Screening of herbal medicines for potential allopathic antidiabetic adulterants: An analytical study

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Abstract

Background: There are several reports worldwide on adulteration of herbal medicines (HMs) with allopathic drugs. In India, only a few studies have reported adulteration of HMs with antidiabetics and there are no systematic studies. **Aims:** To develop a rapid and validated method for detection of allopathic antidiabetic adulterants and to explore the extent of adulteration in HMs sold in South India. **Materials and Methods:** Standards and solvents were purchased from Sigma-Aldrich. Different brands of antidiabetic HM samples with manufacturing licenses were procured from dispensaries. Spiked drug free psyllium husk as solid and flask seed oil as liquid herbal matrices were used for method development. The spiked matrices with different concentrations were extracted with methanol and subjected to centrifugation. The supernatant was collected and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Isocratic elution was carried on a C18 column with 0.1% (v/v) formic acid:methanol (3:7, v/v) as a mobile phase. All drugs were monitored for two ion products in positive electrospray ionization mode using multiple reaction monitoring scans. **Results:** The retention time was 9 min. Limit of detection is 10 Pictograms (pg) for all analytes except for metformin, which was 370 pg. Recoveries of analytes range from 96% to 117%. Forty different brands of antidiabetic HMs were analyzed. Adulterant peaks were not observed in the mass chromatograms of HMs. **Conclusions:** A single-run method was developed by LC-MS/MS for the detection of proposed antidiabetics in HMs from licensed manufacturing units and online sold HMs across herbal dispensaries in Puducherry union territory, India. None of the HMs was found to be adulterated with proposed allopathic antidiabetic adulterants.

Keywords: Adulteration, antidiabetic drugs, Indian traditional medicines, liquid chromatography-triple quadrupole detector, mass spectrometry

Introduction

Herbal medicines (HMs) have gathered increasing recognition in recent years, as one of the treatment options. Nearly 65% of the rural population in India depends on the HMs and about 40%–50% of people in Germany, 42% in the USA, 48% in Australia and 49% in France are using traditional medicine.[1] HMs originated from natural sources such as medicinal plants, minerals, animal products and their combinations are sometime presumed to be devoid of adverse effects.[2] Various brands of pharmaceutical formulations which include proprietary and classical types of HMs are available for the treatment of diabetes as over-the-counter medicines.

The safety of HMs is questionable. It has been reported that HMs are adulterated with allopathic drugs.[3-7] A few reports of HMs reveals the presence of chlorpropamide, glibenclamide, and metformin as allopathic adulterants.[3-5] A study conducted by Bogusz *et al*. found sildenafil, tadalafil, testosterone and glibenclamide as adulterants in various herbal remedies.[6] A retrospective study was done by Ching *et al.* in Hong Kong and found registered and banned antidiabetic allopathic adulterants such as glibenclamide, phenformin, metformin, rosiglitazone, gliclazide, glimepiride, nateglinide and repaglinide in herbal antidiabetic products.[7] Unaware consumption of adulterated HMs may lead to unwanted effects. Phenformin and

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rosiglitazone are banned for the treatment of type-2 diabetes due to lactic acidosis and heart failure.

The development of novel chromatographic methods for the detection of drugs is an ongoing process.[8] Unlike conventional chromatographic techniques, LC-MS detects the analytes based on their ratio of mass to charge (*m*/z). Hence, there are fewer chances of false positive.[9] Tandem mass spectrometry (tandem means coupling of two mass spectrometers) (MS/MS) uses both parent and product ion m/z for the detection and also avoids the crosstalk between the analytes, unlike in a solely equipped mass spectrometry, where the detection is based only on the precursor ion m/z.

Solid-phase extraction is one of the extraction methods, which involves the use of more resources and later vaporization techniques make the method cumbersome as liquid chromatography-tandem mass spectrometry (LC-MS/MS) needs a pure form of sample to minimize the difficulty in interpretation.[10] Use of non-deuterated compounds as internal standards is reported and has found no variation in method development. Several methods were developed and reported for the extraction and detection of antidiabetic drugs in human plasma and urine in individuals or other combinations of drugs using different techniques.[11-16] LC-MS/MS is one among them. Electrospray ionization (ESI) is one of the soft ionization techniques, which is commonly used for the ionization of small molecules. Very few studies have reported on the detection of antidiabetic drugs in the herbal matrices.[6,17] These studies had limitations such as the usage of more solvents and relatively consume more run time. As of our knowledge, there are no systematic studies on the adulterations of HMs with antidiabetics in India. Hence, the current study aims to develop a rapid and validated LC-MS/MS method for the detection of potential allopathic antidiabetic adulterants (metformin, glibenclamide, glimepiride, glipizide, gliclazide and rosiglitazone) in herbal matrices and to examine the problem of adulteration of herbal antidiabetic products which are available in the Puducherry union territory (PUT) region and through web stores.

Materials and Methods

Materials and reagents

The reference standards (rosiglitazone, glimepiride, metformin HCl, glipizide, glibenclamide and gliclazide), internal standard (IS) (clomipramine HCl) and formic acid were obtained from Sigma-Aldrich, Bengaluru, India. LC-MS grade methanol was purchased from Honeywell, India. Milli–Q water was used, from an in-house water purification system Milli-Q, Merck, Darmstadt, Germany.

Instrumentation

LC-MS/MS analyses were performed on a Waters e2695 separation module and Waters Acquity TQ detector (Waters, Milford, CT, USA). Nebulizer gas (nitrogen) was generated by the NM32 LA Peak (Peak scientific, Billerica, MA, USA). Argon gas was used as collision gas (INOX, Chennai, TN, INDIA). ESI was operated in the positive and negative mode.

Chromatographic parameters

Symmetry C18 reverse-phase column (specifications: 150 mm × 3.9 mm i.d., particle size 5 µm) by Waters was used for the analyses. The mobile phase was composed of Milli-Q water containing 0.1% formic acid as solvent A and 100% of methanol as solvent B. An isocratic elution was done throughout the analyses, which contained 30% solvent A and 70% solvent B. The LC column temperature was set at 30°C and the flow rate was 0.6 ml/min.

