

Simultaneous Analysis of 27 multi-class Antidiabetic Drugs in Blood Using LC-MS/MS

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Introduction & Objectives

There are many classes of antidiabetic drugs, and their chemical structures are increasingly diverse. Recently, inappropriate use of antidiabetic drugs as “weight-loss drugs” has been frequently reported, and this has caused health problems. Therefore, rapid identification of these drugs is important in clinical and forensic settings. This study evaluated the LC–MS/MS method for the analysis of structurally diverse antidiabetic drugs in whole blood.

Materials & Methods

Thirty antidiabetic drugs were targeted, including nine DPP-4 inhibitors, six SGLT2 inhibitors, five sulfonylureas, three glinides, three α glucosidase inhibitors, two biguanides, a thiazolidinedione and a glimin. Calibration standards (0.1–500 ng/mL) were prepared in water using mixed working standard solutions containing the 30 target analytes and were processed using the same QuEChERS procedure as whole-blood samples (non–matrix-matched, procedural calibration) to standardize sample preparation. For accuracy assessment, blank whole blood (100 μL) was fortified with the mixed standard solution (10 μL) to a nominal concentration of 20 ng/mL, and diazepam-d5 (0.2 μg/mL, 20 μL) was added as the internal standard. For the QuEChERS extraction, the Micro Volume QuEChERS Kit (Shimadzu Corporation) was used. LC/MS grade water (200 μL) and acetonitrile (300 μL) were added to the kit, mixed, and centrifuged. The supernatant was evaporated under a nitrogen stream and reconstituted in 100 μL water/methanol (80:20, v/v) (Figure 1). LC–MS/MS analysis was performed using an LCMS-8050RX (Shimadzu Corporation). Table 1 shows the LC-MS/MS analytical conditions.

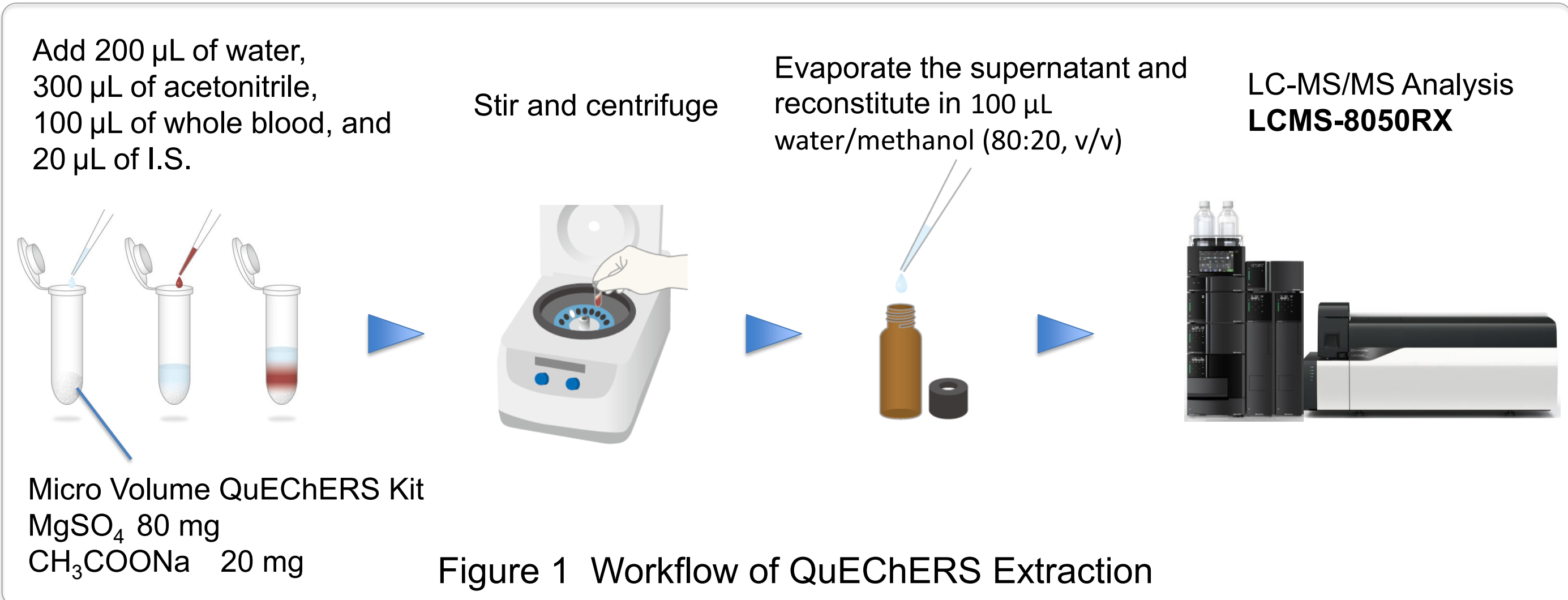


Table 1 LC-MS/MS System and Conditions	
System	: Nexera X3
Column	: Kinetex XB-C18 (100 mm×2.1 mm I.D., 2.6 μm)
Temperature	: 40 °C
Injection volume	: 1 μL
Mobile phases	: 10 mM Ammonium Formate + 0.1 % Formic acid Water 10 mM Ammonium Formate + 0.1 % Formic acid MeOH
Flow rate	: 0.3 mL/min
Time program (%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

