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Application News

Liquid Chromatograph Mass Spectrometer LCMS-8060

Analysis of Antidepressants in Serum / Plasma Using RECIPE® ClinMass® TDM Kit System with Fully Automated Sample Preparation LC/MS/MS System

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User Benefits

- Full solution provided by Shimadzu and RECIPE®
- Fully automated sample preparation
- Verified method for RECIPE® ClinMass® TDM Kit System for Antidepressants in Serum / Plasma

■ Introduction

Major depressive disorder (MDD) affects many adults, often treated initially in primary care with medications. Standard treatments include classical tricyclic antidepressants (TCAs) and newer options like selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), and tetracyclic antidepressants (TeCAs). Therapeutic drug monitoring (TDM) is essential for optimizing doses and minimizing side effects, particularly in cases of uncertain adherence, suboptimal tolerability, or drug interactions.

RECIPE's fully validated analytical method provides the quantification of of 37 Antidepressants and metabolites (Table 2) for TDM using LC-MS/MS.[1]

By addition of the Shimadzu CLAM (Clinical Laboratory Automated sample preparation Module) in front of the LC-MS/MS system (Figure 1) the required sample preparation could be fully automated which achieves results on a fast and high-precision analytical workflow.

To prove that the automated sample preparation leads to reliable results a method verification procedure was evaluated according to the CLSI Guidelines EP06-A, EP15-A3, EP17-A2.

Fig. 1 CLAM LCMS TQ

■ Materials and Methods

Fast, sensitive and robust LC-MS/MS systems provide the basis for routine analysis in clinical laboratories. For the described verification, a Shimadzu CLAM-2040 coupled with a Nexera X3 UHPLC system and a LCMS-8060 triple-quadrupole mass spectrometer was used.

Thirty-seven Antidepressants in serum were verified using the ClinMass® TDM Platform (order no. MS9000) in combination with the ClinMass® Add-on Set for Antidepressants in Serum / Plasma (order no. MS9400) (RECIPE®,Germany).

Lyophilized, matrix-based calibrator and control samples were reconstituted, aliquoted and stored until use.

Then the samples were loaded directly into the CLAM-2040. It was programmed to perform protein precipitation using Precipitant P including internal standards from the ClinMass® TDM Kit System for Antidepressants followed by filtration and sample collection. The sample is then transported using an arm from the CLAM-2040 to the LC without human intervention for LC-MS/MS analysis. Due to overlapped sample preparation (Figure 2) and analysis the throughput was one complete analysis each 5.0 min. Analytical conditions are listed in Table 1. The optimized MRM transitions are summarized in Table 2.

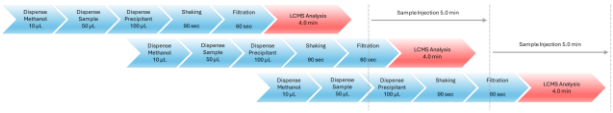

Fig. 2 Scheme fully automated sample preparation and analysis

Table 1 Analytical conditions

Mass Spectrometer	: LCMS-8060
Ionization	: Electrospray Ionization (ESI), positive
Interface Voltage	: 4 kV
Heating Gas	: 10 L/min
DL Temp.	: 250 ˚C
Interface Temp.	: 300 ˚C
Nebulizing Gas	: 3 L/min
Drying Gas	: 10 L/min
Heat Block	: 400 ˚C
CID	: 270 kPa

UHPLC	: Nexera X3
Column Oven	: 40 ˚C
Injection Volume	: 2.0 µL
Flow rate	: 0.7 mL/min
Time Programme	: Binary gradient

Time [min]	Mobile Phase A [%]	Mobile Phase B [%]
Initial	95	5
0.10	95	5
0.20	75	25
1.50	50	50
2.50	45	55
2.60	20	80
3.00	20	80
3.10	95	5
3.70	95	5

Table 2 MRM transitions and parameters of the analytes and isotope-labelled substances

