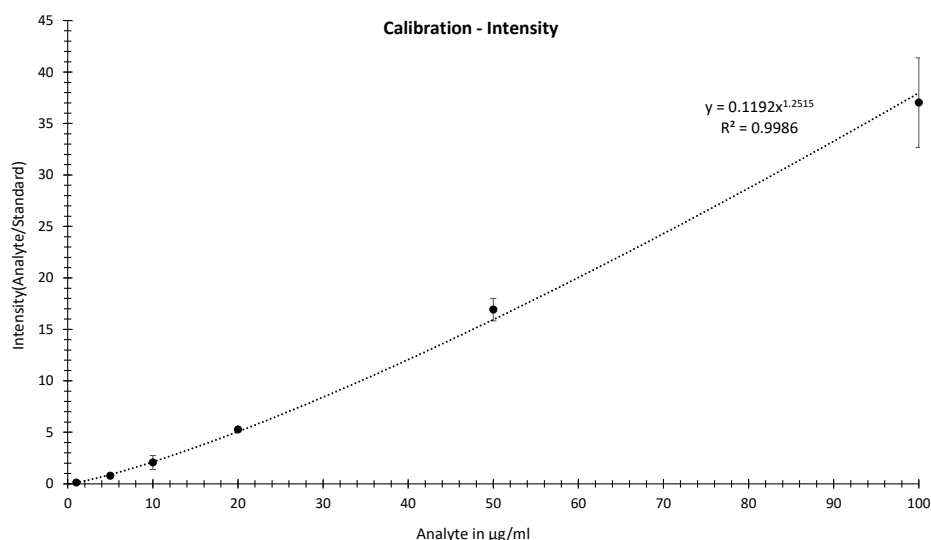


Figure S3: Calibration curve of isobaric pair 2 via intensity.

Table S2: Raw data for creation of calibration curve in Figure S1 via signal intensity (An = analyte; IS = internal standard).

Calibration I - Intensity								
Measurement	c(An)	I(An)	$\bar{X}(\text{An})$	I(IS)	$\bar{X}(\text{IS})$	ratio[I(An)/I(IS)]	$\bar{X}[I(\text{An})/I(\text{IS})]$	$\sigma[I(\text{An})/I(\text{IS})]$
1	1 µg/ml	154057	135573	1617497	1126962	0.095244072	0.126702593	0.027824794
2		133743		1325702		0.100884663		
3		133849		989243		0.13530447		
4		127185		898843		0.141498571		
5		129031		803525		0.160581189		
1	5 µg/ml	693420	715918.4	1005906	900669	0.689348707	0.798185865	0.06409279
2		730382		862749		0.846575308		
3		725998		890747		0.815044002		
4		730693		867337		0.842455701		
5		699099		876607		0.79750561		
1	10 µg/ml	876607	1429500.4	955745	724830	0.917197579	2.073244697	0.678583397
2		1605261		688744		2.330707781		
3		1649151		654173		2.520970752		
4		1468108		720764		2.036877536		
5		1548375		604723		2.560469835		
1	20 µg/ml	2716210	2726256.6	521979	516120	5.203676776	5.28276988	0.068559113
2		2749185		515384		5.334245921		
3		2745403		511908		5.363078913		
4		2675771		505960		5.288503044		
5		2744714		525370		5.224344748		
1	50 µg/ml	5616577	5588423.6	324213	331428	17.32372545	16.92546552	1.080295373
2		5730654		312552		18.33504185		
3		5804163		375270		15.46663202		
4		5580308		341458		16.34258972		
5		5210416		303649		17.15933858		
1	100 µg/ml	6899815	6996595.2	196576	191035	35.09998677	37.03400166	4.359006821
2		6961989		209434		33.24192347		
3		7167270		179563		39.91507159		
4		7141847		212019		33.68493861		
5		6812055		157584		43.22808788		

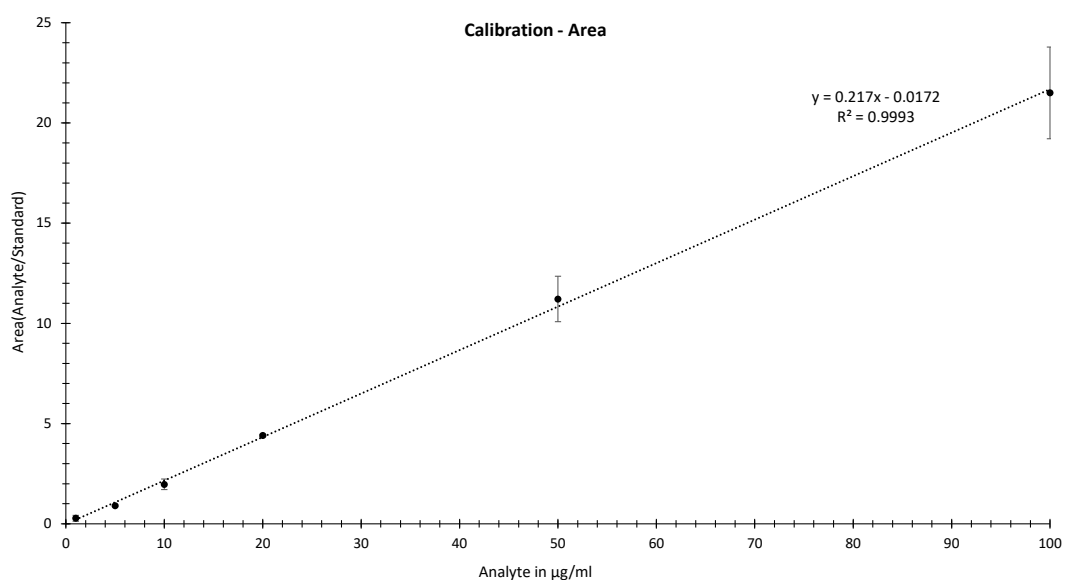


Figure S4: Calibration curve of isobaric pair 2 via MRM TIC area.

