SCREENING OF ADULTERANTS IN ANTI-DIABETIC HERBAL FORMULATION BY HPTLC AND FTIR ANALYSIS

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Abstract: HPTLC analytical methods coupled with FTIR spectra analysis were used to ascertain the existence of synthetic pharmaceutical formulation in Indian Herbal anti-diabetic medicines. To fulfill this objective twenty herbal medicines was collected from local stores, internet and also from road side shops having handmade herbal drugs. In this study, total five herbal samples showed presence of three pharmaceutical adulterants which were Metformin,Glibenclamide and Glimepiride. Out of five samples one sample was found adulterated with Metformin and Glibenclamide both and one with Glimepiride and Glibenclamide

***Index Terms*–Herbal drug, synthetic pharmaceutical, FTIR, Thin Layer Chromatography, HPTLC**

1. INTRODUCTION

Indian herbal medicines in these days are preferred over the conventional medicine because people believe that these medicines are totally natural and obtained by extracting different parts of plants, some other reasons also make them more preferable over the synthetic medicines such as their affordability, and a general perception that they are safe. In comparison to prescription medicines, herbal medicines seem to be safer because they are originated from natural sources or plant extracts.

There have been reports recently that these herbal medicinal products found to be adulterated with undeclared synthetic pharmaceutical or their structurally modified analogues. Many times adulteration or mixing of any synthetic formulation in herbal medicine was done knowingly with the intention of commercial benefit and then it is called intentional adulteration, this type of adulteration found mainly in those cases when the supply of particular herbal product is low or the product is high in cost or if the supplier wants to increase a particular pharmacological effect. Herbal medicines usually are one element of a comprehensive system of medication which involves the utilization of plant and mineral-based medicines to stop and treat sicknesses. Traditional medicines are characterized in many alternative ways throughout the world.

They are mainly sold either as a prescription or over-the-counter (OTC) medicine or as self-medication.[6]. Although herbal medicines in India has been used from ancient times, but still there is lack of knowledge related to its safety and efficacy among the user. Herbal medicines in India has high demand but their safety and quality measures are not well regulated because they are freely available from health-food stores, in supermarkets, on the internet and as well as there is no quality control measures for hand-made products neither in the production and nor in distribution. Unfortunately hand-made herbal drugs are not well regulated in terms of their quality and safety prospect and this issue doesn't have preference for supplier or manufacturer due to it's high cost. For the purpose of determination of adulteration an easy, fast and more reliable screening method is required. FTIR as a non-destructive technique can be used to detect the functional group and provide a high quality spectral data and for further confirmation HPTLC with UV densitometric detection is an easy and rapid method to find out or confirm the identity and to determine the quantity of component detected .A survey and health-risk analysis from 2002 - 2007 was performed in the Netherlands showed that many dietary supplements were adulterated with synthetic drugs and their analogues that were not mentioned on the label [10].This study was performed to check the quality of anti-diabetic herbal medicines available in Indian market for the presence of any possible synthetic adulterant with the use of a method which is easy, fast and that can provide data with higher sensitivity.

2. MATERIALS AND METHODS

20 Samples of Anti-Diabetic herbal drugs were purchased from local stores, online shopping and some open herbal samples were also obtained which did not have ingredient list. Reference Standard of Metformin, Gliblenclamide and Glimiperide was purchased from Indian Pharmacopoeia Commission, Ghaziabad.

2.1 FTIR ANALYSIS:

i. Perkin-Elmer FTIR spectrophotometer and Potassium Bromide for sample preparation was used for FTIR spectra analysis in the wave number range of 400-4000 cm-1. The KBr pellet was prepared used for this study. During analysis some samples were analyzed in SICART Gujarat and some were analyzed from ECC Allahabad.

ii. First weight the 1mg of dried powder of herbal medicines and 500mg of KBr and then dried powder extract was encapsulated in KBr pellet to prepare translucent sample discs. Then KBr pellet scanned between the range of 4000cm-1 -400cm-1

iii. Sample preparation requires minimal processing, samples were in the forms of dry powder, tablets and capsules and prepared by grinding them until obtaining a homogeneous powder for FTIR analysis.

iv. Identification of the type of chemical bonds (functional groups) present in samples can be done by Fourier Transform Infrared Spectrophotometer. By analyzing the peaks of particular FTIR spectrum, the functional group present in compound can be determined. Dried powder of different herbal samples was used for FTIR analysis.