Analyte / IS	Quantifier MRM		Dwell Time	Q1 Pre Bias	CE	Q3 Pre Bias
	Precursor [m/z]	Product [m/z]	msec	[V]	[V]	[V]
Agomelatine	244	185.1	5	-27	-27	-19
Atomoxetine	256.1	117.1	5	-24	-35	-11
Bupropion	240	184.1	5	-16	-12	-12
Citalopram	325.2	109.1	5	-12	-15	-11
Clomethiazole	162	126.2	5	-28	-20	-24
Desmethylcitalopram	311	109.1	5	-11	-10	-11
Desmethylfluoxetine	296	134.1	5	-26	-7	-22
Desmethylmianserine	251.2	208.1	5	-10	-20	-21
Desmethylmirtazapine	252.2	195.1	5	-17	-35	-19
Desmethylsertraline	291.9	158.9	5	-29	-25	-29
Dosulepin	296	202	5	-14	-40	-21
Duloxetine	298	154	5	-19	-6	-15
Dihydro-Bupropion*	242	168.1	5	-17	-3	-30
Fluoxetine	310.1	148.1	5	-17	-9	-15
Fluvoxamine	319	71.2	5	-16	-33	-13
Guanfacine	245.9	60	5	-12	-16	-23
Hydroxybupropion	256	139	5	-17	-40	-14
Methylphenidate	234	84	5	-23	-21	-16
Mianserine	265	58	5	-30	-23	-22
Milnacipran	247.2	100.1	5	-29	-30	-10
Mirtazapine	266	195.1	5	-19	-15	-20
Moclobemide	269	182.1	5	-18	-40	-19
Nefazodone	470.1	274.2	5	-17	-40	-28
O-Desmethyltramadol	250.1	58.1	5	-40	-5	-40
O-Desmethylvenlafaxine	264.2	107	5	-30	-32	-10
Opipramol	364.1	171.1	5	-11	-40	-17
Paroxetine	330	70.1	5	-10	-29	-12
Reboxetine	314	176.1	5	-22	-5	-25
Ritalinic acid	220	84.1	5	-23	-40	-14
Sertraline	306.1	159	5	-15	-15	-25
Tianeptine	437	292	5	-16	-35	-20
Tramadol	264.2	58	5	-30	-5	-21
Tranylcypromine	134	117.1	60	-10	-14	-23
Trazodone	372	120.1	5	-24	-54	-21
Venlafaxine	278.1	58.1	5	-13	-10	-23
Vortioxetine	299	150	5	-20	-24	-15
d3-Agomelatine	247	185.1	5	-17	-16	-12
d3-Atomoxetine	259.2	117	5	-10	-13	-21
d9-Bupropion	249.1	185.1	5	-10	-12	-21
d6-Citalopram	331.2	109.1	5	-35	-40	-22
d3-Dosulepin	299.2	203.1	5	-11	-35	-18
d7-Duloxetine	305.3	154.1	5	-15	-6	-18
d3-Desmethylcitalopram	314.2	109	5	-11	-22	-22
d5-Desmethylfluoxetine	301.1	139.1	5	-11	-7	-29
d4-Desmethylsertraline	295.9	159.9	5	-11	-28	-18
d5-Fluoxetine	315.1	153.1	5	-12	-8	-29
d6-O-Desmethyltramadol	256.2	64	5	-40	-5	-40
d3-Fluvoxamine	322.2	74.1	5	-12	-32	-14
d6-Hydroxybupropion	262.1	139	5	-10	-35	-16
d9-Methylphenidate	243.2	93	5	-27	-21	-16
d3-Mianserine	268.2	61.2	5	-10	-24	-24
d10-Milnacipran	257.2	110.1	5	-18	-30	-20
d3-Mirtazapine	269.2	195.1	5	-10	-15	-22
d8-Moclobemide	277	183.1	5	-10	-30	-21
d6-Nefazodone	476.2	280.1	5	-15	-29	-20
d4-Opipramol	368.1	175.1	5	-25	-16	-19
d4-Paroxetine	334.2	74.1	5	-10	-30	-14
d5-Reboxetine	319.2	176.1	5	-12	-5	-13
d3-Sertraline	309.1	159	5	-15	-25	-18
d6-Tramadol	270.1	64	5	-30	-5	-21
d9-threo-Dihydro-Bupropion	251.1	169	5	-16	-25	-19
d5-Tranylcypromine	139.1	122.1	60	-16	-34	-25
d6-Venlafaxine	284.2	58.2	5	-29	-34	-24
d8-Vortioxetine	307.1	153	5	-11	-24	-17

Table 2 MRM transitions and parameters of the analytes and isotope-labelled substances (continued)

