Printed in the Republic of Korea
ANALYTICAL SCIENCE & TECHNOLOGY
Vol. 32 No. 2, 35-47, 2019
https://doi.org/10.5806/AST.2019.32.2.35

Development and validation of an LC-MS/MS method for the simultaneous analysis of 26 anti-diabetic drugs in adulterated dietary supplements and its application to a forensic sample

Nam Sook Kim†, Geum Joo Yoo†, Kyu Yeon Kim, Ji Hyun Lee, Sung-Kwan Park, Sun Young Baek, and Hoil Kang★

Division of Advanced Analysis, National Institute of Food and Drug Safety Evaluation, Ministry of Food and Drug Safety, Osong Health Technology Administration Complex, 187 Osongsaengmyeong2-ro, Osongeup, Heungdeok-gu, Cheongju-si, Chungcheongbuk-do 363-700, Korea

(Received September 18, 2018; Revised February 21, 2019; Accepted March 7, 2019)

Abstract In this study, high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) was employed to detect 26 antidiabetic compounds in adulterated dietary supplements using a simple, selective method. The work presented herein may help prevent incidents related to food adulteration and restrict the illegal food market. The best separation was obtained on a Shiseido Capcell Pak® C18 MG-II (2.0 mm × 100 mm, 3 μm), which improved the peak shape and MS detection sensitivity of the target compounds. A gradient elution system composed of 0.1 % (v/v) formic acid in distilled water and methanol at a flow rate of 0.3 mL/min for 18 min was utilized. A triple quadrupole mass spectrometer with an electrospray ionization source operated in the positive or negative mode was employed as the detector. The developed method was validated as follows: specificity was confirmed in the multiple reaction monitoring mode using the precursor and product ion pairs. For solid samples, LOD ranged from 0.16 to 20.00 ng/mL and LOQ ranged from 0.50 to 60.00 ng/mL, and for liquid samples, LOD ranged from 0.16 to 20.00 ng/mL and LOQ ranged from 0.50 to 60.00 ng/mL. Satisfactory linearity was obtained from calibration curves, with $R^2 > 0.99$. Both intra and inter-day precision were less than 13.19 %. Accuracies ranged from 80.69 to 118.81 % (intra/inter-day), with a stability of less than 14.88 %. Mean recovery was found to be 80.6-119.0 % and less than 13.4 % RSD. Using the validated method, glibenclamide and pioglitazone were simultaneously determined in one capsule at concentrations of 1.52 and 0.53 mg (per capsule), respectively

Key words: adulteration, antidiabetes drugs, dietary supplement, LC–MS/MS, validation, monitoring

★ Corresponding author
Phone : +82-(0)42-480-8799 Fax : +82-(0)42-480-8790
E-mail : joo819@korea.kr
†These authors contributed equally to this article

1. Introduction

Diabetes is a chronic disease characterized by high blood sugar levels. It is classified into three major types: Type one (I), type two (II), and gestational diabetes. Type II diabetes accounts for 90 % of the cases,[1] and oral antidiabetic drugs constitute the main therapy for most patients required to control their blood glucose.[2] Because of concerns over some of the potential side effects of these drugs, e.g., obesity, weakened immunity, aggravated inflammation, and death caused by hypoglycemic shock from the misuse of medication, dietary supplement and herbal medicine sales have increased as they are considered safer than medication (e.g., synthetic drugs) for treating health in an "all-natural" manner.[3-6] Nevertheless, some manufacturers add illegal adulterants to their products to achieve drastic effects in a short time period. Several unlabeled illegal adulterants have been detected in dietary supplements, which can potentially cause serious risks to public health.[7]

Several antidiabetics have been reportedly detected. In Saudi Arabia, 7.5 mg of glibenclamide has been detected from tablets and 4.5 mg from powders.[8] In China, metformin, glimepiride, and phenformin have been detected in counterfeit dietary and herbal supplements.[9] Furthermore, in Singapore,[10] an outbreak of severe hypoglycemia has been reported, caused by the contamination of illegal sexual enhancement drugs with glyburide. As much as 13-100 mg of glyburide has been detected in blood and urine from 127 non-diabetic patients (among 127, 4 people died). In 2008, similar cases of glibenclamide-induced hypoglycemia have been reported in Hong Kong. Other illegal sexual enhancement brands were implicated in these studies.[11] Hence, it is imperative to develop a simultaneous method to comprehensively screen rapidly. The most widely applied methodologies for pharmaceutical analysis include high-performance liquid chromatography (HPLC),[12-18] high-performance liquid chromatography–tandem mass spectrometry (LC-MS/MS), and spectroscopic methods.[19-23] Almost the whole studies have reported the analysis for the screening of adulterants in urine, blood, body fluids, and plasma. Some of studies described such a method that procedure for screening, identification, and quantification of several antidiabetic drugs in foods and herbal products by LC-MS/MS[8-11] Using LC-MS/MS, typically a particular peak from the mass spectrum is selected and isolated and collisions are induced within the mass spectrometer to force a characteristic fragmentation of the selected ion. The LC-MS/MS can detect slight amount than UPLC and further increase the specificity.

This paper proposed an LC-MS/MS method for the rapid, reliable detection of 26 antidiabetic adulterants in dietary supplements. Multiple reaction monitoring (MRM) was employed to monitor the LC effluent by simultaneously using selected transitions for each compound, where reliability can be improved based on the fact that the relative peak areas maintain good stability. To the best of our knowledge, such a procedure, involving the analysis of such a comprehensive list of compounds, has not been reported thus far. Therefore, there is a clear requirement for comprehensive screening of illegal adulterants 26 antidiabetic adulterants in dietary supplement.

2. Materials and Methods

2.1. Chemicals and reagents

Reference standards of sulfonylureas or meglitinides (carbutamide, chlorpropamide, glibenclamide, glibornuride, gliclazide, glimepiride, glipizide, gliquidone, glymidine, tolazamide, tolbutamide, mitiglinide, nateglinide, repaglinide, alogliptin benzoate), biguanides or thiazolidinedianes (buformin, metformin, phenformin, pioglitazone, rosiglitazone, troglitazone), Dipeptidyl Peptidase (DPP)-4 inhibitors (sitagliptin and vildagliptin), and SGLT(The sodium/glucose cotransporter) 2 inhibitors (canagliflozin, empagliflozin, ipragliflozin) were purchased from USP (Rockville, MD, USA), Sigma-Aldrich (St. Louis, MO, USA), Toronto Research Chemicals (Toronto, ON, Canada), and Santa Cruz (Dallas, TX, USA). HPLC-grade methanol was purchased from Burdick and Jackson (Muskegon, MI, USA), and formic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA). Milli-Q water

(18.1 mΩ) from a Milli-Q purification system (Millipore, Bedford, MA, USA) was used throughout.

2.2. Sample preparation

Stock solutions were prepared by dissolving standards in methanol at approximately 1 mg mL^{-1} and stored in a refrigerator (2-8 °C) until use.

Dietary supplements were pulverized into powders starting from various samples (i.e., tablets, hard capsules, soft capsules, powders, liquids, and pills), and 1 g of the powder sample was dissolved in 70 % methanol. Capsule and soft gel shells were excluded. After 30 min of sonication for complete dissolution, the sample solution was added into a 50 mL volumetric flask, and its volume was made up to the mark. The sample was then filtered through a 0.22 μm polytetrafluoroethylene (PTFE) filter (Millipore, Milford, USA) before analysis.