Tandem mass spectrometry

ESI was used for ionization. TQD type of tandem mass spectrometry was used and it was performed in multiple reaction monitoring mode, to detect the multiple parents and ion products. The resolution was set at 1 Da. The cone voltage (CV) and collision energy (CE) settings for these drugs ranged from 20 to 42 V and 12–36 eV respectively. Argon gas was used as an inert gas in collision-induced dissociation. A full-scan MS acquisition of the mass range between 100 and 600 was used.

Software

MassLynx V4.1 software was used for data acquisition and processing which was provided by the Waters. IntelliStart, which is a part of it, and was used for the development of methods.

Stock solutions and herbal matrices

Stock solutions (1 mg/ml) of reference standards and IS were prepared by adding in methanol (LC-MS grade) as a solvent. Psyllium husk (manufactured by Organic India Pvt. Ltd., Lucknow, India), which is commonly used as laxative, was taken as a solid matrix.[6] Flaxseed oil (packaged by Loose Square Marketing solutions, Haryana, India), which is commonly used as a weight-loss supplement, was taken as a liquid matrix.[18] Both these matrices were confirmed to be devoid of adulterants (glibenclamide, glimepiride, glipizide, gliclazide, metformin and rosiglitazone) by LC-ESI-MS/MS. An equal amount of IS (400 ng) spiked in all standards.

Preparation of calibration standards in a solid herbal matrix

Calibration standards were prepared by appropriate dilutions of stock solution in methanol. Ten µl of the working standards was used to prepare different concentrations of all the drugs (metformin, glibenclamide, glimepiride, glipizide, gliclazide and rosiglitazone) to yield 100 ng, 200 ng, 400 ng, 800 ng and 1000 ng in 1 g of the solid matrix. All standard solutions were stored at −30°C.

Preparation of calibration standards in a liquid herbal matrix

Calibration standards were prepared by appropriate dilutions of stock solution in methanol. Ten µl of the working standards was used to prepare a different concentration of all the

drugs (metformin, glibenclamide, glimepiride, glipizide, gliclazide and rosiglitazone) to yield 100 ng, 200 ng, 400 ng, 800 ng and 1000 ng in 1 ml of liquid matrix. All standard solutions were stored at −30°C.

Extraction

The 1 g or 1 ml of blank matrices and spiked matrices (with standards and IS) were extracted by dissolving with 10 ml of LC-MS grade methanol in 15 ml tarson tube and then centrifuged using Sorvall Legend XTR centrifuge for 5 min at 3600 RPM. Two ml of the supernatant was collected and again centrifuged by using Mikro 22R zentrifugen for 3 min at 16,000 RPM. Finally, 1 ml of the supernatant was collected and used for LC-ESI-MS/MS analysis. Solid samples such as tablets or capsules were powdered using an agate mortar and pestle and followed the same extraction procedure.

Validation

The solutions of pure reference drugs and extracted solutions of spiked solid and liquid herbal matrices were subjected to intraday and interday analysis. From this analysis, the mean retention time and relative standard deviations were calculated. Recoveries in both matrices were calculated by AUC (spiked matrix) divided by AUC (standard) and multiplied by 100. Limit of detection (LOD) and limit of quantification (LOQ) was determined by the standard deviation of low concentration to the slope of the calibration curve signal to noise ratio 3 and 10, respectively.

Methodology

This is an analytical experimental study on the analysis of HMs for the presence of potential allopathic antidiabetic adulterants. The adulterants selected for the study are metformin, glibenclamide, glimepiride, glipizide, gliclazide, and rosiglitazone and were based on the literature. After obtaining the necessary permission from the Institute scientific advisory committee and Institute ethics committee, the HM samples were procured from licensed dispensaries. All samples were collected in triplicates from various herbal dispensaries in the PUT region of India and through online stores with an intact seal. PUT consists of four districts Puducherry, Karaikal, Mahe and Yanam. The medicines that have a manufacturing license were included for analyses. The solid and liquid herbal matrices were first checked for the absence of proposed antidiabetic drugs using LC-ESI-MS/MS and were considered as blanks. Spiked HM samples with standards and IS were extracted using 10 ml of LS-MS grade methanol. This step was followed by centrifugations, as mentioned in the extraction subheading.

Formic acid was used as one of the mobile phases at 0.1% concentration. It acts as a proton source, that is useful in the ionization of the analytes into $[M+H]^+$ ions, which is also useful for the analysis of analytes in positive ionization mode.[19] It has also an advantage of not being a strong ion-pair agent, unlike trifluoroacetic acid, and it does not suppress MS ionization. The injection volume was 25 µl. and 0.6 ml/min kept as flow rate. All mobile phases were sonicated before use. Isocratic elution was applied using 30% of 0.1% formic acid in Milli-Q H_20 (A) and 70% of methanol in Milli-Q H_20 (B). This elution method was selected by trial and error method starting from 50% of A and B solvents, 40% of A and 60% of B, 30% of A and 70% of B and 35% of A and 65% of B (data not shown). Monoisotopic mass was considered as an initial mass of all the analytes since all were small molecules.[20]

Results

A total of forty different brands of antidiabetic HMs were purchased. Samples purchased from each district and through web stores are mentioned in Table 1. Out of which, thirty seven were solid and 3 were liquid dosage formulations. The price range of the samples was between 23 and 622 INR, which includes both proprietary (35 samples) and classical (5 samples) HMs. Percentage of recoveries is in the range of 98%–117% and 99%–110% in liquid and solid matrices, respectively [Table 2]. Intraday and interday analyses were done for both spiked solid and liquid matrices in five different concentrations. The percentage of relative standard deviation of the five different calibration standards was under 15% [Table 3]. A difference in the retention time of metformin was observed, which was higher in the solid matrix than in the liquid matrix, but there was no change in the mean runtime. Total runtime was 12 min, which includes 3 min as an extra runtime to avoid carryover effect [Table 4]. LOD and LOQ are given in Table 2.

