Validation of an LC-MS/MS method for analysis of anti-diabetic drugs in botanical dietary supplements labelled for blood sugar management

Jun Ma*, Rahul S. Pawar, and Erich Grundel

Office of Regulatory Science, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, 5001 Campus Drive, College Park, MD 20740, USA

* Correspondence to: Jun Ma, Office of Regulatory Science, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, 5001 Campus Drive, College Park, MD 20740, USA. E-mail: jun.ma@fda.hhs.gov

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Abbreviations used: LC-MS/MS, liquid chromatography-tandem mass spectrometry; RSD, relative standard deviation.

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ABSTRACT: We developed and validated a liquid chromatography tandem mass spectrometry (LC-MS/MS) method to detect and quantitate 14 anti-diabetic, 2 anti-obesity and 3 cholesterol-lowering drugs in botanical dietary supplements marketed for blood sugar management. Many botanical dietary supplements which carry label statements related to blood sugar management are available over the internet. Potential adulteration of such dietary supplements with anti-diabetic and other prescription drugs, some of which have been removed from the market due to adverse events, is of concern. No significant matrix effects were observed and mean recoveries of all 19 analytes from a single product matrix were 88 to 113% at spiking concentrations from 500 to 2000 µg/g. Mean recoveries of metformin, phenformin, and sibutramine from matrices prepared from multiple product composites ranged from 93 to 115% at a spiking concentration of 100 µg/g. The relative standard deviations (RSD) (%) of intra-day analyses ranged from 0.2 to 13 for all recovery studies. Eighty dietary supplements obtained in the U.S. and carrying label statements related to blood sugar management were analyzed using this method and none were found to be adulterated with the above 19 drugs. Two products obtained outside of the U.S. and known to be adulterated were also analyzed by this method and found to contain phenformin, glibenclamide, and sibutramine. This method provided satisfactory selectivity, linearity, accuracy, precision, and sensitivity for rapid determination of 19 drugs and has broad applicability for the analysis of dietary supplements for possible adulteration with these compounds.

Keywords: botanical dietary supplement; anti-diabetic; adulteration; LC-MS/MS

Introduction

In the United States, dietary supplements are regulated by the U.S. Food and Drug Administration (FDA) under the Federal Food, Drug, and Cosmetic Act (FD&C Act). The Dietary Supplement Health and Education Act of 1994 (DSHEA) amended the FD&C Act to provide a new regulatory framework for dietary supplements while retaining applicable food and drug provisions.[1] DSHEA provided for several types of "statements of nutritional support", including structure/function claims which describe the role of a nutrient or dietary ingredient in affecting the structure or function in humans or characterize the means by which the constituent acts to maintain such structure or function. Statements of nutritional support can be made without pre-market approval from the FDA.[2] DSHEA requires that manufacturers have substantiation for such label statements and notify the FDA within 30 days after marketing a product with a statement of nutritional support.[3] The label must also carry the disclaimer "This statement has not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure or prevent any disease."[4] However, recent studies indicate that consumer understanding of FDA's role in regulating dietary supplements and the purpose of the disclaimers is often lacking.[5]

Dietary supplements that claim to regulate blood sugar levels are increasingly available. The National Institutes of Health's Dietary Supplement Label Database currently lists about 800 products that carry label statements related to blood sugar management[6] (e.g., "blood sugar support", "maintain healthy blood sugar levels". Many contain botanical ingredients (e.g., bitter melon, cinnamon, gymnema, fenugreek). Potential adulteration of such dietary supplements with prescription drugs, including those that have been removed from the market due to adverse events, is of concern because diabetic patients taking such adulterated products may be at risk of developing serious adverse effects (e.g., hypoglycemia, organ damage, lactic acidosis).[7-9]

The presence of synthetic anti-diabetic drugs (e.g., metformin, phenformin, rosiglitazone,gliclazide, glibenclamide, glimepiride) in Chinese proprietary medicines, anti-diabetic herbal medicines, and products identified as dietary supplements and labelled for blood sugar management is well documented.[7-19] A variety of analytical methods have been developed for detecting synthetic drugs in traditional herbal medicines and these reports are usually accompanied by analyses of proprietary medicines or dietary supplements sold for blood sugar management.[7,8,10-20] [Table S1 (Supporting Information)].