2.3. Instrumentation

Analytes were separated on an Agilent 1200 series HPLC instrument (Agilent Technologies, Palo Alto, CA, USA). The LC system consisted of a quaternary pump, a vacuum degasser, and an autosampler. Data in the positive and negative modes were obtained by an electrospray ionization (ESI) source via an API 4000 triple quadrupole mass spectrometer (AB Sciex, Concord, ON, Canada).

Table 1. The condition of LC-MS/MS

LC System	Agilent DE/1200 HPLC		
Column	Capcell Pak C_{18} MGII (2.1 mm × 100 mm, 3.5 μm)		
Column Temp.	40 °C		
Mobile phase	(A) 0.1% Formic acid in Water (B) 0.1% Formic acid in Methanol		
	Time	A (%)	B (%)
	0.0	95.0	5.0
	0.7	95.0	5.0
	1.0	60.0	40.0
	3.0	50.0	50.0
	8.0	10.0	10.0
	12.0	10.0	10.0
	12.1	95.0	5.0
	18.0	95.0	5.0
Flow	0.3 mL/min		
Inj. Volume	2.0 μL		
MS system	AB sciex Qtrap 4000		
Ionization mode	ESI (+)	ESI (−)	
Ion Voltage	5.0 kV	4.5 kV	
Source temp.	500 °C	500 °C	
Curtain gas	30 psi	30 psi	
Collision gas	9 psi	9 psi	

2.4. Analytical method

2.4.1. LC-MS/MS operating conditions

Compounds were separated by a Shiseido Capcell pak® C18 MG-II column (2.0 mm × 100 mm, 3 μm particle size) to improve the peak shape and MS detection sensitivity of the target compounds. A linear gradient system of mobile phase A (0.1 % formic acid in distilled water) and mobile phase B (0.1 % formic acid in MeOH) was used at a flow rate of 0.3 mL/min. *Table* 1 summarizes the gradient program and MS/MS conditions.

3. Method Validation

The following method parameters were evaluated to validate the reliability of the proposed method: specificity, linearity, limit of detection (LOD), limit of quantitation (LOQ), precision, accuracy, recovery, and stability. The range of linearity was tested by analyzing six standard calibration solutions in triplicate at concentrations set at 1, 2, 4, 8, 10, and 20 times of the LOQ. According to the recommended guidelines, LODs and LOQs were determined by spiked samples based on the signal-to-noise ratios of 3:1 and 10:1, respectively. The accuracy of intra- and inter-day experiments were evaluated in triplicate at low (near the LOQ), medium (~5-fold above the LOQ), and high (~10-fold above the LOQ) concentrations to a blank sample containing a dietary supplement. Intra- and inter-day precision was evaluated three times by analysis on the same day and on three days, and the relative standard deviation (% RSD) values were determined for each compound. Recovery was determined as the standard area as compared to the blank sample area spiked with 26 antidiabetic compounds. The blank sample was analyzed along with six types of samples

(powders, pills, hard and soft capsules, liquid, and tablets, respectively) containing three concentrations of the mixed standard solutions. The stability of the 26 antidiabetic compounds solution was evaluated by quantitative determination at several time points over 48 h. The stability of 26 compounds was assessed by processing solutions after 6 h storage at room temperature. Then, the autosampler stability was determined by keeping the reconstituted samples for approximately 24 and 48 h in an autosampler at 4 °C before analysis. Method validation was performed according to the requirements published by the ICH guidelines.[27]

4. Method Application

Seventy-eight samples collected from Korean online or offline markets and eight samples requested from the criminal investigation public office were used, including those from tablets (9), hard capsules (10), soft capsules (12), powders (14), liquids (26), and pills (15).

5. Results and Discussion

5.1. Optimization of mass spectrometric and chromatographic conditions

All diabetic compounds were evaluated using the LC-MS/MS method. Standard solutions containing 20-100 ng/mL in methanol were infused directly into the ESI source of the mass spectrometer. Prominent protonated molecular ions $[M+H]^+$ and $[M+NH_4]^+$ in the positive mode, as well as prominent protonated molecular ions $[M-H]^-$ in the negative mode, were observed in the full scan mass spectra. Ammonium adducts ion $[M+NH_4]^+$ in positive mode was adopted for Canagliflozin and ipragliflozin identification. In previous reported studies, ammonium adducts ions

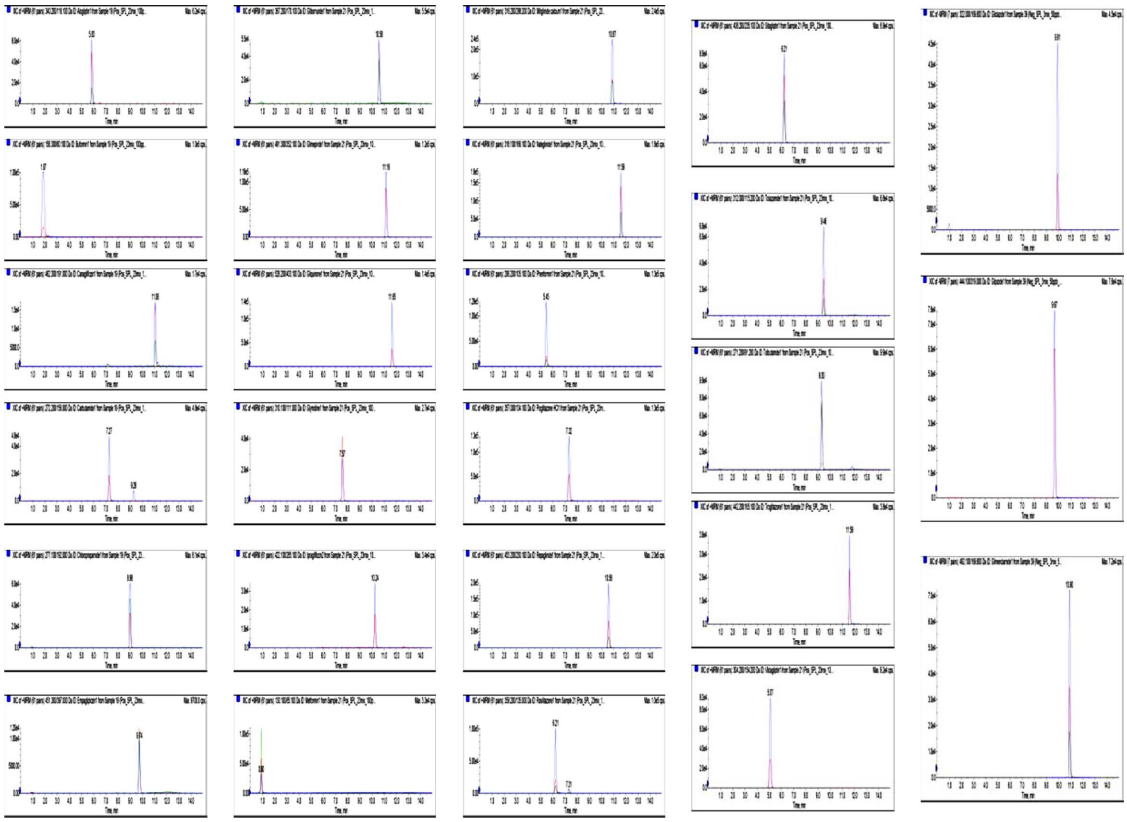

Fig. 1. Individual TIC of 26 anti-diabetic compounds.

