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Targeted Comprehensive Screening of Neuropsychiatric Drugs in Serum by LC-MS/MS: Method Development and Three-Year Clinical Utility

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Background: The increasing prevalence of neuropsychiatric medication misuse highlights the critical need for efficient drug screening in clinical laboratories. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) application for broad serum drug screening remains limited in many clinical settings. Serum analysis is particularly valuable for identifying current drug exposure, confirming the cause of intoxication, and monitoring patient medication adherence.

Purpose: This study aimed to develop and validate an LC-MS/MS method using multiple reaction monitoring followed by information-dependent acquisition-triggered enhanced product ion scans for the simultaneous qualitative identification of 77 neuropsychiatric drugs, and to present its large-scale clinical application over three years.

Methods: Validation included precision, accuracy, recovery, matrix effects, selectivity, and interference. A minimal serum volume (20 µL) underwent simple protein precipitation, with preparation time under 15 minutes per batch. This method was applied to 1,021 clinical serum specimens.

Results: Chromatographic separation was completed in 15 minutes. The precisions (CV) for calculated concentrations and retention times were < 30% and < 1.5%, respectively. Accuracy was assessed for 60 of the 77 analytes (100% agreement). Mean recovery and matrix effects were 93.1% and 99.9%, respectively. Most test orders originated from psychiatry and neurology outpatients. Although fewer orders came from the Department of Emergency Medicine (EM), the EM patients exhibited a higher positivity rate. The most frequently detected drug classes were benzodiazepines (37.9%), antidepressants (30.7%), and antipsychotics (19.7%). Common specific analytes included clonazepam/7-aminoclonazepam (10.2%) and lorazepam (10.0%).

Conclusion: We developed and validated a LC-MS/MS method for the simultaneous detection of a wide range of neuropsychiatric drugs. The method proved essential for verifying medication history, identifying non-adherence, and diagnosing acute intoxication. Our findings highlight the critical role of broad-spectrum serum screening in managing medication-related emergencies and optimizing long-term therapeutic care.

Keywords: drug screening, mass spectrometry, serum, benzodiazepines, antidepressants, antipsychotics

Introduction

Certain drugs, particularly those targeting the central nervous system, such as benzodiazepines, antidepressants, antipsychotics, and anticonvulsants, carry a high risk of overdose and abuse if not properly prescribed. The misuse and abuse

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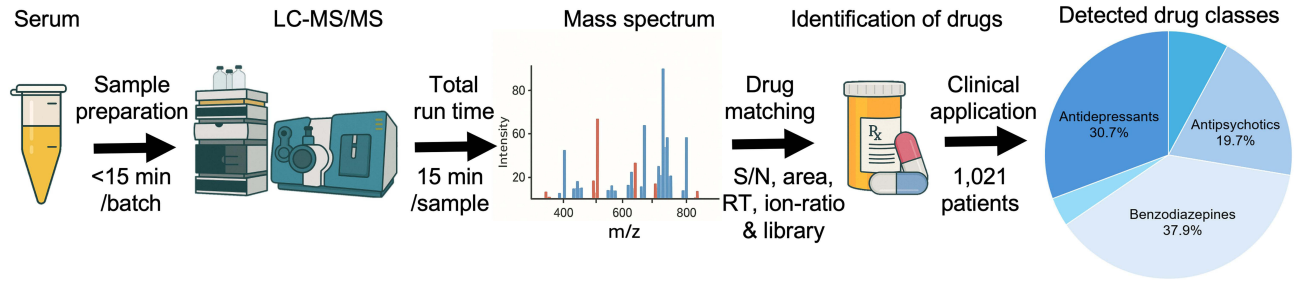
Graphical Abstract



of these drugs have been shown to cause psychological and physical dependence, which can be life-threatening.[1–5] To ensure safe and effective medication management, it is necessary to develop a comprehensive drug screening method that can accurately and timely detect the various medications a patient is currently taking. Immunoassays have been widely employed to detect the presence of drugs due to their ease of use and automation.[6] However, these methods are susceptible to many cross-reactivities and often include limited compounds in the scope of testing.[7,8] Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has emerged as the platform of choice for comprehensive drug screening in many laboratories.[9–15] Its widespread adoption is primarily due to enhanced sensitivity, selectivity, and suitability for high-throughput analysis of multiple analytes.[9]

Comprehensive drug screening using MS/MS methods in clinical laboratories is limited, especially in Korea, where most tests are performed on urine specimens for several illicit drugs.[16] To address this, our laboratory developed an LC-MS/MS drug screening method that specifically uses serum samples. Drug screening tests using serum have distinct purposes from those using urine. Urine drug tests are primarily used to detect illicit drug exposure because drug metabolites accumulate in urine and can be detected for several days after initial use.[17] In contrast, a serum drug screening test reflects a more recent phase, making it useful for identifying current drug exposure, confirming patient adherence to prescribed medication, and verifying abstinence from non-prescribed drugs.[18]

Despite the clinical necessity, the development of comprehensive serum screening assays presents significant analytical challenges. Human serum contains a high concentration of proteins and lipids that can induce substantial matrix effects, potentially compromising the sensitivity and accuracy of LC-MS/MS assays.[19,20] Furthermore, many neuropsychiatric medications circulate at low therapeutic concentrations and are characterized by high protein-binding affinities.[21]

In this study, we present a method for developing and validating an LC-MS/MS method for simultaneous detection of 77 neuropsychiatric medications and metabolites in serum, and share our experience from its large-scale clinical application over three years.

Materials and Method

Chemicals and Reagents

Formic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA), and HPLC-grade methanol was obtained from Burdick & Jackson (Muskegon, MI, USA). All analytes and internal standards (IS) were purchased from Chromsystems (Munich, Germany). These included the following MassCheck® series: Antidepressant 1/EXTENDED Plasma Control (Level 2), Antiepileptic Drugs/EXTENDED Plasma Control (Level 1), Neuroleptics 1/EXTENDED Plasma Control (Level 2), Tricyclic Antidepressants TCA 1 Plasma Control (Level 1), Drugs of Abuse Testing Urine Control Set (Level 2), and Internal Standard Set Neuroleptics 2/Extended 2.

Preparation of Standard and Clinical Specimens

A single-level calibrator with positive and negative quality control (QC) materials was used. The blank serum, which was verified as drug-free (no history of drug administration and confirmed negative for all target analytes tested), was used as

the negative QC and pooled for the preparation of the calibrator and positive QC samples. The calibrator and positive QC were prepared by spiking the drug analytes into pooled blank serum. The concentrations of the calibrator and the positive QC samples are provided in Table 1. The IS working solution was prepared by spiking 1 mL of the 4 IS solutions (IS-A to IS-D) into 300 mL of methanol. The specific multiple reaction monitoring (MRM) transition and retention time (RT) for these IS were: IS-A (RT 8.0 min, m/z 456.1→293.0); IS-B (RT 6.6 min, m/z 331.0→109.1); IS-C (RT 5.9 min, m/z 270.1→195.1); and IS-D (RT 4.0 min, 274.0→256.0). All reagents were stored at −30°C prior to use.

Table 1 Analyte List (77 Drugs and Metabolites) with Corresponding Concentrations of Calibrator and Positive Quality Control (QC)

Analyte	Concentration		
	Calibrator	Positive QC	Unit
10-Hydroxycarbazepine	0.8	0.6	μg/mL
7-Aminoclonazepam	21.3	14.2	ng/mL
7-Aminoflunitrazepam	25.4	16.9	ng/mL
7-Aminonitrazepam	28.8	19.2	ng/mL
9-OH-risperidone	6.7	4.5	ng/mL
Allobarbital	0.081	0.054	μg/mL
Alpha-Hydroxyalprazolam	25.5	17.0	ng/mL
Alpha-Hydroxymidazolam	19.4	12.9	ng/mL
Alpha-Hydroxytriazolam	25.3	16.9	ng/mL
Alprazolam	26.0	17.3	ng/mL
Amitriptyline	26.5	17.7	ng/mL
Amobarbital	0.069	0.046	μg/mL
Aripiprazole	29.9	19.9	ng/mL
Barbital	0.099	0.066	μg/mL
Bromazepam	26.5	17.7	ng/mL
Brotizolam	30.6	20.4	ng/mL
Butalbital	0.089	0.059	μg/mL
Carbamazepine	0.3	0.2	μg/mL
Chlordiazepoxide	26.2	17.5	ng/mL
Citalopram	13.4	8.9	ng/mL
Clobazam	23.3	15.5	ng/mL
Clonazepam	26.3	17.5	ng/mL
Clozapine	55.8	37.2	ng/mL
Demoxepam	29.4	19.6	ng/mL
Desalkylflurazepam	25.8	17.2	ng/mL

(*Continued*)

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Table 1 (Continued).

