

Waters

*Taposh G, Bhaskar K, Veeranjanyulu P, Tirupateswar rao B, Rajesh PMN, Anil Kurup
Waters India Applications Laboratory, Bangalore, 560058*

Clinical background:

In healthy population the coenzyme form of vitamin B12 participates in two key metabolic pathways. One is the conversion of methylmalonyl-coenzyme- A to succinyl-CoA & second one is formation of methionine from homocysteine (Hcy) .

OBJECTIVE:

CHALLENGES:

Simple and fast LC-MS/MS, MRM based assay was developed for the simultaneous determination of MMA & tHcy in plasma and serum samples.



Sample preparation: To the plasma/serum sample, two Internal standard was added with reduction reagent and vortexed for 5 minutes. Extraction solution was added and centrifuged, the supernatant was taken for LC -MS/MS analysis.

Compound	Parent m/z	Daughter m/z	Cone (V)	CE
Homocysteine	136.08	90.03	10	10
Homocysteine- d4	140.08	94.03	10	10
Methylmalonic acid	117.00	73.00	15	12
Methylmalonic acid -d3	120.00	76.00	15	12

MS System	: Xevo TQD
Mode	: ESI +Ve & -Ve
Cone gas	: 50 L/Hr
Desolvation Temp	: 300 °C
Source Temp	: 150 °C
Desolvation gas flow	: 600 L/hr
Data processed through	TargetLynx

Compound name: Homocysteine												
#	Name	ID	Type	RT	Area	IS RT	IS Area	Response	Std. Conc.	Conc.	%	
1	Homocysteine MMA_120416_PA_2_001	EXT BLK		0.70	113011	0.70	68	1657.896		90966...		
2	Homocysteine MMA_120416_PA_2_002	EXT BLK IS		0.70	104093	0.70	552380	0.190				
3	Homocysteine MMA_120416_PA_2_003	EXT STD1	Standard	0.70	156441	0.70	507049	0.309	500.00	508.64	100.00	
4	Homocysteine MMA_120416_PA_2_004	EXT STD2	Standard	0.70	182149	0.70	520434	0.350	750.00	736.17	98.16	
5	Homocysteine MMA_120416_PA_2_005	EXT STD3	Standard	0.70	216867	0.70	547054	0.396	1000.00	990.97	99.09	
6	Homocysteine MMA_120416_PA_2_006	EXT STD4	Standard	0.70	313746	0.70	544657	0.576	2000.00	1976.62	98.83	
7	Homocysteine MMA_120416_PA_2_007	EXT STD5	Standard	0.70	400657	0.70	523307	0.765	3000.00	3015.93	100.53	
8	Homocysteine MMA_120416_PA_2_008	EXT STD6	Standard	0.70	511152	0.70	542655	0.942	4000.00	3984.55	99.61	
9	Homocysteine MMA_120416_PA_2_009	EXT STD7	Standard	0.70	626872	0.70	547024	1.146	5000.00	5102.12	102.04	