Table 2. MRM conditions for 26 anti-diabetic compounds

Compound	Folmula	Ion mode	Precusor ion	Product ion	DP*	CE** (eV)	CXP*** (V)
Alogliptin	$C_{18}H_{21}N_5O_2$	+	340.2	116.1	35	47	22
				323.1	35	27	22
				266.2	35	31	18
Buformin	$C_6H_{15}N_5$	+	158.0	59.90	66	21	10
				42.80	66	59	20
Canagliflozin	$C_{24}H_{25}FO_5S$	–	443.1	365.0	55	20	8
				353.0	55	28	10
				153.0	55	50	10
Carbutamide	$C_{11}H_{17}N_3O_3S$	+	272.2	156.0	68	25	10
				108.1	68	40	10
Chlorpropamide	$C_{10}H_{13}ClN_2O_3S$	+	277.1	192.0	55	19	12
				110.9	55	43	10
				175.0	55	25	14
Empagliflozin	$C_{23}H_{27}ClO_7$	+	451.1	397	50	13	10
				355.1	50	17	8
				71.2	50	50	13
Glibenclamide	$C_{23}H_{28}ClN_3O_5S$	+	492.1	169.9	70	35	15
				367	70	28	13
				127	70	65	10
Glibornuride	$C_{18}H_{26}N_2O_4S$	+	367.2	170.1	25	20	10
				152.2	25	30	10
				349.0	25	20	25
Gliclazide	$C_{15}H_{21}N_3O_3S$	-	322.2	169.8	30	35	10
				105.9	30	50	8
Glimepiride	$C_{24}H_{34}N_4O_5S$	-	489.2	224.9	55	45	10
				364.1	55	30	10
				349.8	55	25	10
Glipizide	$C_{21}H_{27}N_5O_4S$	-	444.1	319.0	23	30	15
				169.9	23	40	8
Gliquidone	$C_{27}H_{33}N_3O_6S$	+	528.2	403.1	45	15	10
				386.0	45	31	14
				165.1	45	63	14
Glymidine	$C_{13}H_{15}N_3O_4S$	+	310.1	111.0	90	35	20
				252.0	90	28	15
Ipragliflozin	$C_{21}H_{21}FO_5S$	+	422.1	151.2	30	30	10
				285.1	30	20	18
				309.1	30	20	20
Metformin	$C_4H_{11}N_5$	+	130.1	85.1	45	20	5
				70.9	45	25	10
				60.0	45	20	10
Mitiglinide	$C_{19}H_{25}NO_3$	+	316.2	298.2	43	22	12
				145.1	43	36	10
				126.2	43	33	10
Nateglinide	$C_{19}H_{27}NO_3$	+	318.1	166.1	50	18	10
				125.2	50	22	7
				120.1	50	25	14
Phenformin	$C_{10}H_{15}N_5$	+	206.2	105.1	60	37	20
				164.2	60	25	10
				189.2	60	23	12

Table 2. Continued

Compound	Folmula	Ion mode	Precusor ion	Product ion	DP*	CE** (eV)	CXP*** (V)
Pioglitazone	$C_{19}H_{20}N_2O_3S$	+	357.0	134.1	91	39	12
				119.0	91	65	20
Repaglinide	$C_{27}H_{36}N_2O_4$	+	453.2	230.1	55	38	12
				162.0	55	33	12
				86.0	55	33	13
Rosiglitazone	$C_{18}H_{19}N_3O_3S$	+	358.2	135.0	48	30	10
				119.0	48	78	11
				107.0	48	60	16
Sitagliptin	$C_{16}H_{15}F_6N_5O$	+	408.2	235.0	45	27	15
				174.0	45	37	10
				193.0	45	35	12
Tolazamide	$C_{14}H_{21}N_3O_3S$	+	312.0	115.2	35	30	5
				141.1	35	30	10
				157.1	35	20	10
Tolbutamide	$C_{12}H_{18}N_2O_3S$	+	271.2	91.20	61	47	17
				155.05	61	25	15
				74.20	61	20	13
Troglitazone	$C_{24}H_{27}NO_5S$	+	443.1	165.0	25	25	10
				367.0	25	25	8
				291.0	25	25	20
Vildagliptin	$C_{17}H_{25}N_3O_2$	+	304.2	154.2	65	25	10
				151.1	65	30	9
				133.2	65	45	10

*Declustering potential
**Collision energy
***Collision cell exit potential

for canagliflozin and ipragliflozin in the presence of formic acid were more predominate and sensitive then hydrogen adduct ions $[M+H]^+$.[24-26] Collision energies were optimized for each analyte to obtain the most intense fragment ions. Multiple reaction monitoring (MRM) transitions were initially monitored for each analyte. During the collision of the precursor ions in tandem MS, more than two daughter ions of the 26 antidiabetic compounds were obtained, highest peak for quantitation and another for confirmation purpose (*Fig.* 1, *Table* 2). To optimize peak shape with appreciable retention times, various columns were investigated. Three columns, HILIC, C18, and C8, were used. The best resolution and intensity were observed with the Shiseido Capcell pak® C18 MG-II column (2.0 mm × 100 mm, 3 μm particle size), without excessive tailing in 18 min. In addition, the separation of these antidiabetic compounds was attempted using various combinations of acetonitrile, methanol, and water with different percentages of buffers. The best separation was achieved using 0.1% formic acid with methanol and water. Because of the high selectivity and efficiency of LC-MS/MS, the simultaneous separation of 26 antidiabetic compounds has been reported for the first time, to the best of our knowledge.

5.2. Method validation

Specificity was confirmed by the MRM transition parameters using the precursor and product ion pairs of UPLC-MS/MS for the determination of the 26 compounds summarized in *Fig.* 2 and 3. The blank and spiked samples of selected ions from the mass spectra obtained using LC/MS/MS, no interfering

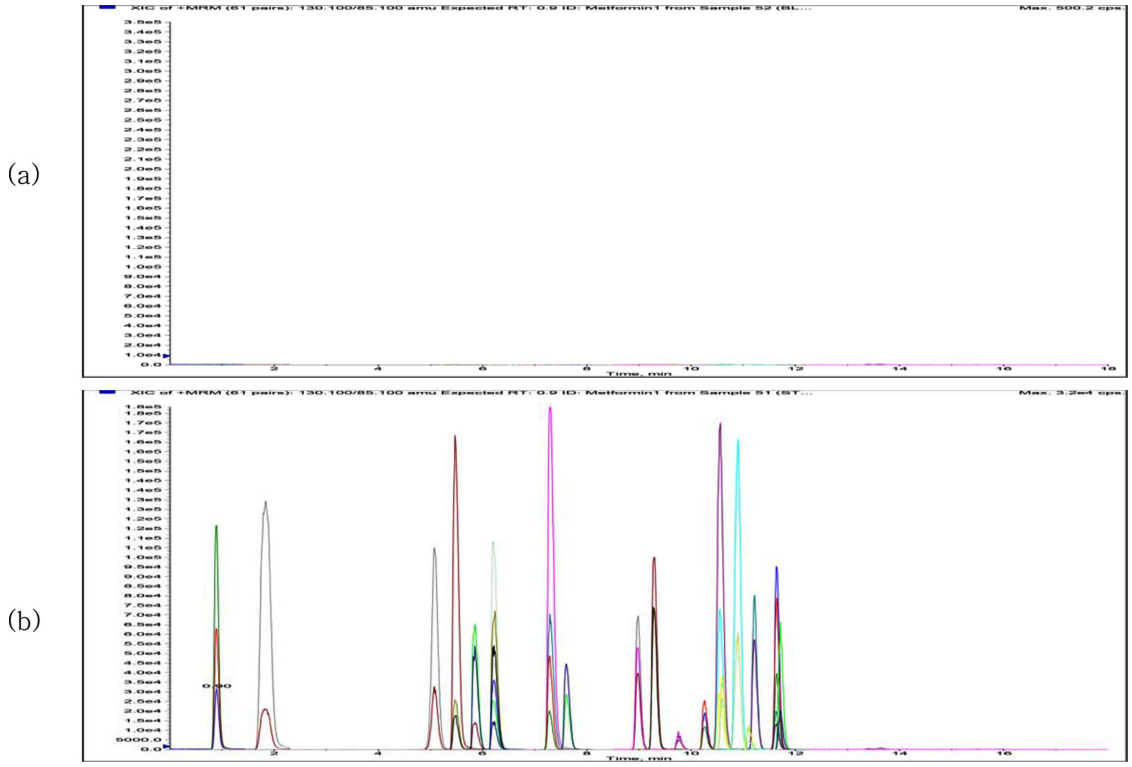

Fig. 2. TIC of (a) blank (b) anti-diabetic compounds in positive mode.