Results

With the QuEChERS procedure, the three highly hydrophilic α-glucosidase inhibitors (Miglitol, Voglibose, Acarbose) were not detected at ≤ 200 ng/mL. However, satisfactory results were obtained for the other twenty-seven antidiabetic drugs. Table 2 shows the retention times and transitions for each detected compound. Figure 2 shows the chromatograms of 50 ng/mL standard solution. Table 3 shows the validation results. Acceptable linearity (R > 0.995) was obtained at 0.1–100 ng/mL for three compounds, 0.1–200 ng/mL for nine compounds, and 0.1–500 ng/mL for the other fifteen compounds. The accuracy for these 27 compounds was within 80–120%. Repeatability (%RSD) was ≤ 7.9% (n = 5), indicating that this method provides sufficient precision for the analysis of whole blood. Figure 3 shows MRM chromatograms of representative drugs in each class in spiked whole blood (20 ng/mL).

Table 2 Retention Time and Transition List of Target Compounds					
#	Name	Drug class	R.T. (min)	Precursor ion (m/z)	Product ion (m/z)
1	Metformin	Biguanide	0.777	[M+H] ⁺ 131.1	> 60.0 (CE -14 V)
2	Imeglimin	Glimin	1.810	[M+H] ⁺ 156.1	> 113.1 (CE -19 V)
3	Buformin	Biguanide	2.210	[M+H] ⁺ 158.1	> 60.0 (CE -15 V)
4	Vildagliptin	DPP-4 inhibitor	2.618	[M+H] ⁺ 304.2	> 154.1 (CE -18 V)
5	Anagliptin	DPP-4 inhibitor	3.630	[M+H] ⁺ 384.2	> 160.0 (CE -31 V)
6	Alogliptin	DPP-4 inhibitor	3.840	[M+H] ⁺ 340.0	> 116.1 (CE -33 V)
7	Saxagliptin	DPP-4 inhibitor	3.871	[M+H] ⁺ 316.2	> 180.1 (CE -20 V)
8	Trelagliptin	DPP-4 inhibitor	3.965	[M+H] ⁺ 358.2	> 341.1 (CE -20 V)
9	Omarigliptin	DPP-4 inhibitor	4.146	[M+H] ⁺ 399.1	> 153.1 (CE -29 V)
10	Sitagliptin	DPP-4 inhibitor	4.403	[M+H] ⁺ 408.1	> 235.2 (CE -18 V)
11	Teneligliptin	DPP-4 inhibitor	5.042	[M+H] ⁺ 427.2	> 243.2 (CE -28 V)
12	Linagliptin	DPP-4 inhibitor	5.216	[M+H] ⁺ 473.2	> 420.2 (CE -23 V)
13	Chlorpropamide	Sulfonylurea	5.838	[M+H] ⁺ 277.0	> 111.0 (CE -31 V)
14	Pioglitazone	Thiazolidinedione	5.978	[M+H] ⁺ 357.1	> 134.0 (CE -28 V)
15	Acetohexamide	Sulfonylurea	6.081	[M+H] ⁺ 325.1	> 243.0 (CE -12 V)
16	Empagliflozin	SGLT2 inhibitor	6.416	[M+NH4] ⁺ 468.2	> 71.1 (CE -27 V)
17	Gliclazide	Sulfonylurea	6.538	[M+NH4] ⁺ 324.1	> 127.1 (CE -18 V)
18	Tofogliflozin	SGLT2 inhibitor	6.656	[M+H] ⁺ 387.2	> 267.1 (CE -15 V)
19	Ipragliflozin	SGLT2 inhibitor	6.721	[M+NH4] ⁺ 422.1	> 285.1 (CE -15 V)
20	Luseogliflozin	SGLT2 inhibitor	6.789	[M+NH4] ⁺ 452.2	> 135.0 (CE -21 V)
21	Dapagliflozin	SGLT2 inhibitor	6.831	[M+NH4] ⁺ 426.2	> 135.1 (CE -21 V)
22	Glibenclamide	Sulfonylurea	7.193	[M+H] ⁺ 494.1	> 369.0 (CE -14 V)
23	Mitiglinide	Glinide	7.215	[M+H] ⁺ 316.2	> 298.1 (CE -16 V)
24	Canagliflozin	SGLT2 inhibitor	7.315	[M+NH4] ⁺ 462.2	> 191.0 (CE -25 V)
25	Glimepiride	Sulfonylurea	7.437	[M+H] ⁺ 491.2	> 352.1 (CE -14 V)
26	Nateglinide	Glinide	7.564	[M+H] ⁺ 318.2	> 166.1 (CE -13 V)
27	Repaglinide	Glinide	8.006	[M+H] ⁺ 453.3	> 230.2 (CE -30 V)