2.2 HPTLC ANALYSIS METHOD AND MATERIAL USED

This analysis was performed on CAMAG HPTLC system (Switzerland). It is equipped with a Linomat-5 applicator, 100 µl sample syringe and TLC Scanner III, TLC Scanner with CAT Software was used for densitometric evaluation of TLC plates with spectral range of 190-800 nm. Pre-coated silica gel 60 F254 TLC plates (10x10 cm) were used as stationary phase. With the use of 100 µl sample syringe reference standard bands of 6mm length were applied on HPTLC plates. Distance traversed by the mobile phase was about 7cm and chamber saturation time was 20 minutes . CAMAG TLC scanner with win CATS Software was used to perform densitometric analysis

Table:1Showing Mobile phases and prepration of herbal sample and reference standard

Reference Standard	**Mobile Phase**	**Preparation of Samples**
Metformin	Methanol: Chloroform: Ammonium acetate (5.5:3:1.5 v/v/v)	Standard:10mg in 10ml methanol Test sample:100mg in 10 ml
Glibenclamide	Toluene: Ethyl acetate: Methanol (**7:1.5:1 v/v/v**).	Standard:25mg in 10ml methanol Test sample:25mg in 10 ml
Glimiperide	Toluene:Ethyl acetate:MeOH-(**5.2:4.3:0.5 v/v**v).	Standard:10mg in 10ml methanol Test sample:50mg in 10 ml

3. RESULT

3.1 FTIR RESULTS

Metformin was found in Herbal drug formulation as A1, A3 and A6 . FTIR spectra of these samples have shown N-H (primary amine) stretching at around 3350 cm-1 , N-H (secondary amine) stretching at 3174 cm^{-1}, C-H (aliphatic) stretching at 2971 cm^{-1} and N-H bending at 1626 cm^{-1} .

Glimiperide was found in Herbal drug formulation as A2 and A7 .FTIR spectra of these samples have shown N-H stretching at 3422 cm^{-1} , C-H (aliphatic) stretching at 2930 cm^{-1} , N-C=O stretch at around 1674 and O=S=O at around 1347 cm^{-1} .

Gliblenclamide was found in Herbal drug formulationas A4, A2. FTIR spectra of these samples have shown C-H stretching at around 2931 cm-1, N-H stretching at around 3121 cm-^{1} , O-H stretching at around 1619 cm-^{1} and C-N stretching at around 1159 cm-^{1}.

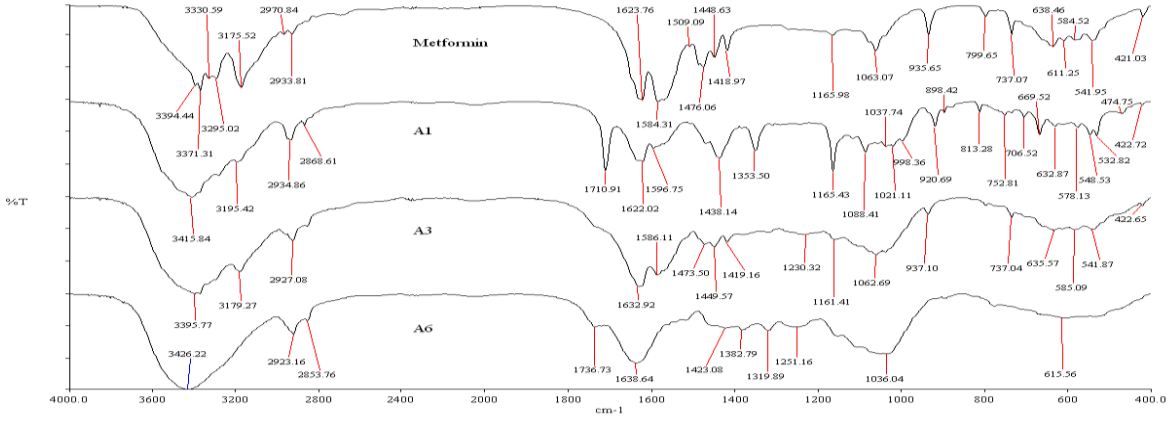


Fig:1. FTIR overlapping spectra of Metformin with A1,A3 and A4 anti-diabetic herbal samples