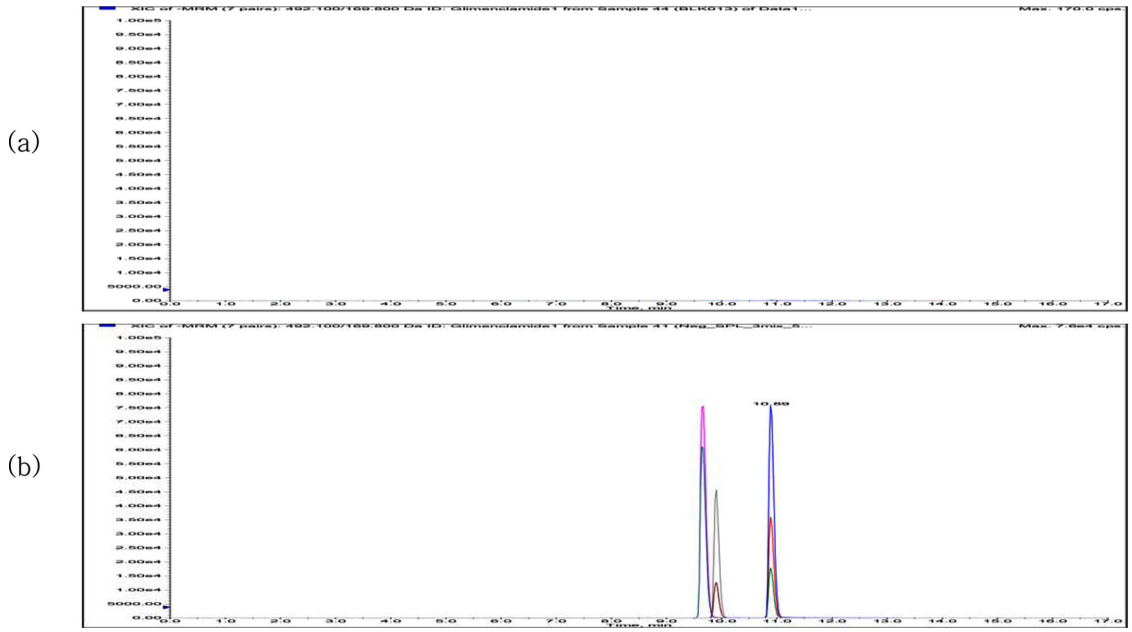

Fig. 3. TIC of (a) blank (b) anti-diabetic compounds in negative mode.

Table 3. The linearity, LOD and LOQ of 26 anti-diabetic compounds

Compound	Calibration curve	Linear range (ng/mL)	R^2	LOD (ng/mL)		LOQ (ng/mL)	
				Solid	Liquid	Solid	Liquid
Alogliptin	y = 4052x − 3456.4	3.10-61.92	0.999	1.00	2.00	3.00	6.00
Buformin	y = 17564x + 9284.8	3.02-60.48	0.999	1.00	1.00	3.00	3.00
Canagliflozin	y = 7177.1x + 3286.9	53.40-1068.00	0.999	20.00	9.00	60.00	27.00
Carbutamide	y = 2078.5x + 1550.5	2.44-48.84	1.000	1.00	5.00	3.00	15.00
Chlorpropamide	y = 11888x − 75.758	9.05-181.08	1.000	3.00	5.00	9.00	15.00
Empagliflozin	y = 3831.6x + 709.9	63.00-1260.00	1.000	20.00	10.00	60.00	30.00
Glibenclamide	y = 1160.1x + 8.1818	5.16-103.20	1.000	0.16	5.00	0.50	15.00
Glibornuride	y = 2362.6x + 11373	6.37-127.44	0.996	2.00	20.00	6.00	60.00
Gliclazide	y = 3723.4x − 884.04	3.07-61.44	1.000	1.00	10.00	3.00	30.00
Glimepiride	y = 2319.8x − 794.44	1.53-30.54	0.999	0.50	0.16	1.50	0.50
Glipizide	y = 3443.4x − 18.586	1.51-30.20	1.000	0.50	0.30	15.00	1.00
Gliquidone	y = 3767.4x + 132.02	1.53-30.63	0.999	0.50	3.00	1.50	9.00
Glymidine	y = 705.08x − 18.586	15.12-302.40	1.000	5.00	3.00	15.00	9.00
Ipragliflozin	y = 792.84x + 4962.6	60.36-1207.20	1.000	20.00	20.00	60.00	60.00
Metformin	y = 7707.5x + 2588.9	3.03-60.57	1.000	1.00	3.00	3.00	9.00
Mitiglinide	y = 8201.2x + 6139.4	3.06-61.17	1.000	1.00	1.00	3.00	3.00
Nateglinide	y = 4572.8x − 247.47	1.50-30.09	0.999	1.00	1.00	3.00	3.00
Phenformin	y = 5096.6x + 2745.5	1.56-31.20	1.000	0.50	2.00	1.00	6.00
Pioglitazone	y = 21430x + 10420	3.03-60.69	1.000	1.00	0.20	3.00	0.50
Repaglinide	y = 19721x + 2643.4	1.05-20.91	1.000	0.30	0.50	1.00	1.50
Rosiglitazone	y = 6222.6x − 5575.8	3.20-63.96	0.997	1.00	3.00	3.00	9.00
Sitagliptin	y = 3736x + 481.82	3.08-61.50	1.000	1.00	0.30	3.00	1.00
Tolazamide	y = 3013.1x − 816.16	15.32-306.30	1.000	5.00	2.00	15.00	6.00
Tolbutamide	y = 2581.3x + 8052.5	31.49-629.70	1.000	10.00	10.00	30.00	30.00
Troglitazone	y = 753.76x + 1665.1	9.16-183.24	1.000	3.00	3.00	9.00	9.00
Vildagliptin	y = 13061x + 405.05	0.86-17.14	1.000	0.30	0.50	1.00	1.50

peaks were observed at the retention times. Under the optimized conditions, a linear relationship with a good correlation coefficient ($R^2 > 0.99$, $n = 3$) was observed between the peak area ratios and the concentrations of 26 compounds in the range of 0.5~1200 ng/mL. The limits of detection and quantitation (LOD/LOQ) were mearsured in solid sample (LOD, 0.16~20.00 ng/mL; LOQ, 0.50~60.00 ng/mL) and in liquid sample (LOD, 0.16~20.00 ng/mL; LOQ, 0.50~60.00 ng/mL), respectively (*Table* 3). The accuracy values from the intra-day analysis was 81.99~112.64 %, whereas the values for the inter day analysis were 80.69~118.81 %. The RSD of precision was ≤ 10.25 % in intra-day and ≤ 13.19 % in inter-day (*Table* 4). Extraction recovery was also investigated by analyzing one of the commercial products that had been spiked with the standard analytes. The mean overall recoveries (with the precision) of all the analytes were summarized in *Table* 5. The stability of 26 antidiabetic compounds up to 48 hr was less than 14.88 % (*Table* 6). The results showed that the method met the desired level of acceptance criteria and hence was considered accurate and precise for the analysis of 26 antidiabetic adulterants in dietary supplement and other herbal products in the area of forensic science.