Findings of therapeutic or higher-than-therapeutic concentrations of anti-diabetic drugs in products labelled as traditional medicines or herbal supplements illustrate the need to determine whether botanical dietary supplements available in the U.S. that carry label statements related to blood sugar management may be adulterated with prescription drugs. We developed and single laboratory validated[21] an LC-MS/MS method to quantitate 14 anti-diabetic compounds (metformin, phenformin, rosiglitazone, pioglitazone, glipizide, chlorpropamide, tolbutamide, acetohexamide, mitiglinide, repaglinide, nateglinide, glimepiride, glibenclamide, gliquidone) (Figure 1) in botanical dietary supplements carrying label statements related to blood sugar management. Because such products may also carry label statements related to weight-management (e.g., "support weight loss") and cholesterol-reduction (e.g., "lowers cholesterol"), we extended the applicability of the method by including the weight loss compounds ephedrine and sibutramine and cholesterol-lowering compounds atorvastatin, lovastatin, and simvastatin in the method (Figure 1), which have been found in products sold as dietary supplements.[22-26] The method was validated for selectivity, linearity, accuracy, sensitivity and precision and is applicable to the quantitation of these 19 analytes at concentrations of ≥ 50 µg/g.

Experimental

Materials

Standards and reagents: Gliquidone was purchased from Selleck Chemicals (Houston, TX, USA). Metformin hydrochloride, phenformin hydrochloride, rosiglitazone, pioglitazone hydrochloride, glipizide, chlorpropamide, tolbutamide, acetohexamide, mitiglinide calcium, repaglinide, nateglinide, glimepiride, glibenclamide, ephedrine sulfate, sibutramine hydrochloride monohydrate, atorvastatin calcium, lovastatin, and simvastatin, all of >95% purity, were purchased from Sigma-Aldrich (St. Louis, MO, USA). LC-MS grade acetonitrile, methanol, and water were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Formic acid was purchased from Sigma-Aldrich.

Dietary supplement samples: A Google search was conducted using the key words "supplement", "blood sugar", and "blood glucose". Inclusion criteria required that a product carry the Supplement Facts panel, be a botanical dietary supplement, carry a claim for blood sugar management (e.g., "blood sugar support", "maintain healthy blood sugar levels") or a name that suggested blood sugar control (e.g., "reducing sugar pill"). Non-botanical products (e.g., supplements of α-lipoic acid, chromium) and, those advertised as traditional or herbal medicines, or those claiming to treat or cure diabetes were excluded. The websites of more than 100 products were reviewed and 20 products that did not meet the inclusion criteria were excluded. Seventy-seven products were ordered from www.amazon.com and all were received. One was excluded because it did not carry the Supplements Facts panel. Five products were purchased from local supermarkets using the inclusion criteria described above. One was excluded because the label did not list any botanical material. Eighty (80) products were thus available for the study.

The products fell into five broad groups (Supporting Information, Table S2): (1) single ingredient products (36% of products) consisting of individual botanicals which have

been associated with blood sugar management; (2) multiple ingredient products (19%) consisting of combinations of botanical materials and extracts; (3) Trademarked (™), Registered (®) and proprietary blend products (19%) whose labels also listed botanical materials, extracts and blends of botanicals; (4) mixtures of botanicals, botanical extracts, vitamins, and minerals (23%) which contained botanical materials as well as minerals (e.g., zinc, chromium) or vitamins (e.g., vitamin C, biotin); and (5) mixtures of botanical materials and animal products or blends of vitamins (5%). Twenty-six (26) of the products (33%) carried manufacturer information; 19 were manufactured in the U.S., five in India, and one each in the United Kingdom and Canada. Two additional botanical dietary supplement products which had been found to be adulterated with anti-diabetic and anti-obesity compounds were kindly provided by Dr. Feng Wei from the China Food and Drug Administration (China FDA, Beijing, P. R. China). These samples were identified as "A" and "B".

Sample Preparation

Twenty (20) pills from a single bottle were ground to form single product homogenates. 0.5 g of the homogenate was sonicated in 40 mL of methanol for 30 min followed by centrifugation (15 min; 3,041 RCF). Samples were diluted 1:100 with methanol; further dilutions were prepared as needed. All sample solutions were filtered through a 0.45 µm nylon membrane prior to injection for LC-MS/MS analysis. A blank matrix extract was prepared from a single dietary supplement product that contained no detectable amounts of the target analytes. The product was prepared and extracted as above and diluted 1:100 to prepare a blank matrix extract. A quality control (QC) sample was prepared by spiking the blank matrix extract with standard compounds at 125 ng/mL each.