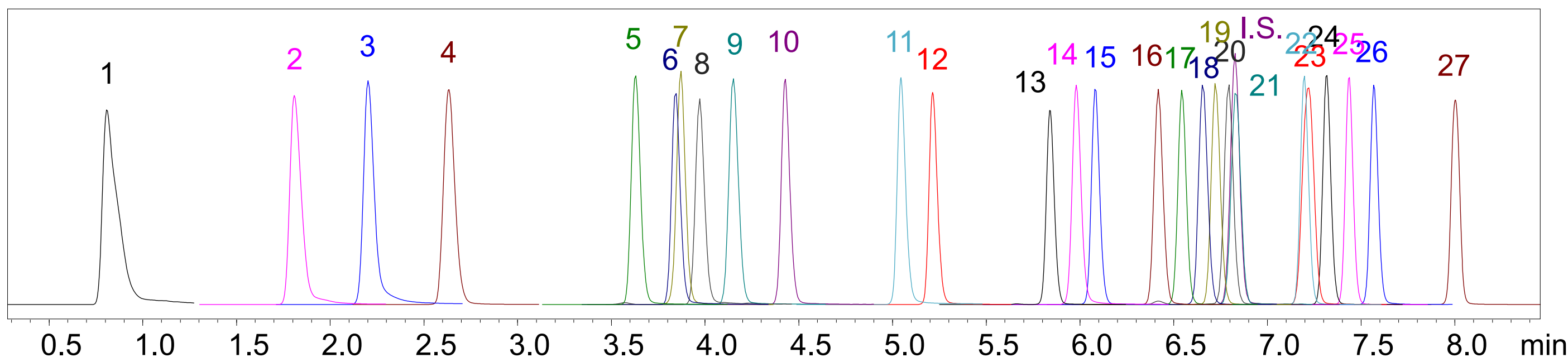


Figure 2 MRM Chromatograms of 27 Target Compounds and The Internal Standard
1: Metformin, 2: Imeglimin, 3: Buformin, 4: Vildagliptin, 5: Anagliptin, 6: Alogliptin, 7: Saxagliptin, 8: Trelagliptin, 9: Omarigliptin, 10: Sitagliptin, 11: Teneligliptin, 12: Linagliptin, 13: Chlorpropamide, 14: Pioglitazone, 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

Table 3 Validation Results for Target Compounds in Spiked Whole Blood (n = 5)					
#	Name	Calibration range ng/mL	R	Accuracy %	Repeatability RSD%
1	Metformin	0.1-500	0.9956	94.7	3.8
2	Imeglimin	0.1-500	0.9991	102.1	5.6
3	Buformin	0.1-500	0.9986	103.0	5.1
4	Vildagliptin	0.1-500	0.9991	119.6	4.6
5	Anagliptin	0.1-200	0.9998	104.8	5.0
6	Alogliptin	0.1-200	0.9990	93.5	5.7
7	Saxagliptin	0.1-200	0.9976	90.0	5.2
8	Trelagliptin	0.1-200	0.9987	90.4	6.0
9	Omarigliptin	0.1-100	0.9958	117.8	5.3
10	Sitagliptin	0.1-200	0.9999	102.2	5.9
11	Teneligliptin	0.1-500	0.9992	103.2	5.4
12	Linagliptin	0.1-500	0.9980	118.0	5.6
13	Chlorpropamide	0.1-500	0.9993	90.4	4.9
14	Pioglitazone	0.1-100	0.9998	92.3	5.1
15	Acetohexamide	0.1-200	0.9999	93.5	5.9
16	Empagliflozin	0.1-500	0.9999	100.8	4.4
17	Gliclazide	0.1-500	0.9995	117.0	5.3
18	Tofogliflozin	0.1-500	0.9997	98.4	5.7
19	Ipragliflozin	0.1-500	0.9983	88.7	2.6
20	Luseogliflozin	0.1-500	0.9964	104.0	7.5
21	Dapagliflozin	0.1-500	0.9994	97.9	5.6
22	Glibenclamide	0.1-200	0.9995	108.9	3.9
23	Mitiglinide	0.1-200	0.9998	85.2	6.3
24	Canagliflozin	0.1-500	0.9995	88.1	7.7
25	Glimepiride	0.1-500	0.9986	106.1	5.1
26	Nateglinide	0.1-200	0.9993	90.2	6.4
27	Repaglinide	0.1-100	0.9975	98.9	3.7

