XOURNALS

Academic Journal of Forensic Sciences

ISSN: 2581-4273 | Volume 03 | Issue 01 | April-2020

Detection of Unclaimed Metformin and Glimepiride in Anti-diabetic Ayurvedic Medicines

Arti Varshney[1] Munish Kumar Mishra[1]

Available online at: www.xournals.com

Received 05th March 2020 | Revised 25th March 2020 | Accepted 06th April 2020

Abstract:

Ayurveda has been proven a boon to our country and because of its lesser side effects; people are shifting to herbal medicines. This has led to increased demands of these herbal products and hence, the purity and efficiency of these products become a matter of concern. This poses the problem of adulteration and adding compounds not been claimed in their ingredients for increasing the effectiveness and fast functioning of the herbal medicine. When referred to diabetes, a major chronic disease, patients with diabetes conform to herbal medications almost every time for overall welfare and better hold of the disease. This study focuses on the detection of two such allopathic compounds that are metformin and glimepiride in anti-diabetic herbal medicines. For this, 15 samples of herbal medicines were collected from local stores of Prayagraj and Aligarh. For metformin, samples were subjected to the color test, followed by thin-layer chromatography and FT-IR spectroscopy for both metformin and glimepiride. Out of 15 samples, 2 samples were detected with the presence of metformin which was confirmed by Thin Layer Chromatography (TLC) and Fourier transform Infrared Spectroscopy (FT-IR).

Keywords: *Metformin, TLC, FT-IR, Glimepiride, Ayurvedic Medicine, Forensic Science*

Authors:

1. *Department of Forensic Science, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, Uttar Pradesh, INDIA.*

Introduction

From long time, dependency of ancient people on nature to cure their diseases and illness and subsequently need which resulted into a solution from the nature has made the use of herbs for treating diseases and illnesses. Now days, other pharmaceuticals have created a sense of dissatisfaction in people's mind and have led to a substantial shift on ancient natural and herbal products as they pose lesser side effects to the body **(Khare *et al.*, 2018)**. Since, consumers assess herbal drugs as "natural" and hence safe, harmless and free from adverse side effects, the use of herbal medicines is gradually being used in both deterrent as well as treatment-based medicines and tonics. Current scenario seeds in the adulteration of herbal and has led to exploitation of the patient's trust on herbal medicines by the counterfeit drug manufacturers blooming to success. Hence, the escalated demands of the herbal medicinal products have raised concerns on the effectiveness and the purity of the drug and adulteration of the same becomes a challenge as days passes by. Adulteration refers to an activity of supplanting the original crude herb partially or totally with other substance having lower or no therapeutic value to achieve financial gain by trading the inferior quality drugs or by adding allopathic drug administrated for treating the same in it. Referring to diabetes, one of the major chronic diseases, patients with diabetes conform to herbal medicines almost all the time for overall welfare and better hold of the disease making counterfeiters' aim easily to the anti-diabetic herbal drugs. Sometimes the bioactive molecules of the herb can interact with the prescribed oral hypoglycemic drug, an issue that patients fail to comprehend and can cause severe ill effects. Additionally, multiple drug therapy required by the diabetic patients let them out at greater risk of unwanted drug interactions. In the worst-case scenario, administration of adulterated herbal drugs with synthetic oral hypoglycemic along with prescribed oral hypoglycemic can inflict serious issues such as leading to overdose and acute hypoglycemia followed by death **(Chowdhary *et al.*, 2018)**. One such illness i.e. Type II diabetes, here, is been looked into where allopathic compounds such as metformin and glimepiride are being added into the ayurvedic medicine of the illness.