Analyte	Concentration		
	Calibrator	Positive QC	Unit
Desipramine	26.8	17.9	ng/mL
Desmethylflunitrazepam	26.7	17.8	ng/mL
Diazepam	26.6	17.7	ng/mL
Duloxetine	11.2	7.5	ng/mL
Estazolam	25.8	17.2	ng/mL
Flunitrazepam	26.4	17.6	ng/mL
Fluoxetine	36.5	24.3	ng/mL
Flurazepam	25.4	16.9	ng/mL
Fluvoxamine	19.7	13.1	ng/mL
Gabapentin	0.4	0.3	μg/mL
Haloperidol	1.8	1.2	ng/mL
Imipramine	6.2	4.1	ng/mL
Lacosamide	0.2	0.1	μg/mL
Lamotrigine	0.3	0.2	μg/mL
Levetiracetam	1.6	1.1	μg/mL
Lorazepam	28.5	19.0	ng/mL
Lormetazepam	28.2	18.8	ng/mL
Medazepam	30.4	20.3	ng/mL
Midazolam	22.7	15.1	ng/mL
Mirtazapine	20.2	13.5	ng/mL
Nitrazepam	29.4	19.6	ng/mL
Norclobazam	26.9	17.9	ng/mL
Nordiazepam	25.3	16.9	ng/mL
Norfluoxetine	39.4	26.3	ng/mL
Nortriptyline	27.8	18.5	ng/mL
Norvenlafaxine	35.8	23.9	ng/mL
Olanzapine	7.1	4.7	ng/mL
Oxazepam	24.6	16.4	ng/mL
Paroxetine	7.5	5.0	ng/mL
Phenylethylmalonamide	0.2	0.1	μg/mL
Pentobarbital	0.074	0.049	μg/mL
Perampanel	19.4	12.9	ng/mL

(*Continued*)

Table 1 (Continued).

Analyte	Concentration		
	Calibrator	Positive QC	Unit
Phenobarbital	0.080	0.053	μg/mL
Phenytoin	0.6	0.4	μg/mL
Prazepam	26.7	17.8	ng/mL
Pregabalin	0.2	0.1	μg/mL
Primidone	0.5	0.3	μg/mL
Quetiapine	77.3	51.5	ng/mL
Risperidone	1.4	0.9	ng/mL
Rufinamide	0.8	0.5	μg/mL
Secbutabarbital	0.086	0.057	μg/mL
Secobarbital	0.087	0.058	μg/mL
Sertraline	9.9	6.6	ng/mL
Temazepam	30.0	20.0	ng/mL
Thiopental	0.068	0.045	μg/mL
Topiramate	0.3	0.2	μg/mL
Triazolam	28.4	18.9	ng/mL
Venlafaxine	13.2	8.8	ng/mL
Zaleplon	27.3	18.2	ng/mL
Zolpidem	28.0	18.7	ng/mL
Zonisamide	1.0	0.6	μg/mL
Zopiclone	27.2	18.1	ng/mL

For a fast and simple sample preparation, 20 μL of patient serum sample was mixed with 60 μL of methanol containing IS. The mixed solution was then centrifuged at 12,500 rpm for 10 min. A 30 μL volume of the supernatant was transferred directly to the autosampler of the LC-MS/MS system for analysis. The final injection volume of each sample was 3 μL.

Instrumentation and Analytical Conditions of LC-MS/MS

LC-MS/MS analysis was performed on a SCIEX QTrap 5500+ mass spectrometer (SCIEX, Foster City, CA, USA) coupled with an Agilent 1290 Infinity II HPLC system (Agilent Technologies, Santa Clara, CA, USA). Chromatographic separation was achieved on a Kinetex Biphenyl column (150 × 2.1 mm, 2.6 μm; Phenomenex, Torrance, CA, USA) maintained at 50 °C. The mobile phases consisted of 0.1% formic acid in distilled water (mobile phase A) and 0.1% formic acid in methanol (mobile phase B). The gradient elusion was initiated with 90% A, followed by a linear decrease to 70% A over 0.1 min, 45% A over next 3.9 min, held for 3 min, and re-equilibrated at 90% A from 11.1 to 15 min at a rate of 0.3 mL/min.

An electrospray ionization (ESI) was employed operating in both positive and negative ion modes. The positive ion spray voltage was set at 5500 V, and the negative ion spray voltage was set at −4500 V. The optimized ESI source parameters were as follows: source temperature, 550 °C; curtain gas (N_2), 35 psi; collision gas (N_2), 10 psi; ion source gas 1 (N_2), 50 psi; and ion source gas 2 (N_2), 55 psi.

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The detection was performed using MRM mode as survey scan, followed by an information-dependent acquisition (IDA)-triggered enhanced product ion (EPI) scan. The characteristic MRM transitions for 77 analytes are provided in Table 2. The IDA scan intensity threshold was set to 500 counts per second (CPS) to ensure the detection of low-concentration analytes. To mitigate the risk of false-positive triggers from background noise, the system was configured for MRM-triggered IDA; an EPI

Table 2 MRM Transitions and Optimized Parameters for Analytes Detection

Analyte	RT (Min)	Precursor Ion (m/z)	Product Ion 1 (m/z)	DP (V)	CE (V)	CXP (V)	Product Ion 2 (m/z)	DP (V)	CE (V)	CXP (V)
10-Hydroxycarbazepine	5.3	255.1	194.1	70.0	70.0	12.0	192.0	120.0	90.0	12.0
7-Aminoclonazepam	4.6	286.1	121.1	110.0	37.0	13.0	250.1	130.0	29.0	13.0
7-Aminoflunitrazepam	5.7	284.0	135.1	90.0	40.0	12.0	227.0	130.0	34.0	12.0
7-Aminonitrazepam	3.3	252.0	121.0	130.0	40.0	7.0	94.1	90.0	50.0	14.0
9-OH-risperidone	5.6	427.2	207.2	80.0	55.0	12.0	179.2	80.0	60.0	12.0
Allobarbital	4.3	207.0	42.0	−110.0	−45.0	−8.0	164.0	−110.0	−15.0	−11.0
Alpha-Hydroxyalprazolam	7.8	325.1	297.1	130.0	40.0	10.0	205.0	130.0	45.0	10.0
Alpha-Hydroxymidazolam	7.0	342.1	168.1	90.0	50.0	11.0	203.1	90.0	52.0	10.0
Alpha-Hydroxytriazolam	7.7	359.1	331.1	150.0	40.0	12.0	176.1	150.0	35.0	12.0
Alprazolam	8.2	309.1	281.1	160.0	35.0	10.0	205.1	130.0	50.0	10.0
Amitriptyline	7.1	278.2	233.0	90.0	25.0	12.0	91.1	90.0	35.0	12.0
Amobarbital	6.1	225.0	42.0	−130.0	−60.0	−7.0	182.0	−110.0	−20.0	−10.0
Aripiprazole	7.5	448.1	285.2	140.0	35.0	12.0	176.1	140.0	45.0	12.0
Barbital	3.3	183.0	42.0	−110.0	−45.0	−8.0	140.0	−120.0	−10.0	−9.0
Bromazepam	7.1	316.0	182.0	70.0	45.0	8.0	209.2	80.0	40.0	8.0
Brotizolam	8.5	393.0	314.0	90.0	35.0	8.0	279.2	110.0	35.0	6.0
Butalbital	5.4	223.0	42.0	−130.0	−45.0	−7.0	155.0	−130.0	−35.0	−10.0
Carbamazepine	7.1	237.0	194.0	65.0	60.0	12.0	192.0	90.0	85.0	12.0
Chlordiazepoxide	6.4	300.0	227.0	70.0	36.0	12.0	282.1	130.0	31.0	8.0
Citalopram	6.0	325.0	109.1	80.0	35.0	12.0	262.0	80.0	30.0	12.0
Clobazam	8.0	301.0	259.0	90.0	31.0	6.0	224.0	110.0	35.0	6.0
Clonazepam	7.5	316.0	270.0	90.0	39.0	11.0	214.0	130.0	45.0	15.0
Clozapine	5.6	327.0	270.0	110.0	35.0	12.0	192.0	110.0	60.0	12.0
Demoxepam	7.3	287.0	269.0	90.0	45.0	10.0	105.0	110.0	30.0	15.0
Desalkylflurazepam	7.7	289.1	140.0	110.0	40.0	10.0	226.0	110.0	38.0	16.0
Desipramine	6.8	267.2	72.1	50.0	20.0	4.0	193.2	50.0	50.0	4.0
Desmethylflunitrazepam	7.3	300.0	254.0	130.0	30.0	11.0	198.0	130.0	45.0	11.0
Diazepam	8.6	285.1	154.0	130.0	35.0	8.0	193.0	170.0	40.0	10.0
Duloxetine	6.9	298.1	44.0	50.0	45.0	12.0	154.0	50.0	15.0	12.0

(*Continued*)

Table 2 (Continued).