10	Homocysteine MMA_120416_PA_2_010	EXT blk		0.70	112650	0.70	74	1517.415		83257...		
11	Homocysteine MMA_120416_PA_2_011	EXT LOQ	QC	0.70	158727	0.70	506299	0.310	500.00	516.10	103.20	
12	Homocysteine MMA_120416_PA_2_012	EXT LOQ	QC	0.70	155664	0.70	507974	0.308	500.00	497.17	99.43	
13	Homocysteine MMA_120416_PA_2_013	EXT LOQ	QC	0.70	155591	0.70	507187	0.309	500.00	509.81	101.96	
14	Homocysteine MMA_120416_PA_2_014	EXT LOQ	QC	0.70	157770	0.70	505067	0.312	500.00	529.72	105.84	
15	Homocysteine MMA_120416_PA_2_015	EXT LOQ	QC	0.70	154538	0.70	507588	0.304	500.00	486.28	97.25	
16	Homocysteine MMA_120416_PA_2_016	EXT LOQ	QC	0.70	161430	0.70	532301	0.303	500.00	479.76	95.95	
17	Homocysteine MMA_120416_PA_2_017	EXT LQC	QC	0.70	184851	0.70	528802	0.350	750.00	733.81	97.84	
18	Homocysteine MMA_120416_PA_2_018	EXT LQC	QC	0.70	181306	0.70	524864	0.345	750.00	711.15	94.82	
19	Homocysteine MMA_120416_PA_2_019	EXT LQC	QC	0.70	182991	0.70	518841	0.353	750.00	750.97	100.13	
20	Homocysteine MMA_120416_PA_2_020	EXT LQC	QC	0.70	187513	0.70	538208	0.348	750.00	727.43	97.00	
21	Homocysteine MMA_120416_PA_2_021	EXT LQC	QC	0.70	186725	0.70	531736	0.351	750.00	742.57	99.01	
22	Homocysteine MMA_120416_PA_2_022	EXT LQC	QC	0.70	184749	0.70	520217	0.355	750.00	764.39	101.91	
23	Homocysteine MMA_120416_PA_2_023	EXT MQC	QC	0.70	327290	0.70	570503	0.574	2000.00	1963.69	98.19	
24	Homocysteine MMA_120416_PA_2_024	EXT MQC	QC	0.70	323760	0.70	557936	0.580	2000.00	1969.88	98.49	
25	Homocysteine MMA_120416_PA_2_025	EXT MQC	QC	0.70	326166	0.70	563121	0.579	2000.00	1994.01	99.70	
26	Homocysteine MMA_120416_PA_2_026	EXT MQC	QC	0.70	316217	0.70	545132	0.580	2000.00	1998.75	99.94	
27	Homocysteine MMA_120416_PA_2_027	EXT MQC	QC	0.70	315447	0.70	548591	0.575	2000.00	1970.98	98.54	
28	Homocysteine MMA_120416_PA_2_028	EXT MQC	QC	0.70	318248	0.70	554655	0.574	2000.00	1964.18	98.24	
29	Homocysteine MMA_120416_PA_2_029	EXT HQC	QC	0.70	521038	0.70	542389	0.961	4000.00	4087.28	102.17	
30	Homocysteine MMA_120416_PA_2_030	EXT HQC	QC	0.70	514772	0.70	548128	0.939	4000.00	3969.17	99.24	
31	Homocysteine MMA_120416_PA_2_031	EXT HQC	QC	0.70	510723	0.70	540101	0.946				

