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Simultaneous determination of selected antidiabetic pharmaceutical drugs as adulterants in herbal medicines sold in Kenya using liquid chromatography-tandem mass spectrometry (LC-MS/MS)

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Abstract

Herbal medicines are usually marketed as natural products with multiple beneficial health claims. However, several studies have established that these products are usually adulterated with pharmaceuticals to increase their treatment effectiveness. Adulterating herbal products with pharmaceuticals can result in adverse herb-drug interactions, undermining their safety. Therefore, it is important to routinely analyse herbal products to determine if they have been adulterated with pharmaceuticals. A liquid chromatography coupled with tandem mass spectroscopy (LC-MS/MS) method was developed and validated for simultaneous identification and quantification of four commonly used antidiabetics – metformin (MET), gliclazide (GLZ), glibenclamide (GLC), and glimepiride (GLP) – in herbal medicines. Chromatographic separation of these analytes was done using a C-18 column and a gradient elution program of 0.1% formic acid in acetonitrile. The developed method showed detection limits and quantification limits ranging from 2.86 to 7.67

ng/mL and 8.64 to 23.24 ng/mL, respectively. The accuracy was above 80% for all analytes except MET (52%). This method was then applied to analyse 24 powdered herbal drugs sourced from the Kenyan market to check for adulteration. MET was detected in 17% of the samples, with concentrations ranging from 900 ng/g to 1969 ng/g. GLC, GLZ, and GLP were not detected in any sample. This method can therefore be used to analyse antidiabetic herbal medicines for adulteration using the four pharmaceuticals.

Keywords: LC-MS/MS; MRM; ESI; Adulteration; Antidiabetic Herbal Medicines.

1 Introduction

The number of diabetic patients is on the rise globally [1]. As a result, a huge increase in the number of diabetic patients using herbal drugs to treat their illnesses is being experienced as patients seek safer treatment options with fewer side effects compared to conventional pharmaceuticals [2]. However, these drugs may be unsafe as several studies have reported that antidiabetic herbal drugs are commonly adulterated with synthetic oral antidiabetic drugs leading to adverse patient events [3]–[6]. Antidiabetic drugs commonly used to adulterate herbal medicines include metformin (MET), gliclazide (GLZ), glibenclamide (GLC), rosiglitazone, glimepiride (GLP), nateglinide, phenformin, and repaglinide [6]–[9]. Therefore, it is critical to analyse antidiabetic herbal medicines for adulteration using antidiabetic pharmaceuticals to promote patient safety.

A wide range of analytical techniques has been utilized to identify and quantify pharmaceuticals used to adulterate herbal remedies, including capillary electrophoresis, infrared spectroscopy, high-performance liquid chromatography (HPLC), and thin layer chromatography [10]–[15]. Hyphenated techniques, particularly HPLC coupled with tandem mass spectroscopy (LC-MS/MS) or mass spectroscopy (LC-MS) are most commonly used to analyse for adulteration of herbal products because they meet the majority of the essential requirements of analysis for food samples and herbal products [7]. Notably, LC-MS/MS allows for a wide range of potential adulterants to be analysed and quantified simultaneously in one run using multiple reaction monitoring (MRM) because this technique has high selectivity and sensitivity for pharmaceuticals [16]–[20].

The World Health Organization (WHO) estimates that about 80% to 90% of Africans rely on herbal medicine to treat their illnesses [21]. The situation is similar in Kenya, especially in rural areas where communities heavily rely on herbal remedies for their primary healthcare needs

because of their accessibility and affordability [22]. However, no strong regulatory mechanisms exist to evaluate herbal medicines sold in the country for their effectiveness and safety [23]. Therefore, it is likely that these herbal remedies are being adulterated with pharmaceuticals without the knowledge of patients and regulatory bodies. As such, this study aims to develop and validate a rapid, simple, selective, and sensitive LC-MS/MS method to identify and quantify adulterants in herbal products, and then apply it to antidiabetic herbal medicines sold in Nairobi and Uasin Gishu Counties, Kenya. To the best of our knowledge, no similar study has been conducted in Kenya that focuses on adulteration in antidiabetic herbal products. GLZ, GLC, GLP, and MET are listed as essential antidiabetic medicines by the Ministry of Health, Kenya, and hence were chosen as the analytes for this study [24].

2 Materials and Methods

2.1 Reagents and Chemicals

HPLC grade formic acid, acetonitrile, and methanol were purchased from Merck (Germany). Ultrapure water was obtained from a Milli-Q water apparatus. Analytical standards of MET (>99% purity) were provided by Kenya Medical Research Institute (KEMRI) while those of GLZ (>99% purity), GLC (>99% purity), and GLP (>99%) were provided by Cosmos Limited, Kenya.

2.2 Sample Collection

Herbal samples were randomly purchased from herbal clinics, herbal product manufacturers, herbalists, and local retailers (supermarkets, nutrition stores, and street vendors) in Uasin Gishu and Nairobi Counties, Kenya. Nairobi County was chosen for this study because it is a cosmopolitan area with many communities with diverse cultures and customs; thus, many herbal products end up here. Uasin Gishu County has a wealth of ethnobotanical knowledge; hence, most of the local herbal products sold in the country are sourced from this region. Ethical clearance was

granted by KEMRI Independent Scientific and Ethical Review Unit (SERU): (KEMRI/SERU/CTMDR/CSC068/3566).

2.3 Preparation of Standard Solutions

Individual stock solutions were prepared by dissolving accurately weighted standards in methanol to make 1 mg/mL concentration. These stock solutions were refrigerated at -20 ℃, awaiting further analysis. A mixed standard solution (0.02 mg/mL) was prepared in methanol and refrigerated at -20 ℃. Working standard solutions were prepared by diluting the mixed standard solution with the initial mobile phase composition (5% acetonitrile) to concentrations of 5 – 250 ng/mL.