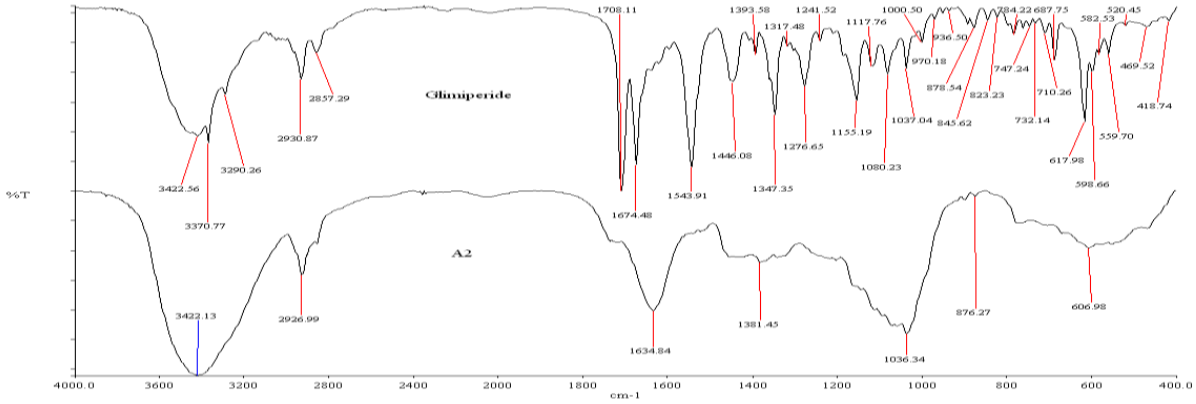


Fig:2. FTIR overlapping spectra of Glimiperide with A2 anti-diabetic herbal samples

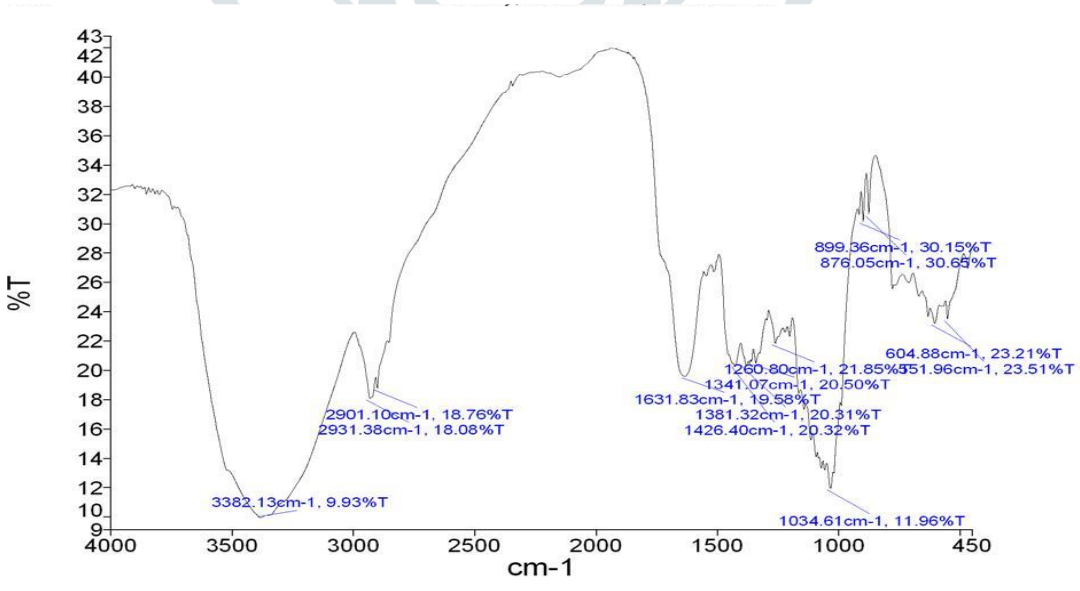


Fig:3.FTIR spectra of A7 Anti-diabetic herbal samples showing same peaks as in Glimiperide Standard