Analyte / IS	Qualifier MRM		Dwell Time	Q1 Pre Bias	CE	Q3 Pre Bias
	Precursor [m/z]	Product [m/z]	msec	[V]	[V]	[V]
Agomelatine	244	141.1	5	-27	-47	-14
Atomoxetine	256.1	65	5	-24	-55	-13
Bupropion	240	131.1	5	-16	-25	-13
Citalopram	325.2	262.1	5	-12	-20	-18
Clomethiazole	162	70.9	5	-28	-44	-17
Desmethylcitalopram	311	234.1	5	-11	-26	-24
Desmethylfluoxetine	296	105.2	5	-26	-23	-19
Desmethylmianserine	251.2	118.1	5	-10	-25	-23
Desmethylmirtazapine	252.2	209.1	5	-17	-23	-22
Desmethylsertraline	291.9	275	5	-29	-12	-13
Dosulepin	296	178.1	5	-14	-47	-18
Duloxetine	298	97.1	5	-19	-53	-19
Dihydro-Bupropion*	242	116.1	5	-17	-20	-11
Fluoxetine	310.1	65.1	5	-17	-80	-14
Fluvoxamine	319	200	5	-16	-20	-22
Guanfacine	245.9	123.2	5	-12	-46	-21
Hydroxybupropion	256	238.1	5	-17	-3	-40
Methylphenidate	234	56	5	-23	-50	-20
Mianserine	265	77.1	5	-30	-55	-14
Milnacipran	247.2	230.2	5	-29	-22	-15
Mirtazapine	266	72.1	5	-19	-21	-13
Moclobemide	269	139	5	-18	-10	-13
Nefazodone	470.1	246.1	5	-17	-35	-16
O-Desmethyltramadol	250.1	232.1	5	-40	-10	-40
O-Desmethylvenlafaxine	264.2	58	5	-30	-5	-22
Opipramol	364.1	143.1	5	-11	-45	-14
Paroxetine	330	192.1	5	-10	-21	-20
Reboxetine	314	91	5	-22	-20	-19
Ritalinic acid	220	56.1	5	-23	-46	-20
Sertraline	306.1	275.1	5	-15	-18	-18
Tianeptine	437	228	5	-16	-38	-15
Tramadol	264.2	42	5	-30	-55	-15
Tranylcypromine	134	65	60	-16	-34	-25
Trazodone	372	78.1	5	-24	-30	-14
Venlafaxine	278.1	260.2	5	-13	-12	-18
Vortioxetine	299	109	5	-20	-40	-22

*Dihydro-bupropion is determined as the sum of erythro- and threo-dihydro-bupropion

■ Results

The trueness was determined by 4-fold analysis of two different quality control (QC) samples in a single analysis sequence. The results (precision in CV% and deviation from the target in % Bias) are summarized in Table 3.

To determine the precision two different levels of QC samples were prepared in 8-fold and analyzed in a single analysis sequence. The intraassay precision for each level is summarized in Table 4.

For determination of the linearity and the lower limit of quantification (LLOQ) several dilutions of the ClinCal® Serum Calibrator Set lyophil. for Antidepressants (order no. MS9413, RECIPE® ,Germany) were prepared in 3-fold and analyzed in a single analysis sequence. The results for linearity evaluation and for the LLOQ are summarized in Table 5.

Table 3 Trueness of measurement

Analyte	Target value [µg/L]	Measured value [µg/L]	CV	Bias	Target value [µg/L]	Measured value [µg/L]	CV	Bias
	MS9482 Level I	Mean (n=4)	[%]	[%]	MS9482 Level II	Mean (n=4)	[%]	[%]
Agomelatine	32.9	29.3	3.2	-10.9	315	313	1.8	-0.6
Atomoxetine	392	405	9.0	3.4	917	953	5.2	4.0
Bupropion	39.4	41.6	3.5	5.5	92.5	98.4	1.6	6.4
Citalopram	44.6	43.3	2.9	-2.9	104	108	2.9	3.5
Clomethiazole	450	482	4.7	7.1	2764	2840	2.4	2.8
Desmethylcitalopram	51.6	49.4	2.1	-4.2	121	114	1.5	-6.0
Desmethylfluoxetine	124	114	3.6	-7.9	292	282	2.7	-3.6
Desmethylmianserine	28.3	31.6	2.9	11.5	69.1	76.9	2.5	11.4
Desmethylmirtazapine	37.8	41.0	2.6	8.5	86.0	92.0	3.5	6.9
Desmethylsertraline	39.2	39.4	2.0	0.4	87.3	94.9	5.5	8.8
Dosulepin	44.3	42.0	3.3	-5.2	103	97.5	2.0	-5.3
Duloxetine	53.0	54.9	2.0	3.7	123	127	2.1	3.5
Dihydro-Bupropion*	268	260	1.4	-3.2	627	622	3.0	-0.8
Fluoxetine	101	99.4	3.7	-1.6	237	239	3.5	1.0
Fluvoxamine	109	101	2.8	-7.4	242	245	2.2	1.4
Guanfacine	2.62	2.39	6.3	-8.9	6.26	6.0	1.1	-4.1
Hydroxybupropion	418	414	1.9	-0.9	972	977	2.0	0.5
Methylphenidate	9.26	9.20	2.4	-0.6	21.7	21.7	3.1	0.0
Mianserine	32.4	31.2	2.0	-3.9	76.3	74.2	0.8	-2.7
Milnacipran	65.1	62.6	3.8	-3.8	153	160	5.2	4.7
Mirtazapine	35.5	34.4	0.7	-3.2	83.4	79.8	2.1	-4.3
Moclobemide	497	464	3.4	-6.6	1041	1059	2.8	1.7
Nefazodone	105	99.5	1.0	-5.2	250	231	1.8	-7.4
O-Desmethyltramadol	233	206	2.2	-11.6	501	487	4.2	-2.7
O-Desmethylvenlafaxine	109	107	2.7	-2.0	251	252	1.6	0.3
Opipramol	109	105	2.6	-3.8	258	253	1.6	-2.1
Paroxetine	42.1	40.8	2.8	-3.0	97.9	99.9	1.9	2.0
Reboxetine	143	134	2.3	-6.5	330	320	2.9	-3.1
Ritalinic acid	70.7	72.0	2.7	1.9	161	168	4.4	4.3
Sertraline	29.2	30.0	1.9	2.7	152	152	2.1	-0.2
Tianeptine	29.0	31.0	1.3	6.7	70.8	73.8	2.4	4.3
Tramadol	237	229	1.7	-3.2	529	521	3.4	-1.5
Tranylcypromine	19.4	18.9	7.8	-2.7	47.8	46.6	3.7	-2.5
Trazodone	555	547	2.5	-1.4	1247	1305	1.8	4.7
Venlafaxine	61.6	61.7	1.5	0.1	142	149	2.1	5.1
Vortioxetine	22.8	22.6	0.8	-0.8	49.8	52.6	1.2	5.7