2.4 LC-MS/MS Instrumentation and Optimization of Operating Conditions

A Waters Alliance 2975 LC system (LC, Milford, MA, USA) coupled to a Waters Quattro Micro mass spectrometer (Micromass, UK) was used. The system was operated using Masslynx 4.1 software. Separation was conducted on an Xbridge^{TM} C_{18} column (3.5 µm * 2.1 mm * 100 mm) fitted with a Vanguard ® pre-column (2.1 mm * 5 mm). The collision and desolvation gases were argon and nitrogen, respectively. The source temperature was set at 120 ℃ while the desolvation temperature was set at 350 ℃. The cone and desolvation gas flow rates were 50 and 700 L/min, respectively. The mass spectrometer was operated in the MRM mode.

MS parameters were optimized using direct infusion, whereby a syringe pump was used to inject 400 ng/mL standard solutions directly into the mass spectrometer. For each standard, both the negative and positive electrospray ionization (ESI) modes were tested. The most abundant m/z for each compound was chosen as the precursor ion, and the intensity of the resultant signal was optimized by changing the cone voltage. The cone voltage with the most intense signal was selected. The collision energies were optimized for each transition, and the two most intense product ions were chosen for qualification and quantification.

For the organic mobile phase, acetonitrile and methanol acidified with 0.1% formic acid were tested to determine which gave better chromatographic separation. Different compositions of the mobile phase were evaluated to improve chromatographic separation. Different separation temperatures (30 ℃ to 40 ℃) and flow rates (0.35 mL/min to 0.45 mL/min) were tested to optimize peak shapes, chromatographic resolution, and analysis time.

2.5 Sample Preparation Optimization

The sample preparation process involved three key steps: extraction, evaporation, and filtration. The absolute recoveries for each step and the entire process were evaluated to determine the optimal conditions. The absolute recoveries were determined by comparing the mean peak area of the spiked blanks (50 ng/mL) with that of the neat standards of similar concentration, as shown in equation 1[25]. A previously-analysed herbal product with no detectable levels of the targeted pharmaceuticals was used as the blank sample.

$$Absolute\ recovery\ (\%) = \frac{mean\ peak\ area_{spiked\ blank}}{mean\ peak\ area\ _{neat\ standard}} * 100 \quad (1)$$

2.6 Method Validation

The performance of the developed method was validated with reference to the International Council for Harmonization (ICH) guidelines for analytical techniques [26].

Linearity was determined by constructing calibration curves and determining the coefficient of determination for all the analytes [27]. Accuracy was calculated by comparing the peak area of the blank spiked to 50 ng/mL prior to extraction to that of the neat standard of a similar concentration. Precision was determined by making three consecutive injections of the mixed standard solution at 10 ng/mL, 100 ng/mL, and 200 ng/mL and calculating the relative standard deviation (RSD) of the mean peak areas obtained.

The LOD was calculated as three times the ratio of the blank's standard deviation, approximated using the y-intercept's standard deviation, to the slope of the calibration curve [28]. The LOQ was calculated as ten times the ratio of the blank's standard deviation to the slope of the calibration curve. The matrix effect was measured by comparing the mean peak area of the blank extract spiked to a concentration of 50 ng/mL after filtration to that of the neat standard of similar concentration [29].

2.7 Data analysis

Microsoft Windows Excel 2019 was used in data entry and analysis. An independent two-tailed t-test was performed to compare the experimental values for the conditions tested during the sample optimization process. A confidence interval of 95% (p= 0.05) was used.

3 Results and Discussion

3.1 Optimized LC-MS/MS operating conditions

The optimal mobile phase was made up of 0.1% formic acid in ultrapure water as solvent A, and 0.1% formic acid in acetonitrile as solvent B. 0.1% formic acid in acetonitrile had sufficient analyte retention, adequate response, and ideal peak shapes for all the antidiabetics, except MET that had a slightly broad peak as illustrated in Fig. 1. Conversely, methanol as the organic mobile phase resulted in a broader peak for MET and insufficient response for the other analytes, consistent with the findings of similar studies [29, 30].

The separation was conducted at a flow rate of 0.40 mL/min and temperature of 40 ℃, as the peaks were better resolved for all the analytes. The following gradient program was used: 0-0.5 min (5% B); 0.5-1.5 min (increase to 30% B); 1.5-2.0 min (increase to 40% B); 2.0-6.0 min (increase to 60% B); 6.0-7.0 min (60% B); 7.0-8.0 min (decrease to 40% B); 8.0-9.0 min (40% B); 9.0-9.5 min (5% B); 9.5-10.0 min (5% B).

The total ion chromatograms (TIC) obtained under these LC conditions for each analyte at 50 ng/mL are shown in Fig. 1.

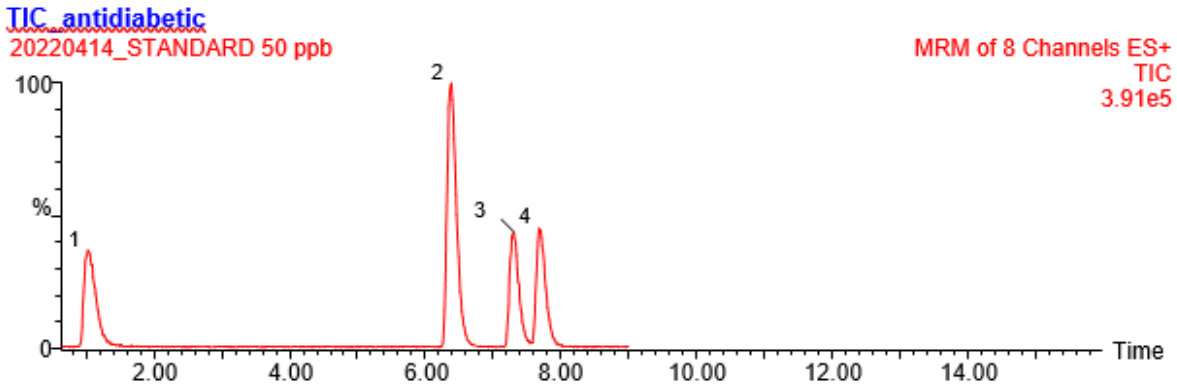
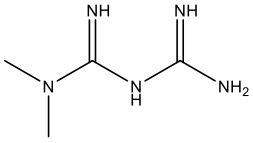
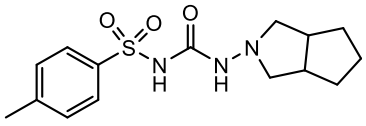
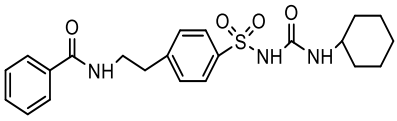
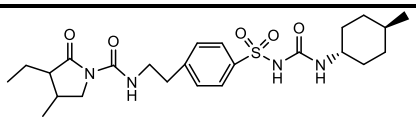


Fig. 1 TIC for the chosen antidiabetics (1 - MET, 2 - GLZ, 3 - GLC, and 4 - GLP)

The MS/MS conditions were also optimized for each analyte. The positive ESI mode was used because it gave optimal results for all the analytes. The MRM transitions for each analyte were optimized, as shown in Table 1.