Analyte	RT (Min)	Precursor Ion (m/z)	Product Ion 1 (m/z)	DP (V)	CE (V)	CXP (V)	Product Ion 2 (m/z)	DP (V)	CE (V)	CXP (V)
Estazolam	8.1	295.0	267.1	110.0	37.0	14.0	205.0	90.0	56.0	5.0
Flunitrazepam	8.1	314.0	268.0	130.0	37.0	12.0	211.0	110.0	43.0	8.0
Fluoxetine	6.4	310.1	148.1	30.0	15.0	14.0	88.0	30.0	25.0	8.0
Flurazepam	6.4	388.0	315.0	110.0	34.0	10.0	288.0	90.0	34.0	8.0
Fluvoxamine	6.3	319.2	71.0	80.0	35.0	8.0	258.1	80.0	20.0	8.0
Gabapentin	2.6	172.2	154.0	70.0	36.0	8.0	137.0	70.0	42.0	10.0
Haloperidol	6.2	376.1	123.0	130.0	60.0	4.0	165.1	130.0	35.0	4.0
Imipramine	6.9	280.9	85.9	50.0	25.0	12.0	58.1	50.0	70.0	12.0
Lacosamide	4.3	252.1	74.1	60.0	40.0	8.0	92.0	60.0	55.0	8.0
Lamotrigine	3.8	256.0	211.0	110.0	60.0	12.0	166.0	110.0	65.0	12.0
Levetiracetam	2.7	171.0	126.0	70.0	49.0	8.0	69.0	70.0	70.0	16.0
Lorazepam	7.4	321.1	275.1	90.0	30.0	10.0	229.1	90.0	45.0	10.0
Lormetazepam	8.1	335.0	298.0	90.0	30.0	15.0	317.0	30.0	20.0	8.0
Medazepam	6.9	271.1	242.2	70.0	25.0	11.0	165.1	70.0	60.0	11.0
Midazolam	6.7	326.1	291.1	50.0	45.0	18.0	249.1	50.0	49.0	12.0
Mirtazapine	5.3	266.1	195.1	130.0	40.0	12.0	72.0	130.0	25.0	12.0
Nitrazepam	7.5	282.1	236.2	90.0	34.0	12.0	180.1	90.0	61.0	10.0
Norclobazam	7.4	287.0	245.0	90.0	31.0	8.0	210.0	110.0	40.0	8.0
Nordiazepam	7.9	271.1	140.1	90.0	37.0	10.0	158.0	130.0	35.0	14.0
Norfluoxetine	6.3	296.0	30.2	30.0	40.0	14.0	134.0	30.0	20.0	12.0
Nortriptyline	7.0	264.1	233.2	50.0	20.0	12.0	105.1	50.0	30.0	12.0
Norvenlafaxine	3.4	264.2	58.0	30.0	50.0	8.0	246.0	30.0	20.0	8.0
Olanzapine	2.5	314.1	256.2	90.0	30.0	12.0	198.1	90.0	55.0	12.0
Oxazepam	7.6	287.0	241.0	70.0	36.0	11.0	268.9	30.0	25.0	8.0
Paroxetine	6.9	330.0	192.0	100.0	30.0	12.0	123.0	100.0	35.0	12.0
Phenylethylmalonamide	3.2	207.0	162.0	100.0	30.0	8.0	91.0	110.0	85.0	8.0
Pentobarbital	6.1	225.1	42.0	−130.0	−45.0	−7.0	182.0	−130.0	−25.0	−10.0
Perampanel	8.7	350.1	219.1	160.0	55.0	14.0	77.1	160.0	80.0	6.0
Phenobarbital	5.1	231.0	42.0	−110.0	−100.0	−7.0	188.0	−90.0	−10.0	−7.0
Phenytoin	6.5	253.1	104.0	161.0	80.0	12.0	182.0	161.0	40.0	8.0
Prazepam	9.0	325.1	271.0	90.0	40.0	8.0	140.1	90.0	49.0	8.0
Pregabalin	2.3	160.1	142.1	70.0	30.0	10.0	124.0	70.0	35.0	8.0
Primidone	4.0	219.1	162.0	121.0	30.0	10.0	91.1	150.0	25.0	8.0

(*Continued*)

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Table 2 (Continued).

Analyte	RT (Min)	Precursor Ion (m/z)	Product Ion 1 (m/z)	DP (V)	CE (V)	CXP (V)	Product Ion 2 (m/z)	DP (V)	CE (V)	CXP (V)
Quetiapine	6.6	385.2	280.1	110.0	40.0	12.0	221.1	110.0	65.0	12.0
Risperidone	6.2	411.1	191.1	100.0	40.0	12.0	69.0	100.0	80.0	12.0
Rufinamide	5.0	239.1	127.0	100.0	85.0	8.0	101.0	115.0	95.0	8.0
Secbutabarbital	5.2	211.0	42.0	−130.0	−50.0	−7.0	168.0	−90.0	−20.0	−9.0
Secobarbital	6.5	237.0	42.0	−130.0	−60.0	−7.0	194.0	−130.0	−10.0	−10.0
Sertraline	7.4	305.9	159.1	50.0	40.0	12.0	274.9	50.0	20.0	12.0
Temazepam	8.2	301.1	255.1	90.0	45.0	8.0	283.0	90.0	20.0	10.0
Thiopental	7.0	241.0	57.7	−110.0	−60.0	−8.0	101.0	−110.0	−25.0	−8.0
Topiramate	5.6	357.1	264.0	50.0	35.0	6.0	282.0	50.0	30.0	18.0
Triazolam	8.1	343.0	308.2	80.0	40.0	8.0	315.1	80.0	40.0	8.0
Venlafaxine	5.1	278.2	58.0	30.0	55.0	8.0	121.0	30.0	30.0	8.0
Zaleplon	7.9	306.2	236.3	45.0	45.0	11.0	264.2	25.0	25.0	11.0
Zolpidem	5.8	308.1	235.1	60.0	60.0	12.0	263.0	35.0	35.0	8.0
Zonisamide	4.0	211.0	119.0	−110.0	−45.0	−10.0	147.1	−110.0	−40.0	−10.0
Zopiclone	4.8	389.1	245.2	23.0	23.0	6.0	112.0	75.0	75.0	13.0

Abbreviations: CE, collision energy; CXP, collision cell exit potential; DP, declustering potential; m/z, mass-to-charge ratio; MRM, multiple reaction monitoring; RT, retention time.

scan was only initiated upon the detection of a specific target analyte's pre-defined MRM transition. Up to eight most intense peaks exceeding this threshold were evaluated by dependent EPI scan. The EPI scans were performed at a rate of 10,000 Da/s after a fixed filled time of 25 ms. The identification was further ensured by requiring a spectral library match for each triggered EPI spectrum. SCIEX OS software, version 3.4.5.828, was used for data acquisition and analysis.

Method Validation

Precision, accuracy, extraction recovery, matrix effect, carryover, selectivity, and interference were evaluated according to the Clinical and Laboratory Standards (CLSI) C62-A.[22] Precision was assessed using a positive QC sample. The RT and calculated concentrations were used to determine the coefficient of variation (CV) for within-run, between-run, and total precision. Within-run precision was assessed by the analysis of 6 replicates in a single assay, and between-run precision was evaluated over a period of 20 days. Accuracy was evaluated using 31 samples consisting of proficiency testing (PT) materials from the College of American Pathologists (CAP) or LGC Standards and verified patient specimens with known medication histories. Extraction recovery was assessed in 10 replicate analyses by comparing the ratio of the peak area of the analytes in pre-spiked samples to those in post-spiked samples. Matrix effect was evaluated in 6 analyses by comparing the peak area of analytes spiked in the solution mobile phase with that in post-spiked samples. Carryover was evaluated by injecting a blank sample after running the high-concentration spiked sample. Selectivity was evaluated using 30 independent blank serum samples. Potential interference from 86 compounds, including antiarrhythmics, TCA, drugs of abuse, and psychostimulants, was evaluated across 5 different blank serum sources.

The limits of identification (LOI) was determined as the lowest concentration for which the following criteria are present: (1) Signal-to-noise ratio (S/N) ≥10; (2) acquired spectra matched library spectra with a purity greater than 70%; (3) RT deviation was less than 2.5% of the expected value; (4) ion ratio deviation was less than 30% of the expected value; and (5) area intensity exceeded 10,000.