Instrumentation and experimental conditions

LC-MS/MS analysis was performed using a Shimadzu Prominence UFLC XR HPLC system (Columbia, MD, USA) and an AB Sciex Qtrap 5500 mass spectrometer (Foster City, CA, USA). The data were collected and processed using Analyst® version 1.6.2 software with Analyst® Classic integration algorithm. The source parameters were: curtain gas 20 au, collision gas medium (9 au), ionspray voltage 5500 V, source temperature 550 °C, ion source gas **1** 50 au, ion source gas **2** 50 au, entrance potential 10 V. For the MRM (Multiple Reaction Monitoring) transitions, excluding those involving the loss of a methyl group or water, the most abundant transitions were selected for quantification and the second most abundant were selected for confirmation. The parameters for the precursor ($[M+H]^+$) and two product ions (*m*/*z*) for the analytes are summarized in Supporting Information Table S3. The chromatographic separation was carried out using a Luna PFP (2) column (150 x 2.0 mm, 3 µm) (Phenomenex, Torrance, CA, USA) with a linear gradient of water (A) and acetonitrile (B) both containing 0.1% formic acid, from 5% B to 85% B in 15 min at a flow rate of 0.30 mL/min. The column temperature was set at 25 °C, and the autosampler temperature was set at 4 °C. A 1 µL injection was made and data were acquired in ESI positive mode for each analysis. Separations on an XBridge BEH HILIC column (100 x 2.1 mm, 3.5 µm) (Waters, Milford, MA, USA) were also evaluated.

Standard solutions

Stock solutions (200 µg/mL) were volumetrically prepared by dissolving individual standards in methanol. The stock solutions for each standard were mixed in equal volumes to prepare a working stock solution (4 µg/mL). The working stock solution was further diluted to provide concentrations of 6.25 – 2000 ng/mL for each analyte. The standards were analyzed using the LC-MS/MS method above with the Luna PFP (2) column and calibration curves were generated by plotting the peak areas versus concentrations of the individual

compounds. The concentrations of analytes in each sample were calculated from the calibration curves. The blank matrix extract and the QC sample were analyzed after every 20 injections for quality control purposes. Carryover was removed by injecting three blank matrix extracts following injection of samples containing higher amounts of the target analytes (i.e., > 2000 ng/mL, data not shown).

Method Validation

Selectivity and matrix effects: The selectivity of the method was investigated by comparing the LC-MS/MS chromatograms of the blank matrix extract with those of the blank matrix extract spiked with 19 target analytes. Matrix effects were measured for each standard at 25, 100, and 400 ng/mL in methanol and compared to a post-extraction fortified matrix.[14]

Linearity: Linearity was evaluated by analyzing five concentrations of standard solutions from 6.25 to 2000 ng/mL. Calibration lines were generated by plotting the peak areas versus concentrations of the individual target analytes. The calibration lines were fitted using Microsoft Excel Version 2010 and the residuals were plotted as a function of target analyte concentration.

Accuracy and recovery: *Recoveries from a single product matrix:* Solid standards (0.25, 0.5, and 1 mg of analytes) were weighed and added to 0.5 g portions of the blank matrix and mixed thoroughly to prepare spike concentrations of 500, 1000, and 2000 µg/g (n = 3 at each concentration). All spiked blank matrices were allowed to stand at room temperature for one hr. They were then extracted, centrifuged, diluted 1:100 or further and analyzed using the methods above. Recoveries were also evaluated by spiking higher concentrations (40 mg/g) of metformin (Retention time, (RT), 1.3 min), rosiglitazone (RT, 7.9 min) and gliquidone (RT, 13.9 min) representing high, medium and low polarity compounds into 0.5 g portions of the blank matrix and treating them as above. Recoveries

were calculated by the formula: Recovery (%) = (100) (Aos/As), where Aos = Observed amount; As = Spiked amount.

Recoveries from mixtures of multiple product matrices: Recoveries were also evaluated in more complex matrices prepared from mixtures of four or five dietary supplement products. Five products were selected from each of Groups 1 through 4 (Table S2 (Supporting Information)). All four products from Group 5 were selected. Ten capsules or tablets from a single bottle were homogenized to prepare a composite. Two g of each product composite were combined to form a group composite (G1-G5). Metformin (RT, 1.3 min), phenformin (RT, 5.5 min), and sibutramine (RT, 12.8 min), which are frequently reported as adulterants in botanical dietary supplements, were selected for the spike recovery studies. Fifty µg of each analyte (0.5 mL of 0.1 mg/mL standard solution) was added to 0.5 g portions of each group composite and mixed thoroughly. The final spike concentration was 100 µg/g for each analyte and spiked group composites were prepared in triplicate. The spiked composites were allowed to stand at room temperature for 24 hr and were then extracted, centrifuged, filtered, diluted 1:100 and analyzed using the method above.

Precision: Intra-day precision was calculated from the results of the single product matrix and group composite recovery studies.

LOQs: For the purpose of this study the lowest concentration of calibration standard (6.25 ng/mL) was used as the default limit of quantitation (LOQ) for all of the analytes.