Table 1 Optimized MRM parameters for the 4 antidiabetics

Compound Name	Structure	Precursor ion	Product ion	Cone voltage (V)	Collision energy (eV)
MET		130	71[q] 60	23	22 24
GLZ		324	127[q] 110	30	28 28
GLC		494	369[q] 169	22	20 38

GLP		491	352[q] 126	22	17 39

[q] Indicates the product ion used for quantification

3.2 Optimization of the sample preparation steps

3.2.1 Extraction step

Different concentrations of 20 mL of methanol in ultrapure water (30%, 60%, 90%) were used to extract 2 g of the blank sample spiked to a final analyte concentration of 50 ng/mL using the mixed standard solution. The extraction was conducted using a shaker at 200 rpm and a sonicator for 20 minutes, and the absolute recoveries compared, as shown in Fig. 2. 90% methanol as the extraction solvent using a sonicator compared to a shaker significantly enhanced the absolute recoveries for GLC (73.4% vs 47.5%, p=0.03) and GLP (78.1% vs 27.9%, p=0.002). It also enhanced the absolute recoveries of MET (52.0% vs 42.4%, p=0.11) and GLZ (129.7% vs 125.2%, p=0.72), but the increases were not statistically significant. Although the absolute recoveries of GLZ were the highest, they were within the acceptable range of 70% to 130% for routine analysis of products with complex matrices, such as herbal products [32]. One possible explanation of the absolute recoveries of GLZ being so high is the presence of matrix influences, particularly ion enhancement, as GLZ is the only analyte in the study whose matrix effect was above 100%. Similar studies on GLZ have reported high recoveries [3], [33-34]. Hence, it is recommended that the pre-and post-spiking approach is used when optimizing the sample preparation process to ensure that the method accounts for matrix influences. As for MET, several studies have reported low recoveries for it due to its high polarity [30], [38].

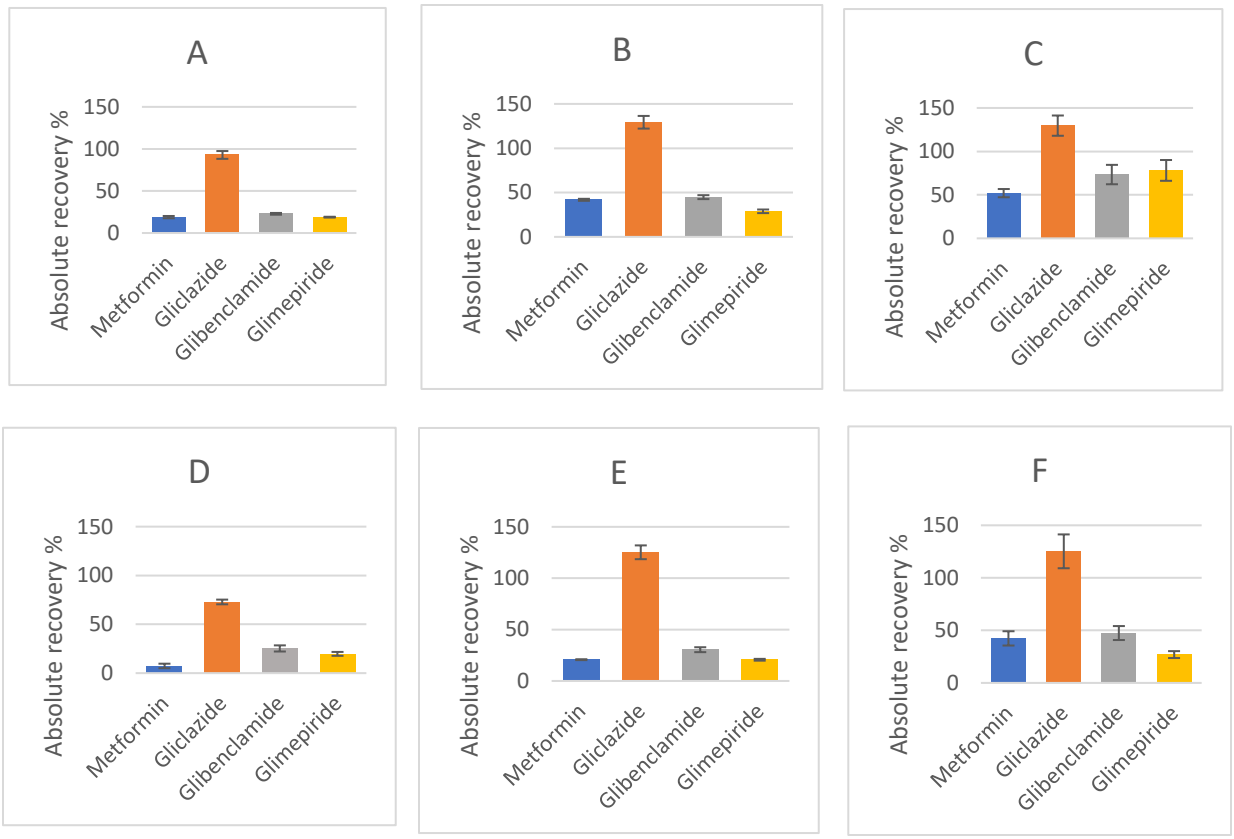


Fig. 2 Absolute recoveries for the entire sample preparation process (A, B and C using a sonicator for extraction with 30%, 60% and 90% methanol as the extraction solvent, respectively; and D, E and F using a shaker for extraction with 30%, 60%, and 90% methanol as the extraction solvent, respectively)