Clinical Application

From March 2022 to March 2025, 1,045 serum drug screening tests from 1,021 patients were performed at Samsung Medical Center. Only the first test result of each patient was included in the data analysis. Demographic and clinical information, including age, gender, visit type, department, indication for testing, diagnosis, and past medical conditions, was obtained by reviewing the patients' electronic medical records alongside the drug screening test results.

Results

Method Validation

The sample preparation time was less than 15 minutes per batch, with a total chromatographic run time of 15 minutes per sample. Total ion chromatograms (TIC) of the calibrator and a patient serum sample are presented in Figure 1.

The LOI and analytical performance for each analyte are summarized in Table 3. For total precision of calculated concentrations, all 77 analytes had a CV of less than 30%, with 84% of analytes remaining under 20%. The total precision for the RT was less than a 1.5% CV. The mean extraction recovery was 93.1%. The mean IS-normalized matrix effect of the analytes ranged from 83.9% (for phenytoin) to 119.4% (for citalopram), with an average of 99.9%. The assay demonstrated no endogenous interfering peaks across all 30 independent blank serum samples. Furthermore, no significant carryover or exogenous interference was detected from the 86 evaluated drug compounds. For the qualitative accuracy assessment, 60 analytes, (some of which were included multiple times; Table 4), were evaluated, and all of them showed 100% agreement. The remaining 17 analytes could not be evaluated due to a lack of appropriate testing materials.

Clinical Application

A total of 1,021 cases were analyzed over a three-year period. The test was predominantly ordered by the Departments of Psychiatry (PSY), accounting for 731 cases (71.6%), followed by Neurology (NR) with 263 cases (25.8%), with almost all cases involving outpatients (97.5%). The Department of Emergency Medicine (EM) accounted for 27 ordered cases (2.6%).

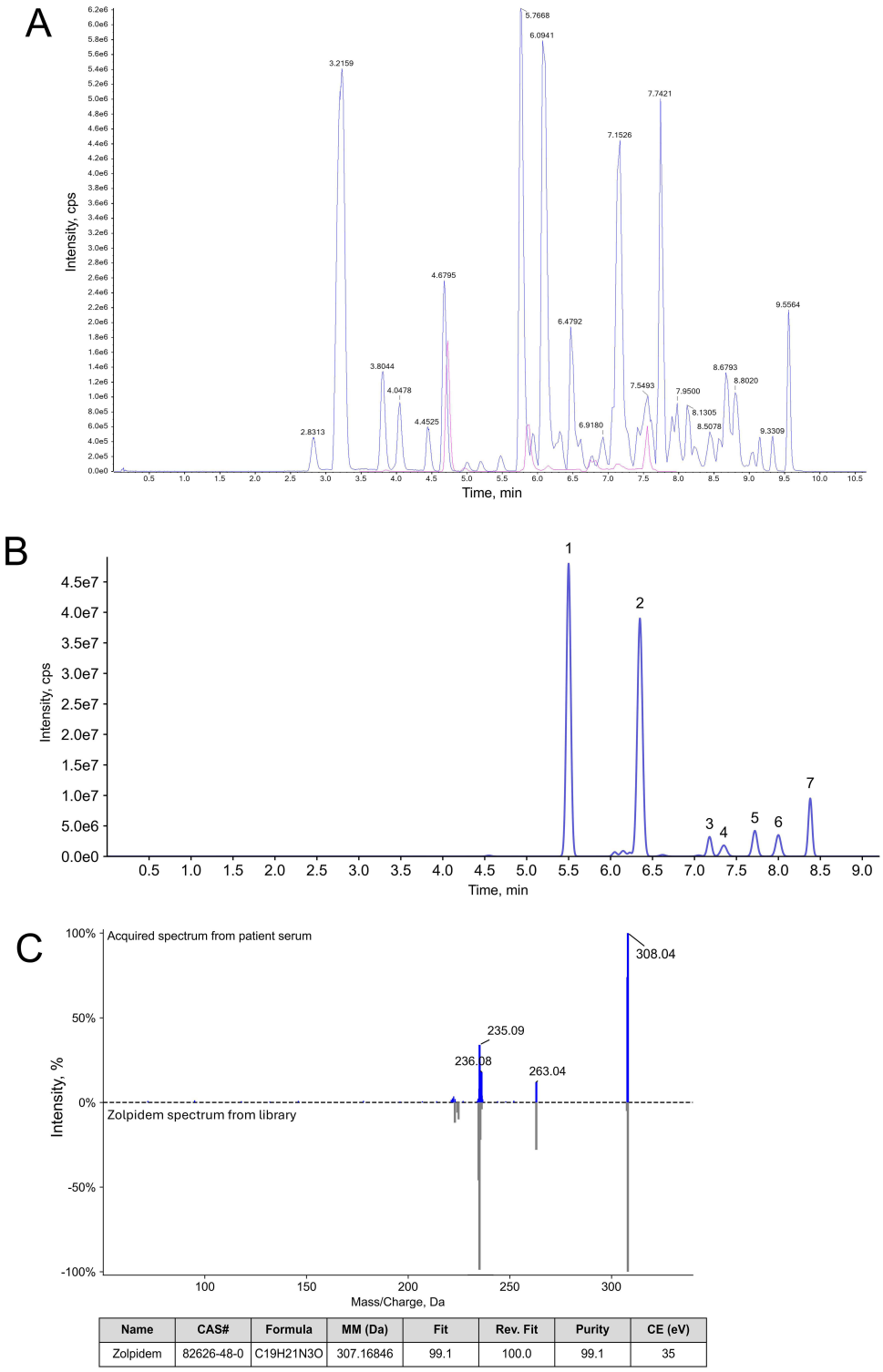
Patients tested in the EM had a higher positive test rate (85.2%) compared to outpatients (59.5%; Figure 2A). Detailed information for the 27 patients tested in the EM is presented in Table 5. The most common test indication was suspected drug intoxication, accounting for 17 cases (63%). Of these, 14 had underlying psychiatric disorders, primarily depressive disorder and bipolar affective disorder. Notably, all patients with such underlying psychiatric disorders tested positive on the drug screening test.

The frequently detected drug compounds were: clonazepam and 7-aminoclonazepam (159 detections, 10.2%), lorazepam (155, 10.0%), and alprazolam and alpha-hydroxyalprazolam (136, 8.8%) (Table 6). Regarding drug classes, benzodiazepines were the most frequently detected, accounting for 37.9% of detections. They were followed by antidepressants (30.7%), antipsychotics (19.7%), and anticonvulsants (7.9%). This detection trend was similarly observed in both outpatients and EM patients, except that antipsychotics were detected more commonly than antidepressants in EM patients (Figure 2B and C).

Discussion

In this study, we developed a simple and fast LC-MS/MS method appropriate for clinical laboratory use, and shared our experience of its large-scale clinical application. LC-MS/MS procedures have been introduced for screening for multiple drugs in serum (Table 7). These previously published methods included a number of drug compounds ranging from 39 to 700 items, with the most frequently included drug classes being antipsychotics, antidepressants, and drugs of abuse. For this study, we selected antipsychotics and antidepressants, as in the existing studies, along with anticonvulsants, and sedative-hypnotics including benzodiazepines and barbiturates.

For sample preparation prior to drug detection, liquid-liquid extraction (LLE) or solid-phase extraction (SPE) was dominantly utilized,[9,12–15] both of which require multiple manual steps. The present study used a protein precipitation (PPT) procedure to minimize the laborious manual steps for faster and simpler preparation. While PPT is efficient for



Name	CAS#	Formula	MM (Da)	Fit	Rev. Fit	Purity	CE (eV)
Zolpidem	82626-48-0	C19H21N3O	307.16846	99.1	100.0	99.1	35

Figure 1 (**A**) Total ion chromatograms (TIC) of the calibrator containing 77 analytes with their respective retention time (RT). The color-coded peaks represent the polarity of the Multiple Reaction Monitoring (MRM) transitions: blue lines indicate positive ionization mode and pink lines indicate negative ionization mode. (**B**) TIC of a patient serum specimen positive for multiple neuropsychiatric medications. The numeric labels correspond to the following identified compounds: (1) zolpidem, (2) quetiapine, (3) lorazepam, (4) oxazepam, (5) nordiazepam, (6) temazepam, and (7) diazepam. (**C**) Enhanced Product Ion (EPI) spectrum confirmation for zolpidem. The upper panel shows the acquired spectrum from the patient's serum, while the lower panel (mirrored) displays the reference library spectrum.

