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Research Article

HPLC-DAD ESTIMATION OF UNDECLARED SYNTHETIC COMPOUNDS IN SELECTED INDIAN HERBAL FORMULATIONS

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ABSTRACT

The present investigation was carried out to detect the most common synthetic compounds in selected Indian herbal formulations using high-pressure liquid chromatography-diode array detector (HPLC-DAD) and to compile an ultraviolet (UV) library to detect more than 50 synthetic compounds by comparing the analytical data (UV spectra, retention time, relative retention time) with those of the standards for future use. Twenty nine Indian herbal formulations comprising analgesics, anti-diabetics and slimming products were subjected for HPLC-DAD analysis followed by UV spectra comparison with the standard compound. Results revealed that marketed formulations with no specification of undeclared drugs on their label were analysed out of which three formulations were found to contain synthetic compounds. Overlapped UV spectra were found to be > 97 %. It is concluded that development and implementation of more such analytical methods for the quality control of herbal formulations is strongly recommended. Additionally, implementation of stricter regulatory requirements regarding labelling of herbal formulations is suggested.

Keywords: *undeclared synthetic compounds, HPLC-DAD, Validation*

INTRODUCTION

Herbal formulations (HFs) are popular worldwide due to the belief that they are safer than synthetic drugs. However, nowadays HFs have not remained trustworthy as lots of evidence are coming into literature about adulteration of these products with synthetic drugs or their congeners, in order to enhance the claims stated on the label. This poses a health threat to patients who unknowingly consume a compound which may be untested for safety [1].

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Adulteration of herbal formulations with undeclared synthetic drugs or by mixing the analogues of prescription drugs that are created by replacing or adding functional groups to the original chemical are the recent major problems since they may cause adverse side effects [2].

In India, diabetes, pain and obesity are becoming serious health disorders due to poor food habits and irrational life style. As a result, there are a number of alternative medicines available in the India market selling like hot cakes. These products are often sold without prescription of a qualified doctor with an established presumption that these formulations do not have any side effects being herbal [3,4]. There are increasing evidences of introducing pharmaceutical adulterants associated with a proliferation of

herbal products that may pose threat to human health because these are not declared on the label [5]. Several analysis had been previously reported in other countries like Singapore, Hongkong, Saudi Arabia etc. asserting the presence of codeine [6], sibutramine, rimonabant [7], sildenafil [8], tadalfil, testosterone, glibenclamide [9]. in herbal remedies. But no valuable study is yet reported on the status of adulteration of anti-diabetic Indian herbal formulations with synthetic adulterants. Analgesics and slimming formulations are the other two categories which are taken into consideration since proficient analysis work on adulteration had been not done on major pharmacological classes in India.

The main objective of the present study was detection of most common and logic synthetic adulterants in herbal remedies using high performance liquid chromatography (HPLC) and to compile an ultraviolet (UV) library of more than 50 compounds that can be referred in future for research work for solute identification by comparing the analytical data. Since intentional adulteration of "natural herbal medicines" with unknown synthetic drugs or chemicals can be fatal for the health of the consumer, it was felt important to modify and validate effective analytical tools to monitor and evaluate these herbal drugs.

MATERIALS AND METHODS

Materials and reagents

Twenty nine herbal formulations belonging to three different pharmacological classes i.e. analgesics, anti-diabetics and slimming formulations were collected from retail outlets. The formulations were coded from 1-29 to hide the identity. The reference standard compounds i.e. beclomethasone, betamethasone, dexamethasone, hydrocortisone, diclofenac free acid, diclofenac sodium, diclofenac potassium, nimuselide, paracetamol, aspirin, acelofenac, ibuprofen, piroxicam, chloroxazone, elitriptan, naratriptan, mefenamic acid, metformin, glibenclamide, nateglimide, glimiperide, pioglitazone, rimonabant and sibutramine were obtained from different nearby pharmaceutical industries as gift samples. These standards were duly subjected for analysis for their identity and percent purity before usage. Acetonitrile and methanol (HPLC grade) were purchased from Rankem Reagent Industry Ltd. HPLC grade water used throughout the experiment was obtained from Milli-Q-water purification system (Ranken Rion Ltd, India).

Equipment

HPLC from HITACHI Elite lachrom attached with quaternary Gradient Pump (L2130), Autosampler (L2200), Photo diode array detector (PDA) and software EZ chromlite data station for LC system. The samples were weighed using analytical balance of model no. AUX220 manufactured by Shimadzu Corporation Ltd. and sonicated for proper dissolution of contents using sonicator of model no. UCB5200D supplied by Macro Scientific Pvt. Ltd.