Table S3: Raw data for creation of calibration curve in Figure S2 via MRM TIC area (An = analyte; IS = internal standard).

Calibration I - Area								
Measurement	c(An)	A(An)	$\bar{X}(\text{An})$	A(IS)	$\bar{X}(\text{IS})$	ratio[A(An)/A(IS)]	$\bar{X}[\text{A(An)/A(IS)}]$	$\sigma[\text{A(An)/A(IS)}]$
1	1 µg/ml	109182	104207.6	205609	446840	0.531017611	0.272369817	0.147154383
2		106338		648002		0.164101345		
3		105162		507913		0.20704727		
4		100292		451075		0.222339966		
5		100064		421601		0.237342891		
1	5 µg/ml	401332	411919.2	497189	456910	0.80720209	0.903747505	0.055320398
2		410811		452577		0.907715151		
3		419650		450533		0.931452302		
4		414047		444193		0.932133104		
5		413756		440056		0.940234879		
1	10 µg/ml	759033	772196.6	502451	399379	1.510660741	1.964864987	0.26400505
2		776828		397018		1.956656877		
3		784070		374830		2.091801617		
4		783000		366380		2.137125389		
5		758052		356214		2.128080311		
1	20 µg/ml	1314852	1320227	296480	300086	4.434875877	4.400322135	0.059931782
2		1323744		296061		4.471186681		
3		1314994		298234		4.409269232		
4		1311572		300075		4.370813963		
5		1335973		309578		4.315464923		
1	50 µg/ml	2598585	2640745.2	234620	237599	11.07571818	11.21233832	1.134267506
2		2656863		251891		10.54766943		
3		2678581		273823		9.7821622		
4		2633155		218454		12.05359023		
5		2636542		209207		12.60255154		
1	100 µg/ml	2858083	3052872.4	144820	142145	19.73541638	21.63515943	2.285727571
2		3054761		164088		18.61660207		
3		3120077		135443		23.03608898		
4		3122559		132795		23.5141308		
5		3108882		133580		23.27355892		

3. Method Qualification – Linearity

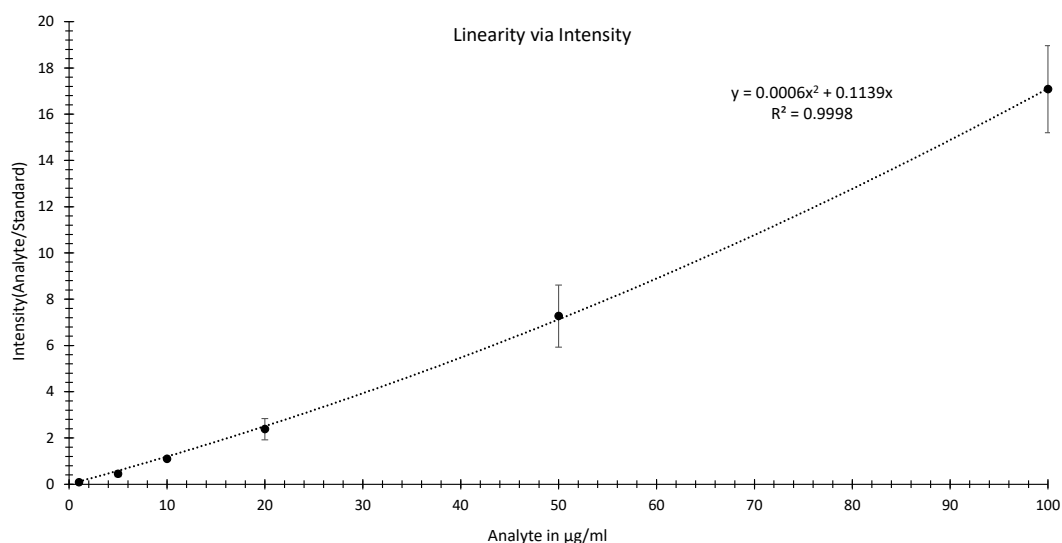


Figure S5: Calibration curve of isobaric pair 1 via intensity.

Table S4: Raw data for creation of calibration curve in Figure S3 signal intensity (An = analyte; IS = internal standard).

Calibration - Intensity								
Measurement	c(An/IS)	I(An)	$\bar{X}(\text{An})$	I(IS)	$\bar{X}(\text{IS})$	ratio[I(An)/I(IS)]	$\bar{X}[I(\text{An})/I(\text{IS})]$	$\sigma[I(\text{An})/I(\text{IS})]$
1	1 µg/ml	359632	310617.4	17266	24091	0.048010188	0.078623605	0.019480198
2		293342		20974		0.07150016		
3		266859		22771		0.085329706		
4		252227		24081		0.095473522		
5		381027		35361		0.092804447		
1	5 µg/ml	351922	342928.2	88149	154251	0.250478799	0.448405117	0.12350485
2		305989		124616		0.40725647		
3		317342		169966		0.535592515		
4		388051		212191		0.546812146		
5		351337		176331		0.501885654		
1	10 µg/ml	524686	573733	550636	628575	1.049458152	1.096042124	0.04944966
2		542737		612089		1.127781964		
3		581392		660633		1.136295305		
4		589309		666868		1.131610072		
5		630541		652651		1.035065127		
1	20 µg/ml	614719	499098	1006443	1164925	1.637240756	2.381091934	0.462632854
2		488201		1087562		2.227693102		
3		485666		1274230		2.62367553		
4		454413		1255901		2.763787568		
5		452491		1200487		2.653062713		
1	50 µg/ml	369988	300981.6	1966505	2141802	5.315050758	7.26751094	1.341390023
2		313725		2070741		6.600497251		
3		287541		2163250		7.523274942		
4		265523		2297043		8.651013283		
5		268131		2211469		8.247718466		
1	100 µg/ml	180932	162403	2621674	2757273	14.48983043	17.08066615	1.884692985
2		167111		2651840		15.86873396		
3		155860		2744157		17.60655075		
4		155327		2975424		19.15587116		
5		152785		2793268		18.28234447		