Stability: Stability of the 19 target analytes was evaluated in the QC sample solution (125 ng/mL of each analyte in the blank matrix extract) after storage at room temperature and at -20°C. One portion of the QC sample was stored at room temperature and analyzed at 0, 9, and 24 hr after preparation with three injections at each time point. Another portion of the QC sample was stored at -20°C and analyzed at monthly intervals for six months with three injections at each interval.

Results and discussion

Method development

Selection of extraction solvent: The QuEChERS (quick, easy, cheap, effective, rugged, and safe) method is a popular sample extraction method for LC-MS analysis.[27] However, low recovery was reported when QuEChERS was used to extract the high polarity analyte metformin from sample matrices.[28] Because of the high affinity of metformin for the aqueous phase, it could not be completely transferred to the acetonitrile phase during salt-induced phase separation. Since methanol has been frequently used as a solvent to extract pharmaceutical adulterants from botanical dietary supplement matrices,[20, 29] we used this solvent with sonication to extract these compounds.

Column selection: Yang *et al.* reported that metformin (log $P_{\text{octanol/water}}$ = -2.64), is poorly retained on a conventional reversed-phase liquid chromatographic column such as C18.[30] Liu *et al.* reported that a hydrophilic interaction liquid chromatography (HILIC) column (Intersil HILIC column, GL Sciences, Torrance, CA) provided adequate retention of metformin.[31] However, we found that an XBridge BEH HILIC column (100 x 2.1 mm, 3.5 µm) (Waters, Milford, MA, USA) did not provide sufficient separation for target analytes such as acetohexide, mitiglinide, and repaglinide (medium polarity) and lovastatin, gliquidone, and simvastatin (low polarity) (data not shown). A Luna PFP (2) column (see above) provided satisfactory separation and was selected for the analysis. The chromatograms of the 19 standards are shown in Supporting Information, Figure S1.

Instrument parameters: The tune and MS/MS parameters were optimized by infusing solutions of the standard compounds into the mass spectrometer. Use of ESI in positive mode with solvents containing 0.1% formic acid modifier provided adequate response for the ionization of the target analytes ($[M + H]^+$). Precursor ions were selected for fragmentation by collision-induced dissociation. The MS/MS parameters were optimized to achieve

adequate response for the precursor and product ions. Two transitions from each compound were simultaneously monitored and ion ratios and retention times were compared to confirm the identities of the target analytes. The ratio of the primary transition to the secondary transition matched within ± 10% of the average value of the calibration standard and the retention time of the suspected analyte peak matched within ± 5% of the average retention time of the calibration standard.[32]

Method validation

Selectivity and matrix effects: Possible interference from matrix components with the measurement of 19 target analytes was investigated by comparing the LC-MS/MS chromatograms of the blank matrix extract with those of the blank matrix extract spiked with 19 target analytes. The responses of the compounds at three concentrations in the blank matrix extract were compared with those of the standard solutions in methanol at the same concentrations (i.e., response ratios). These ratios ranged from 94 to 111% (Supporting Information, Table S4), indicating that the co-extracted matrix did not produce significant effects on the ionization of target analytes. Based on these observations, our method demonstrated adequate selectivity for the 19 analytes of interest.

Linearity: The suitability of linear calibration curves for evaluation of instrument signal response versus analyte concentration was determined. Regression equations, correlation coefficients, sum of residuals, and mean of residuals for calibration curves are shown in Supporting Information, Table S5. Based on these results, the calibration data were considered acceptable for a quantitative method.

Accuracy and recovery: The recoveries of target analytes from a single product matrix at concentrations from 500 to 2000 µg/g ranged from 88 to 113% (Table 1). The lowest recovery observed (88%) was that for tolbutamide at a concentration of 500 µg/g. Acceptable

recoveries were obtained for all analytes at concentrations of 500 μg/g and above. Recoveries were also evaluated at 40 mg/g, a concentration approaching a therapeutic dose of the pharmaceutical.[28] Mean recoveries of metformin, rosiglitazone and gliquidone at spiking concentrations of 40 mg/g were 106%, 107%, and 106%, respectively (Table 1).

Precision: Intra-day precision of the method was calculated from the data in Table 1. RSD (%) are shown with the recovery data. The RSDs ranged from 0.2 to 13%, showing acceptable precision. Additional intra-day precision values are shown in Table 2. Taken together, these data demonstrate acceptable method precision.

