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Application News

Triple Quadrupole Liquid Chromatograph Mass Spectrometer

Simultaneous Analysis of 27 Antidiabetic Drugs in Whole Blood by LC–MS/MS

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User Benefits

- 27 antidiabetic drugs with diverse chemical structures can be quantified in a single run.
- Antidiabetic drugs can be detected with high sensitivity at concentrations of 0.1 ng/mL in whole blood
- Analysis can be run on the same workflow and analytical conditions of "LC/MS/MS Forensic Toxicology Database."

■ Introduction

Antidiabetic (glucose-lowering) drugs, mainly prescribed for the treatment of diabetes, include many classes with different mechanisms of action, such as biguanides, sulfonylureas (SUs), DPP-4 inhibitors, and SGLT2 inhibitors. Overdose or polypharmacy use of these drugs causes severe hypoglycemia. It can lead to the loss of consciousness and may result in falls, traffic accidents, and other serious incidents. In recent years, some antidiabetic drugs have been promoted on social media as "weight-loss drugs," and their non-medical use for beauty care or diet purposes has become a social concern. In addition, these drugs have become easier to obtain through personal importation, raising concerns about adverse health effects from overdose or polypharmacy without medical supervision[1)].

This study introduces the evaluation results of a simultaneous LC-MS/MS method using the LCMS-8050RX for 27 antidiabetic drugs in blood.

Fig. 1 Nexera™ X3 + LCMS-8050RX

■ Sample Pretreatment

Human whole blood and ultrapure water were spiked with 27 antidiabetic drugs to prepare spiked samples and standard samples. Compound information is summarized in Table 1. Sample pretreatment was performed using a Micro Volume QuEChERS kit (P/N: S225-37870-91). Diazepam-*d*5 (0.2 µg/mL) was prepared in methanol and used as the internal standard (ISTD). The workflow of sample pretreatment is shown in Fig. 2.

200 µL of water, 300 µL of acetonitrile, 100 µL of whole blood, and 20 µL of ISTD were added to the Micro Volume QuEChERS kit and mixed thoroughly. After centrifugation, the supernatant was collected and evaporated to dryness under a stream of nitrogen. The residue was reconstituted in 100 µL of 20% methanol, and the resulting solution was used as the analytical sample.

Fig. 2 Flow of Sample Pretreatment Using Micro Volume QuEChERS Kit

Table 1 Target Antidiabetic Drugs

#	Name	Formula	M.W.	Drug class
1	Metformin	C4H11N5	129.10	Biguanide
2	Imeglimin	C6H13N5	155.12	Glimin
3	Buformin	C6H15N5	157.13	Biguanide
4	Vildagliptin	C17H25N3O2	303.19	DPP-4 inhibitor
5	Anagliptin	C19H25N7O2	383.21	DPP-4 inhibitor
6	Alogliptin	C18H21N5O2	339.17	DPP-4 inhibitor
7	Saxagliptin	C18H25N3O2	315.19	DPP-4 inhibitor
8	Trelagliptin	C18H20FN5O2	357.16	DPP-4 inhibitor
9	Omarigliptin	C17H20F2N4O3S	398.12	DPP-4 inhibitor
10	Sitagliptin	C16H15F6N5O	407.12	DPP-4 inhibitor
11	Teneligliptin	C22H30N6OS	426.22	DPP-4 inhibitor
12	Linagliptin	C25H28N8O2	472.23	DPP-4 inhibitor
13	Chlorpropamide	C10H13ClN2O3S	276.03	Sulfonylurea
14	Pioglitazone	C19H20N2O3S	356.12	Thiazolidinedione
15	Acetohexamide	C15H20N2O4S	324.11	Sulfonylurea
16	Empagliflozin	C23H27ClO7	450.14	SGLT2 inhibitor
17	Gliclazide	C15H21N3O3S	323.13	Sulfonylurea
18	Tofogliflozin	C22H26O6	386.17	SGLT2 inhibitor
19	Ipragliflozin	C21H21FO5S	404.11	SGLT2 inhibitor
20	Luseogliflozin	C23H30O6S	434.18	SGLT2 inhibitor
21	Dapagliflozin	C21H25ClO6	408.13	SGLT2 inhibitor
22	Glibenclamide	C23H28ClN3O5S	493.14	Sulfonylurea
23	Mitiglinide	C19H25NO3	315.18	Glinide
24	Canagliflozin	C24H25FO5S	444.14	SGLT2 inhibitor
25	Glimepiride	C24H34N4O5S	490.22	Sulfonylurea
26	Nateglinide	C19H27NO3	317.20	Glinide
27	Repaglinide	C27H36N2O4	452.27	Glinide