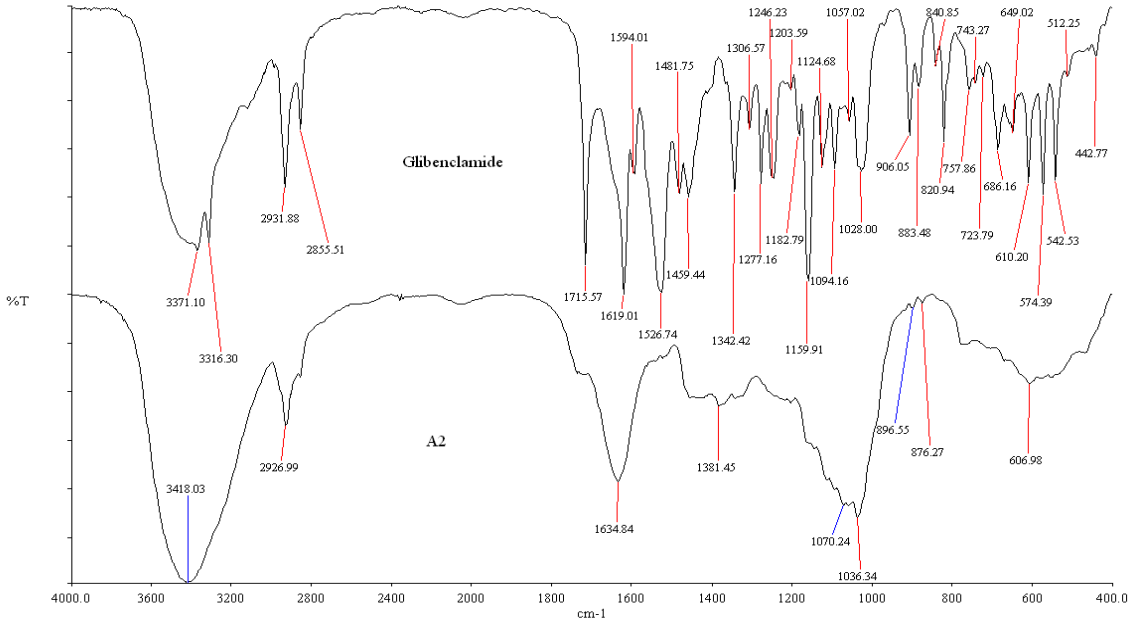


Fig:4. FTIR overlapping spectra of Glibenclamide with A2 Anti-diabetic herbal samples

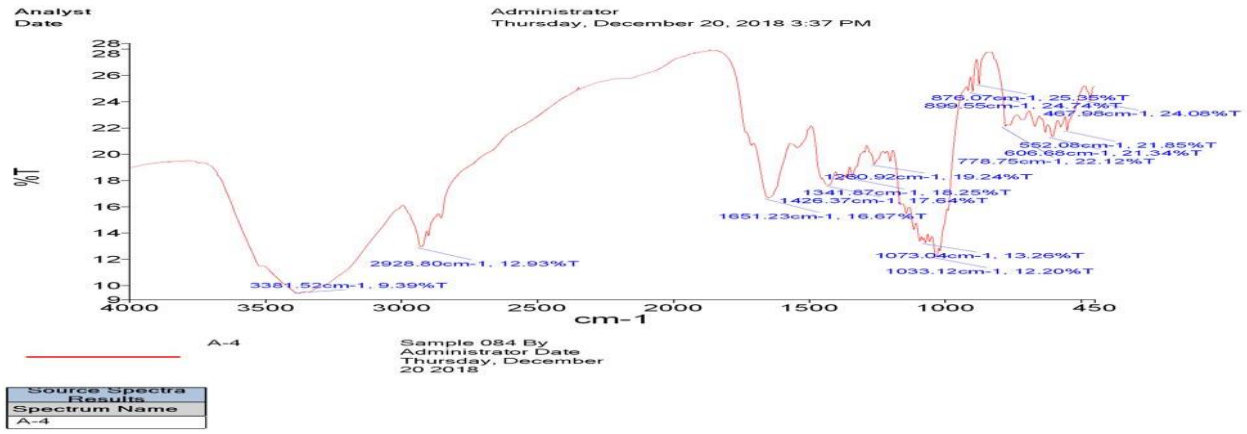


Fig:5. FTIR spectra of A4 Anti-diabetic herbal samples showing similar peaks as in Glibenclamide

3.2 HPTLC Result and Discussion

Metformin: HPTLC analysis through using Mobile phase as methanol: chloroform: ammonium acetate (5.5:3:1.5 v/v/v) gave good resolution with Rf value of 0.66. Metformin showed linear regresssion in the range of 1000-6000 ng/spot. Crisp, dark band was visible when chromatogram was viewed under 254 nm. Herbal drug formulation A3, A4 and A6 showed presence of Metformin.