Compound name: MMA

#	Name	ID	Type	RT	Area	IS RT	IS Area	Response	Std. Conc.	Conc.
1	Homocysteine MMA, 120416_Pa2_001	EXT BLK		0.74	102	0.75	1	87.647	55882	
2	Homocysteine MMA, 120416_Pa2_002	EXT BLK IS		0.74	100	0.74	945	0.106		
3	Homocysteine MMA, 120416_Pa2_003	EXT STD1	Standard	0.74	177	0.74	876	0.202	50.00	49.03
4	Homocysteine MMA, 120416_Pa2_004	EXT STD2	Standard	0.74	222	0.74	894	0.248	75.00	78.47
5	Homocysteine MMA, 120416_Pa2_005	EXT STD3	Standard	0.74	241	0.74	873	0.277	100.00	96.64
6	Homocysteine MMA, 120416_Pa2_006	EXT STD4	Standard	0.74	412	0.74	907	0.454	200.00	209.87
7	Homocysteine MMA, 120416_Pa2_007	EXT STD5	Standard	0.74	521	0.74	902	0.578	300.00	288.87
8	Homocysteine MMA, 120416_Pa2_008	EXT STD6	Standard	0.74	654	0.74	874	0.748	400.00	397.43
9	Homocysteine MMA, 120416_Pa2_009	EXT STD7	Standard	0.74	820	0.74	901	0.909	500.00	500.52
10	Homocysteine MMA, 120416_Pa2_010	EXT blk		0.74	105	0.73	1	124.430	79398	
11	Homocysteine MMA, 120416_Pa2_011	EXT LOQ	QC	0.74	169	0.74	857	0.197	50.00	45.64
12	Homocysteine MMA, 120416_Pa2_012	EXT LOQ	QC	0.74	172	0.74	864	0.199	50.00	46.74
13	Homocysteine MMA, 120416_Pa2_013	EXT LOQ	QC	0.75	160	0.74	877	0.182	50.00	36.41
14	Homocysteine MMA, 120416_Pa2_014	EXT LOQ	QC	0.74	163	0.74	855	0.191	50.00	41.70
15	Homocysteine MMA, 120416_Pa2_015	EXT LOQ	QC	0.74	163	0.74	882	0.185	50.00	38.17
16	Homocysteine MMA, 120416_Pa2_016	EXT LOQ	QC	0.74	169	0.74	822	0.205	50.00	51.06
17	Homocysteine MMA, 120416_Pa2_017	EXT LOQ	QC	0.74	197	0.74	879	0.225	75.00	63.38
18	Homocysteine MMA, 120416_Pa2_018	EXT LOC	QC	0.74	191	0.74	872	0.219	75.00	59.86
19	Homocysteine MMA, 120416_Pa2_019	EXT LOC	QC	0.74	226	0.74	905	0.250	75.00	79.38
20	Homocysteine MMA, 120416_Pa2_020	EXT LOC	QC	0.74	200	0.74	849	0.236	75.00	70.66
21	Homocysteine MMA, 120416_Pa2_021	EXT LOC	QC	0.74	200	0.74	857	0.234	75.00	69.14
22	Homocysteine MMA, 120416_Pa2_022	EXT LOC	QC	0.74	203	0.74	867	0.234	75.00	69.20
23	Homocysteine MMA, 120416_Pa2_023	EXT MQC	QC	0.74	379	0.74	848	0.447	200.00	205.46
24	Homocysteine MMA, 120416_Pa2_024	EXT MQC	QC	0.74	365	0.74	906	0.403	200.00	177.03
25	Homocysteine MMA, 120416_Pa2_025	EXT MQC	QC	0.74	377	0.74	876	0.430	200.00	194.39
26	Homocysteine MMA, 120416_Pa2_026	EXT MQC	QC	0.74	402	0.74	916	0.439	200.00	200.12
27	Homocysteine MMA, 120416_Pa2_027	EXT MQC	QC	0.74	374	0.74	943	0.397	200.00	173.18
28	Homocysteine MMA, 120416_Pa2_028	EXT MQC	QC	0.74	393	0.74	905	0.434	200.00	196.86
29	Homocysteine MMA, 120416_Pa2_029	EXT HQC	QC	0.74	620	0.74	872	0.712	400.00	374.25
30	Homocysteine MMA, 120416_Pa2_030	EXT HQC	QC	0.74	617	0.74	860	0.717	400.00	377.90
31	Homocysteine MMA, 120416_Pa2_031	EXT HQC	QC	0.75	602	0.75	845	0.712	400.00	374.68
32	Homocysteine MMA, 120416_Pa2_032	EXT HQC	QC	0.74	652	0.74	882	0.739	400.00	392.00
33	Homocysteine MMA, 120416_Pa2_033	EXT HQC	QC	0.74	601	0.74	895	0.671	400.00	348.51
34	Homocysteine MMA, 120416_Pa2_034	EXT HQC	QC	0.74	640	0.74	871	0.735	400.00	380.34

The figure displays three plots related to the analysis of Homocysteine:

- Top Left Chromatogram:** Shows the chromatogram for Homocysteine (MMA_120416_16_2_003). The peak is labeled with a retention time of 0.70 minutes and an area of 156441. The x-axis represents time in minutes (0.5 to 3.5), and the y-axis represents intensity in AU (-5 to 35).
- Bottom Left Chromatogram:** Shows the chromatogram for Homocysteine D4 (EXT STD1 EXT STD1). The peak is labeled with a retention time of 0.70 minutes and an area of 507049. The x-axis represents time in minutes (0.5 to 3.5), and the y-axis represents intensity in AU (-5 to 35).
- Right Plot:** A calibration curve for Homocysteine. The x-axis is labeled "Conc" (Concentration) and ranges from 0 to 5000. The y-axis is labeled "Area" and ranges from 0.0 to 1.10. The curve is linear, with the equation $y = 0.00019227x + 0.215462$ and a correlation coefficient $r^2 = 0.999502$. The plot also indicates the response type as "Internal Std (Ref 2), Area" and the curve type as "Linear, Origin Exclude, Weighting: 1/x^2, Axis trans: None".

Figure 3: Representative chromatogram of tHcy and tHcy-d4.