LOQs: The lowest concentration of calibration standard (6.25 ng/mL) was used as the default LOQ for all of the analytes tested. This was based on the response of the lowest calibration standard, the response of the fortified blank matrix extract, and the recoveries of the group composites. All 19 analytes showed responses well above S/N requirements traditionally used for LOQ determinations, with metformin (S/N =23) showing the lowest response. Additionally, a fortified blank matrix extract was used to determine an instrumental LOQ and the values ranged from 4 to 2000 ng/g across all analytes. Finally, the group composites all fortified at 100 μg/g show acceptable recoveries for the analytes evaluated (Table 2). Review of the literature regarding adulteration of Chinese proprietary medicines and herbal supplements with anti-diabetic drugs revealed that such adulterants, when present, are found at mg/g (≥ 0.1%) concentrations. These are often in the range of therapeutic doses or at concentrations that will likely have some pharmacological effect. Based on such high concentrations, a determination of the lowest possible values for LOQs, often in the ng/g range, is not critical to establish that the method is "fit for purpose" in quantitating these 19 adulterants in dietary supplements. Therefore, the lowest calibration standard was used as the method LOQ for all analytes.

LODs and LOQs are most often useful for control of undesirable impurities and for low level contaminants (e.g., those specified as "not more than"). However, LODs and LOQs are often not necessary for compositional specifications or for high level adulterants. In such cases, an alternative approach is to treat the lowest calibration standard as an LOQ and to assume that the LOD is 1/3 of this concentration. The rationale for this approach is that therapeutic doses (mg/g) of the adulterants, if present, are expected to be significantly higher (orders of magnitude) than the concentration of the lowest calibration standard. In these cases, knowledge of the actual LODs and LOQs is not necessary. For this reason, we used 6.25 ng/mL as the LOQ for this method. Using an LOQ of 6.25 ng/mL, it is possible to calculate that a sample adulterated at a concentration of 50 µg adulterant/g would be identified as such by this method if the sample was subjected to the standard extraction procedure of the analysis (e.g., 0.5 g sample weight, 40 mL extractant, centrifugation, 1:100 dilution, filtration,). Thus, the method is applicable to the determination of adulteration at concentrations of ≥ 50 µg/g.

Stability: The 19 target analytes in the QC sample solution remained stable (three analyses, RSD < 10%) at room temperature for at least 24 hours, demonstrating short term stability. They also remained stable (six analyses, RSD < 10%) at -20°C for at least six months, demonstrating long term stability (data not shown).

When comparing our method with previously published methods for analysis of anti-diabetic compounds in botanical dietary supplements,[10, 12-14, 16, 18, 33] we note that our method provides adequate selectivity, sensitivity, accuracy, and precision and simultaneously analyzes a wider range of target compounds including anti-diabetic, anti-obesity, and cholesterol-lowering compounds. However, the instrument was not run in full scan mode and therefore, would not likely detect any adulterants other than the 19 targeted analytes. Vaclavik *et al*. developed an ultra-high performance liquid chromatography-quadrupole-

orbital ion trap mass spectrometry (UPLC-Q-orbitrap MS) method for simultaneous determination of 96 possible adulterants including pharmaceuticals, plant toxins and other secondary metabolites in botanical dietary supplements.[28] While the work of Vaclavik *et al*. is the first report of an integrated analysis of an extremely wide range of compounds, limitations in the quantitative aspects of the analysis suggest that triple quadrupole instruments are currently more suitable for studies such as those described in this paper.[34]

Dietary supplements

Labeling: All products met our inclusion criteria. Seventy-seven (77) of the products (96%) carried an explicit statement related to blood sugar man2010agement and product names of the remaining three suggested this function. Seventy-four of the 77 products (96%) that carried statements regarding blood sugar management carried the full FDA disclaimer. The remaining three (4%) carried partial disclaimers (i.e., one or the other but not both of the two sentences above).

Analysis: All products were analyzed for adulteration with 19 compounds using the developed LC-MS/MS method. Both transitions were free of any interference from components in the 80 samples tested. None of the target analytes were detected in any of these products.

This unexpected finding prompted us to extend the recovery studies to additional matrices. The second recovery study in multiple product composites was performed to provide a more comprehensive evaluation of recovery since the initial recovery studies were performed in a single product matrix. In addition, composites in the second study were exposed to the analytes for 24 hours before extraction to allow time for analytes to bind with matrix components. The results of the second study showed recoveries of 93 to 115% for

metformin, phenformin and sibutramine (100 µg/g each) from five complex mixtures of matrices (Table 2). Taken together, these findings demonstrate that the method provides satisfactory recoveries of analytes over a wide range of concentrations from a variety of botanical matrices.