Sample preparation

1.0 g of sample was weighed and mixed with 20.0 mL of ethanol (95% denaturated). The samples were sonicated for 30.0 minutes and filtered using a 0.45 µm membrane. The volume of the filtered supernatant was completed to 5.0 mL with methanol.

Sample analysis

The HPLC conditions listed below were used for both purity control and quantitative determination using UV detection at a wavelength of 220, 254 and 280nm as per method described by Liu et al. 2001. The injection volume was 10µL and the column temperature was maintained at 40°C. A chromatographic separation was performed using a Reliasil reversed phase C18, 5µm, 250×4.6 mm column with a mobile phase consisting of disodium hydrogen phosphate buffer (A), pH adjusted to 3.2andacetonitrile (B), with a flow rate of 1.0 mL/min. A gradient elution technique was followed as step gradient begin from 10% to 30% of B over 10 min, then to 50% of B over another 10 min, and finally to 70% of B over 10 min and maintained for another 5 min. Caffeine was taken as internal standard. A UV library was

built up on compilation of UV spectra of standard drugs. Solute identification in samples was carried out by library search and with data comparison with those in library. Library matches of UV spectra automatically calculated for each peak and similarity of 100% represents a perfect overlay.

Validation

The HPLC-DAD method was validated by limit of detection (LOD), limit of quantification (LOQ), determined from calibration curve using concentrations ranging from 0.05-1mg/ml. According to ICH,Q2(R1) [9] a signal giving three times higher response than noise was regarded as the detection limit where signal giving ten times higher response than noise was determined to be the limit of quantification.

RESULTS

The obtained retention times (RT) and relative retention times (RRT) of standard compounds are presented in table 1. RT was used for the qualitative estimation of any particular synthetic adulterant in any sample herbal formulation. After comparing the HPLC chromatogram and UV spectra, it was revealed that out of 29 samples, three samples showed presence of synthetic adulterants as presented in fig. 1to fig. 3. The RT of the standards differed from the library data by ≤ 10% which compiled well with a previous report which quoted 15%. RRT was also considered in drug identification. The UV library match indicated how closely the unknown spectrum matched the library data. Overlapping of greater than 97% was achieved in the present findings which are well acceptable. Moreover, validation studies were found satisfactory and in order as per analytical requirements.

Table I: Retention time and relative retention time of reference standard drugs by HPLC-DAD method

STANDARD COMPOUNDS	*RETENTION TIME*	*RRT W.R.T CAFFEINE*
Analgesics		
Beclomethasone	30.453	3.69
Betamethasone	29.48	3.57
Dexamethasone	20.11	2.43
Hydrocortisone	20.99	2.54
Diclofenac free acid	26.12	3.16
Diclofenac Na+	26.10	3.16
Diclofenac K+	26.14	3.16
Nimuslide	23.85	2.89
Paracetamol	5.28	0.64
Aspirin	11.38	1.37
Acelofenac	24.66	2.98
Ibuprofen	26.78	3.24
Piroxicam	19.52	2.36
Chloroxazone	16.12	1.95
Elitriptan	18.21	2.20
Naratriptan	10.54	1.27
Mefenamic acid	28.53	3.45
Anti –diabetics:		
Metformin	2.57	0.31
Glibenclamide	26.60	3.22
Nateglimide	7.32	0.88
Glimiperide	27.62	3.34
Pioglitazone	18.29	2.21
Slimming drugs:		
Rimonabant	29.63	3.59
Sibutramine	33.04	4.00

Table II: Validation of HPLC-DAD method

DRUG NAME	*REGRESSION EQUATION*	*COEFFICIENT OF DETERMINATION(R^2)*	*LOD (MG/ML)*	*LOQ (MG/ML)*
Ibuprofen	y = 1885x- 18.25	0.9946	0.12	0.38
Rimonabant	y = 2125x -16.25	0.9941	0.13	0.45
Glibenclamide	y = 2175x + 13.25	0.9972	0.27	0.89