■ Analytical Conditions

The LC–MS/MS analytical conditions are shown in Table 2, and the transitions for each compound are shown in Table 3. Fig. 3 shows the MRM chromatograms obtained under these conditions.

Table 2 LC-MS/MS System and Conditions

System	:	Nexera X3
Column	:	Kinetex XB-C18 (100 mm × 2.1 mm I.D., 2.6 µm) (Phenomenex, P/N : 00D4496-AN)
Temperature	:	40 ˚C
Injection volume	:	1 µL
Mobile phases	:	10 mM Ammonium Formate + 0.1 % Formic acid Water 10 mM Ammonium Formate + 0.1 % Formic acid MeOH
Flow rate	:	0.3 mL/min
Time program (%B)	:	Refer to LC/MS/MS Forensic Toxicology Database
System	:	LCMS-8050RX (Corespray ESI)
Nebulizing gas	:	3 L/min
Drying gas	:	10 L/min
Heating gas	:	10 L/min
DL temp.	:	250 ˚C
Heat block temp.	:	400 ˚C
Interface temp.	:	300 ˚C

Table 3 Retention Times and MRM Transitions of Antidiabetic Drugs

#	Name	Retention time (min)	Precursor ion (*m/z*)		Product ion (*m/z*)
1	Metformin	0.777	$[M+H]^+$ 131.1	>	60.0 (CE -14 V) , 71.1 (CE -23 V) , 85.0 (CE -14 V)
2	Imeglimin	1.810	$[M+H]^+$ 156.1	>	113.1 (CE -19 V) , 71.0 (CE -30 V) , 68.0 (CE -35 V)
3	Buformin	2.210	$[M+H]^+$ 158.1	>	60.0 (CE -15 V) , 57.0 (CE -24 V) , 116.2 (CE -18 V)
4	Vildagliptin	2.618	$[M+H]^+$ 304.2	>	154.1 (CE -18 V) , 97.0 (CE -32 V) , 151.1 (CE -22 V)
5	Anagliptin	3.630	$[M+H]^+$ 384.2	>	160.0 (CE -31 V) , 231.1 (CE – 18 V) , 132.0 (CE -51 V)
6	Alogliptin	3.840	$[M+H]^+$ 340.0	>	116.1 (CE -33 V) , 323.1 (CE -18 V) , 266.6 (CE -21 V)
7	Saxagliptin	3.871	$[M+H]^+$ 316.2	>	180.1 (CE -20 V) , 119.2 (CE -45 V) , 163.0 (CE -37 V)
8	Trelagliptin	3.965	$[M+H]^+$ 358.2	>	341.1 (CE -20 V) , 107.1 (CE -60 V) , 134.1 (CE -35 V)
9	Omarigliptin	4.146	$[M+H]^+$ 399.1	>	153.1 (CE -29 V) , 127.0 (CE -58 V) , 382.1 (CE -19 V)