Abbreviations: CAS#, Chemical Abstracts Service registry number; CE, collision energy; CPS, counts per second; MM (Da), monoisotopic mass in Daltons; Rev. Fit, reverse fit score.

Table 3 LOI, Precision, Extraction Recovery, and Matrix Effect for Target Analytes

Analyte	LOI (ng/mL)	Precision (% CV)						Recovery (SD), % (n=10)	Matrix Effect (SD), % (n=6)
		Total (n=26)		Within-Run (n=6)		Between-Run (n=20)			
		RT	Calculated Concentration	RT	Calculated Concentration	RT	Calculated Concentration		
10-Hydroxycarbazepine	100	0.2	13.9	0.1	8.7	0.2	15.3	86.4 (6.4)	92.5 (7.4)
7-Aminoclonazepam	2.1	0.9	6.2	0.1	2.8	0.9	6.7	74.8 (5.8)	104.8 (3.5)
7-Aminoflunitrazepam	2.5	0.7	12.5	0.2	13.7	0.8	11.7	86.0 (7.8)	101.3 (5.2)
7-Aminonitrazepam	3.0	1.1	6.4	0.2	2.8	1.1	7.1	80.8 (10.3)	98.3 (1.4)
9-OH-risperidone	0.7	0.5	12.4	0.2	11.3	0.6	13.0	86.6 (7.6)	98.6 (3.3)
Allobarbital	50.0	0.3	9.4	0.2	8.0	0.3	9.9	97.1 (17.7)	99.0 (4.4)
Alpha-Hydroxyalprazolam	2.5	0.1	17.6	0.0	13.6	0.1	17.7	88.9 (4.8)	110.3 (14.4)
Alpha-Hydroxymidazolam	7.0	0.7	21.5	0.0	11.0	0.8	23.9	93.7 (18.2)	95.7 (8.7)
Alpha-Hydroxytriazolam	8.0	0.1	10.4	0.1	9.9	0.1	10.6	86.4 (5.6)	109.9 (11.6)
Alprazolam	2.0	0.1	15.3	0.0	6.5	0.1	15.5	92.3 (6.3)	103.9 (14.6)
Amitriptyline	2.7	0.5	18.6	0.1	16.7	0.5	18.2	88.7 (5.1)	103.0 (13.2)
Amobarbital	50.0	0.2	14.9	0.2	12.4	0.2	15.6	94.9 (6.5)	96.1 (6.8)
Aripiprazole	3.0	0.5	12.8	0.1	9.8	0.5	11.6	84.3 (7.3)	104.2 (10.0)
Barbital	70.0	0.3	7.2	0.2	4.7	0.3	7.9	98.0 (24.1)	114.3 (12.8)
Bromazepam	2.7	0.2	13.4	0.1	15.6	0.2	12.3	91.1 (11.3)	101.4 (12.7)
Brotizolam	3.1	0.1	17.3	0.1	14.4	0.1	17.0	93.2 (12.7)	89.1 (10.8)
Butalbital	60.0	0.2	17.1	0.2	18.7	0.3	15.0	103.9 (9.9)	106.1 (9.1)
Carbamazepine	30.0	0.1	10.7	0.1	11.3	0.1	10.7	90.8 (4.9)	101.0 (12.2)
Chlordiazepoxide	2.5	0.9	17.6	0.1	16.5	1.0	18.1	88.7 (7.2)	99.8 (9.0)
Citalopram	1.3	0.5	14.9	0.1	11.1	0.5	15.7	85.2 (5.1)	119.4 (7.6)

(*Continued*)

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Table 3 (Continued).

Analyte	LOI (ng/mL)	Precision (% CV)						Recovery (SD), % (n=10)	Matrix Effect (SD), % (n=6)
		Total (n=26)		Within-Run (n=6)		Between-Run (n=20)			
		RT	Calculated Concentration	RT	Calculated Concentration	RT	Calculated Concentration		
Clobazam	2.3	0.1	20.3	0.1	15.1	0.1	20.9	101.6 (6.0)	88.7 (10.8)
Clonazepam	3.0	0.1	14.4	0.1	7.7	0.1	16.0	98.3 (6.3)	107.0 (12.4)
Clozapine	6.0	0.6	12.1	0.1	12.3	0.7	11.4	86.3 (7.2)	100.1 (9.5)
Demoxepam	20.0	0.1	21.3	0.1	15.1	0.1	21.1	93.5 (8.1)	92.7 (11.8)
Desalkylflurazepam	3.0	0.1	12.3	0.0	13.0	0.1	12.1	95.0 (6.8)	93.1 (11.8)
Desipramine	3.0	0.4	18.7	0.1	16.7	0.5	19.0	86.7 (10.2)	93.6 (5.4)
Desmethylflunitrazepam	9.0	0.1	13.6	0.1	13.5	0.1	13.4	93.8 (7.0)	94.3 (11.4)
Diazepam	3.0	0.1	11.4	0.1	12.1	0.1	10.9	87.9 (13.9)	92.6 (13.8)
Duloxetine	11.0	0.4	28.0	0.1	23.8	0.5	22.3	86.6 (20.2)	108.2 (15.3)
Estazolam	3.0	0.1	13.0	0.0	11.4	0.1	13.4	92.7 (4.5)	107.3 (9.5)
Flunitrazepam	3.0	0.1	17.9	0.1	12.8	0.1	19.3	97.2 (3.9)	96.9 (15.0)
Fluoxetine	12.0	0.4	24.3	0.1	19.5	0.4	19.1	91.8 (15.5)	88.6 (11.8)
Flurazepam	3.0	0.5	18.8	0.1	18.3	0.5	17.4	89.8 (8.0)	97.5 (15.8)
Fluvoxamine	6.0	0.4	18.2	0.1	20.5	0.4	18.0	93.9 (18.2)	102.4 (3.2)
Gabapentin	40.0	0.4	5.7	0.1	3.6	0.5	6.2	87.8 (4.4)	103.6 (3.1)
Haloperidol	0.6	0.4	18.9	0.1	15.4	0.5	19.8	86.2 (10.6)	111.4 (11.3)
Imipramine	3.0	0.5	19.0	0.1	11.4	0.5	21.0	87.7 (14.5)	100.8 (10.5)
Lacosamide	60.0	0.2	10.6	0.2	11.9	0.2	10.1	82.4 (13.8)	103.0 (4.7)
Lamotrigine	30.0	0.6	7.8	0.2	3.3	0.6	8.2	85.5 (12.0)	101.1 (4.1)
Levetiracetam	150	0.2	6.3	0.2	3.0	0.2	7.0	93.1 (6.5)	101.1 (4.0)

(*Continued*)

Table 3 (Continued).

Analyte	LOI (ng/mL)	Precision (% CV)						Recovery (SD), % (n=10)	Matrix Effect (SD), % (n=6)
		Total (n=26)		Within-Run (n=6)		Between-Run (n=20)			
		RT	Calculated Concentration	RT	Calculated Concentration	RT	Calculated Concentration		
Lorazepam	3.0	0.1	13.4	0.1	11.9	0.1	14.2	93.2 (12.7)	106.0 (10.6)
Lormetazepam	10.0	0.1	20.0	0.1	15.8	0.1	21.5	92.3 (3.6)	91.5 (11.4)
Medazepam	3.0	0.4	13.1	0.1	7.8	0.5	14.4	89.5 (7.3)	98.4 (114)
Midazolam	2.0	0.4	15.8	0.1	14.3	0.5	16.3	84.4 (8.7)	90.5 (3.8)
Mirtazapine	2.0	0.6	12.3	0.1	14.8	0.7	11.7	81.7 (8.4)	100.8 (7.1)
Nitrazepam	3.0	0.1	15.6	0.1	11.1	0.1	16.9	96.1 (6.2)	98.0 (10.6)
Norclobazam	2.5	0.1	13.9	0.1	7.5	0.1	15.1	99.2 (10.1)	101.2 (15.3)
Nordiazepam	2.5	0.2	12.0	0.0	17.6	0.2	10.3	90.2 (4.9)	97.0 (7.4)
Norfluoxetine	13.0	0.3	17.5	0.1	16.4	0.4	18.2	93.5 (7.6)	99.2 (11.5)
Nortriptyline	3.0	0.5	15.6	0.1	9.9	0.5	17.2	82.4 (8.5)	95.7 (9.3)
Norvenlafaxine	5.0	0.5	7.8	0.2	5.0	0.5	7.9	82.9 (9.4)	103.2 (1.3)
Olanzapine	7.1	0.7	16.5	0.2	8.3	0.8	17.8	111.7 (29.0)	116.7 (7.1)
Oxazepam	9.0	0.1	12.8	0.1	12.6	0.1	12.8	86.4 (5.6)	106.3 (12.8)
Paroxetine	5.0	0.4	25.6	0.1	20.6	0.4	23.0	84.7 (11.6)	87.9 (7.8)
Phenylethylmalonamide	20.0	0.2	6.2	0.1	2.3	0.2	6.8	85.8 (7.9)	102.5 (5.0)
Pentobarbital	50.0	0.2	15.8	0.2	14.0	0.2	15.6	92.3 (6.7)	96.7 (14.7)
Perampanel	2.0	0.1	9.1	0.0	11.3	0.1	8.7	107.9 (10.1)	96.1 (10.8)
Phenobarbital	50.0	0.2	16.8	0.1	14.9	0.2	17.6	97.3 (9.3)	87.5 (4.8)
Phenytoin	60.0	0.1	22.0	0.1	18.9	0.1	17.8	99.5 (25.8)	83.9 (11.5)
Prazepam	3.0	0.1	11.0	0.1	10.1	0.1	11.1	89.7 (8.5)	114.9 (15.2)

(*Continued*)

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Table 3 (Continued).