Metformin is a crystalline white colored powder, hygroscopic in nature and has a bitter taste. Chemically, metformin is 1, 1 dimethyl-biguanide hydrochloride with similar mode of action and uses as that of other biguanides. Metformin is believed to lower the glucose concentration and hence, an anti-hyperglycemic drug for type II diabetes patients without causing hypoglycemia. Metformin is commonly labeled as an insulin sensitizer leading to a decreased insulin resistance and a clinically significant drop of plasma fasting insulin levels **(Bretnall, 1998)**. Metformin in polycystic ovary syndrome or PCOS has been proved helpful in its management as many women have high insulin levels resulting in cell resistant to insulin's action. The high levels of insulin cause a surge in the male hormone testosterone and displaying its related manly symptoms. Although not licensed, metformin is used to control its symptoms. Metformin cause lactic acidosis that can occur due to a buildup of metformin in body. It also leads to anemia as it can decrease the levels of vitamin B-12 in body. Additionally it leads to hypoglycemia if other factors like diet and exercise are not properly followed and to various kidney problems and liver failure when taken for long **(Bretnall, 1998)**. Glimepiride (original trade name Amaryl) is a long acting sulfonylurea anti-diabetic drug taken orally. Glimepiride creates a surge in insulin production by pancreas and hence, is helpful in treating type 2 diabetes mellitus; its mode of action is to increase insulin secretion by the pancreas. However, it requires suitable insulin synthesis as precondition to treat appropriately **(Basit *et al.*, 2012)**. Metformin can cause lactic acidosis, anemia and hypoglycemia in some patients who have been on its treatment for long. It may also lead to several heart, kidney and liver problems. Glimepiride may lead to low sodium levels in body and may cause liver damage.

Theory

The techniques used in the study, chemical color test and thin layer chromatography are feasible and yield valid results. The chemical color test using sodium nitroprusside, sodium ferricynide and sodium hydroxide yields promising results and has achievable requirements **(Indian Pharmacopoeia 2010)**. Thin layer chromatography administers propitious results as well as is accessible for carrying out separation of components **(Ramesh *et al.*, 2015)**. Also, Infrared spectroscopy has yielded positive results in determining the components based on its structure and has successfully identified the substances in question **(Kumar *et al.*, 2011)**.

Materials and Methods

[I] Sample Collection

Samples were collected from local medicinal stores of herbal medicine of Prayagraj and Aligarh. 15 herbal samples of anti-diabetes were collected, 12 of which were branded and three of them were collected loosefinger, whereas radial loop opens the thumb. A loop must have atleast a core and a delta respectively.

Patent samples of metformin and glimepiride were also collected from a medicinal store.

[II] Chemical Technique: Color Test: Sample was taken and crushed it finely using mortar and pestle. Weighed 1-2 grams of the sample using spatula into a watch glass upon a weighing balance. The sample was transferred into a test tube and was dissolved in 10mL of distilled water. After, added 10ml of a solution prepared by mixing equal volumes of a 10 percent w/v solution of sodium nitroprusside, a 10 per cent w/v solution of potassium ferricyanide and a 10 percent-w/v solution of sodium hydroxide into the test tube. The solution was allowed to stand for 20 minutes. A red wine color was developed within 3 minutes.

[III] Chromatographic Technique: Thin Layer Chromatography: TLC plates of Merck KgeA (TLC silica gel 60 F_{254}) were used to isolate the compounds from herbal drugs. Preparation of sample solution: Small amount of sample was mixed in 1-2 ml solvent methanol. Mobile phase- 20ml of methanol and water mobile phase was prepared of 60:40%v/v. The prepared mobile phase was saturated for 30 min. The prepared TLC apparatus was run and detection was carried out in iodine chamber.

[IV] Instrumental Technique: FT-IR: The samples, which confirmed the presence of metformin and glimepiride, were analyzed by PerkinElmer's (Spectral Version 10.03.06) Fourier transform - infrared absorption spectroscopy. Briefly, the samples were pressed into a potassium bromide pellet, and the spectra were detected over a range of 4000-5000 cm-1.

Results

[I] Color Test: Out of 15 samples, only two samples, S5 and S11 showed the presence of metformin.

Figure No. 1: Similar Color Gradient (Red Wine color) of Patent Metformin and Sample S5

Figure No. 2: Similar Color Gradient (Red Wine color) of Patent Metformin and Sample S11

[II] Thin Layer Chromatography

1. Metformin

a. Calculation of R_f value of patent metformin

R_f value = Distance travelled by solute / Distance travelled by the solvent

= 5.1/6.2 = 0.85

b. Calculation of R_f value of sample S5

R_f value = Distance travelled by solute / Distance travelled by solvent

= 4.9/5.8 = 0.84

c. Calculation of R_f value of sample S11

R_f value = Distance travelled by solute / Distance travelled by solvent

= 5.0/5.8 = 0.86

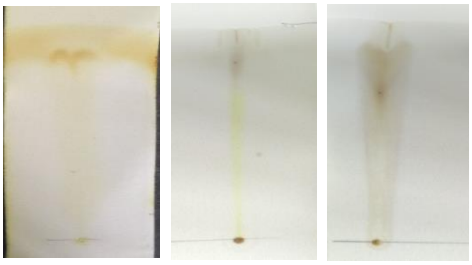

Figure No. 3: Chromatogram of Patent Metformin, Sample S5 and S11.