10	Sitagliptin	4.403	$[M+H]^+$ 408.1	>	235.2 (CE -18 V) , 174.0 (CE -28 V) , 193.0 (CE -25 V)
11	Teneligliptin	5.042	$[M+H]^+$ 427.2	>	243.2 (CE -28 V) , 68.1 (CE -50 V) , 267.2 (CE -28 V)
12	Linagliptin	5.216	$[M+H]^+$ 473.2	>	420.2 (CE -23 V) , 403.1 (CE -28 V) , 158.1 (CE -61 V)
13	Chlorpropamide	5.838	$[M+H]^+$ 277.0	>	111.0 (CE -31 V) , 175.0 (CE -19 V) , 192.0 (CE -20 V)
14	Pioglitazone	5.978	$[M+H]^+$ 357.1	>	134.0 (CE -28 V) , 119.0 (CE -47 V) , 106.0 (CE -47 V)
15	Acetohexamide	6.081	$[M+H]^+$ 325.1	>	243.0 (CE -12 V) , 119.1 (CE -28 V) , 183.0 (CE -20 V)
16	Empagliflozin	6.416	$[M+NH4]^+$ 468.2	>	71.1 (CE -27 V) , 355.0 (CE -14 V) , 397.0 (CE -15 V)
17	Gliclazide	6.538	$[M+NH4]^+$ 324.1	>	127.1 (CE -18 V) , 110.0 (CE -22 V) , 91.0 (CE -40 V)
18	Tofogliflozin	6.656	$[M+H]^+$ 387.2	>	267.1 (CE -15 V) , 119.0 (CE -30 V) , 369.1 (CE -10 V)
19	Ipragliflozin	6.721	$[M+NH4]^+$ 422.1	>	285.1 (CE -15 V) , 151.0 (CE -24 V) , 369.1 (CE -12 V)
20	Luseogliflozin	6.789	$[M+NH4]^+$ 452.2	>	135.0 (CE -21 V) , 417.2 (CE -12 V) , 269.1 (CE -24 V)
21	Dapagliflozin	6.831	$[M+NH4]^+$ 426.2	>	135.1 (CE -21 V) , 355.1 (CE -14 V) , 166.9 (CE -26 V)
22	Glibenclamide	7.193	$[M+H]^+$ 494.1	>	369.0 (CE -14 V) , 169.1 (CE -33 V) , 304.0 (CE -26 V)
23	Mitiglinide	7.215	$[M+H]^+$ 316.2	>	298.1 (CE -16 V) , 126.1 (CE -23 V) , 145.0 (CE -30 V)
24	Canagliflozin	7.315	$[M+NH4]^+$ 462.2	>	191.0 (CE -25 V) , 267.2 (CE -20 V) , 249.0 (CE -19 V)
25	Glimepiride	7.437	$[M+H]^+$ 491.2	>	352.1 (CE -14 V) , 126.0 (CE -27 V) , 103.1 (CE -44 V)
26	Nateglinide	7.564	$[M+H]^+$ 318.2	>	166.1 (CE -13 V) , 120.1 (CE -22 V) , 125.2 (CE -22 V)
27	Repaglinide	8.006	$[M+H]^+$ 453.3	>	230.2 (CE -30 V) , 162.1 (CE -21 V) , 174.1 (CE -45 V)