LOD = Limit of detection; LOQ= Limit of quantification

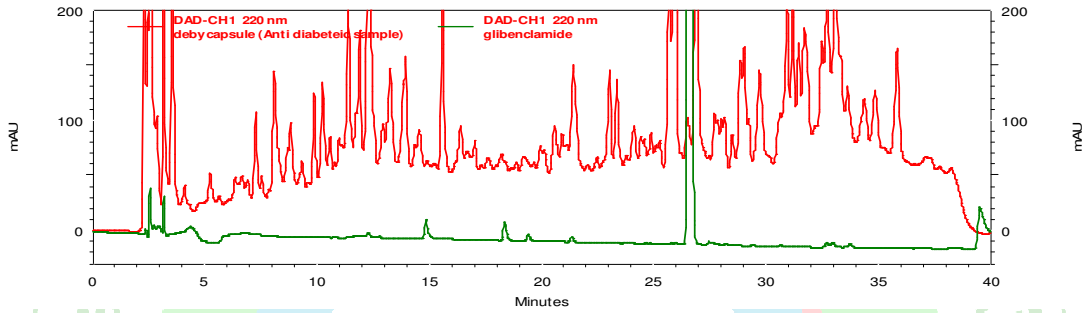

Figure 1: Overlapped HPLC Chromatogram of standard drug Glibenclamide with an antidiabetic formulation.

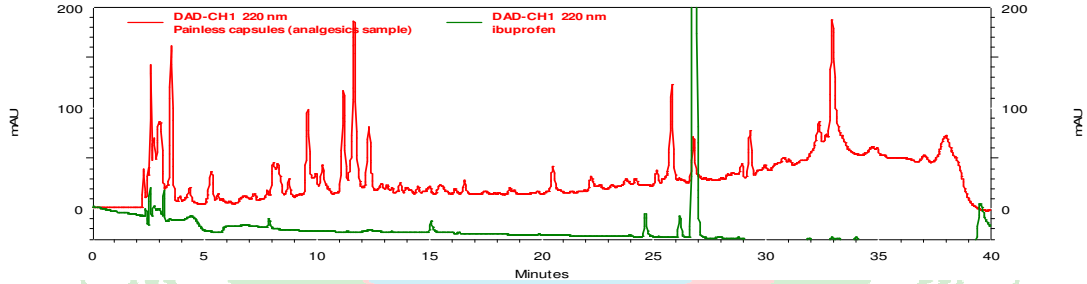

Figure 2. Overlapped HPLC Chromatogram of standard drug Ibuprofen with an analgesic formulation

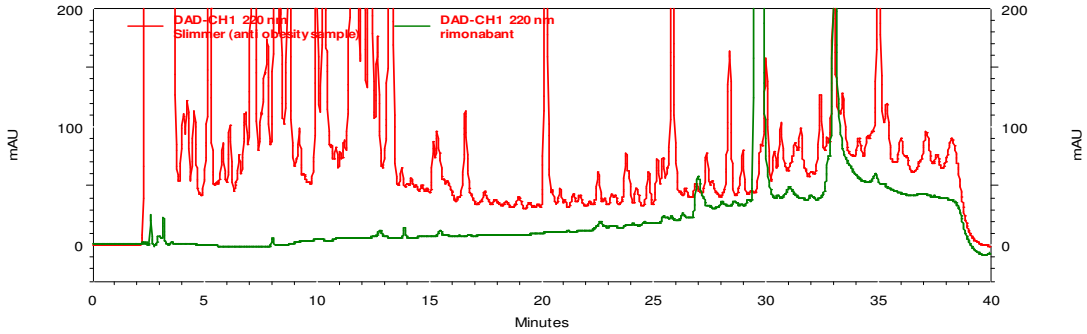

Figure 3. Overlapped HPLC Chromatogram of standard drug Rimonabant with an antiobesity formulation.

DISCUSSION

There is an increasing concern over the quality and safety aspects of herbal formulations all over the world in the recent past. Regulatory agencies are putting their best efforts to control the problem of adulteration at levels ranging from cultivation of medicinal plant to marketing of herbal formulations. In India, regulatory affairs of herbal drugs are mainly controlled by Indian Drugs and Cosmetic Act 1940 which has provisions for storage, manufacturing, labelling and marketing etc. There are different sections for each category of drugs such as section 33E for misbranded drugs, section 33 EE for adulterated drugs and section 33EEE for spurious drugs. Labelling provisions of herbal drugs under rule 161 must include the name of the formulation, true list of the ingredients used in the formulation together with the quantity of each ingredient and if the list is long, a separate list should be enclosed with the packing and reference be made on the label. Violation of the act by the manufacturer is punishable under section 33J [10]. But in the present case it seems the implementation of these regulations are lacking in India. Moreover, labelling requirements seem insufficient and need to be modified in which there should be mandatory declarations regarding quality check parameters to ensure safety and efficacy of a formulation.

CONCLUSION

The HPLC-DAD conditions were successfully applied for the determination of undeclared herbal adulterants which has a scope to be adopted by the manufacturing industries. Through this study, need for stricter regulations to be framed and implemented by the regulatory authorities is also accelerated.

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