5.3. Method application

The established UPLC-MS/MS method was successfully applied to the determination of 26 antidiabetic compounds in six dietary supplement samples, used to prevent diabetics, collected from a criminal investigation public office and online or offline Korean markets. Sample preparation was carried out as described in Sample Preparation in the

Table 4. Intra- and inter-day validation of the developed method

Compound	Accuracy (%)						Precision (% RSD)					
	Intra-day			Inter-day			Intra-day			Inter-day		
	Low[1)]	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High
Alogliptin	98.92	93.14	93.36	94.38	87.83	88.90	8.85	9.23	7.26	11.48	8.95	8.96
Buformin	102.46	95.66	105.63	104.05	99.39	102.95	5.61	2.79	3.41	2.65	3.93	3.36
Canagliflozin	87.81	96.35	98.43	81.07	84.32	84.88	6.20	8.68	2.10	7.20	12.80	14.72
Carbutamide	111.25	112.89	107.01	112.03	113.85	118.81	5.36	3.49	2.91	4.85	3.20	9.25
Chlorpropamide	87.64	95.32	96.93	92.08	92.48	92.49	3.52	2.93	1.13	12.65	8.18	8.91
Empagliflozin	86.06	99.06	86.59	95.02	96.59	92.16	8.71	9.30	9.33	8.48	2.56	6.95
Glibenclamide	82.21	86.45	92.32	93.20	89.11	93.00	4.27	8.65	1.49	10.65	4.64	6.72
Glibornuride	81.99	98.96	112.64	86.98	97.32	105.00	7.13	0.97	4.38	10.10	5.62	6.42
Gliclazide	87.38	93.54	94.45	92.28	94.14	97.73	3.39	2.98	0.92	4.62	0.59	3.26
Glimepiride	104.86	98.93	98.20	101.08	102.40	98.23	7.70	6.99	3.30	3.34	2.98	2.17
Glipizide	99.33	97.74	97.19	97.95	95.63	97.10	1.36	4.03	2.43	5.43	2.13	4.01
Gliquidone	100.27	92.64	86.57	98.88	95.99	94.06	5.91	8.21	8.81	3.49	3.23	7.00
Glymidine	92.07	95.20	102.02	92.82	94.23	93.37	2.26	2.86	1.34	8.21	9.12	10.01
Ipragliflozin	90.30	96.97	95.57	93.46	95.91	97.04	3.34	1.88	2.46	2.99	2.06	3.65
Metformin	102.19	97.16	105.40	105.01	104.77	104.79	5.12	7.69	3.00	3.22	6.66	5.00
Mitiglinide	83.73	92.82	100.62	88.21	91.42	94.63	5.02	4.41	3.72	9.37	5.89	6.31
Nateglinide	102.00	100.95	99.50	109.31	106.61	103.74	5.66	3.17	7.45	6.26	4.60	3.65
Phenformin	97.65	88.29	96.28	99.96	93.75	97.97	8.05	6.72	1.66	5.06	7.84	3.76
Pioglitazone	98.85	95.32	99.67	106.05	109.24	107.03	5.75	1.93	2.79	9.50	11.86	7.00
Repaglinide	85.04	99.24	101.33	89.42	98.59	100.63	5.70	0.98	0.28	6.19	4.95	3.98
Rosiglitazone	109.63	106.11	97.61	99.78	96.83	95.38	3.26	4.34	5.67	12.24	10.95	6.14
Sitagliptin	112.99	109.25	100.26	80.69	80.79	84.52	10.25	8.38	5.78	8.90	3.23	3.71
Tolazamide	102.13	103.22	111.64	101.93	100.66	101.07	2.25	4.49	3.80	2.27	7.30	11.38
Tolbutamide	88.89	91.61	95.35	90.42	91.42	93.68	1.78	2.03	2.71	6.87	6.42	6.58
Troglitazone	94.23	103.68	91.76	86.44	91.71	92.15	7.90	8.76	5.13	7.81	11.31	4.00
Vildagliptin	110.17	109.34	99.40	109.02	82.06	80.38	6.38	4.17	1.04	6.57	13.19	12.38

[1)]Concentration (ng/mL): low (near the LOQ), medium (~5-fold above the LOQ), and high (~10-fold above the LOQ

Materials and Methods section. Of the 26 antidiabetic compounds, glibenclamide and pioglitazone were simultaneously detected in one capsule and confirmed (*Fig.* 4) at concentrations of 1.52 and 0.53 mg (per capsule), respectively. Based on this result, the proposed method is rapid, reliable, and accurate, with good applicability.

6. Conclusions

In this study, an accurate, reproducible method based on liquid chromatography coupled with electrospray ionization tandem mass spectrometry was developed and validated for the simultaneous determination of 26 antidiabetic compounds in dietary supplements. In addition, this study exerts significant advantages over earlier reported methods, e.g., simultaneous quantification of 26 antidiabetic analytes, shorter run time, wider linearity range with high sensitivity, and simple reproducible extraction. The developed method was successfully validated. Using the validated method, glibenclamide as an insulin secretagogues and pioglitazone as an insulin action enhancer were simultaneously detected from a capsular dietary supplement. These medicine compounds can be dangerous for consumers without a doctor's prescription. From the results of validation parameters, the developed simultaneous analysis method can be used for the routine analysis of antidiabetic compounds in various forms of dietary supplements. This advanced

Table 5. Extraction recovery of 26 anti diabetic compounds in dietary supplement samples (six types) by LC-MS/MS