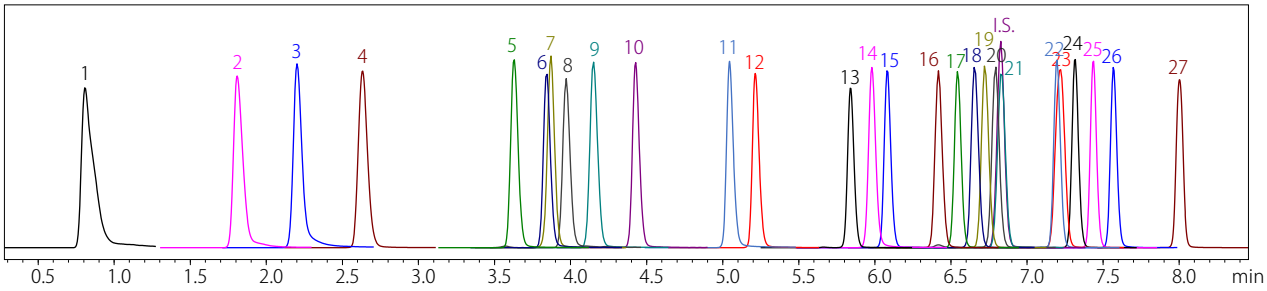

Fig. 3 MRM Chromatograms of 27 Antidiabetic Drugs and diazepam-d5 (ISTD)
1: Metformin, 2: Imeglimin, 3: Buformin, 4: Vildagliptin, 5: Anagliptin, 6: Alogliptin, 7: Saxagliptin, 8: Trelagliptin, 9: Omarigliptin, 10: Sitagliptin, 11: Teneligliptin, 12: Linagliptin, 13: Chlorpropamide, 14: Pioglitazone, 15: Acetohexamide, 16: Empagliflozin, 17: Gliclazide, 18: Tofogliflozin, 19: Ipragliflozin, 20: Luseogliflozin, 21: Dapagliflozin, 22: Glibenclamide, 23: Mitiglinide, 24: Canagliflozin, 25: Glimepiride, 26: Nateglinide, 27: Repaglinide, I.S.: Diazepam-d5

■ Validation Results

Calibration standards were spiked at whole-blood equivalent concentrations of 0.1, 0.5, 1.0, 5.0, 10, 50, 100, and 500 ng/mL to evaluate linearity and sensitivity. The calibration ranges and correlation coefficients are summarized in Table 4.

Good linearity (R > 0.995) was obtained over 0.1–100 ng/mL for three compounds (Omarigliptin, Pioglitazone, and Repaglinide), 0.1–200 ng/mL for nine compounds (Anagliptin, Alogliptin, Saxagliptin, Trelagliptin, Sitagliptin, Acetohexamide, Glibenclamide, Mitiglinide, and Nateglinide), and 0.1–500 ng/mL for the other 15 compounds. In addition, peaks were detected for all compounds even at the low concentration of 0.1 ng/mL. The MRM chromatograms for each compound are shown in Fig. 4.

Standard solutions were spiked to whole blood at a concentration of 20 ng/mL to evaluate the accuracy and repeatability (n = 5). The accuracy for all compounds are within 80–120% (Fig. 5). Repeatability, expressed as %RSD, was ≤ 7.9%, indicating that this method provides sufficient precision for the analysis of whole blood.

Table 4 Calibration range and Linearity

Name	Calibration range (ng/mL)	R	R^2
Metformin	0.1–500	0.9956	0.9912
Imeglimin	0.1–500	0.9991	0.9981
Buformin	0.1–500	0.9986	0.9972
Vildagliptin	0.1–500	0.9991	0.9982
Anagliptin	0.1–200	0.9998	0.9996
Alogliptin	0.1–200	0.9990	0.9979
Saxagliptin	0.1–200	0.9976	0.9953
Trelagliptin	0.1–200	0.9987	0.9974
Omarigliptin	0.1–100	0.9958	0.9916
Sitagliptin	0.1–200	0.9999	0.9999
Teneligliptin	0.1–500	0.9992	0.9983
Linagliptin	0.1–500	0.9980	0.9961
Chlorpropamide	0.1–500	0.9993	0.9986
Pioglitazone	0.1–100	0.9998	0.9995
Acetohexamide	0.1–200	0.9999	0.9997
Empagliflozin	0.1–500	0.9999	0.9999
Gliclazide	0.1–500	0.9995	0.9990
Tofogliflozin	0.1–500	0.9997	0.9993
Ipragliflozin	0.1–500	0.9983	0.9966
Luseogliflozin	0.1–500	0.9964	0.9928
Dapagliflozin	0.1–500	0.9994	0.9988
Glibenclamide	0.1–200	0.9995	0.9989
Mitiglinide	0.1–200	0.9998	0.9996
Canagliflozin	0.1–500	0.9995	0.9989
Glimepiride	0.1–500	0.9986	0.9971
Nateglinide	0.1–200	0.9993	0.9985
Repaglinide	0.1–100	0.9975	0.9950