*Dihydro-bupropion as a sum of erythro and threo-dihydro-bupropion

Table 4 Intraassay results [CV%]

Analyte	Sample	Measured value [µg/L]	CV	Sample	Measured value [µg/L]	CV
		Mean (n=8)	[%]		Mean (n=8)	[%]
Agomelatine	MS9482 Level I	30.3	4.4	MS9482 Level II	311	1.8
Atomoxetine	MS9482 Level I	395	7.4	MS9482 Level II	947	3.6
Bupropion	MS9482 Level I	41.2	2.6	MS9482 Level II	98.5	2.1
Citalopram	MS9482 Level I	43.7	2.5	MS9482 Level II	108	2.0
Clomethiazole	MS9482 Level I	475	4.9	MS9482 Level II	2823	1.9
Desmethylcitalopram	MS9482 Level I	48.3	3.7	MS9482 Level II	116	2.9
Desmethylfluoxetine	MS9482 Level I	115	3.4	MS9482 Level II	283	3.3
Desmethylmianserine	MS9482 Level I	31.3	2.8	MS9482 Level II	75.4	3.2
Desmethylmirtazapine	MS9482 Level I	41.1	2.3	MS9482 Level II	91.6	2.8
Desmethylsertraline	MS9482 Level I	39.0	3.1	MS9482 Level II	93.7	4.5
Dosulepin	MS9482 Level I	42.1	3.0	MS9482 Level II	98.5	1.9
Duloxetine	MS9482 Level I	54.0	4.1	MS9482 Level II	127	2.1
Dihydro-Bupropion*	MS9482 Level I	260	1.4	MS9482 Level II	617	2.2
Fluoxetine	MS9482 Level I	101	3.5	MS9482 Level II	240	3.1
Fluvoxamine	MS9482 Level I	99.9	2.2	MS9482 Level II	243	2.4
Guanfacine	MS9482 Level I	2.47	6.5	MS9482 Level II	6.08	2.6
Hydroxybupropion	MS9482 Level I	410	2.6	MS9482 Level II	974	1.4
Methylphenidate	MS9482 Level I	9.21	2.3	MS9482 Level II	21.6	2.7
Mianserine	MS9482 Level I	31.0	1.4	MS9482 Level II	74.3	0.6
Milnacipran	MS9482 Level I	63.6	3.7	MS9482 Level II	159	4.4
Mirtazapine	MS9482 Level I	34.1	2.2	MS9482 Level II	80.5	2.0
Moclobemide	MS9482 Level I	467	2.6	MS9482 Level II	1055	2.8
Nefazodone	MS9482 Level I	98.9	1.1	MS9482 Level II	231	1.4
O-Desmethyltramadol	MS9482 Level I	206	2.3	MS9482 Level II	492	4.1
O-Desmethylvenlafaxine	MS9482 Level I	106	2.9	MS9482 Level II	249	1.6
Opipramol	MS9482 Level I	105	2.2	MS9482 Level II	250	2.5
Paroxetine	MS9482 Level I	41.2	3.4	MS9482 Level II	98.5	2.1
Reboxetine	MS9482 Level I	132	2.5	MS9482 Level II	318	2.1
Ritalinic acid	MS9482 Level I	73.0	3.2	MS9482 Level II	167.2	3.9
Sertraline	MS9482 Level I	29.7	2.2	MS9482 Level II	150.8	2.3
Tianeptine	MS9482 Level I	31.2	2.1	MS9482 Level II	72.7	2.5
Tramadol	MS9482 Level I	231	2.7	MS9482 Level II	525	2.7
Tranylcypromine	MS9482 Level I	19.3	7.6	MS9482 Level II	47.0	4.0
Trazodone	MS9482 Level I	550	2.1	MS9482 Level II	1322	1.8
Venlafaxine	MS9482 Level I	61.5	1.1	MS9482 Level II	149	1.7
Vortioxetine	MS9482 Level I	22.3	1.7	MS9482 Level II	52.6	1.6