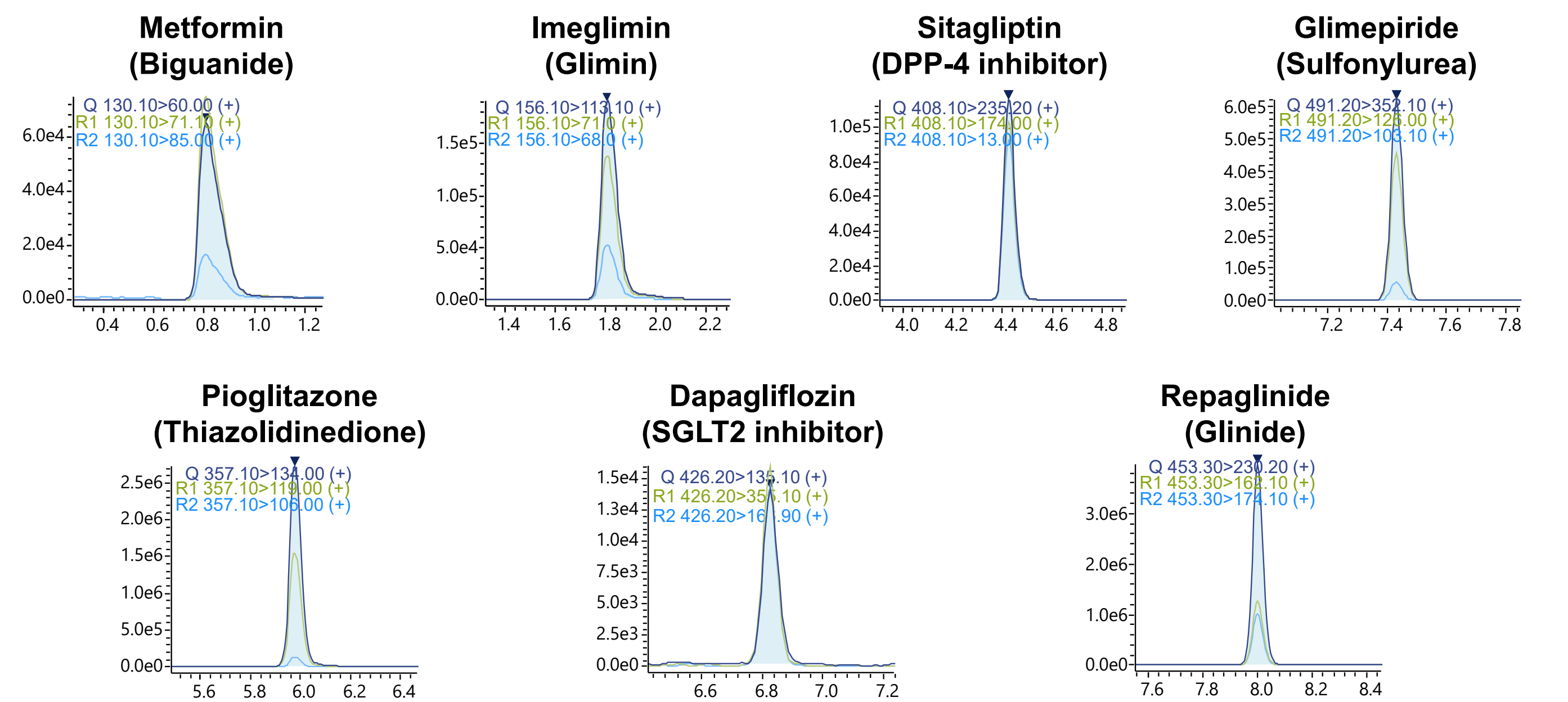


Figure 3 MRM Chromatograms of Representative Compounds in Spiked Whole Blood (20 ng/mL)

Summary

This method enabled the quantification of 27 antidiabetic drugs in whole blood. This QuEChERS extraction supports the rapid identification of diverse antidiabetic drugs in forensic toxicology. However, the method showed limited applicability to highly polar compounds (e.g., α-glucosidase inhibitors) due to insufficient recovery. In addition, the demand for high-molecular-weight drugs, GLP-1 inhibitors, is also increasing. Future work will focus on optimizing the extraction to improve the recovery of these analytes.

Check the detail with Application News!



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