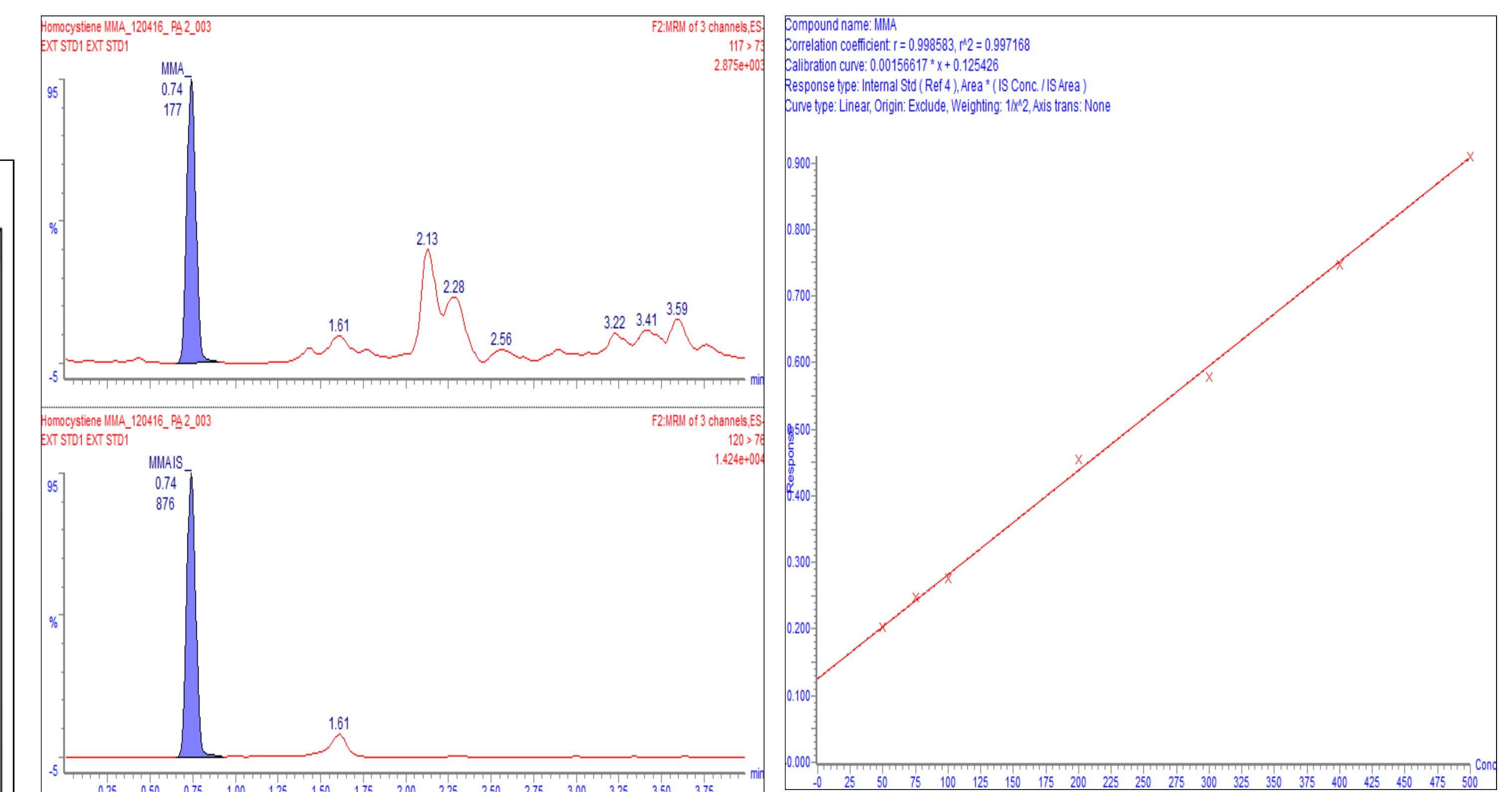


Figure 5: Representative chromatogram of MMA and MMA-d3.

1) Demonstrated simultaneous quantification method for MMA and tHcy, which is reliable, sensitive, precise, accurate and reproducible in serum and plasma matrix.

2) The method demonstrated showed less matrix effect and good sensitivity up to 50 ppb for MMA & 500 ppb for tHcy.

3) Opportunity for this method to be validated and used or routine analysis.

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4. <https://pubchem.ncbi.nlm.nih.gov/compound/L-Homocysteine>.

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