*Dihydro-bupropion as a sum of erythro and threo-dihydro-bupropion

Table 5 Linearity evaluation, including LLOQ / LOD

	Linear Range [µg/L]	R^2	LLOQ [µg/L]	LOD [µg/L]
Agomelatine	4.95 - 683	0.999	4.95	1.65
Atomoxetine	69.0 - 2065	0.992	69.0	23.0
Bupropion	3.40 - 224	1.000	3.40	1.13
Citalopram	6.75 - 233	1.000	6.75	2.25
Clomethiazole	110 - 6336	0.995	110	36.7
Desmethylcitalopram	8.45 - 271	0.997	8.45	2.82
Desmethylfluoxetine	20.1 - 672	0.996	20.1	6.7
Desmethylmianserine	10.7 - 185	0.998	10.7	3.57
Desmethylmirtazapine	12.4 - 194	0.993	12.4	4.13
Desmethylsertraline	12.6 - 199	0.999	12.6	4.2
Dosulepin	3.80 - 234	0.999	3.80	1.27
Duloxetine	17.5 - 274	0.991	17.5	5.83
Dihydro-Bupropion*	25.5 - 1578	0.999	25.5	8.50
Fluoxetine	31.2 - 535	0.995	31.2	10.4
Fluvoxamine	31.7 - 527	0.999	31.7	10.6
Guanfacine	0.834 - 13.7	0.998	0.834	0.278
Hydroxybupropion	130 - 2075	0.996	130	43.3
Methylphenidate	0.705 - 47.8	0.999	0.705	0.235
Mianserine	5.20 - 173	1.000	5.20	1.73
Milnacipran	22.4 - 397	0.993	22.4	7.47
Mirtazapine	5.65 - 179	0.999	5.65	1.88
Moclobemide	53.3 - 2691	0.997	53.3	17.8
Nefazodone	7.40 - 465	0.999	7.40	2.47
O-Desmethyltramadol	73.9 - 1185	0.994	73.9	24.6
O-Desmethylvenlafaxine	18.9 - 579	0.999	18.9	6.3
Opipramol	8.80 - 590	0.997	8.80	2.93
Paroxetine	12.6 - 220	0.997	12.6	4.2
Reboxetine	23.6 - 752	0.999	23.6	7.87
Ritalinic acid	13.6 - 382	0.991	13.6	4.53
Sertraline	4.70 - 293	0.999	4.70	1.57
Tianeptine	2.88 - 165	0.992	2.88	0.96
Tramadol	75.4 - 1138	0.998	75.4	25.1
Tranylcypromine	6.15 - 108	0.999	6.15	2.05
Trazodone	144 - 2552	0.994	144	48.0
Venlafaxine	20.6 - 349	1.000	20.6	6.87
Vortioxetine	0.751 - 118	0.999	0.751	0.250

*Dihydro-bupropion as a sum of erythro and threo-dihydro-bupropion

■ Conclusion

The ClinMass® TDM Kit System for Antidepressants in Serum / Plasma (order no. MS9000 and MS9400) was successfully verified on the CLAM-2040 with the analytical system LCMS-8060 from Shimadzu.

All 37 analytes passed the acceptance criteria for accuracy (trueness, precision) and linearity.

The lower limit of quantification (LLOQ) was below published clinical reference ranges.

■ References

1. Instruction Manual, ClinMass® TDM Kit System, Antidepressants in Serum / Plasma, RECIPE ® Chemicals + Instruments GmbH

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Shimadzu Corporation
www.shimadzu.com/an/

SHIMADZU Europa GmbH,
www.shimadzu.eu