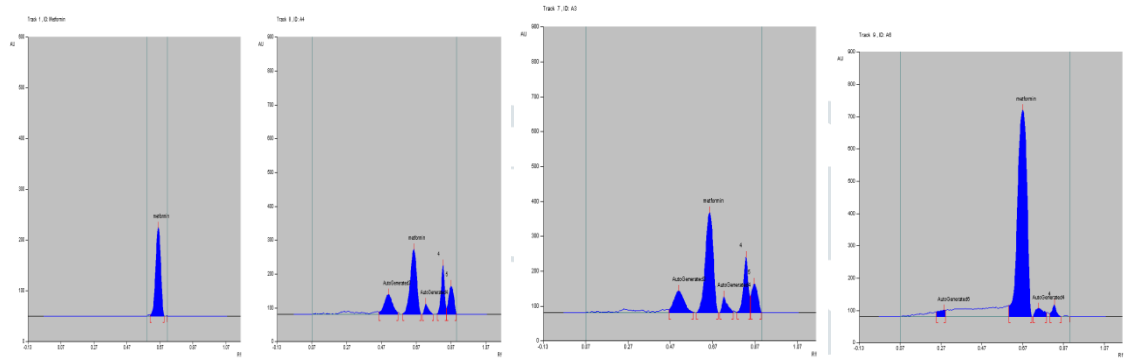
Gliblenclamide: HPTLC analysis was performed by using toluene: ethyl acetate: methanol (7:1.5:1 v/v/v).as mobile phase and given a good resolution with Rf value of 0.48. Gliblenclamide showed linear regression in the range of 1000-6000 ng/spot. Bands were visible when chromatogram was viewed under 254 nm. Herbal drug formulation A2 and A4 showed the presence of Gliblenclamide.

Glimiperide :To detect the presence of this pharmaceutical in herbal samples HPTLC analysis was done by using toluene:ethyl acetate:MeOH--(**5**.2:4.3:0.5 v/vv). as a mobile phase and it has also given a good resolution with Rf value of 0.66 It showed linear regression in the range of 500-3000 ng/spot. Herbal drug formulation A2 and A7 showed the presence of Glimiperide.

Validation of performed HPTLC method was accomplished as per ICH guideline. Repeatability, intra-day and inter-day was done to find out the precision of the method. Recovery study was observed by analysing sample at three different levels of 50%, 100% and 150% of sample. The method's specificity was determined by comparing Rf value and spectrum of standard with those of samples. Summary is shown in Table 2.