To further evaluate method performance, two botanical dietary supplement products obtained from the China FDA were also analyzed in triplicate using the same method. Based on the analysis, it was determined that product "A" contained phenformin (23.4 ± 0.2 mg/g or 7.9 ± 0.1 mg/capsule) and glibenclamide (6.5 ± 0 mg/g; 2.2 ± 0 mg/capsule) and product "B" contained sibutramine (35.6 ± 0.8 mg/g; 9.9 ± 0.2 mg/capsule) (Figure 2). These results were consistent with previously reported findings and showed that our method can detect and quantitate adulteration in samples obtained from the market place.

Based on our review of the available literature, our finding that none of the 80 dietary supplement samples we analyzed showed the presence of any of the 19 adulterants was unexpected. However, the set of dietary supplement samples we analyzed were quite different from products which have previously been reported to be adulterated. None of our products were traditional Chinese medicines or herbal medicines. None carried statements about treating or curing diabetes. All products carried the required Supplement Facts panel and 96% carried the full disclaimer required with use of statements of nutritional support. To the best of our knowledge, this is the first study in the U.S. to look for potential adulteration in a large set of dietary supplements specifically labelled for blood sugar management.

While these current findings are encouraging, the products were collected, in part, to evaluate the newly developed LC-MS/MS method and not as a statistical representation of dietary supplements marketed for blood sugar management in the U.S. We emphasize that our conclusions are applicable only to the set of products that we analyzed and cannot be extrapolated to the other anti-diabetic products or other supplement categories.

It is likely that multiple factors contributed to the findings. Such factors may have included recent actions by the FDA (e.g., warning letters) to remove from the market illegal products that claimed to mitigate, treat, cure or prevent diabetes. Such actions may have led to a reduced likelihood of adulteration. Our use of restrictive inclusion/exclusion criteria in product selection may have resulted in our missing adulterated products. Similarly, the lack of randomization in product selection and/or dependence on limited internet search algorithms may also have contributed to our findings. Finally, adulteration of supplements sold for blood sugar management may be relatively infrequent in the U.S. and far less than that seen in weight loss, body-building and sexual performance enhancement products. Several recent reviews have indicated that adulteration of products including those for weight loss, sports, and sexual performance enhancement is far more prevalent than adulteration of supplements sold for blood sugar control.[35-37]. These reviews include data and information from emergency department visits,[35] reviews of cases of toxicity due to chemical adulterants in botanical dietary supplements,[36] and reviews of the classes of drugs frequently seen as being added illegally to specific classes of supplements.[37] Regardless of possible contributing factors, the newly developed method will allow FDA to more effectively monitor such products for the presence of adulterants.

Conclusion

A sensitive LC-MS/MS method was developed and validated for simultaneous analysis of 14 anti-diabetic 2 anti-obesity and 3 cholesterol-lowering drugs in botanical dietary supplements carrying label statements related to blood sugar management. This method provided satisfactory selectivity, linearity, accuracy, precision, and sensitivity for rapid and accurate determination of a variety of synthetic drugs and can be used for

monitoring adulteration of botanical dietary supplements with these compounds at concentrations ≥ 50 μg/g.

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Table 1. Recovery of analytes from a single product matrix at concentrations of 500, 1000, 2000 and 40,000 µg/g[a]

Analyte	Spiking concentration (µg/g)			
	500	1000	2000	40,000
	Recovery (%)			
Metformin	101 (4)	97 (5)	96 (6)	106 (11)
Ephedrine	112 (13)	100 (8)	95 (3)	-
Phenformin	113 (6)	104 (13)	101 (5)	-
Rosiglitazone	102 (5)	110 (4)	98 (6)	107 (5)
Pioglitazone	100 (5)	96 (9)	109 (3)	-
Glipizide	91 (1)	90 (7)	103 (2)	-
Chlorpropamide	94 (4)	97 (4)	105 (1)	-
Tolbutaminde	88 (6)	96 (3)	99 (3)	-
Acetohexamide	99 (3)	100 (5)	98 (2)	-
Mitiglinide	106 (7)	101 (1)	101 (3)	-
Repaglinide	99 (2)	96 (12)	98 (7)	-
Atorvastatin	89 (4)	108 (7)	111 (8)	-
Nateglinide	107 (3)	97 (5)	90 (2)	-
Glimepiride	110 (0.5)	10 (8)	93 (2)	-
Sibutramine	108 (8)	97 (3)	94 (1)	-
Glibenclamide	95 (2)	100 (2)	99 (8)	-
Lovastatin	103 (3)	90 (6)	95 (1)	-
Gliquidone	103 (2)	108 (2)	104 (4)	106 (3)
Simvastatin	101 (3)	98 (2)	102 (0.2)	-

[a]A composite was prepared from a dietary supplement product that contained no detectable amounts of the target analytes. Triplicate 0.5 g portions of the composite were spiked with solid standards of all analytes to provide concentrations from 500 to 2000 µg/g. Three analytes only were spiked at the 40,000 µg/g concentrations. The spiked composites were allowed to stand at room temperature for one hour and were then extracted and analyzed. Recoveries were calculated as described above. Values are means and (%RSD) of three determinations at each concentration.