[III] Fourier Transform- Infrared Spectroscopy

The samples, which gave positive results for both the chemical color test and the chromatographic (TLC) techniques, were subjected to FT-IR and showed positive results as depicted below.

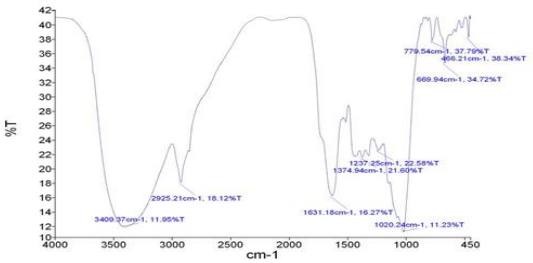

Figure No. 4: Spectrogram Showing IR Absorption Frequencies of Sample S5

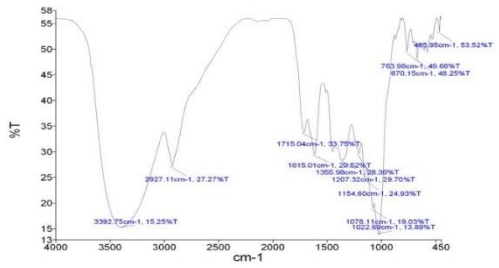

Figure No. 5: Spectrogram Showing IR Absorption Frequencies of Sample S11

Table No. 2: Absorption Frequency of Standard IR, Metformin, and Samples of Functional Groups

S. No.	IR Absorption Range (cm^{-1})	Functional Group	Metformin Absorption Frequency (cm^{-1})	Observed Frequency of Samples (cm^{-1})	
				Sample S5	**Sample S11**
1.	3400-3300 3330-3250	N-H Stretching (primary)	3372.03	**3403.37**	**3392.75**
2.	3000-2000	N-H Stretch (Secondary)	3299.02, 3174.64	**2925.21**	**2927.11**
3.	3000-2840	C-H Stretching (Aliphatic)	2971.31, 2934.42	**2925.21**	**2927.11**
4.	1690-1640	N-H Bending	1626.67, 1583.14, 1567.96	**1631.18**	**1615.01**
5.	1250-1020	C-N Stretching	1166	**1237.25, 1020.24**	**1154.6,1207.32, 1078.11, 1022.69**

Sample S5 showed positive results for the color test, further confirmed by thin-layer chromatography by similar R_f values of the sample and patent metformin that are **0.84 and 0.85** respectively. The FT-IR spectrogram in Fig. 1.4 showed the presence of the same functional groups at similar ranges to that of metformin i.e. **3403.37 cm^{-1}, 2925.21 cm^{-1}, 1631.18 cm^{-1}, 1237.25 cm^{-1}, and 1020.24 cm^{-1}** as listed in table 2.1.

Sample S11 showed positive results for a color test. This was further confirmed by thin-layer chromatography by similar R_f values of the sample and patent metformin that are **0.86 and 0.85** respectively. The FT-IR spectrogram in Fig.1.5 showed presence of the same functional groups at similar ranges to that of metformin i.e. **3392.75 cm^{-1}, 2927.11 cm^{-1}, 1615.01 cm^{-1}, 1207.32 cm^{-1}, 1154.6 cm^{-1}, 1078.11 cm^{-1} and 1022.69 cm^{-1}** as listed in the table 2.1.

Discussion

Now days, counterfeiters, for effective functioning of the herbal drugs, are adulterating the herbal drugs with synthetic compounds which are affecting the all over health of an individual. Such drugs may lead to unfavorable interaction and may cause fatalities. Such observations of adulteration were made by **Khare *et al.*,** who had observed adulterants in various herbal drugs used for treatment of various illness and diseases.