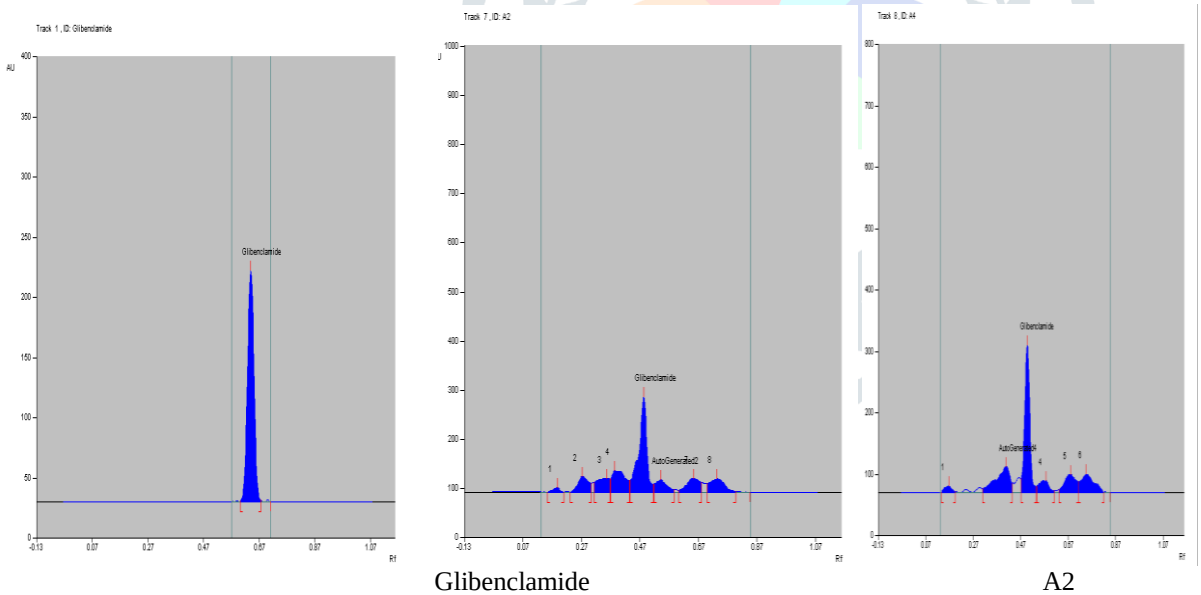
Table 2.Result of quantitaive analysis for Metformin,Glibenclamide and Glimiperide

Parameter	Metformin	Glibenclamide	Glimiperide
Linearity range	1000-6000 ng/spot	1000-6000 ng/spot	500-3000 ng/spot
Correlation coefficient	0.995	0.998	0.997
Limit of detection	351ng/µl	272.30 ng/ µl	195.96 ng/ µl
Limit of quantitation	1066 ng/µl	825.15 ng/µl	248.95 ng/ µl
Recovery (n = 3)	101.92±0.84	103.42±0.84	100.22±1.10
Precision (% RSD) Repeatability of application Inter day (n = 6) Intra day (n = 6)	 0.79 0.73 0.90	 0.94 0.91 1.28	 0.90 0.87 0.98
Robustness	Robust	Robust	Robust



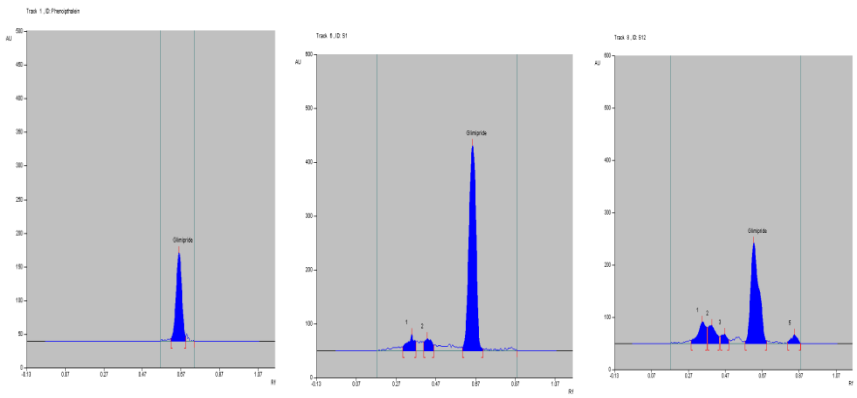
Metformin Standard A3 A4 A6

Fig 6 Metformin standard and sample A3, A4,A6, all samples and reference standard showing same Rf value of 0.66



Glibenclamide A2 A4

Fig 7. Glibenclamide standard and sample A2 and A4 ,the reference standard and test samples showing same Rf value of 0.48



Standard Glimiperide A7 A2

Fig 8. Glimiperide standard and sample A2 and A7 showing same Rf value of 0.66

4. Conclusion

In this study twenty herbal anti-diabetic medicinal products were taken to check the presence of any synthetic formulations, out of which five herbal anti-diabetic products were found to be adulterated with one or two synthetic pharmaceutical .For identification purpose FTIR was used first after that for confirmation HPTLC was applied on samples identified with synthetic adulterant. HPTLC method was applied to check the adulterant in herbal products and all methods was found to be successful to detect the presence of synthetic drug as undeclared ingredient in herbal anti-diabetic products without any interference with other component present in herbal products.

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