Analyte	LOI (ng/mL)	Precision (% CV)						Recovery (SD), % (n=10)	Matrix Effect (SD), % (n=6)
		Total (n=26)		Within-Run (n=6)		Between-Run (n=20)			
		RT	Calculated Concentration	RT	Calculated Concentration	RT	Calculated Concentration		
Pregabalin	20.0	0.3	5.2	0.2	3.3	0.4	5.7	91.7 (6.6)	107.8 (2.7)
Primidone	50.0	0.2	5.8	0.1	2.6	0.2	6.5	96.6 (15.3)	97.0 (4.2)
Quetiapine	3.0	0.4	22.0	0.1	16.5	0.5	21.8	97.0 (13.8)	91.4 (9.8)
Risperidone	0.9	0.5	17.9	0.1	17.7	0.5	18.4	90.8 (12.1)	85.6 (4.3)
Rufinamide	70.0	0.2	13.7	0.2	15.7	0.2	11.4	92.3 (12.1)	103.9 (5.7)
Secbutabarbital	60.0	0.2	15.4	0.2	16.6	0.2	14.8	101.2 (24.3)	97.2 (4.3)
Secobarbital	60.0	0.1	22.8	0.1	21.5	0.2	17.9	98.7 (13.5)	92.1 (14.0)
Sertraline	3.0	0.5	15.4	0.1	12.5	0.5	15.0	90.9 (7.1)	102.6 (14.8)
Temazepam	3.0	0.1	18.2	0.1	17.0	0.1	18.9	91.8 (4.5)	97.1 (14.6)
Thiopental	70.0	0.1	22.3	0.1	13.2	0.1	21.6	98.7 (15.6)	89.0 (14.9)
Topiramate	50.0	0.2	24.7	0.1	18.6	0.2	21.0	99.5 (9.3)	111.4 (5.4)
Triazolam	10.0	0.1	12.9	0.0	11.1	0.1	12.5	91.6 (2.7)	108.0 (16.1)
Venlafaxine	3.0	0.6	17.7	0.1	14.4	0.6	18.3	88.6 (10.8)	99.1 (8.5)
Zaleplon	9.0	0.1	14.1	0.1	11.3	0.1	13.8	102.8 (8.1)	99.5 (7.5)
Zolpidem	3.0	0.5	15.3	0.1	14.6	0.6	15.9	88.7 (8.2)	113.3 (8.9)
Zonisamide	100	0.3	6.0	0.2	4.3	0.3	6.1	78.6 (22.3)	94.4 (5.5)
Zopiclone	9.0	0.5	6.1	0.2	4.7	0.6	6.6	218.8 (70.5)	96.2 (2.7)

Abbreviations: CV, coefficient of variation; LOI, limit of identification; SD, standard deviation.

clinical turnaround times, it is recognized that the PPT-processed extracts contain higher levels of residual phospholipids and proteins compared to those from LLE or SPE. These components can lead to column fouling and ion source contamination, if unmanaged. To mitigate these associated risks, our laboratory adheres to a system-maintenance protocol, which includes monitoring of column backpressure for every batch and performing column washing before and after each analytical run. Furthermore, while the required sample volume for various methods ranged from 50 µL to

Table 4 Qualitative Accuracy Assessment Results

Analyte	% Agreement (Agreement Rate)
10-Hydroxycarbazepine	100% (5/5)
7-Aminoclonazepam	100% (2/2)
7-Aminoflunitrazepam	100% (1/1)
7-Aminonitrazepam	
9-OH-risperidone	100% (1/1)
Allobarbital	100% (2/2)
Alpha-Hydroxyalprazolam	100% (2/2)
Alpha-Hydroxymidazolam	
Alpha-Hydroxytriazolam	
Alprazolam	100% (6/6)
Amitriptyline	100% (2/2)
Amobarbital	100% (3/3)
Aripiprazole	100% (3/3)
Barbital	
Bromazepam	100% (2/2)
Brotizolam	
Butalbital	
Carbamazepine	100% (2/2)
Chlordiazepoxide	
Citalopram	100% (2/2)
Clobazam	100% (4/4)
Clonazepam	100% (4/4)
Clozapine	100% (1/1)
Demoxepam	
Desalkylflurazepam	
Desipramine	100% (1/1)
Desmethylflunitrazepam	100% (1/1)
Diazepam	100% (3/3)
Duloxetine	100% (4/4)
Estazolam	
Flunitrazepam	100% (1/1)
Fluoxetine	100% (2/2)

(*Continued*)

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Table 4 (Continued).

Analyte	% Agreement (Agreement Rate)
Flurazepam	
Fluvoxamine	100% (1/1)
Gabapentin	100% (1/1)
Haloperidol	100% (1/1)
Imipramine	100% (1/1)
Lacosamide	100% (5/5)
Lamotrigine	100% (2/2)
Levetiracetam	100% (4/4)
Lorazepam	100% (7/7)
Lormetazepam	
Medazepam	
Midazolam	100% (3/3)
Mirtazapine	100% (2/2)
Nitrazepam	100% (1/1)
Norclobazam	100% (4/4)
Nordiazepam	100% (3/3)
Norfluoxetine	100% (2/2)
Nortriptyline	100% (2/2)
Norvenlafaxine	100% (3/3)
Olanzapine	100% (3/3)
Oxazepam	100% (2/2)
Paroxetine	100% (2/2)
Phenylethylmalonamide	
Pentobarbital	100% (3/3)
Perampanel	100% (3/3)
Phenobarbital	100% (1/1)
Phenytoin	100% (1/1)
Prazepam	
Pregabalin	100% (3/3)
Primidone	100% (1/1)
Quetiapine	100% (4/4)
Risperidone	100% (1/1)
Rufinamide	100% (1/1)

(*Continued*)

Table 4 (Continued).

Analyte	% Agreement (Agreement Rate)
Secbutabarbital	
Secobarbital	100% (1/1)
Sertraline	100% (1/1)
Temazepam	100% (2/2)
Thiopental	100% (2/2)
Topiramate	100% (3/3)
Triazolam	
Venlafaxine	100% (2/2)
Zaleplon	100% (1/1)
Zolpidem	100% (2/2)
Zonisamide	100% (2/2)
Zopiclone	100% (1/1)
Total	100% (138/138)

1 mL, the present study requires the least amount. The minimal sample volume and streamlined sample preparation offer significant advantages for a clinical laboratory setting.

Even with a simple and rapid sample preparation, the sensitivity of this assay was comparable. A recent study has reported cutoff values of benzodiazepines, antidepressants, anticonvulsants, antipsychotics, and barbiturates in a qualitative LC-MS/MS drug screening method.[9] The reported cutoff values were 5–20 ng/mL, 5.0–50 ng/mL, 100 ng/mL, 5.0–100 ng/mL, and 100 ng/mL for benzodiazepines, antidepressants, anticonvulsants, antipsychotics, and barbiturates, respectively. In contrast, in this study, the cutoff values for these drug classes were 2–20 ng/mL, 1.3–13 ng/mL, 20–150 ng/mL, 0.6–7.1 ng/mL, and 2.0–70 ng/mL, respectively. The present assay showed similar or superior sensitivity compared with the previously published method.[9]

Previous methods[9,10,12–15] were primarily described from a methodological standpoint, with limited examples of their practical application to patient specimens. While a few methods tested patient specimens,[10,14] this was only for the purpose of comparison with a pre-existing validated method. In contrast, the method in the current study was routinely applied to over 1,000 patient serum specimens over three years for the purpose of guiding medical treatment.