As mentioned by **Steyn *et al*.**, conducted a study on the patients suffering from type2 diabetes and were on prescribed allopathic drugs. The patient shifted to herbal drug purchased from India that showed significant amount of two synthetic drugs namely, metformin and glibenclamide. A case report as studied by **Kumar *et al*.**, who observed a component added in locally available anti-diabetic herbal drugs apart from the ones listed in the monograph was metformin analyzing them by UV, IR spectroscopy and HPLC. In this study, the anti-diabetic herbal drugs were acquired from the local market and were subjected firstly to preliminary tests (chemical color test and thin layer chromatography). The assurance for the presence of metformin hydrochloride and glimepiride, the two synthetic drugs under study was done through the Fourier-transform infrared spectroscopy.

Conclusion

From the research work conducted, it is observed and concluded that herbal drugs are adulterated with synthetic compound that are not mentioned in the monograph. Two of the samples showed presences of Metformin through preliminary chemical color test and thin layer chromatography and was confirmed by Fourier Transform Infrared spectroscopy. The study is helpful to take account of food forensic, branch of forensic chemistry and eliminate such illicit practices causing harm to the society and individuals. Along with this many food as well as health related, medicinal herbal products are adulterated, and there is an urgent need to elicit the criminal intentions of the manufacturers.

References:

Basit, Abdul, Musarrat Riaz, and Asher Fawwad. "Glimepiride: Evidence-Based Facts, Trends, and Observations." *Vasc Health Risk Manag* 8 (2012): 436-472. Print.

Bretnall, A. and Clarke, G. "Metformin", *Analytical Profiles of Drug Substances and Excipients*, (1998): 25. Print.

Ching, Chor Kwan, Ying Hoo Lam, Albert Y. W. Chan, and Tony W. L. Mak. "Adulteration of Herbal Antidiabetic Products with Undeclared Pharmaceuticals: A Case Series in Hong Kong." *British Journal of Clinical Pharmacology* 73.5 (2012): 795-800. Print.

Chowdhary, T. A. "Determination of Synthetic Drugs as an Adulterant in Herbal Anti-diabetic Medicines by HPLC", *BRAC University*. (2018). Print.

Dumitrescu, R et al. "Metformin-Clinical Pharmacology in Pcos." *Journal of Medicine and Life* 8.2 (2015): 187-192. Print.

Gunasekaran, S., R K Natarajan, V. Renganayaki, and S. Natarajan. "Vibrational Spectra and Thermodynamic Analysis of Metformin." *Indian Journal of Pure & Applied Physics* (IJPAP) 44.07 (2006): 495-500. Print.

Indian Pharmacopoeia 2010, 6th ed. Ghazibad: Indian Pharmacopoeia Comm, 2010. Print

Khare, Bhawana, Munish Kumar Mishra, and Lav Kesharwani. "Screening of Adulterants in Herbal Formulations for Forensic Considerations." *Journal of Pharmacognosy and Phytochemistry* 7.2v (2018): 532-36. Print.

Kumar, Manish, Vivekananda Mandal, and S. Hemalatha. "Detection of Metformin Hydrochloride In A Traditionally Used Indian Herbal Drug For Antidiabetic: A Case Report." *International Journal of Pharma and Bio Science* 2.2 (2011): 307-13. Print.

Poole, C.F. "Chromatography", *Encyclopedia of Separation Sciences*, (2000): 40-64. Print.

Prakash, Om, Jyoti, Amit Kumar, Pawan Kumar, and Niranjan Kumar Manna. "Adulteration and Substitution in Indian Medicinal Plants: An Overview." *Journal of Medicinal Plants Studies* B 1.4 (2013): 127-32. Print.

Ramesh, L. Sawant, D. Kallyani Tanpure, and A. Jadhav Kalyani. "Simultaneous Estimation of Isoniazid, Metformin Hydrochloride and Glimepiride in Bulk by TLC Method." *Indian Journal of Drugs* 3.4 (2015): 90-93. Print.

Steyn, Mia et al. "A Herbal Treatment For Type 2 Diabetes Adulterated With Undisclosed Drugs." *The Lancet* 391.10138 (2018): 2411. Web. 14 Apr. 2020.