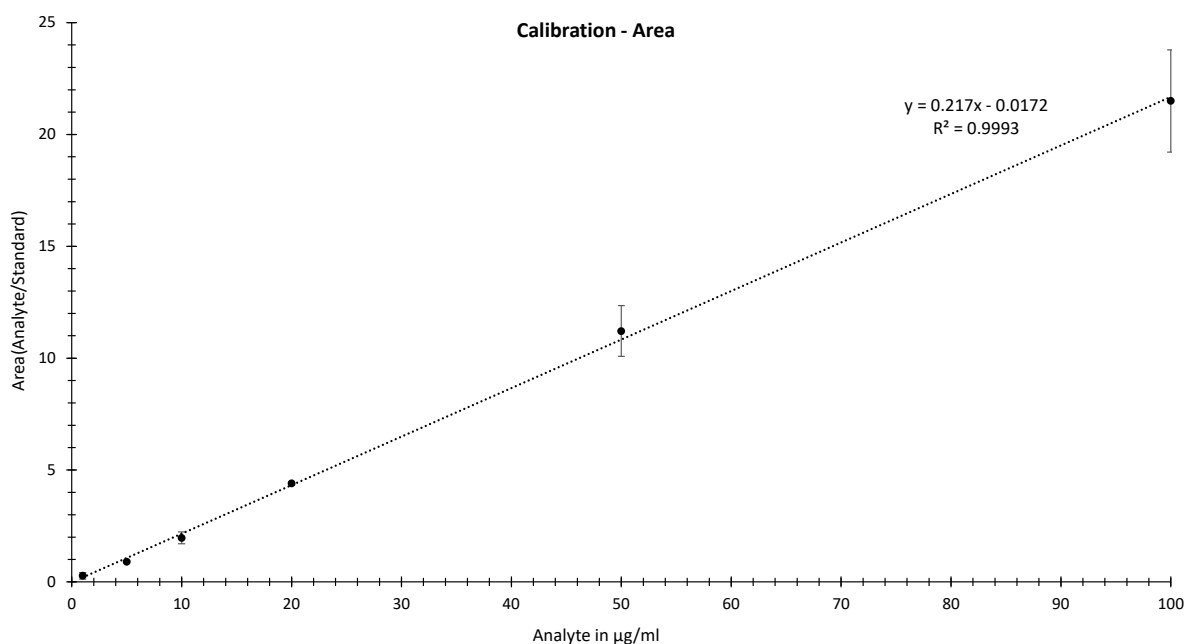


Figure S6: Calibration curve of isobaric pair 1 via MRM TIC area. (see Figure 4)

Table S5: Raw data for creation of calibration curve in Figure S4 via MRM TIC area (An = analyte; IS = internal standard).

Calibration I - Area								
Measurement	c(An)	A(An)	$\bar{X}(\text{An})$	A(IS)	$\bar{X}(\text{IS})$	ratio[A(An)/A(IS)]	$\bar{X}[A(\text{An})/A(\text{IS})]$	$\sigma[A(\text{An})/A(\text{IS})]$
1	1 µg/ml	109182	104207.6	205609	446840	0.531017611	0.272369817	0.147154383
2		106338		648002		0.164101345		
3		105162		507913		0.20704727		
4		100292		451075		0.222339966		
5		100064		421601		0.237342891		
1	5 µg/ml	401332	411919.2	497189	456910	0.80720209	0.903747505	0.055320398
2		410811		452577		0.907715151		
3		419650		450533		0.931452302		
4		414047		444193		0.932133104		
5		413756		440056		0.940234879		
1	10 µg/ml	759033	772196.6	502451	399379	1.510660741	1.964864987	0.26400505
2		776828		397018		1.956656877		
3		784070		374830		2.091801617		
4		783000		366380		2.137125389		
5		758052		356214		2.128080311		
1	20 µg/ml	1314852	1320227	296480	300086	4.434875877	4.400322135	0.059931782
2		1323744		296061		4.471186681		
3		1314994		298234		4.409269232		
4		1311572		300075		4.370813963		
5		1335973		309578		4.315464923		
1	50 µg/ml	2598585	2640745.2	234620	237599	11.07571818	11.21233832	1.134267506
2		2656863		251891		10.54766943		
3		2678581		273823		9.7821622		
4		2633155		218454		12.05359023		
5		2636542		209207		12.60255154		
1	100 µg/ml	2858083	3052872.4	144820	142145	19.73541638	21.63515943	2.285727571
2		3054761		164088		18.61660207		
3		3120077		135443		23.03608898		
4		3122559		132795		23.5141308		
5		3108882		133580		23.27355892		