Varying concentrations of formic acid (0.05% and 0.02% formic acid) were added to improve the absolute recoveries of MET. However, the addition of formic acid to the extraction solvent reduced the extraction efficiency of the extraction solvent for all the analytes, as shown in Fig. 3. Using 90% methanol with 0.05% formic acid as the extracting solvent compared to using 90% methanol only significantly reduced the absolute recoveries of MET (42.7% vs 52.0%, p=0.03), GLZ (104.4% vs 129.7%, p=0.04), GLC (40.9% vs 73.4%, p=0.01), and GLP (26.9% vs 78.1%, p=0.001). Similarly, but to a greater extent, using 90% methanol with 0.02% formic acid as the extracting solvent compared to using 90% methanol only significantly reduced the absolute recoveries of MET (42.0% vs 52.0%, p=0.03), GLZ (97.4% vs 129.7%, p=0.02), GLC (11.3% vs

73.4%, p<0.001), and GLP (9.1% vs 78.1%, p<0.001). This effect can be attributed to formic acid lowering the pH of the extraction solvent, which subsequently reduced the solubility of the analytes. GLZ, GLC, and GLP are sulfonylurea drugs; thus, their solubility is poor in weakly acidic media because they are weakly acidic [31-32]. As such, sonicating the samples with 90% methanol for 20 mins was determined to be the optimal conditions for extracting the selected analytes.

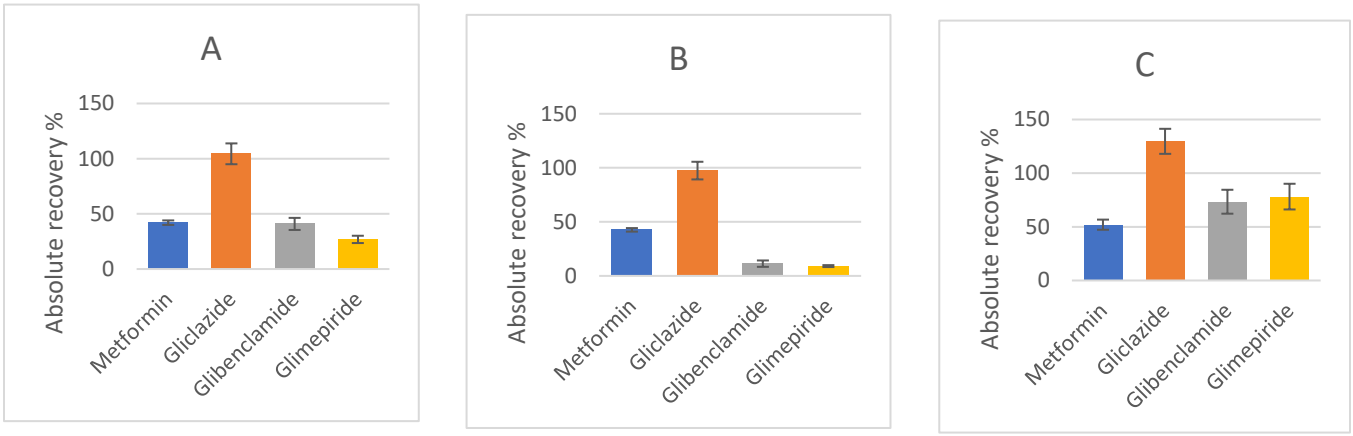


Fig. 3 Effect of adding formic acid to the extraction solvent (A =0.05%; B = 0.02%; and C = No formic acid)

3.2.2 Evaporation step

Since 90% methanol was used to extract the blank herbal sample, switching this organic solvent with an aqueous solvent was necessary to optimize chromatographic separation[38]. Consequently, the extraction solvent was evaporated to near dryness under a gentle nitrogen stream at 40ºC in a nitrogen evaporator (N-EVAP^{TM}111, model 5085, USA) before the aqueous solvent was added. The absolute recoveries for the analytes were calculated in this step to determine the effectiveness of this solvent-switching process. The blank sample was spiked with the mixed standard solution to a final analyte concentration of 50 ng/mL after extraction but before evaporation, and its mean peak areas compared to that of the neat standards of the same

concentration to get the absolute recoveries for this evaporation step. The absolute recoveries were 68% for MET, 107% for GLZ, 96% for GLC, and 80% for GLP.

3.2.3 Filtration step

Samples need to be filtered using a 0.22-micron filter before they are injected into the LC for analysis to protect the column from damage, prolonging its lifetime [39]. However, some studies have reported the adsorption of pharmaceutical compounds on the filter [40]–[42]. Hence, the absolute recoveries for the 0.22-micron cellulose and PTFE filters were calculated to determine their potential to adsorb the four antidiabetics, as illustrated in Fig. 4. The 0.22-micron PTFE filter compared to the 0.22-micron cellulose filter significantly enhanced the recoveries of GLZ (108% vs 58%, $p<0.001$), GLC (88% vs 4%, $p<0.001$), and GLP (74% vs 7%, $p<0.001$). Several studies have established cellulose materials have a high adsorption capacity for sulfonylurea drugs and herbicides [43], [44]. Hence, the 0.22-micron PTFE filter was used.