The mass chromatograms of blank solid and liquid matrices are shown in Figures 1 and 2, respectively. The mass

Table 1: Total number of herbal medicines of different brands collected from different districts in Puducherry union territory and through online

Region/ place	Solid dosage formulations	Liquid dosage formulations	Subtotal
Puducherry	11	01	12
Karaikal	4	0	4
Mahe	5	0	05
Yanam	14	2	16
Online	3	0	3
Subtotal	37	3	Total=40

Table 2: Percentage of recoveries, LOD and LOQ

Antidiabetic standards	Liquid matrix (%)	Solid matrix (%)	LOD (pg)	LOQ (ng)
Glibenclamide	115	110	10	0.1
Glimepiride	111	99	10	0.1
Glipizide	114	107	10	0.1
Gliclazide	114	104	10	0.1
Rosiglitazone	113	96	10	0.1
Metformin	98	101	370	1.67

pg: Picograms, ng - Nanograms LOD: Limits of detection, LOQ: Limits of quantification. Recovery calculated by the formula AUC (matrix) divided by AUC (methanol) and multiplied by 100. AUC: Area under the curve

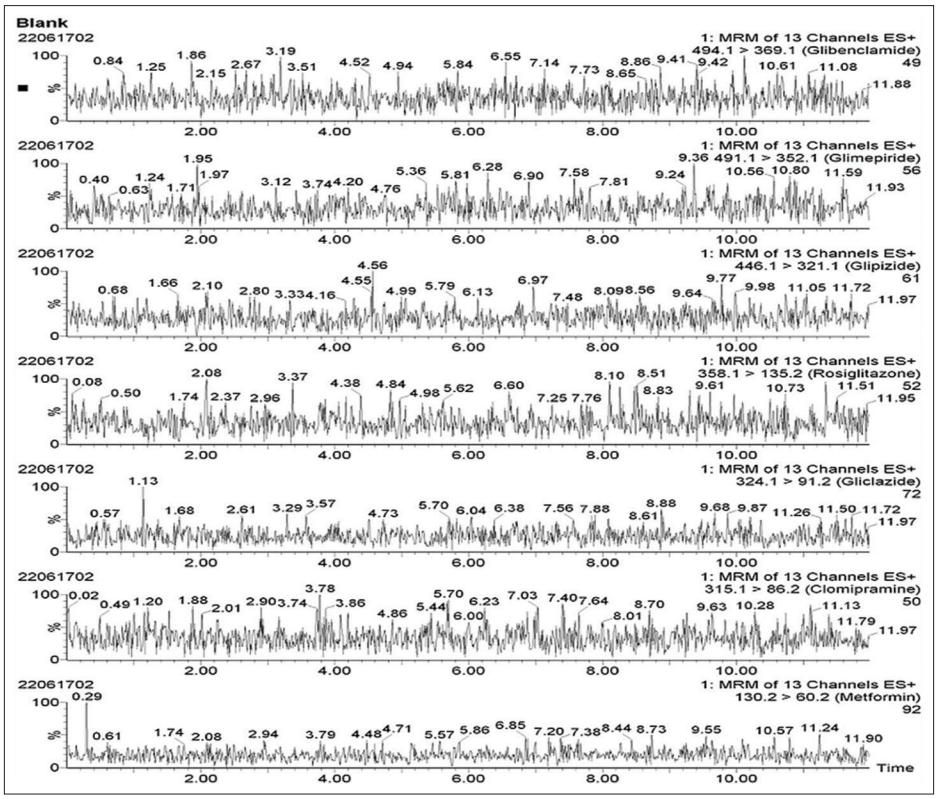

Figure 1: Mass chromatogram of blank solid matrix – Psyllium husk; No peaks of glibenclamide, glimepiride, glipizide, rosiglitazone, gliclazide, clomipramine (IS) and metformin

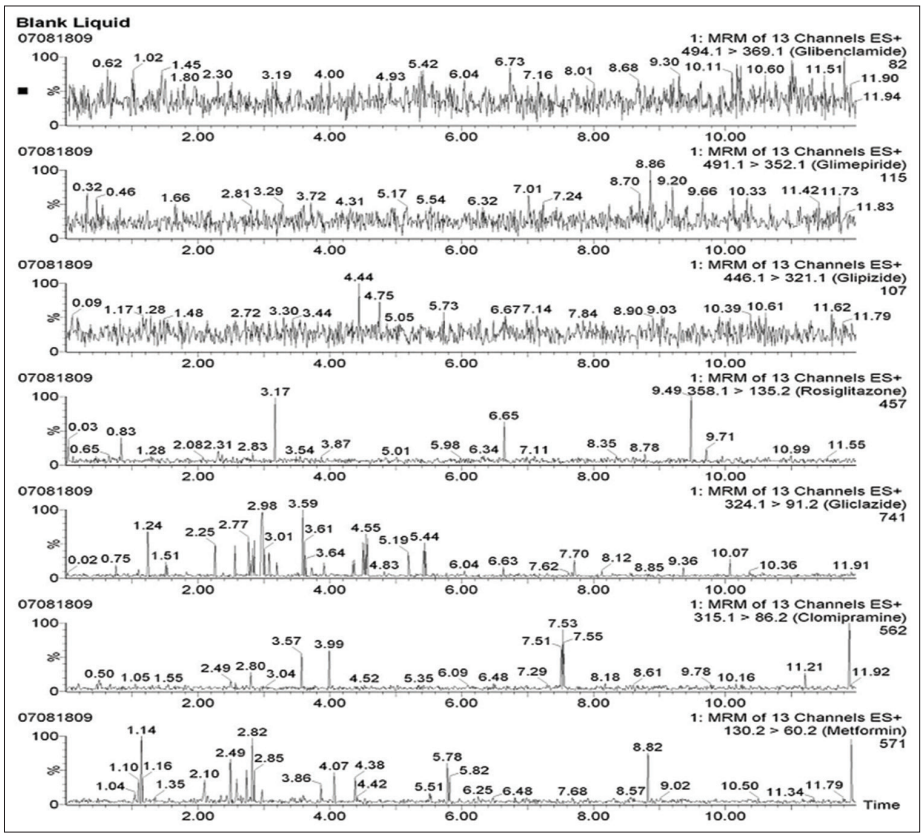

Figure 2: Mass chromatogram of blank liquid matrix – Flax seed oil; No peaks of glibenclamide, glimepiride, glipizide, rosiglitazone, gliclazide, clomipramine (IS) and metformin