4. Method Qualification – Sensitivity

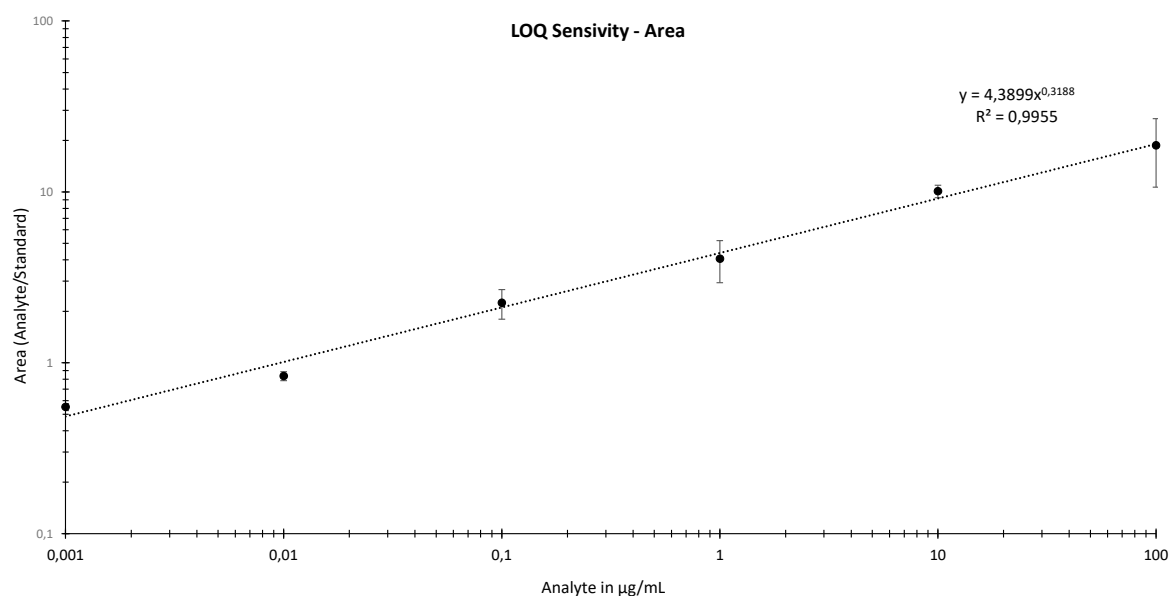


Figure S7: Sensitivity experiments of isobaric pair 1 analyte via MRM TIC area on a logarithmic scale. With LLOQ of 1 ng/ml.

Table S6: Raw data for sensitivity experiment in Figure S5 via MRM TIC area (An = analyte; IS = internal standard).

LOQ Sensitivity - Area								
Measurement	c(An/IS)	A(An)	$\bar{X}(\text{An})$	A(IS)	$\bar{X}(\text{An})$	ratio[A(An)/A(IS)]	$\bar{X}[A(\text{An})/A(\text{IS})]$	$\sigma[A(\text{An})/A(\text{IS})]$
1	1 ng/ml	28072	29049,33333	48313	52809	0,581044439	0,551340966	0,025724094
2		26189		48823		0,536407021		
3		32887		61291		0,536571438		
1	10 ng/ml	50271	50036	63633	60001	0,790014615	0,83603941	0,049650276
2		50121		60427		0,829447101		
3		49715		55944		0,888656514		
1	100 ng/ml	89992	91978	49595	41990	1,814537756	2,239763215	0,43905356
2		90803		41026		2,213303759		
3		95140		35349		2,691448131		
1	1 µg/ml	167267	170100	48518	43635	3,44752463	4,060907553	1,121691319
2		167966		49699		3,379665587		
3		175067		32689		5,355532442		
1	10 µg/ml	288514	292386	31548	29127	9,145239001	10,08156874	0,852228267
2		294439		28621		10,28751616		
3		294204		27211		10,81195105		
1	100 µg/ml	411756	434461	44109	26778	10,01620083	18,72715829	8,063122706
2		445799		22030		20,23599637		
3		445828		17194		25,92927765		

Table S7: Raw data for precision determination of LLOQ in Figure S5 via MRM TIC area (An = analyte; IS = internal standard).

LLOQ Precision - Area								
Measurement	c(An/IS)	A(An)	$\bar{X}(\text{An})$	A(IS)	$\bar{X}(\text{An})$	ratio[A(An)/A(IS)]	$\bar{X}[A(\text{An})/A(\text{IS})]$	$\sigma[A(\text{An})/A(\text{IS})]$
1	1 ng/ml	28072	29049,33333	48313	52809	0,581044439	0,551340966	0,025724094
2		26189		48823		0,536407021		
3		32887		61291		0,536571438		
1	1 ng/ml	27635	26462	44525	49382	0,620662549	0,538372581	0,094227745
2		21763		49963		0,435582331		
3		29988		53658		0,558872861		
1	1 ng/ml	25812	28367,33333	48655	55255	0,530510739	0,511053017	0,043107607
2		23658		51247		0,461646535		
3		35632		65863		0,541001776		