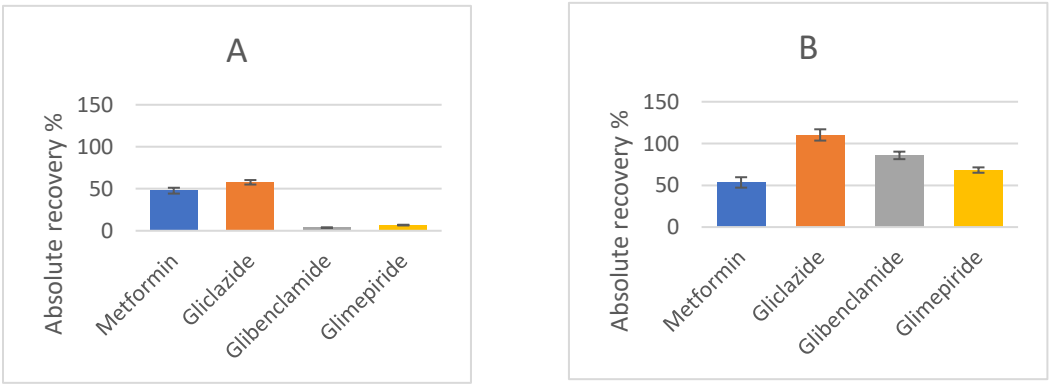


Fig. 4 Absolute recoveries for filtration step (A = 0.22-micron cellulose filter and B = 0.22-micron PTFE filter)

3.2.4 Optimized Sample Preparation Process

The optimized sample preparation process was as follows. About 2 grams of the powdered sample were weighed into a 50-mL centrifuge tube and vortexed for 2 mins for a uniform composition. The sample was then dissolved in 90% methanol in a ratio of 1:10 and vortex-mixed for 1 min,

followed by sonication for 20 minutes before centrifuging at 4500 rpm for 5 min. 1 mL of the supernatant was transferred into a 10 mL vial, and the extraction solvent evaporated to almost dryness under a gentle stream of nitrogen gas at room temperature. It was then reconstituted to 1 mL using Milli-Q water, transferred into a 1.5 mL Eppendorf tube and centrifuged at 15000 rpm for 5 minutes. One mL of the supernatant was filtered using a 0.22-micron PTFE syringe filter and diluted ten times using Milli-Q water. One mL of the filtrate was then placed in an HPLC vial, and 10 µL of it was injected in triplicate into the LC system for analysis.

3.3 Method Validation

Linearity calibration curves in 5 – 200 ng/mL range were determined for GLP and GLC and 5 – 250 ng/mL for MET and GLZ, as shown in supplementary file 1. The coefficients of determination (r^2) were 0.9981, 0.9997, 0.9978, and 0.9985 for MET, GLZ, GLC, and GLP, respectively, indicating good linearity as they were within the acceptable limits of >0.990 [28]. The LOQs and LODs for all the compounds are indicated in Table 2. The accuracy for the analytes, expressed as mean absolute recovery, was at least 80% for all the analytes, except for MET (52%). The accepted level of variation for accuracy recommended by most of the guidelines is ± 20% [45]. However, several studies have also reported low accuracies for MET due to its high polarity [30], [38]. The precision ranged between 8.5% and 16.1%, with the lowest variability at the highest concentration level and vice versa. All the obtained precision values were below the accepted level of variation (15 to 20%) [45]. The matrix effect for each analyte can be found in Table 2. Only GLZ had a matrix effect of over 100%, indicating ion enhancement. The other analytes – MET, GLC, and GLP – had a matrix effect of less than 100%, indicating ion suppression.

Table 2 Method validation for all the analytes

Compound	**Linear regression**	**Linear range (ng/mL)**	**r^2**	**Accuracy (%)**	**LOD (ng/mL)**	**LOQ (ng/mL)**	**Matrix effect (%)**
MET	y = 106.42x + 216.18	5 - 250	0.9981	52.00	7.67	23.24	68.41
GLZ	y = 272.55x – 148.29	5 -250	0.9997	129.66	2.84	8.62	106.96
GLC	y = 122.48x – 285.81	5 - 200	0.9978	79.64	7.11	21.63	96.08
GLP	y = 123.68x – 263.89	5 - 200	0.9985	81.76	5.96	18.07	79.97

3.4 Application of validated method to herbal medicines

After the method was validated, it was used to analyse 24 herbal drugs purchased from Nairobi and Uasin Gishu Counties, Kenya for possible adulteration. GLZ, GLC, and GLP were not detected in any of the analysed samples. MET was detected in 17 % of the samples (n = 4) at concentrations shown in Table 3.

Table 3 Concentrations (mean ±SD) of MET in sampled herbal products (n = 4)

Sample No.	**Concentration (ng/g)**
Sample 5	1781 ± 116
Sample 10	1969 ± 86
Sample 18	1385 ± 174
Sample 22	903 ± 265

Several studies have reported adulteration of herbal drugs with MET [4], [6], [8], [9]. GLZ, GLC, and GLP were not detected in any sample. MET is readily available nationwide because it is the least costly antidiabetic [46]–[48], which could explain why it was the only antidiabetic detected in the samples. Adulterating herbal products with MET is dangerous because of its high potential for adverse drug-herb interactions. Mild hypoglycaemia has been reported in diabetic patients taking MET with herbal products, including melatonin, ginseng, salvia, gobo, and nopal [49], [50]. Additionally, some herbs, including *Gymnema sylvestre,* reduce the MET's bioavailability when taken together, leading to increased blood glucose levels [51].

4 Conclusion

A rapid LC-MS/MS method was developed, validated, and applied to simultaneously detect four common antidiabetic medications present as adulterants in herbal products. This method showed high precision, accuracy, linearity, and sensitivity. Therefore, this method can be used to monitor antidiabetic herbal drugs for adulteration using these four antidiabetic pharmaceuticals. The presence of metformin in 17% of the samples shows that herbal medicines in the country are being adulterated without the knowledge of the public and regulatory bodies. As such, this study recommends routinely testing herbal products in the country for possible adulteration using pharmaceuticals. The method should also be expanded to include other classes of drugs that have been reported as common adulterants in literature.

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Environmental Sciences, University of Jyvaskyla, Finland, for providing the laboratory facilities for conducting the analysis.

Declarations

Conflict of interest: The authors declare that they do not have any conflict of interest in this study.

Ethical statement: This study did not involve any human or animal testing. Nevertheless, ethical clearance was obtained from SERU, KEMRI. The ethics number for the project is KEMRI/SERU/CTMDR/CSC058/3566.

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References

[1] International Diabetes Federation, “IDF Diabetes Atlas,” 2021. Accessed: Sep. 12, 2022. [Online]. Available: https://diabetesatlas.org/idfawp/resource-files/2021/07/IDF_Atlas_10th_Edition_2021.pdf.