Compound	Tablet			Hard-Capsule			Soft-Capsule			Liquid			Pill			Powder		
	mean ± %RSD			mean ± %RSD			mean ± %RSD			mean ± %RSD			mean ± %RSD			mean ± %RSD		
	Low[+]	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High
Alogliptin	100.3±5.5	102.4±12.0	105.3±8.9	100.9±4.8	98.9±12.8	104.3±6.0	101.9±11.4	104.9±10.5	115.1±6.9	114.6±1.5	106.3±11.5	116.9±8.2	96.5±3.0	110.8±11.2	106.6±6.3	95.9±8.5	100.6±9.7	99.5±4.5
Buformin	92.3±8.4	88.9±4.0	85.2±1.3	116.2±7.7	111.6±3.1	119.0±1.9	106.5±10.7	113.3±3.1	112.8±8.9	109.6±11.6	108.4±5.9	109.4±2.3	101.9±1.7	97.4±3.2	95.9±2.0	93.9±5.3	93.1±0.2	94.8±1.9
Canagliflozin	96.4±1.2	102.9±6.8	90.8±2.0	102.1±7.6	96.0±3.8	100.5±5.2	103.2±4.2	97.5±7.2	99.0±4.2	97.4±13.6	94.3±6.0	99.4±4.5	91.5±9.1	92.3±8.6	89.7±3.5	109.3±6.5	102.8±6.5	99.3±8.0
Carbutamide	80.6±7.9	84.7±4.9	100.3±12.0	98.9±11.7	110.3±12.1	106.2±6.3	104.2±7.6	102.1±5.4	114.5±3.1	100.5±9.5	94.7±8.4	108.9±1.4	92.8±6.8	103.0±7.4	107.6±9.7	89.3±5.2	87.6±6.4	93.1±6.4
Chlorpropamide	80.9±6.8	90.9±2.3	84.6±6.5	91.1±1.1	90.2±8.7	86.8±3.6	95.9±2.2	91.8±1.7	98.1±2.3	93.5±3.0	97.4±5.6	106.2±2.8	90.9±9.0	89.5±2.6	85.9±1.4	90.9±7.7	89.5±1.8	91.8±8.3
Empagliflozin	97.5±6.5	95.8±7.7	95.5±8.0	112.6±6.9	98.1±12.4	112.8±10.2	110.3±6.4	97.4±4.3	104.6±7.7	96.5±10.4	97.6±5.8	108.0±9.0	96.9±4.9	97.1±2.9	97.4±7.7	111.2±4.7	100.9±6.7	100.5±3.3
Glibenclamide	106.1±6.5	98.7±0.8	93.1±3.6	108.1±5.6	109.3±6.7	100.3±6.0	114.9±2.2	110.9±7.7	110.4±1.0	111.6±8.5	96.4±1.5	105.2±4.6	116.3±3.8	110.3±11.3	108.5±0.9	93.9±6.0	97.7±6.5	99.0±6.0
Glibornuride	86.2±8.8	99.2±0.7	94.9±1.1	104.4±7.3	109.0±6.9	94.8±5.3	116.8±6.7	114.1±3.6	97.4±7.1	90.3±5.5	100.9±3.0	95.5±7.8	99.3±5.1	108.3±2.6	105.4±11.7	96.1±6.2	102.2±7.0	96.6±4.9
Gliclazide	87.9±8.5	91.2±0.9	88.5±0.3	107.6±8.6	118.6±3.9	112.8±4.3	108.9±9.6	118.8±8.0	118.3±2.5	99.9±8.3	105.9±4.0	112.9±5.8	98.5±3.5	112.1±1.1	113.2±8.5	89.3±7.3	99.1±4.4	101.1±5.3
Glimepiride	91.0±1.2	105.1±11.0	92.4±6.6	84.9±7.3	96.0±10.3	96.6±6.1	96.4±8.4	101.8±5.7	101.9±4.5	90.8±9.2	104.4±6.4	93.4±2.6	81.0±5.3	97.7±0.4	98.9±4.0	87.8±11.1	111.5±9.2	95.0±6.9
Glipizide	106.6±12.3	102.5±2.2	105.9±7.3	110.1±7.6	105.3±1.0	92.3±5.1	115.4±7.7	108.1±4.7	108.0±5.3	103.4±7.4	96.8±6.1	100.0±8.4	103.8±7.3	97.5±4.4	102.4±5.5	93.2±9.8	89.7±3.4	91.5±4.2
Gliquidone	95.0±12.4	104.3±5.1	96.3±3.6	107.7±7.8	101.7±4.6	96.1±4.6	110.9±1.2	106.2±2.1	105.1±7.6	111.2±2.6	109.0±1.4	110.5±4.3	104.2±6.9	99.9±5.4	95.5±4.0	93.1±6.7	92.3±5.6	93.4±1.5
Glymidine	96.9±3.9	96.3±1.3	97.3±2.6	92.9±7.7	99.8±10.7	99.1±11.1	89.1±9.0	98.5±3.3	101.2±4.7	106.2±0.9	97.9±7.7	95.5±6.7	90.5±6.2	92.2±8.1	93.7±2.4	92.5±6.8	104.7±5.8	93.1±5.5
Ipragliflozin	96.5±3.4	89.1±4.4	86.5±9.5	96.0±6.3	92.4±4.1	100.2±0.7	95.6±1.6	91.8±3.0	99.4±2.6	102.0±7.3	100.8±5.1	106.0±2.8	87.1±6.7	87.1±2.7	85.9±2.5	98.2±1.5	95.3±3.7	101.9±6.9
Metformin	100.0±3.2	114.0±1.7	97.9±2.5	116.4±2.0	85.2±7.6	82.8±6.6	113.1±7.7	110.1±7.9	118.6±6.4	102.6±4.5	111.6±8.8	90.7±1.2	91.4±2.3	103.7±4.8	94.3±1.5	94.7±4.5	94.6±6.4	98.4±7.2
Mitiglinide	91.4±3.4	87.2±4.8	91.0±4.6	98.8±7.9	97.4±7.9	87.8±5.2	108.5±2.3	96.6±9.1	104.0±1.2	99.2±8.8	95.1±9.7	101.6±4.2	102.2±5.8	95.5±5.6	98.8±0.2	93.5±2.7	89.4±3.6	97.1±1.5
Nateglinide	88.8±12.2	95.7±4.7	90.4±6.6	85.9±9.6	95.3±6.0	105.6±4.5	91.6±7.0	103.9±3.1	105.1±7.8	94.5±9.1	104.1±6.9	118.0±10.0	90.7±2.4	90.6±4.9	96.0±5.1	95.5±7.8	94.3±7.1	103.0±9.4
Phenformin	108.0±2.9	104.6±2.8	100.5±6.5	114.3±10.0	105.8±8.9	119.0±3.5	115.1±8.7	107.7±7.9	119.0±4.5	108.5±3.0	106.5±4.9	92.7±4.2	96.0±10.5	93.0±5.3	98.8±2.7	91.9±4.6	84.7±1.5	94.5±1.6
Pioglitazone	90.3±3.8	95.9±5.1	104.8±1.7	90.6±4.8	100.7±4.3	92.0±1.7	97.3±5.3	103.0±3.3	103.4±1.3	91.2±6.2	99.8±1.3	101.4±3.5	84.8±1.0	90.2±3.5	86.5±4.3	86.4±7.3	93.6±3.6	92.7±4.3
Repaglinide	106.4±10.5	96.3±9.5	94.1±10.3	94.1±8.1	105.2±1.2	88.3±3.5	101.66.3	106.3±9.0	107.9±3.4	99.2±7.3	105.4±8.0	110.8±1.5	97.6±1.8	99.7±3.7	104.7±1.1	91.4±4.3	93.9±4.5	94.3±3.0
Rosiglitazone	97.0±8.9	88.4±8.0	85.6±3.5	91.0±1.4	87.1±2.9	103.3±2.1	100.8±2.9	98.3±9.9	101.1±2.2	106.2±9.9	107.8±8.2	114.5±9.7	94.7±2.1	91.7±7.4	90.4±1.6	95.1±3.9	99.3±3.1	99.0±3.7
Sitagliptin	107.2±3.7	98.6±10.5	94.7±5.7	95.2±2.9	90.3±4.1	97.1±7.3	105.1±2.7	106.7±5.3	102.5±6.4	102.4±3.3	102.5±3.9	109.5±5.0	93.9±8.4	96.2±7.7	92.7±6.8	95.8±11.4	84.1±5.3	96.1±5.4
Tolazamide	88.8±7.9	85.6±3.7	81.2±4.9	107.0±6.7	110.7±4.4	85.3±6.8	109.5±4.5	110.8±0.5	111.7±5.9	100.7±7.2	96.3±9.6	101.6±2.8	103.3±5.7	104.1±9.1	107.0±7.8	94.5±3.0	103.0±5.7	98.6±3.9
Tolbutamide	113.6±1.4	95.4±4.0	91.4±2.6	94.8±1.5	96.3±6.1	86.1±2.9	100.2±3.0	99.2±5.2	99.6±2.1	100.0±3.0	99.7±6.2	103.1±2.7	95.8±1.7	92.5±3.9	91.2±1.3	96.0±3.5	96.1±4.9	95.5±2.4
Troglitazone	97.3±13.4	89.4±7.9	88.8±4.0	88.8±7.6	88.5±7.2	101.5±7.0	87.4±7.5	87.6±7.1	95.5±4.1	99.3±2.2	95.4±2.5	99.7±3.1	88.2±3.3	91.1±6.5	98.1±8.3	92.1±9.2	94.9±3.9	105.2±8.2
Vildagliptin	94.2±12.2	91.8±1.3	95.0±1.9	108.2±2.2	107.1±6.0	96.9±4.6	85.5±8.8	89.5±5.7	101.3±5.2	102.4±7.7	88.4±6.0	105.5±4.1	111.8±0.8	116.4±1.4	105.5±1.5	97.4±11.3	89.9±2.7	95.2±2.7