5. Method Qualification – Accuracy & Precision

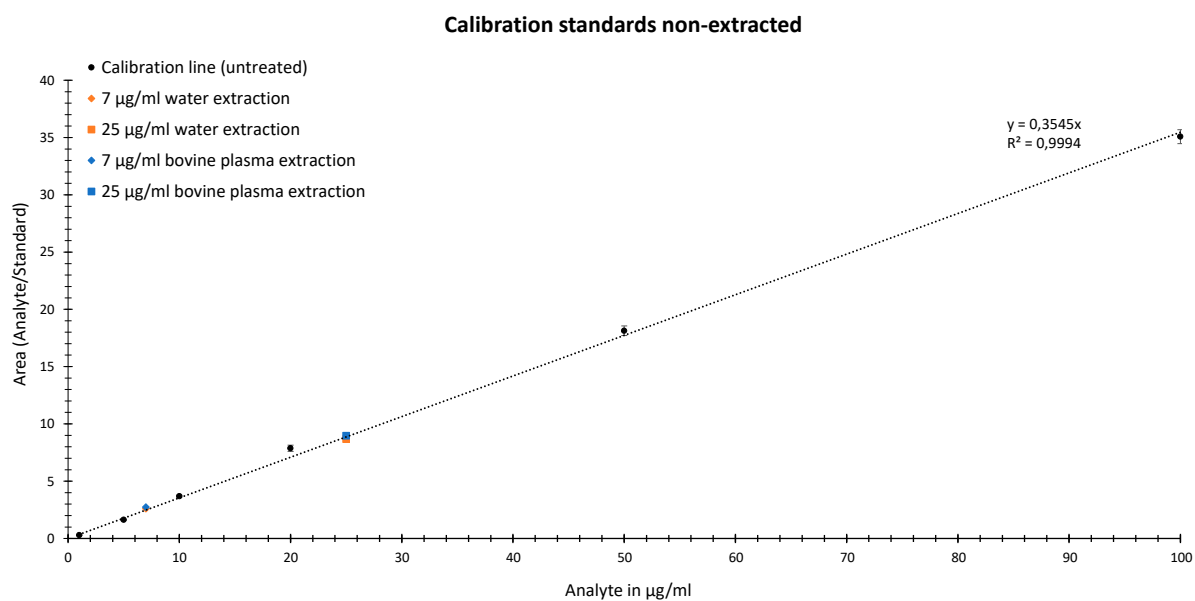


Figure S8: Calibration curve of isobaric pair 1 via MRM TIC area; non-extracted. (see Figure 5)

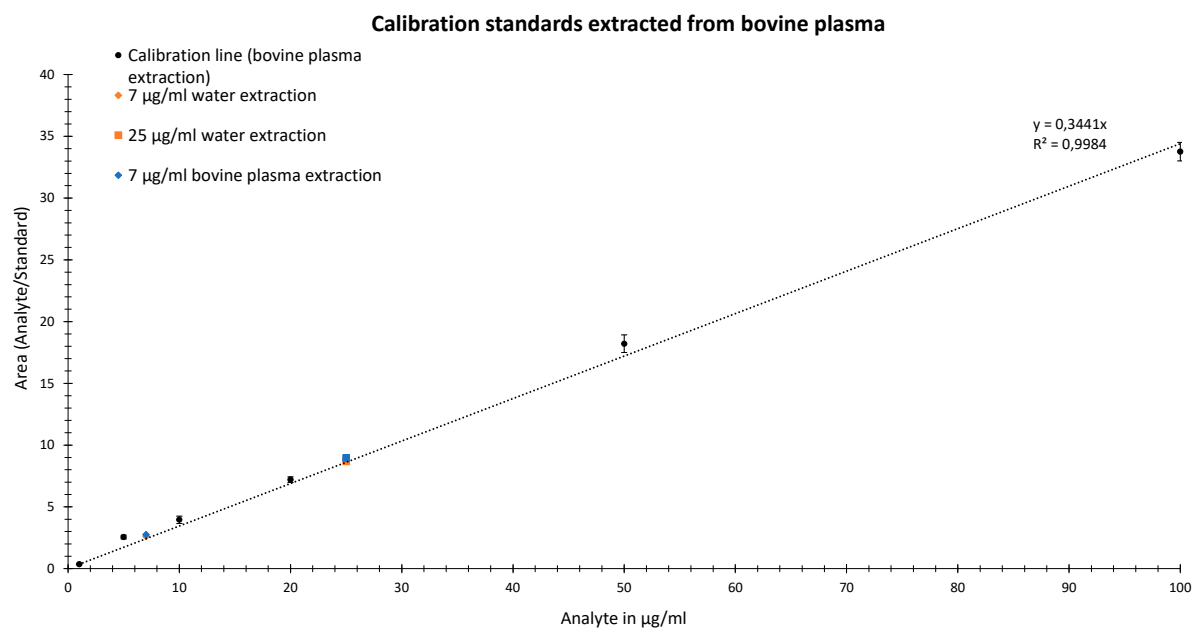


Figure S9: Calibration curve of isobaric pair 1 via MRM TIC area; extracted from bovine plasma. (see Figure 5)

Table S8: Raw data for non-extracted calibration curve in Figure S6 via MRM TIC area (An = analyte; IS = internal standard).

Calibration curve non-extracted - Area								
Measurement	c(An)	A(An)	$\bar{X}(\text{An})$	A(IS)	$\bar{X}(\text{IS})$	ratio[A(An)/A(IS)]	$\bar{X}[A(\text{An})/A(\text{IS})]$	$\sigma[A(\text{An})/A(\text{IS})]$
1	1 µg/ml	1765	1710	5938	5785	0,297238127	0,295571172	0,001976986
2		1677		5716		0,293386984		
3		1688		5701		0,296088406		
1	5 µg/ml	7342	7534	4433	4602	1,656214753	1,637940416	0,039646869
2		7708		4629		1,665154461		
3		7553		4743		1,592452035		
1	10 µg/ml	14368	14222	3805	3854	3,7760841	3,691193796	0,075353749
2		14162		3899		3,632213388		
3		14137		3857		3,665283899		
1	20 µg/ml	31917	32176	4162	4088	7,668668909	7,874877387	0,27422088
2		32466		3966		8,186081694		
3		32144		4137		7,769881557		
1	50 µg/ml	77254	74989	4344	4139,666667	17,78406998	18,12967702	0,416599958
2		72547		3902		18,59226038		
3		75167		4173		18,01270069		
1	100 µg/ml	139568	140361	3934	4002	35,47737672	35,08062098	0,610312064
2		140571		4089		34,37784299		
3		140945		3983		35,38664323		

Table S9: Raw data for calibration curve extracted from bovine plasma in Figure S7 via MRM TIC area (An = analyte; IS = internal standard).