Table 3: Intra-day and inter-day analysis of antidiabetics in solid and liquid matrices

Amount of drug (ng)	RSD (%)			
	Intra-day		Inter-day	
	Solid matrix	Liquid matrix	Solid matrix	Liquid matrix
Glibenclamide				
100	14.2	9.3	11.4	12.0
200	6.2	0.2	7.9	13.8
400	12.0	13.8	12.8	13.1
800	10.8	6.8	7.1	13.2
1000	14.0	3.6	9.3	12.7
Glimepiride				
100	10.8	14.2	11.8	11.9
200	10.2	14.4	12.5	7.4
400	12.3	12.8	12.4	8.2
800	5.5	7.3	13.0	7.8
1000	5.2	12.7	11.4	12.2
Glipizide				
100	12.4	6.2	13.7	9.0
200	4.5	7.1	4.8	5.9
400	6.4	14.2	13.8	8.8
800	8.2	3.8	4.2	13.1
1000	13.9	1.4	8.1	14.0
Gliclazide				
100	11.9	6.9	13.3	2.8
200	9.6	7.0	9.7	11.8
400	11.0	3.3	13.4	11.8
800	12.7	1.1	10.4	6.7
1000	1.3	1.0	5.4	9.0
Rosiglitazone				
100	3.5	9.9	3.8	13.0
200	12.2	13.7	8.4	9.0
400	5.4	6.3	12.9	14.4
800	9.7	8.7	6.2	14.3
1000	13.7	13.2	5.4	7.1
Metformin				
100	13.9	0.4	10.5	11.5
200	6.0	8.1	14.2	10.7
400	5.8	3.8	7.6	11.8
800	8.6	1.9	12.0	8.5
1000	1.1	6.8	9.1	12.6

RSD: Relative standard deviation

chromatograms of the antidiabetic standards are depicted in Figure 3. The mass chromatograms of the antidiabetics spiked in a solid and liquid matrix are shown in Figures 4 and 5, respectively. The representative mass chromatograms of solid and liquid HM spiked with IS are shown in Figures 6 and 7, respectively. Finally, the mass chromatograms of intentionally spiked with antidiabetic standards are depicted in Figures 8 and 9, respectively.

The obtained mass chromatograms from the HMs spiked with IS were compared with mass chromatograms of the herbal matrices spiked with proposed antidiabetic adulterants and IS. The absence of the respective peaks in the mass chromatograms of HMs reveals the absence of the adulterants. None of the mass chromatograms of HMs showed adulterant peaks other than IS peak, which confirms that the samples analyzed are devoid of the proposed adulterants. Finally, HMs were analyzed with the intentionally spiked proposed possible adulterants, to overcome false-negative findings that may happen at the time of extraction due to degradation. All proposed adulterant peaks were seen in mass chromatograms of intentionally spiked HMs [Figure 8].

Discussion

In this study, a total of forty antidiabetic HMs were collected, out of which thirty five were proprietary and the remaining 5 were classical preparations. The details of 5 classical preparations from which they were formulated are as follows:

i. One preparation is of siddha formulation - *Keezhanelli tablets*, formulated with reference to *Vaidyaratna Surukkam* [Table 5].[21,22]
ii. Four preparation were of Ayurvedic formulations - *Nisha Amalaki churnam,* and *Nisha Amalaki* powder are formulated with reference to *Ashtangahridaya*. *Panchabana Rasa* formulated with reference to *Yogaratnakara*. *Abhraka Bhasma* formulated with reference to *Rasarantna samuchaya* [Table 5].[21, 23-25]

Among purchased proprietary medicines there were total 28 Ayurvedic proprietary medicines, 6 Siddha proprietary medicines and one herbal proprietary anti-diabetic medicine were monitored. The method for monitoring was developed as follows,

Chromatographic parameters such as mobile phase consists

Table 4: Parent and product ion pattern of anti-diabetics

Antidiabetic standards	Molecular formula	Monoisotopic mass (Da)	Precursor ion (*m/z*)	Product ion (*m/z*)	CV (V)	CE (eV)	Mean retention time (min)	ESI mode
Glibenclamide	$C_{23}H_{28}ClN_3O_5S$	493.14	494.10	369.10	36	16	6.93	+ve
Glimepiride	$C_{24}H_{34}N_4O_5S$	490.23	491.10	352.10	20	12	8.89	+ve
Glipizide	$C_{21}H_{27}N_5O_4S$	445.18	446.10	321.10	20	16	3.33	+ve
Gliclazide	$C_{15}H_{21}N_3O_3S$	323.13	324.10	91.20	30	36	4.25	+ve
Rosiglitazone	$C_{18}H_{19}N_3O_3S$	357.12	358.10	135.20	42	28	1.21	+ve
Metformin	$C_4H_{11}N_5$	129.10	130.20	60.20	22	12	1.13	+ve
Clomipramine (IS)	$C_{19}H_{23}ClN_2$	314.16	315.10	86.20	24	16	1.43	+ve

CV: Cone voltage, CE: Collision energy, Da: Daltons, ESI: Electrospray ionization, *m/z*: Mass to charge ratio, product ion - though monitored for two product ions shows only the most abundant ion, +ve: Positive mode, IS: Internal standard

Table 5: Details of procured antidiabetic herbal medicines

	Puducherry	Karaikal district	Mahe distric	Yanam	Online marketing websites	Total
Proprietary Herbal anti diabetic medicines						
Ayurveda	3	2	5	15	3	28
Siddha	5	1	0	_	_	6
Herbal	_	1	_	_	_	1
Classical anti-diabetic herbal medicines						
Ayurveda	3	_	_	1	_	4
Siddha	1	_	_	_	_	1
Total	12	4	5	16	3	40