Table 2. Recovery of metformin, phenformin, and sibutramine (high, medium, and low polarity analytes, respectively) from matrices prepared from multiple product composites[a]

Group/Analyte	Spiking concentration, 100 µg/g Recovery, %		
	Metformin	Phenformin	Sibutramine
1. Single botanicals	103 (5)	101 (8)	93 (1)
2. Multiple botanicals	113 (1)	115 (1)	107 (3)
3. Trademarked, registered botanicals	106 (4)	115 (0.3)	100 (2)
4. Botanicals, vitamins, minerals	101 (5)	115 (6)	99 (3)
5. Botanicals, animal products, other constituents	111 (4)	104 (8)	94 (2)

[a]Mixtures of dietary supplement products were prepared from equal weights of four or five products from each of the groups listed in Table S1 (See Supporting information, Table S1). Triplicate 0.5 g portions of the mixtures were spiked with 0.5 mL of 0.1 mg/mL solutions of metformin, phenformin, and sibutramine. The spiking concentration of each analyte was 100 µg/g. Spiked composites were allowed to stand for 24 hours at room temperature and were then extracted and analyzed. Recoveries were calculated as described in the text. Values are means and (%RSD) of three determinations at each concentration.

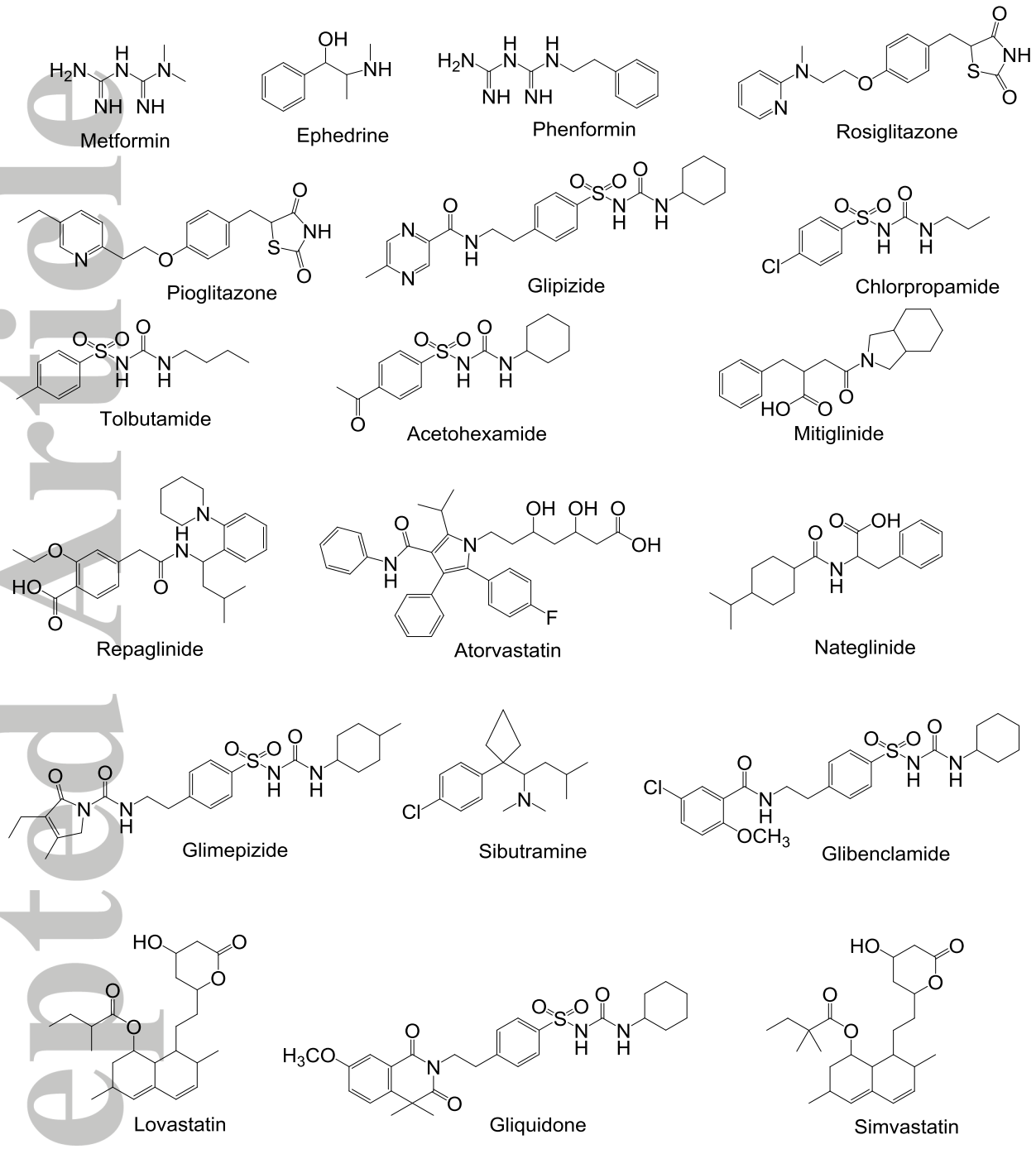

Figure 1. Chemical structures of the 19 anti-diabetic, anti-obesity, and cholesterol-lowering compounds.

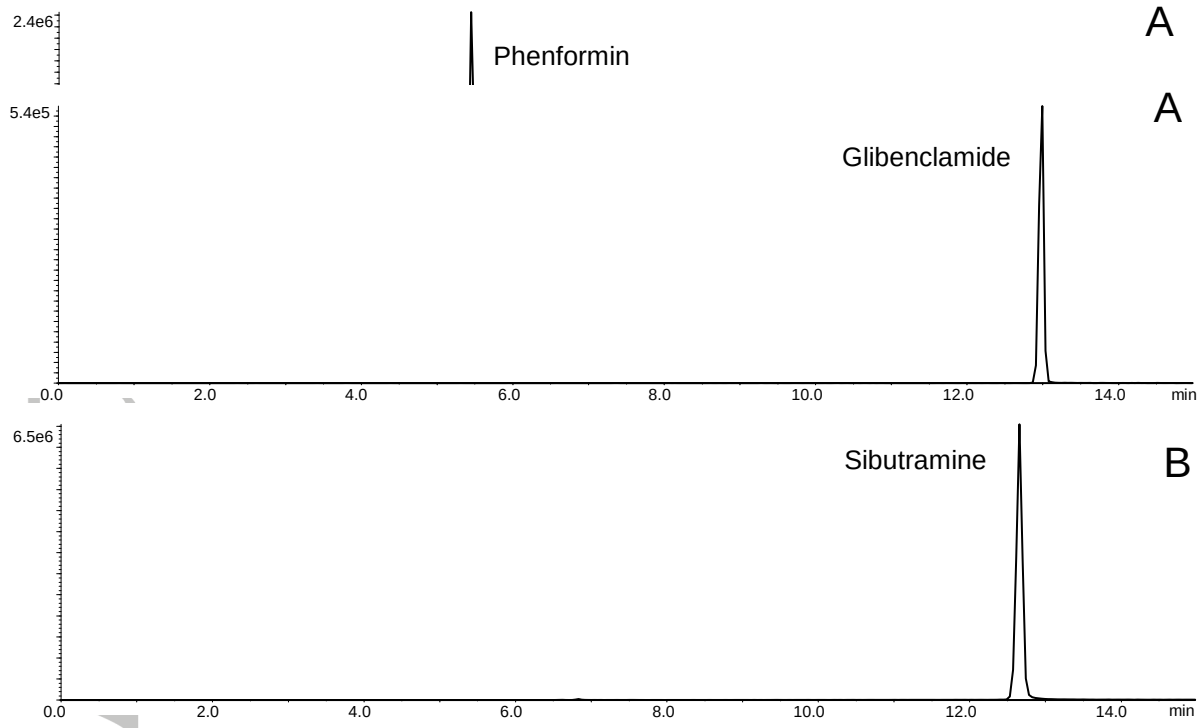

Figure 2. LC-MS/MS chromatograms (quantification transitions) of phenformin and glibenclamide in product A, and sibutramine in product B.

Graphical Abstract:

Validation of an LC-MS/MS method for analysis of anti-diabetic drugs in botanical dietary supplements labelled for blood sugar management

Jun Ma, Rahul S. Pawar and Erich Grundel

Eighty (80) dietary supplements carrying label statements for blood sugar management were analyzed by a validated LC-MS/MS method for the presence of 14 anti-diabetic, two anti-obesity and three cholesterol-lowering drugs. None of the potential adulterants were found in any of the products. Two additional products known to be adulterated with pharmaceuticals were also analyzed and found to contain mg/g quantities of the analytes of interest. The method is applicable to the determination of potential adulterants in dietary supplements.