[1)]Concentration (ng/mL): low (near the LOQ), medium (~5-fold above the LOQ), and high (~10-fold above the LOQ)

Table 6. Stability of anti-diabetic over 24 h, as determined by LC-MS/MS

Compound		0[1]	24	48
		RSD (%)		
Alogliptin	Low[2]	10.74	7.54	0.15
	Medium	1.01	9.30	2.63
	High	3.04	3.25	1.96
Buformin	Low	6.88	1.45	1.29
	Medium	8.58	1.54	0.65
	High	2.86	3.84	2.57
Canagliflozin	Low	4.36	7.50	9.64
	Medium	3.93	12.96	10.45
	High	1.68	13.50	6.32
Carbutamide	Low	11.32	8.98	6.16
	Medium	0.63	13.71	5.07
	High	2.76	3.58	5.85
Chlorpropamide	Low	8.38	9.12	14.44
	Medium	6.92	3.49	11.25
	High	2.71	10.53	14.88
Empagliflozin	Low	1.53	6.35	3.87
	Medium	3.10	3.13	4.95
	High	1.14	0.57	0.00
Glibenclamide	Low	1.83	13.29	10.91
	Medium	3.48	1.44	2.32
	High	0.60	5.20	6.00
Glibornuride	Low	6.80	2.68	9.23
	Medium	5.41	3.10	10.73
	High	0.23	7.54	13.57
Gliclazide	Low	3.05	8.15	6.05
	Medium	2.20	8.19	4.30
	High	0.00	4.11	2.07
Glimepiride	Low	4.96	0.44	9.23
	Medium	0.63	7.80	11.65
	High	0.68	4.76	1.70
Glipizide	Low	2.01	7.74	8.56
	Medium	1.92	9.67	10.88
	High	3.05	12.18	12.02
Gliquidone	Low	10.74	11.84	5.89
	Medium	5.24	6.51	8.55
	High	7.06	2.31	7.51
Glymidine	Low	6.51	9.38	2.95
	Medium	0.25	11.95	3.04
	High	1.07	6.59	1.74
Ipragliflozin	Low	3.82	13.97	7.44
	Medium	0.89	10.71	0.40
	High	0.97	7.98	2.71
Metformin	Low	12.06	6.69	2.18
	Medium	5.08	0.25	5.56
	High	1.31	8.58	11.59
Mitiglinide	Low	4.09	8.34	4.19
	Medium	3.59	9.86	10.44
	High	1.40	8.28	7.72
Nateglinide	Low	8.83	0.35	2.76
	Medium	2.54	11.11	3.96
	High	2.30	5.44	2.77
Phenformin	Low	0.56	0.46	8.36
	Medium	0.11	9.21	11.63
	High	0.27	8.36	3.08
Pioglitazone	Low	3.53	1.77	2.12
	Medium	0.80	1.31	2.88
	High	5.85	7.28	9.23
Repaglinide	Low	8.54	5.54	14.27
	Medium	4.93	1.94	13.39
	High	6.60	2.13	9.89
Rosiglitazone	Low	1.30	2.78	7.85
	Medium	2.94	8.20	11.31
	High	6.25	4.49	2.54
Sitagliptin	Low	6.47	10.31	6.47
	Medium	1.73	8.09	1.31
	High	2.26	9.57	1.00
Tolazamide	Low	7.45	6.26	14.50
	Medium	4.73	4.44	13.58
	High	5.17	8.95	13.98
Tolbutamide	Low	6.28	11.66	7.56
	Medium	0.38	12.23	12.95
	High	1.04	10.46	12.38
Troglitazone	Low	4.59	3.45	1.60
	Medium	0.55	6.29	2.72
	High	5.15	9.87	8.38
Vildagliptin	Low	8.29	11.61	1.36
	Medium	1.18	8.70	2.24
	High	1.44	3.73	6.80

[1]0 hour is stored for 6 hour at the room temperature after making the solution.

[2]Concentration (ng/mL): low (near the LOQ), medium (~5-fold above the LOQ), and high (~10-fold above the LOQ)