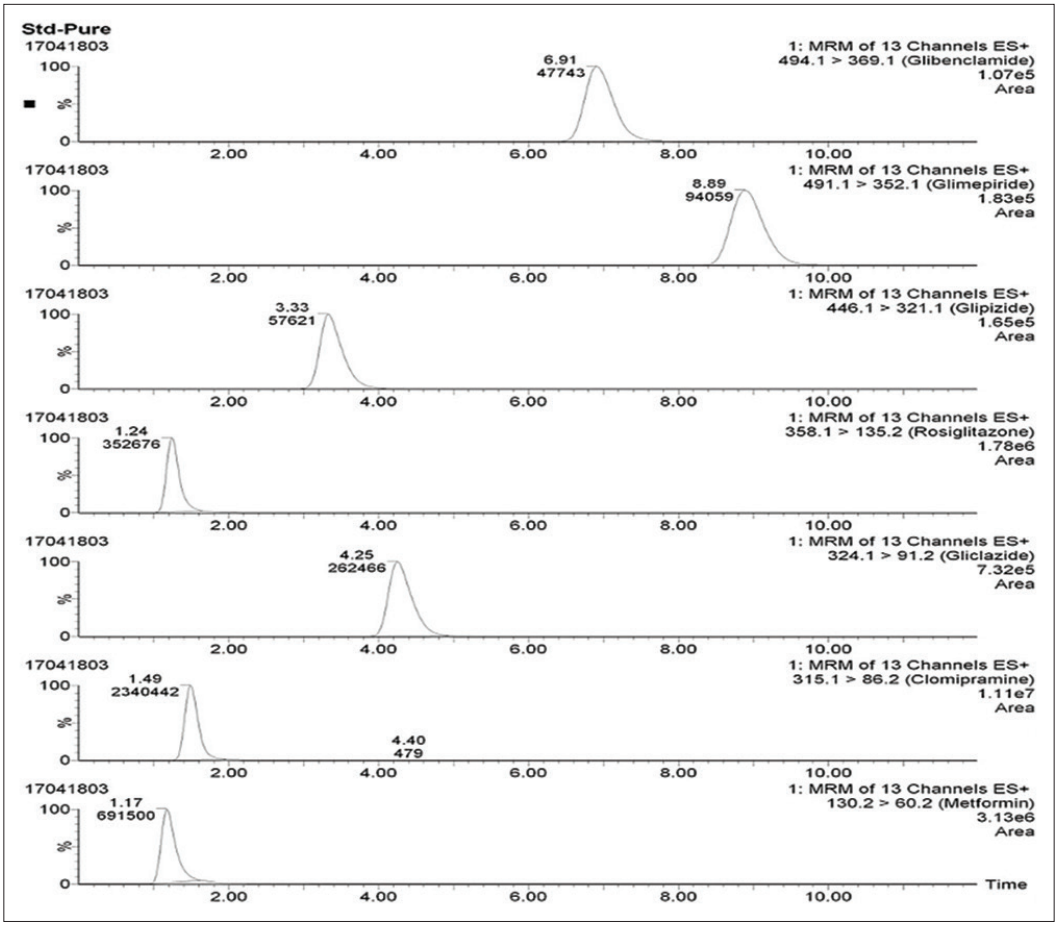

Figure 3: Mass chromatogram of reference standards (in methanol): Retention time and abundance of glibenclamide, glimepiride, glipizide, rosiglitazone, gliclazide, clomipramine (IS) and metformin

of 30% of (0.1% formic acid in milli Q water) as solvent A and 70% of 100% methanol as solvent B was eluted through reverse phase column (150*3.9 mm, particle size 5µm) at 30^0c, flow rate0.6m/min. Common method of sample (solid and liquid) preparation was used as. 1 mg/ml stock solution in 100% methanol. For the extraction method, 1 gm/ml sample in 10 ml methanol was centrifuged at 3600 rpm for 5 mins and 16000 rpm for 3 mins. Internal standard (clomipramine) were spiked in herbal solid matrix (psyllium husk) and liquid matrix (flax seed oil). The adulterants choosen were glibenclamide, glimepiride, glipizide, rosiglitazone, gliclazide and metformin. The method was validated by percentage recovery, LOD, LOQ on the basis of AUC of spiked IS matrix and sample.

For mass spectrometry, initially all the analytic ions in ESI positive and negative modes and considered the positive ESI mode signals, as these signal intensities for all the spikes were higher than those in negative ion mode. A difference of one proton mass between the initial monoisotopic mass and the precursor ion mass suggests the occurrence of protonation [Table 4]. The protonated form of each analyte including IS was used as the precursor to obtain product ion spectra at third quadrupole. All parameters were optimized

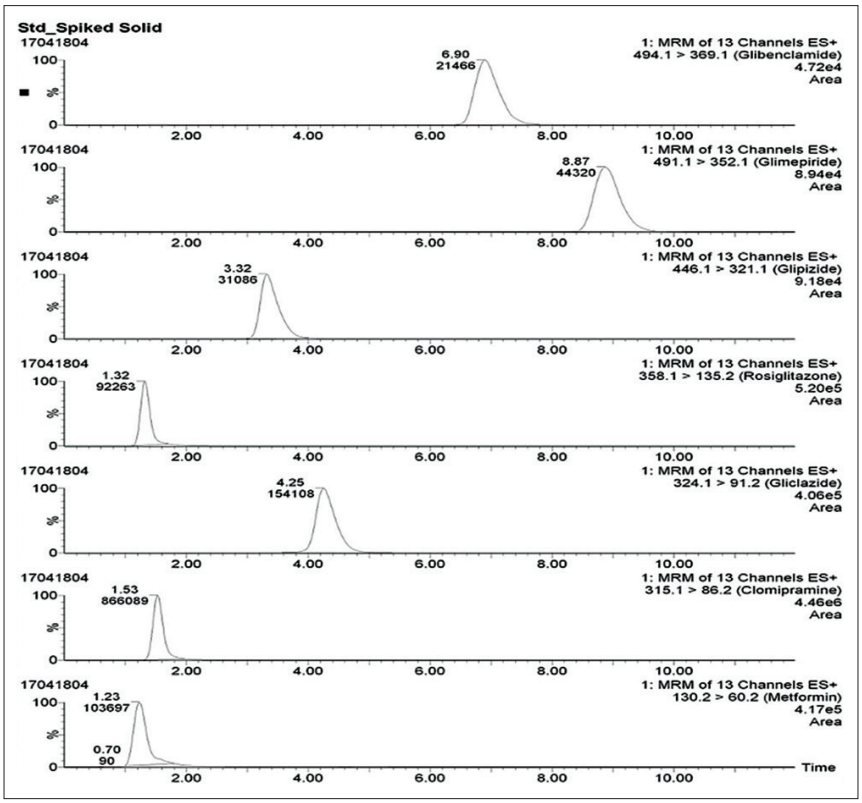

Figure 4: Mass chromatogram of the spiked solid matrix: Retention time and abundance of glibenclamide, glimepiride, glipizide, rosiglitazone, gliclazide, clomipramine (IS), and metformin