Calibration curve extracted from bovine plasma - Area								
Measurement	c(An)	A(An)	$\bar{X}(\text{An})$	A(IS)	$\bar{X}(\text{IS})$	ratio[A(An)/A(IS)]	$\bar{X}[A(\text{An})/A(\text{IS})]$	$\sigma[A(\text{An})/A(\text{IS})]$
1	1 µg/ml	195	195,33	581	560,666667	0,335628227	0,350309416	0,033015161
2		195		596		0,327181208		
3		196		505		0,388118812		
1	5 µg/ml	1577	1502,00	584	590	2,700342466	2,549946978	0,151609526
2		1414		554		2,55234657		
3		1515		632		2,397151899		
1	10 µg/ml	1883	1935,33	520	489,666667	3,621153846	3,962623424	0,29686754
2		1914		466		4,107296137		
3		2009		483		4,15942029		
1	20 µg/ml	5385	5322,67	729	739,666667	7,386831276	7,198537238	0,227641912
2		5353		737		7,263229308		
3		5230		753		6,945551129		
1	50 µg/ml	14583	14766,67	788	811,666667	18,50634518	18,20102772	0,716978415
2		15290		817		18,71481028		
3		14427		830		17,38192771		
1	100 µg/ml	57220	57570,00	1667	1706,333333	34,32513497	33,75545995	0,747896612
2		57209		1681		34,03271862		
3		58281		1771		32,90852626		

Table S10: Raw data for sample testing extracted from water in Figure S6 & S7 via MRM TIC area (An = analyte; IS = internal standard).

Extraction from water - 7 µg/ml - Area								
Measurement	c(An)	A(An)	$\bar{X}(\text{An})$	A(IS)	$\bar{X}(\text{IS})$	ratio[A(An)/A(IS)]	$\bar{X}[A(\text{An})/A(\text{IS})]$	$\sigma[A(\text{An})/A(\text{IS})]$
1	7 µg/mL	6023	5305,333333	2028	2040	2,969921105	2,605322318	0,340716699
2		5023		1969		2,551041138		
3		4870		2122		2,295004713		
Extraction from water - 25 µg/ml - Area								
Measurement	c(An)	A(An)	$\bar{X}(\text{An})$	A(IS)	$\bar{X}(\text{IS})$	ratio[A(An)/A(IS)]	$\bar{X}[A(\text{An})/A(\text{IS})]$	$\sigma[A(\text{An})/A(\text{IS})]$
1	25 µg/mL	18127	17660,66667	1981	2040	9,150429076	8,671379084	0,549367478
2		17751		2019		8,791976226		
3		17104		2119		8,071731949		

Table S11: Raw data for sample testing extracted from bovine plasma in Figure S6 & S7 via MRM TIC area (An = analyte; IS = internal standard).

Extraction from bovine plasma - 7 µg/ml - Area								
Messung	c(An)	A(An)	$\bar{X}(\text{An})$	A(IS)	$\bar{X}(\text{IS})$	ratio[A(An)/A(IS)]	$\bar{X}[A(\text{An})/A(\text{IS})]$	$\sigma[A(\text{An})/A(\text{IS})]$
1	7 µg/mL	6862	7816,66667	3267	2923	2,100397919	2,739246718	0,787221661
2		7411		2966		2,498651382		
3		9177		2536		3,618690852		
Extraction from bovine plasma - 25 µg/ml - Area								
Messung	c(An)	A(An)	$\bar{X}(\text{An})$	A(IS)	$\bar{X}(\text{IS})$	ratio[A(An)/A(IS)]	$\bar{X}[A(\text{An})/A(\text{IS})]$	$\sigma[A(\text{An})/A(\text{IS})]$
1	25 µg/mL	37301	36942,33333	4330	4131	8,614549654	8,963087554	0,504476649
2		36915		4227		8,733144074		
3		36611		3837		9,541568934		

Table S12: Raw data for precision calculation by triplicate replica from analyte sample testing

Precision			
Calibration line (untreated)		Calibration line (Plasma extracted)	
7 µg/ml	25 µg/ml	7 µg/ml	25 µg/ml
2,605	8,6713	2,739	8,963
2,545	8,81	2,699	8,866
2,666	8,556	2,743	8,799

6. Method Qualification – Short term biofluid stability study

Table S13: Raw data for biofluid stability study in bovine plasma via MRM TIC area; set 1 (An = analyte; IS = internal standard).