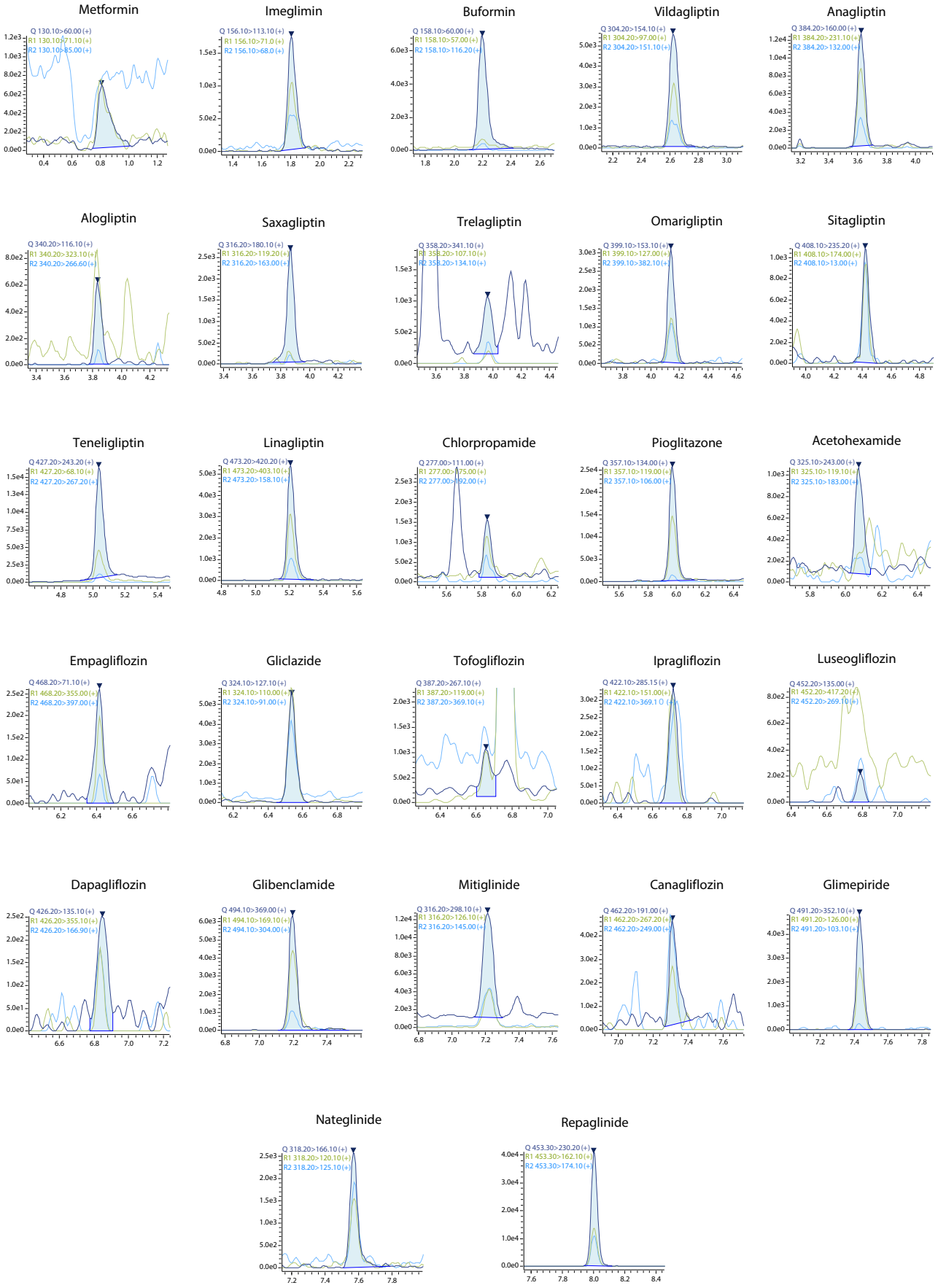

Fig. 4 MRM Chromatograms at 0.1 ng/mL in whole blood

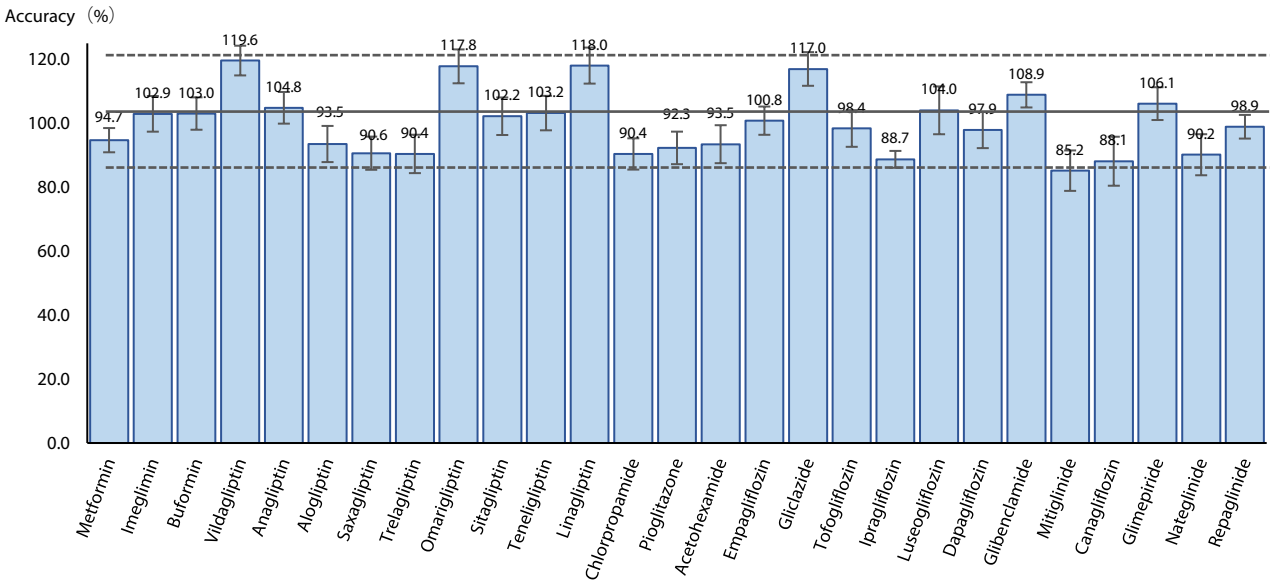

Fig. 5 Accuracy and Repeatability of Antidiabetic Drugs in Spiked Whole Blood (n = 5)

■ Summary

This method enables the quantitation of 27 antidiabetic drugs in a single analysis run. The method provides high sensitivity, allowing detection even at low concentrations around 0.1 ng/mL in whole blood, and enables quantification over a wide concentration range.

The Micro Volume QuEChERS kit can be carried out using the same workflow and same analytical conditions as the "LC/MS/MS Forensic Toxicology Database". This allows parallel analysis alongside other drugs of abuse and supports more efficient, comprehensive toxicological analysis in research and forensic applications.

＜Reference＞

1) Wasim Rauf Kadri, Khalid Raza, "Comparison of Different Antidiabetic Medication Classes: Efficacy and Safety", European Journal of Cardiovascular Medicine. Vol.15, 10. Oct. 2025, p.223-227

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