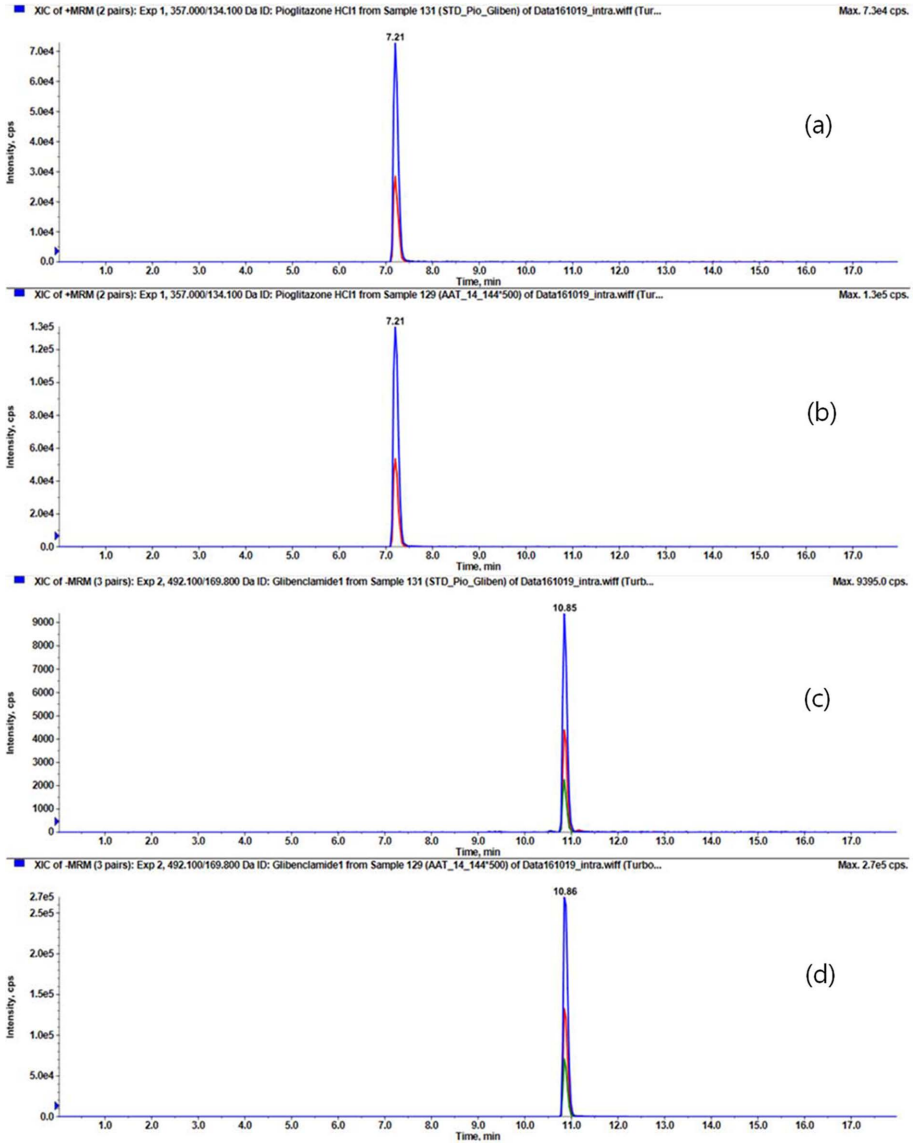

Fig. 4. Mass spectra of a standards and sample that contains the pioglitazone and glibenclamid. (a) pioglitazone STD, (b) pioglitazone in sample, (c) glibenclamid STD, (d) glibenclamid in sample

analytical method might help to restrict the use of illegal adulterants in dietary supplements.

Acknowledgements

This study was supported by a grant (Number: 15181MFDS521) from the Ministry of Food and Drug Safety in 2016.

References

1. G. S. K. Reddy, S. A. Kumar, and V. R. Kumar, *Int. Res. J. Pharm.*, **5**, 283-289 (2014).
2. S. Kai, K. Ishikawa, H. Ito, T. Ogawa, H. Yamashita, Y. Nagata, and H. Kanazawa, *Chromatography*, **36**, 19-24 (2015).
3. S. Singh, B. Prasad, A. A. Savaliya, R. P. Shah, V. M. Gohil, and A. Kaur, *TrAC, Trends Anal. Chem.*, **28**, 13-

28 (2009).
4. E. N. Ediage, J. D. Di Mavungu, M. L. Scippo, Y. J. Schneider, Y. Larondelle, A. Callebaut, J. Robbens, C. Van Peteghem, and S. De Saeger, *J. Chromatogr. A*, **1218**, 4395-4405 (2011).
5. F. Song, A. El-Demerdash, and S. J. Lee, *J. Pharm. Biomed. Anal.*, **70**, 40-46 (2012).
6. F. Shi, C. Guo, L. Gong, J. Li, P. Dong, J. Zhang, P. Cui, S. Jiang, Y. Zhao, and S. Zeng, *J. Chromatogr. A*, **1344**, 91-98 (2014).
7. H. M. Lee and B. J. Lee, *Food Addit. Contam.*, **28**, 396-407 (2011).
8. M. J. Bogusz, H. Hassan, E. Al-Enazi, Z. Ibrahim, and M. Al-Turail, *J. Pharm. Biomed. Anal.*, **41**, 554-564 (2006).
9. B. Guo, M. Wang, Y. Liu, J. Zhou, H. Dai, Z. Huang, L. Shen, Q. Zhang, and B, Chen, *J. Agric. Food. Chem.*, **63**, 6954-6967 (2015).
10. S. L. Kao, C. L. Chan, C. C. Tchoyoson Lim, R. Dalan, D. Gardner, E. Pratt, M. Lee, and K. O. Lee, *N. Engl. J. Med.*, **360**, 734-736 (2009).
11. W. T. Poon, Y. H. Lam, H. H. C. Lee, C. K. Ching, W. T. Chan, S. S. Chan, C. K. Lai, M. L. Tse, A. Y. W. Chan, and T. W. L. Mak, *Hong Kong Medical Journal*, **15**, 196-200 (2009).
12. N. Sultana, M. Saeed Arayne, N. Shafi, F. A. Siddiqui, and A. Hussain, *J. Chromatogr. Sci.*, **49**, 774-779 (2011).
13. M. D. Adullah Al Mahmud, S. Bhadra, A. Haque, Md. Eliaz-Al-Mamun, and S. S. Haider, *Dhaka Univ. J. Pharm. Sci.*, **13**, 51-56 (2014).
14. A. B. Loni, M. R. Ghante, and S. D. Sawant. *Der Pharma Chemica*, **4**, 854-859 (2012).
15. S. Manasa, K. Dhanalakshmi, N. Reddy, and G. S. Sreenivasa, *Int. J. Pharm. Sci Drug Res.*, **6**, 250-252 (2014).
16. B. R. Jani, K. V. Shah, and P. P. Kapupara, *Int. J. Res. Development in Pharm. Life Sci.,* **4**, 1569-1576 (2015).
17. S. Patil Sudarshan, C. G. Bonde, Development, and validation of analytical method for simultaneous estimation of glibenclamide and metformin HCI in bulk and tablets using UV-visible spectroscopy, **1**, 905-909 (2009).
18. N. S. Kim, K. Y. Kim, G. J. Yoo, J. h. Lee, H. N. Park, S. K. Park, and S. Y. Baek, *Food Additives & Containminants: Part A*, **35**, 387-394 (2018).
19. S. R. Polagani, N. R. Pilli, G. Ramakrishna, and G. Venkateswarlu, *J. Pharm. Anal.*, **1**, 9-19 (2013).
20. O. Gonzalez, R. M. Alonso, N. Ferreiros, W. Weinmann, R. Zimmermann, and S. Dresen, *J. Chromatogr. B*, **879**, 243-252 (2011).
21. S. B. Naraharisetti, B. J. Kirby, M. F. Hebert, T. R. Easterling, and J. D. Unadkat, *J. Chromatogr. B*, **860**, 34-41 (2007).
22. S. H. Kim, J. Lee, T. Yoon, J. Choi, D. Choi, D. Kim, and S. W. Kwon, *Biomed. Chromatogr.*, **23**, 1259-1265 (2009).
23. C. de Andrade, G. de Araujo Lock, M. C. Pigatto, S. E. Haas, T. D. Costa, and B. Verlindo de Araujo, *Biomed. Chromatogr.*, **28**, 1722-1727 (2014).
24. A. Kobushi, Y. Ito, K. Yano, and T. Sakaeda, *Journal of chromatography B.*, **1000**, 22-28 (2015).
25. D. Devineni, C. R. Curtin, D. Polidori, M. J. Gutierrez, J. Murphy, S. Rusch, and P. L. Rothenberg, *J. Clin. Pharmacol.*, **53**, 601-610 (2013).
26. R. N. Mamidi, F. Cuyckens, J. Chen, E. Scheers, D. Kalamaridis, R. Lin, J Silva, S. Sha, D. C. Evans, M. F. Kelley, D. Devineni, M. D. Johnson, and H. K. Lim, *Drug Metab. Dispos.*, **42**, 903-916 (2014).
27. International conference on harmonization, 2005. Harmaonized tripartite auideline, validation of analytical procedures: text and methodology Q2(R1). London: European Medicinies Agency (EIMA).

Authors' Positions

Nam Sook Kim : Junior Scientific officer
Geum Joo Yoo : Junior Scientific officer
Kyu Yeon Kim : Resercher
Ji Hyun Lee : Action officer
Sung-Kwan Park : Senior Scientific officer
Sun Young Baek : Direction of Division
Hoil Kang : Direction of Division