Pharmacokinetic - Area - Bio. Rep. Set 1								
Measurement	t [h]	A(An)	$\bar{X}(\text{An})$	A(IS)	$\bar{X}(\text{IS})$	ratio[A(An)/A(IS)]	$\bar{X}[\text{A(An)/A(IS)}]$	$\sigma[\text{A(An)/A(IS)}]$
1	0	60270	56617	11587	10827	5,201518944	5,231400977	0,088918939
2		55014		10659		5,161272164		
3		54567		10235		5,331411822		
1	0,25	51050	45951	11885	10455	4,295330248	4,42082198	0,198768062
2		37744		8117		4,64999384		
3		49060		11364		4,317141851		
1	1	38159	38350	11069	11423	3,447375553	3,358669688	0,0768264
2		38721		11686		3,313451994		
3		38171		11514		3,315181518		
1	4	21106	20115	7798	7696	2,706591434	2,613243927	0,081121063
2		18255		7094		2,573301381		
3		20983		8197		2,559838965		
1	24	37662	38253	13603	13844,6667	2,768653973	2,766873384	0,121972585
2		38645		14616		2,644020252		
3		38453		13315		2,887945926		
1	48	169835	171369	64859	64827	2,618526342	2,643554487	0,025044231
2		172585		65286		2,643522348		
3		171688		64336		2,668614772		
1	96	40551	38918	18947	18561	2,140233282	2,096198211	0,039091893
2		38850		18653		2,082774889		
3		37352		18083		2,065586462		
1	192	21556	21534	14397	14872	1,497256373	1,4487994	0,043273595
2		21640		15304		1,414009409		
3		21405		14915		1,435132417		

Table S14: Raw data for biofluid stability study in bovine plasma via MRM TIC area; set 2 (An = analyte; IS = internal standard).

Pharmacokinetic - Area - Bio. Rep. Set 2								
Measurement	t [h]	A(An)	$\bar{X}(\text{An})$	A(IS)	$\bar{X}(\text{IS})$	ratio[A(An)/A(IS)]	$\bar{X}[A(\text{An})/A(\text{IS})]$	$\sigma[A(\text{An})/A(\text{IS})]$
1	0	53673	54197,33333	9979	10090	5,37859505	5,373984655	0,074223665
2		52394		9621		5,445795655		
3		56525		10670		5,297563261		
1	0,25	56826	54119	11975	11534	4,745386221	4,698791524	0,169850825
2		54523		12088		4,510506287		
3		51009		10538		4,840482065		
1	1	29997	29201	9061	8893	3,310561748	3,283684472	0,047736916
2		28886		8947		3,228568235		
3		28721		8672		3,311923432		
1	4	30447	29805	10182	10430	2,990276959	2,859183991	0,113530149
2		28956		10364		2,793901968		
3		30012		10744		2,793373045		
1	24	168859	170655	51230	53122	3,296096037	3,214252202	0,077841471
2		171062		53365		3,205509229		
3		172044		54771		3,141151339		
1	48	185730	107349	69861	49781,6667	2,658564865	2,248335727	0,682150012
2		45535		17343		2,625554979		
3		90781		62141		1,460887337		
1	96	40427	39754	18899	18647	2,139107889	2,131863777	0,008070719
2		39652		18587		2,133318986		
3		39183		18455		2,123164454		
1	192	25486	24156	16009	14778	1,591979512	1,636330277	0,038774265
2		22662		13708		1,653195214		
3		24320		14617		1,663816105		

Table S15: Raw data for biofluid stability study in bovine plasma via MRM TIC area; set 3 (An = analyte; IS = internal standard).

Pharmacokinetic - Area - Bio. Rep. Set 3								
Measurement	t [h]	A(An)	$\bar{X}(\text{An})$	A(IS)	$\bar{X}(\text{IS})$	ratio[A(An)/A(IS)]	$\bar{X}[A(\text{An})/A(\text{IS})]$	$\sigma[A(\text{An})/A(\text{IS})]$
1	0	56939	59751	11294	11701,66667	5,041526474	5,10472959	0,058693512
2		62963		12208		5,157519659		
3		59351		11603		5,115142636		
1	0,25	48366	51104	11243	11828	4,301876723	4,320499421	0,033488226
2		53072		12341		4,300461875		
3		51874		11900		4,359159664		
1	1	187649	191459	55191	56081	3,399992752	3,413844105	0,02263604
2		192393		56560		3,40157355		
3		194334		56493		3,439966013		
1	4	29244	28074	9748	9584	3	2,931081012	0,119088101
2		27458		9829		2,793570048		
3		27519		9174		2,999672989		
1	24	248305	191901	84560	77012,66667	2,936435667	2,408781655	0,915132888
2		87762		64909		1,352077524		
3		239636		81569		2,937831774		
1	48	125391	119164	45830	42991,33333	2,736002618	2,774235165	0,061373039
2		117914		43008		2,741675967		
3		114188		40136		2,845026909		
1	96	38665	39595	22204	23122,33333	1,741352909	1,718735093	0,086702425
2		42176		25987		1,622965329		
3		37945		21176		1,791887042		
1	192	33653	31566	18553	17203,33333	1,813884547	1,836390964	0,045033186
2		30429		16115		1,888240769		
3		30615		16942		1,807047574		

Table S16: Raw data for calibration curve for biofluid stability study in bovine plasma (An = analyte; IS = internal standard).

Calibration for Biofluid stability study - Area								
Messung	c(An)	A(An)	$\bar{X}(\text{An})$	A(IS)	$\bar{X}(\text{IS})$	ratio(An/IS)	$\bar{X}(\text{An/IS})$	$\sigma(\text{An/IS})$
1	1 µg/ml	14960	14545	122563	123027.667	0.122059675	0.11832101	0.00726142
2		14846		120747		0.122951295		
3		13829		125773		0.109952056		
1	5 µg/ml	49646	48938	118456	111375	0.419109205	0.44349778	0.0542532
2		48255		118937		0.405718994		
3		48914		96732		0.505665137		
1	10 µg/ml	94084	94323	81805	98795	1.15010085	0.9767175	0.17547704
2		94984		118846		0.799219158		
3		93900		95735		0.980832506		
1	20 µg/ml	165417	159832	70232	71029	2.355293883	2.25059475	0.14398214
2		165518		71650		2.310090719		
3		148560		71204		2.08639964		
1	50 µg/ml	386639	395840	77905	76264.6667	4.962954881	5.1940731	0.23065721
2		398394		76688		5.194997914		
3		402486		74201		5.424266519		
1	100 µg/ml	688096	686501	68268	66317	10.07933439	10.3742991	0.61591676
2		688428		62120		11.08222795		
3		682979		68563		9.961334831		