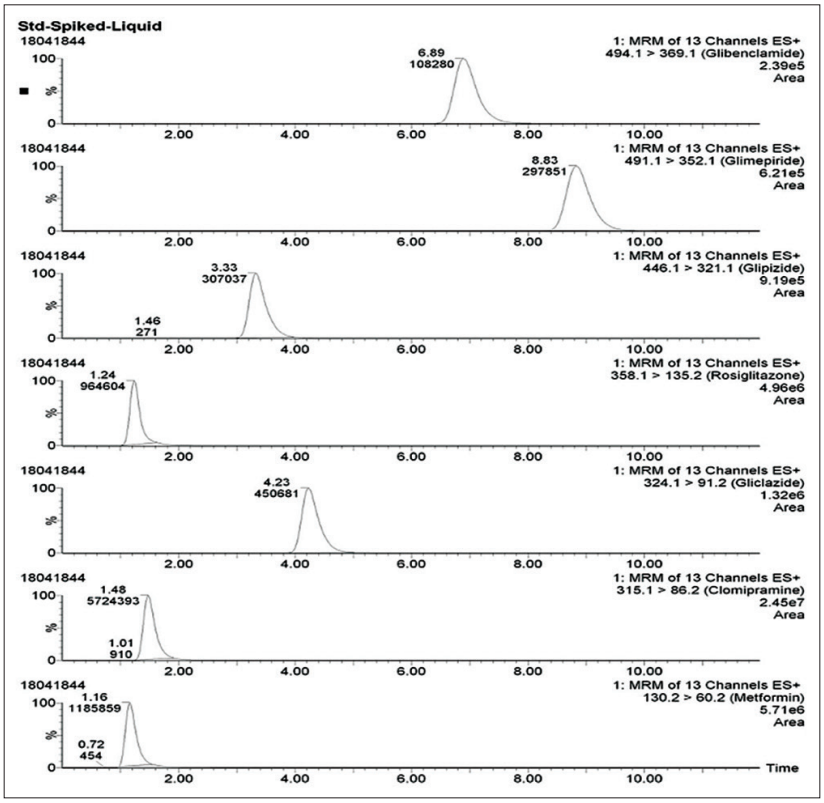

Figure 5: Mass chromatogram of the spiked liquid matrix: Retention time and abundance of glibenclamide, glimepiride, glipizide, rosiglitazone, gliclazide, clomipramine (IS), and metformin (from top to bottom)

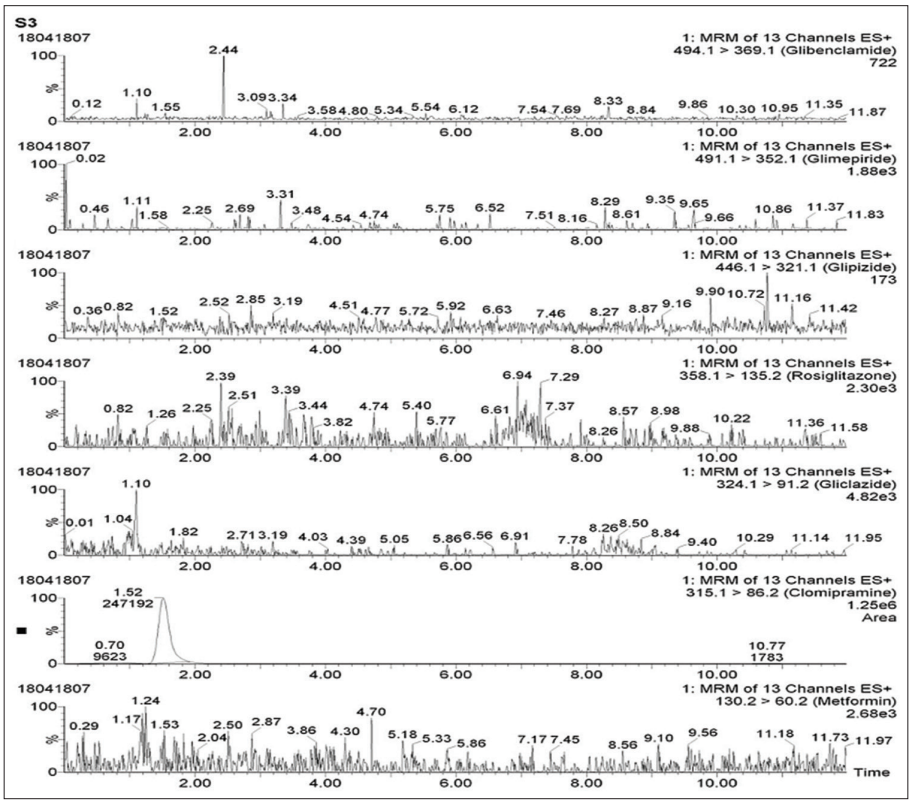

Figure 6: Mass chromatogram of solid HM sample number 3: Presence of clomipramine (IS) peak only and the absence of proposed adulterants peaks