At our institution, the test was predominantly applied for sleeping disorders by the NR, mood disorders by the PSY, and suspected acute drug poisoning by the EM. For patients with sleeping or mood disorders, the test was used to verify their current drug history, as anamnestic information does not always provide enough evidence of all drugs taken. For patients with suspected drug intoxication, the test was ordered to identify the specific drug causing the overdose because the clinical symptoms of intoxication can be non-specific. We observed that some outpatients, despite submitting prescriptions from other hospitals, had negative test results. Such cases suggested medication non-adherence, highlighting the need for re-prescribing and patient education.

The drugs most frequently detected in outpatients were benzodiazepines, followed by antidepressants and antipsychotics. These same drug classes were also the most common findings in EM patients with suspected intoxication. This indicates that drug intoxication can result from commonly prescribed medications, not just illegal drugs, which highlights the need for a detection method that can accurately and timely identify therapeutic drugs.

A critical consideration in multi-target screening is the potential for chromatographic co-elution to interfere with mass spectral purity. In this study, although several analytes exhibited overlapping RT due to the broad nature of the 77-

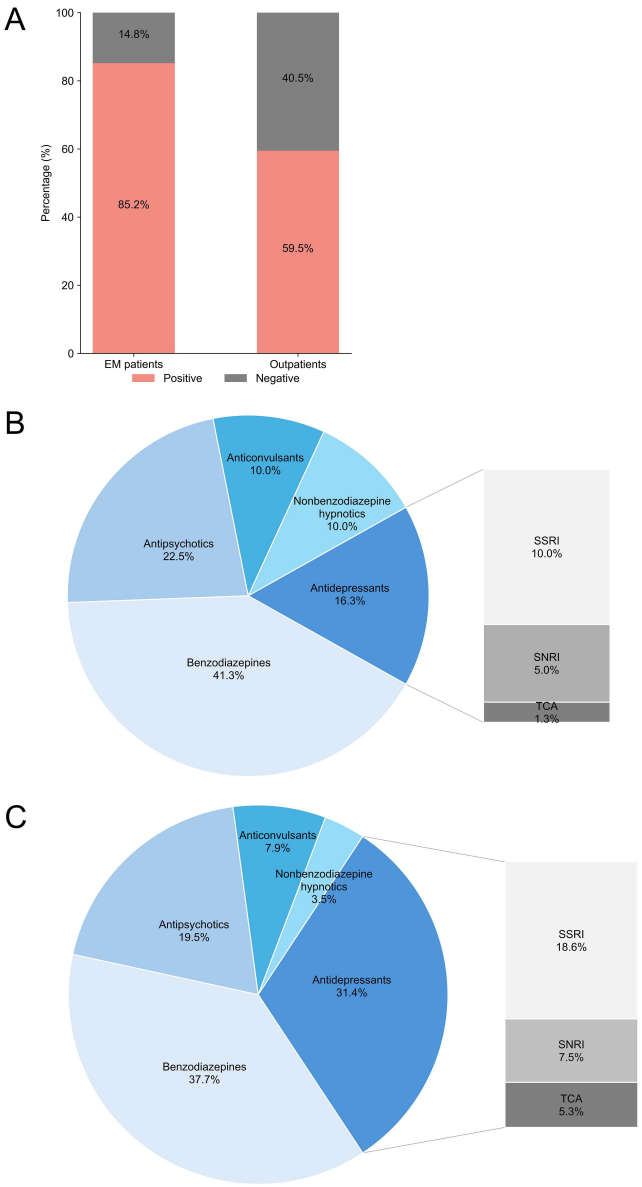

Figure 2 Drug screening test results in the EM patients and outpatients. (**A**) Comparison of the positive and negative rate. (**B**) Distribution of drug classes detected in EM patients. (**C**) Distribution of drug classes detected in outpatients.
Abbreviations: EM, department of emergency medicine; SNRI, serotonin-norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant.

compound panel, the analytical specificity was preserved through a dual-layer confirmation process. The MRM transition acted as a selective filter for the precursor ion, while the subsequent EPI scan provided a distinctive fragmentation profile. By utilizing a library matching strategy, the method successfully isolated target spectra from co-eluting interferences.

Despite the high identification performance provided by this approach, several limitations must be acknowledged. First, the analytical accuracy was not fully evaluated for all compounds due to the unavailability of reference materials. Specifically, accuracy testing could not be performed for 17 analytes, and for 20 analytes, accuracy was verified using only a single reference material. Future studies will prioritize the acquisition of additional reference materials and clinical samples to achieve a more comprehensive validation of the entire 77-analyte panel.

Second, the absence of stable-isotope-labeled internal standards (SIL-IS) for every analyte represents a technical constraint. In this qualitative screening assay, 4 IS were selected to cover the early, middle, and late elution periods; these

Table 5 Characteristics and Drug Screening Results of 27 EM Patients

No.	Sex	Age (Years)	Test Indication	Psychiatric History	Test Result	No. of Detected Drug	Detected Drugs
1	M	23	Dysarthria	ADHD	Positive	3	Clonazepam, 7-Aminoclonazepam, Aripiprazole
2	F	39	Dysarthria	None	Positive	11	Clonazepam, 7-Aminoclonazepam, Flunitrazepam, 7-Aminoflunitrazepam, Desmethylflunitrazepam, Alprazolam, Clobazam, Norclobazam, Fluoxetine, Norfluoxetine, Zolpidem
3	M	46	Mental change	None	Negative	0	None
4	F	72	Mental change	None	Negative	0	None
5	F	83	Mental change	None	Positive	2	Clozapine, Gabapentin
6	F	87	Mental change	None	Positive	3	Triazolam, alpha-Hydroxytriazolam, Nortriptyline
7	F	51	Mental change	None	Positive	5	Lorazepam, Lacosamide, Quetiapine, Sertraline, Zolpidem
8	M	68	Not specified	None	Positive	1	Pregabalin
9	M	63	Paraparesis	None	Positive	1	Zolpidem
10	M	53	Suspected drug intoxication	None	Positive	3	Zolpidem, Pregabalin, 10-Hydroxycarbazepine
11	F	42	Suspected drug intoxication	Depressive disorder	Positive	6	Clonazepam, 7-Aminoclonazepam, Lorazepam, Quetiapine, Zolpidem, Zopiclone
12	F	88	Suspected drug intoxication	Depressive disorder	Positive	5	Clonazepam, 7-Aminoclonazepam, Alprazolam, alpha-Hydroxyalprazolam, Citalopram
13	F	31	Suspected drug intoxication	Depressive disorder	Positive	10	Clonazepam, 7-Aminoclonazepam, Alprazolam, Aripiprazole, Diazepam, Nordiazepam, Duloxetine, Quetiapine, Venlafaxine, Norvenlafaxine
14	F	55	Suspected drug intoxication	Depressive disorder	Positive	2	Haloperidol, Lorazepam
15	M	45	Suspected drug intoxication	Bipolar affective disorder	Positive	2	Lorazepam, Olanzapine
16	F	36	Suspected drug intoxication	Depressive disorder	Positive	7	Clonazepam, 7-Aminoclonazepam, Risperidone, 9-OH-risperidone, Alprazolam, Aripiprazole, Lorazepam
17	F	24	Suspected drug intoxication	Anxiety disorder	Positive	8	Clonazepam, 7-Aminoclonazepam, Aripiprazole, Fluoxetine, Norfluoxetine, Norvenlafaxine, Quetiapine, Sertraline
18	F	38	Suspected drug intoxication	None	Negative	0	None
19	F	45	Suspected drug intoxication	Depressive disorder	Positive	14	Alprazolam, alpha-Hydroxyalprazolam, Clonazepam, 7-Aminoclonazepam, Diazepam, Nordiazepam, Temazepam, Oxazepam, Fluoxetine, Norfluoxetine, Duloxetine, Lorazepam, Quetiapine, Zolpidem
20	F	16	Suspected drug intoxication	Depressive disorder	Positive	2	Aripiprazole, Lamotrigine
21	F	21	Suspected drug intoxication	Bipolar affective disorder	Positive	5	Alprazolam, alpha-Hydroxyalprazolam, Clonazepam, 7-Aminoclonazepam, Sertraline
22	M	38	Suspected drug intoxication	Schizophrenia	Positive	5	Bromazepam, Lorazepam, Olanzapine, Quetiapine, Zolpidem

(*Continued*)

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Table 5 (Continued).

No.	Sex	Age (Years)	Test Indication	Psychiatric History	Test Result	No. of Detected Drug	Detected Drugs
23	F	26	Suspected drug intoxication	Bipolar affective disorder	Positive	2	Lamotrigine, Lorazepam
24	M	31	Suspected drug intoxication	None	Positive	2	Flurazepam, Desalkylflurazepam
25	F	29	Suspected drug intoxication	Schizophrenia	Positive	2	Haloperidol, Lorazepam
26	F	32	Suspected drug intoxication	Bipolar affective disorder	Positive	4	Haloperidol, Lamotrigine, Lorazepam, Norfluoxetine
27	M	54	Syncope	None	Negative	0	None

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; EM, Department of Emergency Medicine; No, number.