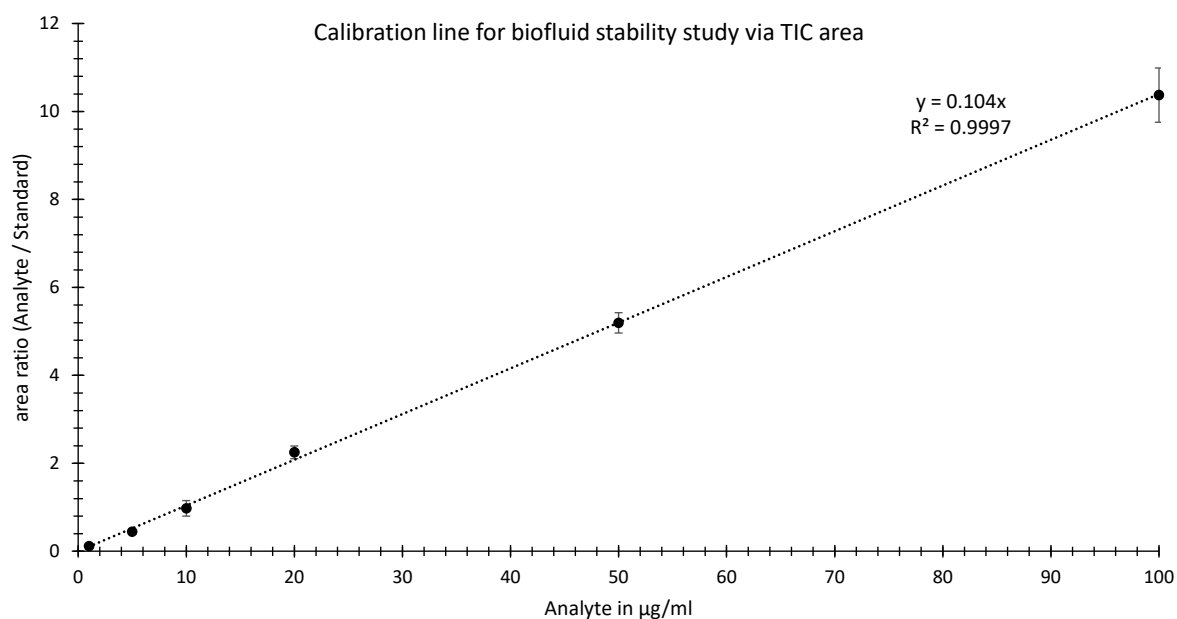


Figure S10: Calibration curve for biofluid stability study from raw data in table S16.

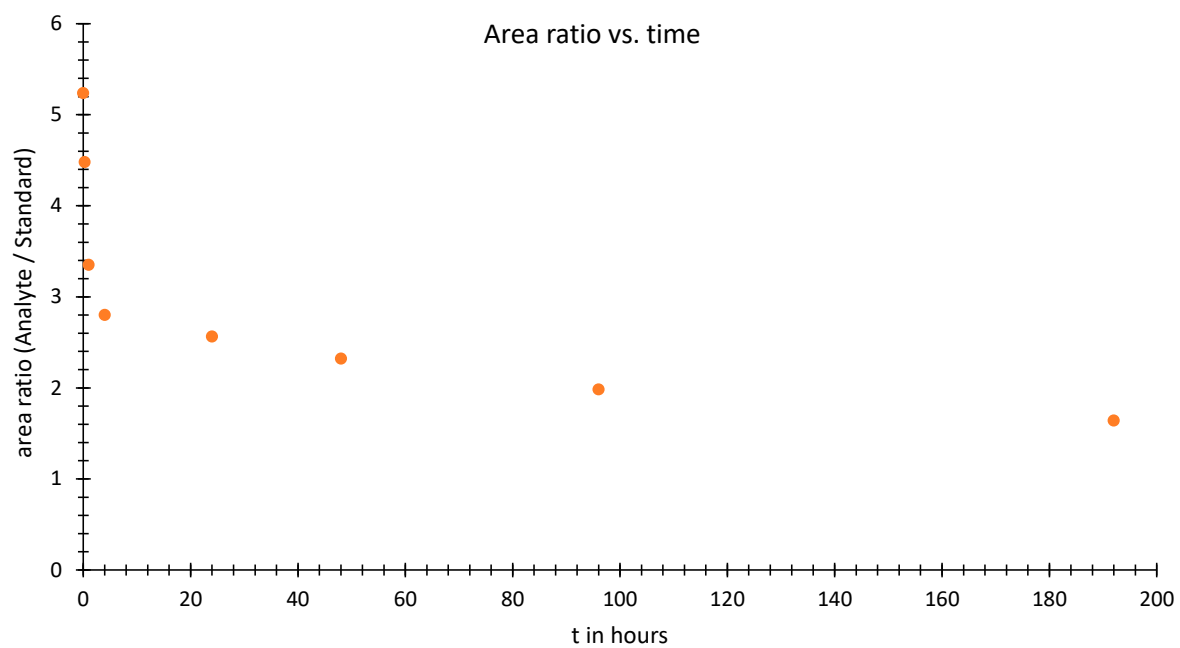


Figure S11: Mean area ratios in relation to degradation time in hours.