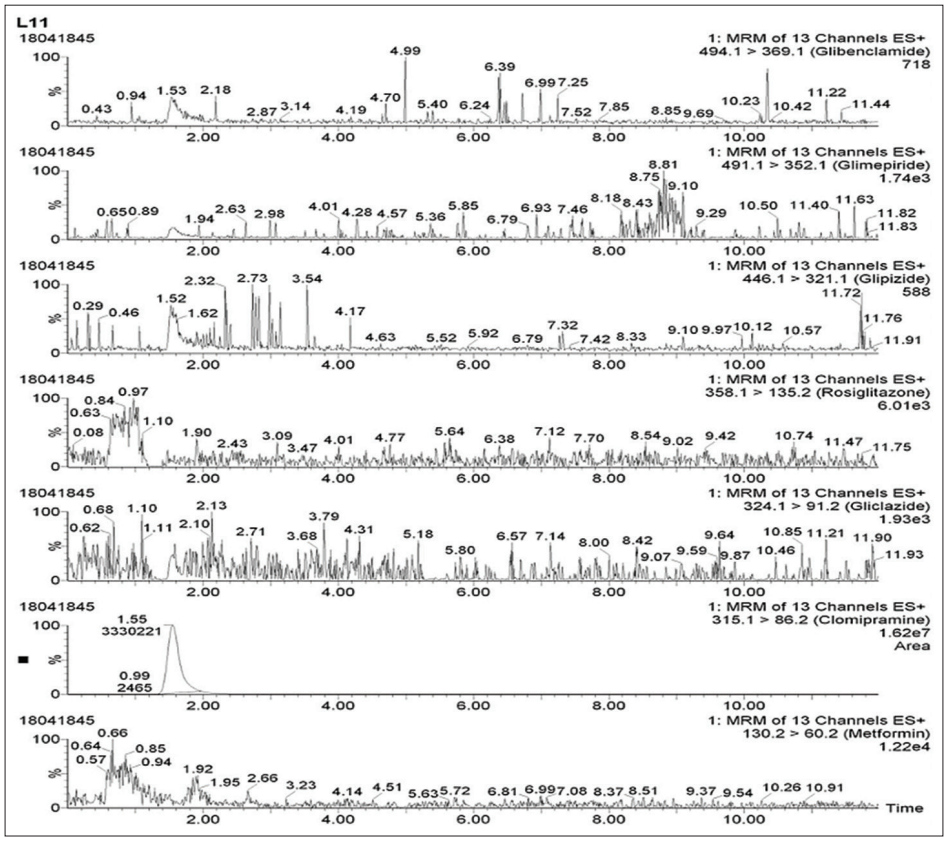

Figure 7: Mass chromatogram of liquid AHM sample number 11. clomipramine (IS) peak only present and the absence of proposed adulterants peak

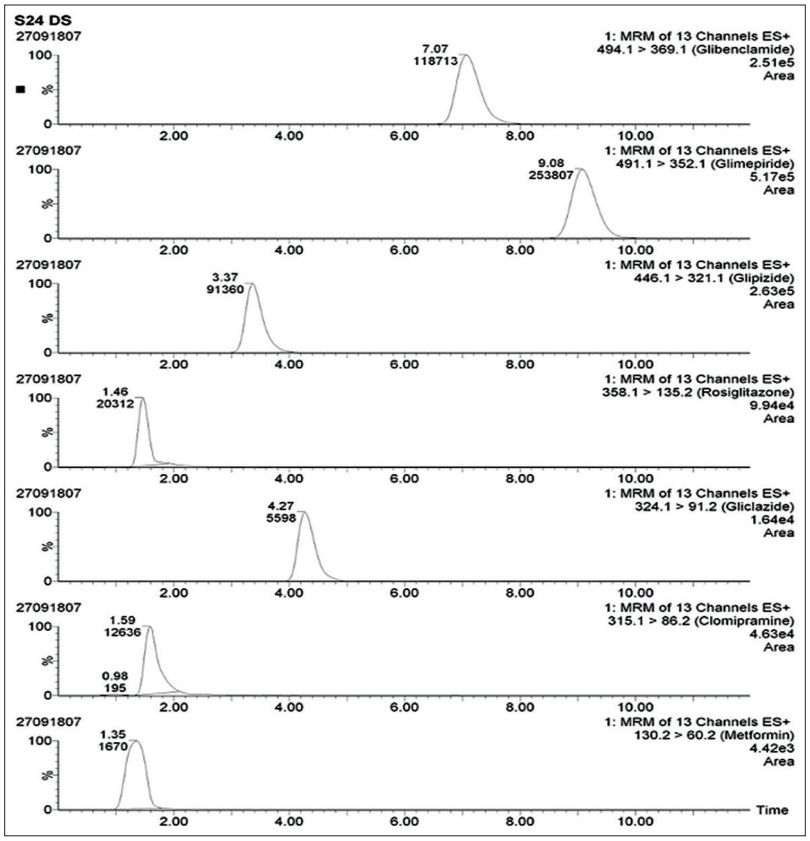

Figure 8: Mass chromatogram of intentionally spiked solid HM sample number 24 shows peaks of all antidiabetic analytes