[2] A. S. J. Edussuriya, S. Y. S. Subhashini, K. D. S. Amarasinghe, G. S. D. Kumari, K. M. O. N. Perera, and K. G. P. K. Munidasa, “Experiences of Patients on Natural Herbal Treatments for Diabetes Mellitus at the Diabetes Clinic in Base Hospital - Matara, Sri Lanka,” *J. Patient Exp.*, vol. 8, 2021, doi: 10.1177/23743735211039313.

[3] W. Pang, H. Yang, Z. Wu, M. Huang, and J. Hu, “LC-MS-MS in MRM Mode for Detection and Structural Identification of Synthetic Hypoglycemic Drugs Added Illegally to ‘Natural’ Anti-Diabetic Herbal Products,” *Chromatographia*, vol. 70, no. 9, pp. 1353–1359, 2009, doi: 10.1365/s10337-009-1344-0.

[4] C. K. Ching, Y. H. Lam, A. Y. W. Chan, and T. W. L. Mak, “Adulteration of herbal antidiabetic products with undeclared pharmaceuticals: A case series in Hong Kong,” *Br. J. Clin. Pharmacol.*, vol. 73, no. 5, pp. 795–800, May 2012, doi: 10.1111/J.1365-2125.2011.04135.X.

[5] E. Ernst, “Adulteration of Chinese herbal medicines with synthetic drugs: a systematic review,” *J. Intern. Med.*, vol. 252, no. 2, pp. 107–113, Aug. 2002, doi: 10.1046/J.1365-2796.2002.00999.X.

[6] A. T. Chowdry, “Determination of synthetic drugs as adulterant in herbal anti-diabetic medicines by HPLC,” BRAC University, 2018.

[7] H. A. J. Al Lawati, I. Al Busaidi, A. M. Kadavilpparampu, and F. O. Suliman, “Determination of Common Adulterants in Herbal Medicine and Food Samples using

Core-shell Column Coupled to Tandem Mass Spectrometry," *J. Chromatogr. Sci.*, vol. 55, no. 3, pp. 232–242, 2017, doi: 10.1093/chromsci/bmw175.

[8] M. Kumar, V. Mandal, and S. Hemalatha, "Detection of metformin hydrochloride in a traditionally used Indian herbal drug for antidiabetic: A case report | Request PDF," *Int. J. Pharma Bio*, vol. 2, no. 2, pp. 307–313, 2011, Accessed: Aug. 28, 2022. [Online]. Available: https://www.researchgate.net/publication/279588573_Detection_of_metformin_hydrochloride_in_a_traditionally_used_Indian_herbal_drug_for_antidiabetic_A_case_report.

[9] M. Steyn, L. Couchman, G. Coombes, K. A. Earle, A. Johnston, and D. W. Holt, "A herbal treatment for type 2 diabetes adulterated with undisclosed drugs," *Lancet*, vol. 391, no. 10138, p. 2411, Jun. 2018, doi: 10.1016/S0140-6736(18)31134-6.

[10] V. Cianchino, G. Acosta, C. Ortega, L. D. Martínez, and M. R. Gomez, "Analysis of potential adulteration in herbal medicines and dietary supplements for the weight control by capillary electrophoresis," *Food Chem.*, vol. 108, no. 3, pp. 1075–1081, Jun. 2008, doi: 10.1016/J.FOODCHEM.2007.11.042.

[11] I. Fejos, G. Neumajer, S. Béni, and P. Jankovics, "Qualitative and quantitative analysis of PDE-5 inhibitors in counterfeit medicines and dietary supplements by HPLC–UV using sildenafil as a sole reference," *J. Pharm. Biomed. Anal.*, vol. 98, pp. 327–333, Sep. 2014, doi: 10.1016/J.JPBA.2014.06.010.

[12] Y. R. Ku, L. Y. Chag, L. K. Ho, and J. H. Lin, "Analysis of synthetic anti-diabetic drugs in adulterated traditional Chinese medicines by high-performance capillary electrophoresis," *J. Pharm. Biomed. Anal.*, vol. 33, no. 2, pp. 329–334, Sep. 2003, doi: 10.1016/S0731-7085(03)00287-5.

[13] D. T. C. Minh, L. A. Thi, N. T. T. Huyen, L. Van Vu, N. T. K. Anh, and P. T. T. Ha, "Detection of sildenafil adulterated in herbal products using thin layer chromatography combined with surface enhanced Raman spectroscopy: 'Double coffee-ring effect' based enhancement," *J. Pharm. Biomed. Anal.*, vol. 174, pp. 340–347, Sep. 2019, doi: 10.1016/J.JPBA.2019.05.043.

[14] S. M. Verbitski, G. T. Gourdin, L. M. Ikenouye, J. D. McChesney, and J. Hildreth, "Detection of Actaea racemosa Adulteration by Thin-Layer Chromatography and Combined Thin-Layer Chromatography-Bioluminescence," *J. AOAC Int.*, vol. 91, no. 2, pp. 268–275, Mar. 2008, doi: 10.1093/JAOAC/91.2.268.

[15] C. Viana *et al.*, "A capillary zone electrophoretic method for the determination of hypoglycemics as adulterants in herbal formulations used for the treatment of diabetes," *Anal. Methods*, vol. 5, no. 8, pp. 2126–2133, Mar. 2013, doi: 10.1039/C3AY26263D.

[16] Y. Chen, L. Zhao, F. Lu, Y. Yu, Y. Chai, and Y. Wu, "Determination of synthetic drugs used to adulterate botanical dietary supplements using QTRAP LC-MS/MS," *http://dx.doi.org/10.1080/02652030802641880*, vol. 26, no. 5, pp. 595–603, 2009, doi: 10.1080/02652030802641880.

[17] P. Jin, S. Xu, W. Xu, X. He, Y. Kuang, and X. Hu, "Screening and quantification of fourteen synthetic antidiabetic adulterants in herbal pharmaceuticals and health foods by HPLC and confirmation by LC-Q-TOF-MS/MS," *Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess.*, vol. 37, no. 1, pp. 11–18, Jan. 2020, doi: 10.1080/19440049.2019.1675910/SUPPL_FILE/TFAC_A_1675910_SM0600.DOC.