Table 6 Detected Drugs in 1,021 Clinical Serum Samples Over a Three-year Period

Drugs with Their Respective Metabolites	Number of Detection	Drug Classification
Clonazepam, 7-Aminoclonazepam	159	Benzodiazepines
Lorazepam	155	Benzodiazepines
Alprazolam, alpha-Hydroxyalprazolam	136	Benzodiazepines
Aripiprazole	129	Atypical antipsychotics
Citalopram	128	SSRI
Quetiapine	103	Atypical antipsychotics
Venlafaxine, Norvenlafaxine	102	SNRI
Fluoxetine, Norfluoxetine	80	SSRI
Diazepam, Nordiazepam, Temazepam, Oxazepam	69	Benzodiazepines
Zolpidem	58	Nonbenzodiazepine hypnotics
Mirtazapine	45	TCA
Sertraline	42	SSRI
Risperidone, 9-OH-risperidone	39	Atypical antipsychotics
Paroxetine	32	SSRI
Amitriptyline, Nortriptyline	31	TCA
Pregabalin	28	Anticonvulsants
Bromazepam	28	Benzodiazepines
Topiramate	25	Anticonvulsants
Lamotrigine	25	Anticonvulsants
Olanzapine	22	Atypical antipsychotics

(*Continued*)

Table 6 (Continued).

Drugs with Their Respective Metabolites	Number of Detection	Drug Classification
Flunitrazepam, 7-Aminoflunitrazepam, Desmethylflunitrazepam	13	Benzodiazepines
Duloxetine	12	SNRI
Gabapentin	12	Anticonvulsants
Flurazepam, Desalkylflurazepam	11	Benzodiazepines
Haloperidol	10	Typical antipsychotics
Clobazam, Norclobazam	9	Benzodiazepines
10-Hydroxycarbazepine	8	Anticonvulsants
Lacosamide	6	Anticonvulsants
Carbamazepine	6	Anticonvulsants
Zonisamide	5	Anticonvulsants
Levetiracetam	4	Anticonvulsants
Imipramine, Desipramine	4	TCA
Triazolam, alpha-Hydroxytriazolam	3	Benzodiazepines
Clozapine	3	Atypical antipsychotics
Chlordiazepoxide	3	Benzodiazepines
Midazolam, alpha-Hydroxymidazolam	2	Benzodiazepines
Phenytoin	2	Anticonvulsants
Perampanel	2	Anticonvulsants
Fluvoxamine	1	SSRI
Zaleplon	1	Nonbenzodiazepine hypnotics
Allobarbital	0	Barbiturates
Amobarbital	0	Barbiturates
Barbital	0	Barbiturates
Brotizolam	0	Benzodiazepines
Butalbital	0	Barbiturates
Demoxepam	0	Benzodiazepines
Estazolam	0	Benzodiazepines
Lormetazepam	0	Benzodiazepines
Medazepam	0	Benzodiazepines
Nitrazepam, 7-Aminonitrazepam	0	Benzodiazepines
Pentobarbital	0	Barbiturates
Prazepam	0	Benzodiazepines
Primidone, Phenylethylmalonamide, Phenobarbital	0	Barbiturates

(*Continued*)

https://doi.org/10.2147/DDDT.S585730

Table 6 (Continued).

Drugs with Their Respective Metabolites	Number of Detection	Drug Classification
Rufinamide	0	Anticonvulsants
Secbutabarbital	0	Barbiturates
Secobarbital	0	Barbiturates
Thiopental	0	Barbiturates
Zopiclone	0	Nonbenzodiazepine hypnotics

Abbreviations: SNRI, serotonin-norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; TCA, Tricyclic Antidepressant.

Table 7 LC-MS(/MS) Procedure for Screening for Multiple Drugs, and/or Their Metabolites in Whole Blood, Plasma, or Serum

Compounds	Sample		IS/Calibration/ QC	Detection Mode	Validation Data	Application	Ref.
	Type (Volume)	Preparation					
78 drugs including anticonvulsants, antipsychotics, antidepressants, and sedative-hypnotics, such as barbiturates and benzodiazepines	S (20 μL)	PPT	4 IS/ 1-point calibration/ positive QC 1 negative QC	LC-MS/MS (Qtrap), RM, IDA	LOI, precision, accuracy, recovery, ME, carryover, selectivity	1021 patient samples	This study
127 drugs including anticonvulsants, antipsychotics, antidepressants, drugs of abuse and sedative-hypnotics such as benzodiazepines	S, U (100 μL)	SPE	122 IS/ 1-point calibration (U)/ 2 positive QC 1 negative QC	LC-MS/MS (QQQ), MRM	Precision, accuracy, ME, reinjection reproducibility, carryover, analyte/ run stability, interference, correlation of multiple instruments, method comparison	None	[9]
42 drugs of abuse	WB, P (50 μL)	PPT and filtration	20 IS/ 6-point calibration (P)/ 2 positive QC	LC-MS/MS (QQQ), MRM, IDA	LLOD, LLOQ, stability, precision, accuracy, recovery, ME, method comparison	43 patient samples	[10]
700 drugs including antidepressants, antipsychotics, drugs of abuse, etc	S, U (1mL)	LLE	2 IS/ Areas of the MRM signals of benzodiazepines used for calibration/ Spectral purity of the 2 IS used for QC	LC-MS/MS (Qtrap), MRM, IDA	None	4 postmortem samples	[15]
54 drugs including antidepressants, antipsychotics, antiarrhythmics, etc	WB, S, U (1mL)	SPE	1 IS/ NA/ NA	LC-MS/MS (Qtrap), MRM, IDA	ME, recovery, method comparison	1 patient sample	[14]
39 sedative-hypnotics including barbiturates and benzodiazepines	S (0.5mL)	SPE	1 IS/ NA/ NA	LC-MS (Q), SIM	LOD, ME, recovery	1 postmortem sample	[12]
301 drugs including drugs of abuse, antidepressants, neuroleptics, cardiac drugs, and sedative-hypnotics including benzodiazepines	S (1mL)	LLE or SPE	3 IS/ NA/ NA	LC-MS/MS (Qtrap), MRM, IDA	None	None	[13]

Abbreviations: IS, internal standard; LLE, liquid-liquid extraction; LLOD, lower limit of detection; LLOQ, lower limit of quantification; LOD, limit of detection; ME, matrix effect; NA, not available; P, plasma; PPT, protein precipitation; Q, single quadrupole; QQQ, triple quadrupole; Qtrap, triple quadrupole-linear ion trap; Ref, reference; S, serum; SIM, selected ion monitoring; SPE, solid-phase extraction; U, urine; WB, whole blood.

were utilized to monitor chromatographic stability and ionization efficiency across the 15-minute run time. While the use of these 4 selected IS compensated for broad matrix effects, the lack of analyte-specific SIL-IS means that potential analyte-specific ion suppression or enhancement cannot be entirely ruled out.

Third, as this method was developed primarily for broad-spectrum qualitative screening, it does not provide precise quantitative concentration data. The lack of quantification may limit the clinical interpretation of exact drug exposure levels and their direct correlation with toxicological symptoms. To address this in clinical practice, our laboratory performs supplementary quantitative LC-MS/MS measurements for specific analytes following a positive screening result to provide definitive confirmatory data.

Finally, the generalizability of our findings may be constrained by the study's scope. The predominance of outpatient samples and the lack of external validation or inter-laboratory reproducibility assessments mean that performance may vary across different clinical populations or laboratory settings.

Conclusion

We developed and validated an LC-MS/MS method for the simultaneous detection of 77 neuropsychiatric drugs and metabolites in serum. This method was validated in accordance with international guidelines and successfully applied to routine clinical analyses, primarily to establish the baseline drug history and to identify acute drug poisoning. By providing rapid and broad-spectrum identification, the assay served as a critical first step in the diagnostic workflow, enabling timely clinical interventions in emergency settings. We anticipate this method will continue to serve as a reliable test for detecting drug compounds in patients and aid in their treatment management.

Data Sharing Statement

All data generated and analyzed during this study are available from the corresponding author upon reasonable request.

Ethics Approval and Informed Consent

This study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Institutional Review Board (IRB) of Samsung Medical Center (SMC 2024-01-032). Due to the retrospective nature of the data analysis, the requirement for informed consent was waived. All data were anonymized to ensure patient privacy and confidentiality.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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