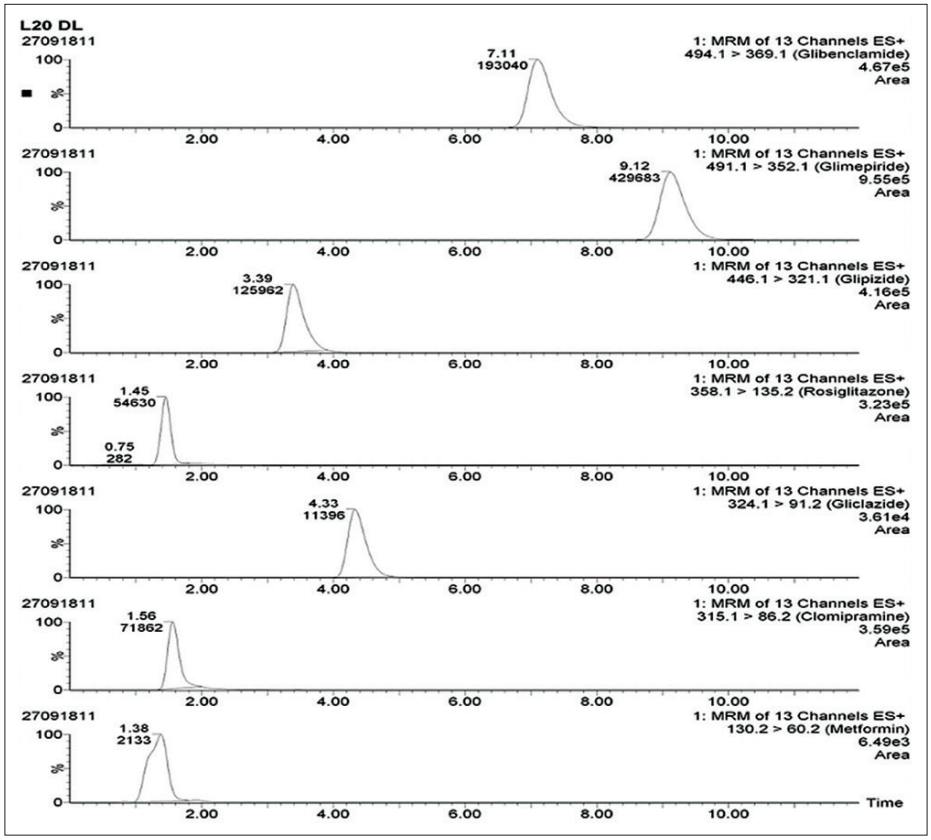

Figure 9: Mass chromatogram of intentionally spiked liquid HM sample number 20 show peaks of all proposed anti-diabetic adulterants

Table 6: Product ion pattern and run time compared with literature

Antidiabetic standards	Observed		Compared		References
	Product ion (*m/z*)	Run time (min)	Product ion (*m/z*)	Run time (min)	
Glibenclamide	369.1	6.93	369	9.49	Ho *et al*., 2004[28]
Glimepiride	352.1	8.89	352	29.2	Bogusz *et al*., 2006[6]
Glipizide	321.1	3.33	321	16	Zhang, 2015[29]
Gliclazide	91.2	4.25	127	27	Bogusz *et al*., 2006[6]
Rosiglitazone	135.2	1.21	134.9	28	Chen *et al*., 2011[10]
Metformin	60.2	1.13	60.1	1	Polagani *et al*., 2012[27]
Clomipramine (IS)	86.2	1.43	86	4.9	Balsevich *et al*., 2015[30]

IS: Internal standard

with the use of MassLynx software like CV and CE. The procedure complies with ICH harmonized tripartite guideline for the validation of analytical procedures.[26] Clomipramine HCl was used as IS as it is not a part of antidiabetic action and therefore cannot be used as antidiabetic adulterants in HMs. All the analytes for two transitions (data not shown) were scanned and considered the most abundant transition for monitoring. The respective m/z ratios of precursor and product ions of metformin (130.2 > 60.2), glibenclamide (494.1 > 369.1), glimepiride (491.1 > 352.1), glipizide (4461.1 > 321.1), rosiglitazone (358.1 > 135.2) and clomipramine (315.1 > 86.20) were considered for monitoring. The observed m/z ratio of product ions in the study was similar to the studies reported in the literature.[6,10,27-30] The respective details are shown in Table 6. However, for gliclazide, the observed transition in the present study was different (324.1 > 91.2 vs. 324.1–127.25) from two other reported studies.[6,16]

The mass chromatograms of HM samples spiked with IS were compared with the mass chromatograms of herbal matrices spiked with antidiabetic standards and IS. Mass chromatograms of HM sample 3 and 11 showed the presence of only IS peak [Figures 6 and 7]. The absence of the respective allopathic antidiabetic peak in the HM sample chromatograms reveals the absence of potential adulterants in the analyzed HM samples. Finally, intentionally spiked HM samples were also analyzed because surrogate matrices were used in the method development process and/or to avoid a chance of unidentified due to degradation of the spiked analytes through the extraction procedure. Mass chromatograms of intentionally spiked solid and liquid HM samples showed all the antidiabetic standard peaks [Figures 8 and 9].

A study by Bogusz *et al.* used LC–ESI–MS-MS for the detection of antidiabetic drug adulterants such as chlorpropamide, glibenclamide, gliclazide, glimepiride, glipizide, pioglitazone, tolazamide, and tolbutamide in the herbal remedies and found glibenclamide as an adulterant.[6] A retrospective study by Ching *et al*. on the analysis of the 29 illicit herbal antidiabetic products revealed the presence of eight registered or banned oral antidiabetic adulterants, namely glibenclamide, phenformin, metformin, rosiglitazone, gliclazide, glimepiride, nateglinide and repaglinide as adulterants.[7] A case report by Wood *et al.,* a diabetic patient on a visit to his native in India for approximately after 1 year and on returning to the clinic in the U.K., found to be excellent glycemic control because of three different HMs used. On the analysis of blood and HM, samples using high-performance liquid chromatography-ultraviolet (HPLC-UV) were found to contain chlorpropamide as an adulterant.[3] Another study by Padinjakara *et al.*, patients who had purchased HMs from India, on the chemical analysis found to contain glibenclamide as an adulterant.[4] A case report by Kumar *et al.* found metformin as an adulterant in the HM by HPLC-UV/IR spectroscopy, which was taken from the local herbal medicinal practitioner.[5] Conversely, in the study, none of the anti-diabetic adulterants in proprietary as well as classical HMs were found which are collected from the local HM dispensaries and through web stores. It might be due to the strict inclusion criteria, i.e., intact samples with manufacturing licenses. The study, thus, indicates the products marketed by licensed manufacturers had no problem of allopathic adulterants and can serve as authentic sources for use.

Conclusions

A single-run LC-ESI-MS/MS method was developed for the detection of potential allopathic anti-diabetics adulterants in herbal solid and liquid matrices. The method was validated by checking recovery, LOD and LOQ. The developed method has a relatively easy extraction process, quick run time and sensitive detection of adulterants. All the samples of herbal antidiabetic medicine were tested for the presence of adulterants glibenclamide, glimepiride, glipizide, rosiglitazone, gliclazide and metformin. No adulteration was found in the analyzed samples with the proposed adulterants. The developed method can be useful for routine screening of HMs for proposed anti-diabetic adulterants.

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Conflicts of interest

There are no conflicts of interest.

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