[18] Q. Liang, J. Qu, G. Luo, and Y. Wang, "Rapid and reliable determination of illegal adulterant in herbal medicines and dietary supplements by LC/MS/MS," *J. Pharm.*

Biomed. Anal., vol. 40, no. 2, pp. 305–311, Feb. 2006, doi: 10.1016/J.JPBA.2005.07.035.

[19] F. Liu *et al.*, "Simultaneous Screening and Determination of Eight Tetracycline Antibiotics Illegally Adulterated in Herbal Preparations Using HPLC–DAD Combined with LC–MS–MS," *Chromatogr. 2017 812*, vol. 81, no. 2, pp. 303–314, Dec. 2017, doi: 10.1007/S10337-017-3450-8.

[20] Y. Zhong, C. Sun, J. Xiong, and Y. Shi, "Simultaneous determination of eight adulterants in weight management supplements and herbs by HPLC–DAD and LC–MS/MS," *J. Liq. Chromatogr. Relat. Technol.*, vol. 40, no. 12, pp. 640–648, Jul. 2017, doi: 10.1080/10826076.2017.1343730.

[21] WHO, "WHO GLOBAL REPORT ON TRADITIONAL AND COMPLEMENTARY MEDICINE 2019," Geneva, 2019. Accessed: Aug. 28, 2022. [Online]. Available: http://apps.who.int/bookorders.

[22] M. Bahmani, H. Golshahi, K. Saki, M. Rafieian-Kopaei, B. Delfan, and T. Mohammadi, "Medicinal plants and secondary metabolites for diabetes mellitus control," *Asian Pacific J. Trop. Dis.*, vol. 4, no. S2, pp. S687–S692, Feb. 2014, doi: 10.1016/S2222-1808(14)60708-8.

[23] W. K. Chebii, J. K. Muthee, and K. Kiemo, "The governance of traditional medicine and herbal remedies in the selected local markets of Western Kenya," *J. Ethnobiol. Ethnomed.*, vol. 16, no. 1, pp. 1–24, Jun. 2020, doi: 10.1186/S13002-020-00389-X/FIGURES/12.

[24] Ministry of Health, "Kenya Essential Medicines List 2019," Nairobi, 2019. Accessed: Mar. 08, 2023. [Online]. Available: https://www.health.go.ke/wp-content/uploads/2020/03/Kenya-Essential-Medicines-List-2019.pdf.

[25] E. Ngumba, P. Kosunen, A. Gachanja, and T. Tuhkanen, “A multiresidue analytical method for trace level determination of antibiotics and antiretroviral drugs in wastewater and surface water using SPE-LC-MS/MS and matrix-matched standards,” *Anal. Methods*, vol. 8, no. 37, pp. 6720–6729, Oct. 2016, doi: 10.1039/C6AY01695B.

[26] ICH, “VALIDATION OF ANALYTICAL PROCEDURES Q2(R2),” 2022. Accessed: Aug. 29, 2022. [Online]. Available: https://database.ich.org/sites/default/files/ICH_Q2-R2_Document_Step2_Guideline_2022_0324.pdf.

[27] P. Kairigo, E. Ngumba, L. R. Sundberg, A. Gachanja, and T. Tuhkanen, “Occurrence of antibiotics and risk of antibiotic resistance evolution in selected Kenyan wastewaters, surface waters and sediments,” *Sci. Total Environ.*, vol. 720, p. 137580, Jun. 2020, doi: 10.1016/J.SCITOTENV.2020.137580.

[28] J. Ouma, A. Gachanja, S. Mugo, and J. Gikunju, “Antibiotic Residues in Milk from Juja and Githurai Markets in Kenya by Liquid Chromatography-Tandem Mass Spectrometry,” *Chem. Africa*, vol. 4, no. 4, pp. 769–775, Aug. 2021, doi: 10.1007/S42250-021-00269-1.

[29] E. Ngumba, A. Gachanja, and T. Tuhkanen, “Occurrence of selected antibiotics and antiretroviral drugs in Nairobi River Basin, Kenya,” *Sci. Total Environ.*, vol. 539, pp. 206–213, Jan. 2016, doi: 10.1016/J.SCITOTENV.2015.08.139.

[30] K. Heinig and F. Bucheli, “Fast liquid chromatographic-tandem mass spectrometric (LC–MS–MS) determination of metformin in plasma samples,” *J. Pharm. Biomed. Anal.*, vol. 34, no. 5, pp. 1005–1011, Mar. 2004, doi: 10.1016/J.JPBA.2003.11.017.

[31] P. A. Shah, J. V. Shah, M. Sanyal, and P. S. Shrivastav, “LC–MS/MS analysis of metformin, saxagliptin and 5-hydroxy saxagliptin in human plasma and its pharmacokinetic study with a fixed-dose formulation in healthy Indian subjects,” *Biomed.*

Chromatogr., vol. 31, no. 3, p. e3809, Mar. 2017, doi: 10.1002/BMC.3809.

[32] D. Steiner, R. Krska, A. Malachová, I. Taschl, and M. Sulyok, "Evaluation of Matrix Effects and Extraction Efficiencies of LC-MS/MS Methods as the Essential Part for Proper Validation of Multiclass Contaminants in Complex Feed," *J. Agric. Food Chem.*, vol. 68, no. 12, pp. 3868–3880, Mar. 2020, doi: 10.1021/ACS.JAFC.9B07706/ASSET/IMAGES/LARGE/JF9B07706_0002.JPEG.

[33] G. Pamidimarri, J. Mathaiyan, S. Manikandan, S. Rajan, and G. Batmanabane, "Screening of herbal medicines for potential allopathic antidiabetic adulterants: An analytical study," *Ayu*, vol. 40, no. 4, p. 262, 2019, doi: 10.4103/AYU.AYU_227_19.

[34] M. J. Bogusz, H. Hassan, E. Al-Enazi, Z. Ibrahim, and M. Al-Tufail, "Application of LC–ESI–MS–MS for detection of synthetic adulterants in herbal remedies," *J. Pharm. Biomed. Anal.*, vol. 41, no. 2, pp. 554–564, May 2006, doi: 10.1016/J.JPBA.2005.12.015.

[35] M. Al Bratty *et al.*, "Development and Validation of LC–MS/MS Method for Simultaneous Determination of Metformin and Four Gliptins in Human Plasma," *Chromatogr. 2017 806*, vol. 80, no. 6, pp. 891–899, Mar. 2017, doi: 10.1007/S10337-017-3288-0.

[36] S. L. Ali, H. H. Blume, and B. S. Schug, "Sulfonylureas: Physico-chemical Properties, Analytical Methods of Determination and Bioavailability," in *Oral Antidiabetics. Handbook of Experimental Pharmacology*, vol. 119, Berlin, Heidelberg: Springer, 1996, pp. 73–128.

[37] D. S. Robinson, "Pharmacokinetic Mechanisms of Drug Interactions," *http://dx.doi.org/10.1080/00325481.1975.11713963*, vol. 57, no. 2, pp. 55–62, 2016, doi: 10.1080/00325481.1975.11713963.

[38] H. C. van de Ven, A. F. G. Gargano, S. J. Van der Wal, and P. J. Schoenmakers, "Switching solvent and enhancing analyte concentrations in small effluent fractions using in-column focusing," *J. Chromatogr. A*, vol. 1427, pp. 90–95, Jan. 2016, doi: 10.1016/J.CHROMA.2015.11.082.

[39] R. X. Li *et al.*, "Screening of pesticide residues in Traditional Chinese Medicines using modified QuEChERS sample preparation procedure and LC-MS/MS analysis.," *J. Chromatogr. B*, vol. 1152, Sep. 2020, doi: 10.1016/J.JCHROMB.2020.122224.

[40] S. Mompelat, A. Jaffrezic, E. Jardé, and B. LeBot, "Storage of natural water samples and preservation techniques for pharmaceutical quantification," *Talanta*, vol. 109, pp. 31–45, May 2013, doi: 10.1016/J.TALANTA.2013.01.042.

[41] W. Buchberger, "Current approaches to trace analysis of pharmaceuticals and personal care products in the environment," *J. Chromatogr. A*, vol. 1218, no. 4, pp. 603–618, Jan. 2011, doi: 10.1016/J.CHROMA.2010.10.040.

[42] L. Chen, H. Lin, H. Li, M. Wang, B. Qiu, and Z. Yang, "Influence of filtration during sample pretreatment on the detection of antibiotics and non-steroidal anti-inflammatory drugs in natural surface waters," *Sci. Total Environ.*, vol. 650, pp. 769–778, Feb. 2019, doi: 10.1016/J.SCITOTENV.2018.08.437.

[43] I. G. Cara, B. G. Rusu, L. Raus, and G. Jitareanu, "Sorption potential of alkaline treated straw and a soil for sulfonylurea herbicide removal from aqueous solutions: An environmental management strategy," *Chemosphere*, vol. 186, pp. 360–366, Nov. 2017, doi: 10.1016/J.CHEMOSPHERE.2017.07.140.

[44] Y. Zhang *et al.*, "Functional biochar for efficient residue treatment of sulfonylurea herbicides by weak molecular interaction," *Biochar*, vol. 3, no. 4, pp. 545–556, Dec. 2021,

doi: 10.1007/S42773-021-00111-4/METRICS.

[45] N. Sveshnikova, T. Yuan, J. M. Warren, and M. D. Piercey-Normore, “Development and validation of a reliable LC-MS/MS method for quantitative analysis of usnic acid in Cladonia uncialis,” *BMC Res. Notes*, vol. 12, no. 1, pp. 1–6, Aug. 2019, doi: 10.1186/S13104-019-4580-X/TABLES/1.

[46] S. Hailu, P. C. Rockers, T. Vian, M. Onyango, R. Laing, and V. J. Wirtz, “Access to diabetes medicines at the household level in eight counties of Kenya,” *J. Diabetol.*, vol. 9, no. 2, p. 45, 2018, doi: 10.4103/JOD.JOD_32_17.

[47] D. Ongarora, J. Karumbi, W. Minnaard, K. Abuga, V. Okungu, and I. Kibwage, “Medicine Prices, Availability, and Affordability in Private Health Facilities in Low-Income Settlements in Nairobi County, Kenya,” *Pharm. J. Pharm. Educ. Pract.*, vol. 7, no. 2, p. 40, Apr. 2019, doi: 10.3390/PHARMACY7020040.

[48] P. C. Rockers, R. O. Laing, P. G. Ashigbie, M. A. Onyango, C. K. Mukiira, and V. J. Wirtz, “Effect of Novartis Access on availability and price of non-communicable disease medicines in Kenya: a cluster-randomised controlled trial,” *Lancet Glob. Heal.*, vol. 7, no. 4, pp. e492–e502, Apr. 2019, doi: 10.1016/S2214-109X(18)30563-1.

[49] A. M. Carella, “Hypoglycemia by Ginseng in type 2 Diabetic Patient: Case Report Now I’working on departmental activity and italian multicenter study on COPD View project MEDICINA-Open Access Journal MDPI Special Issue ‘Circulating MicroRNAs in Overweight and Obese Patients’ View project,” 2017, doi: 10.29328/journal.hodms.1001001.

[50] S. W. Holloway *et al.*, “Adverse interactions between herbal and dietary substances and prescription medications: A clinical survey,” *Altern. Ther.*, vol. 13, no. 2, pp. 30–35, Mar.

2007.

[51] R. C. Gupta, D. Chang, S. Nammi, A. Bensoussan, K. Bilinski, and B. D. Roufogalis, “Interactions between antidiabetic drugs and herbs: An overview of mechanisms of action and clinical implications,” *Diabetol. Metab. Syndr.*, vol. 9, no. 1, pp. 1–12, Jul. 2017, doi: 10.1186/S13098-017-0254-9/TABLES/1.