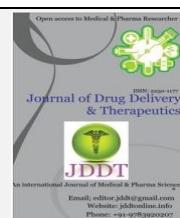


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Research Article

Colorimetric method for determination of corticosteroids by UV Visible Spectroscopy and its application to Ayurvedic formulations

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ABSTRACT

Usage of herbomineral preparations increased drastically these days. Some of the Ayurvedic formulations are been adulterated with steroids. Usage of such formulations can be injurious to health. Hence an attempt has been made to identify and determine steroids in both Ayurvedic and pharmaceutical formulations. In the present study a simple, sensitive and economical visible spectroscopic method has been used which involves the oxidation of corticosteroids by iron(III) and subsequent complexation of iron(II) with potassium hexacyanoferrate (III), forming bluish green colored complex having the maximum absorbance at 780 nm. The method has been applied for the determination of corticosteroids in Ayurvedic formulations. The method has been statistically validated in which the Standard deviation ranging from 0.0008 to 0.0135, Relative standard deviation from 0.08% to 1.35% and Standard error was varying from 0.0018 to 0.0060 for selected Ayurvedic formulations. The studies have shown that the method is fast, reproducible and accurate and can be used in the analysis of marketed formulations. The processed samples were stable up to 2 hours minimizing the error in terms of fluctuating absorbance values.

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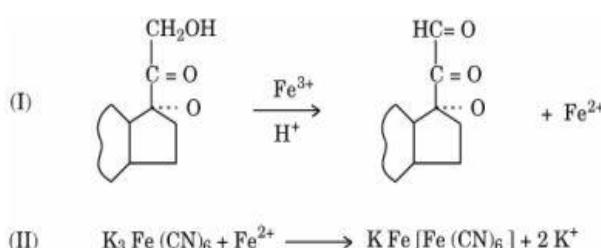
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INTRODUCTION

Medicinal plants play an important role in the development of potent therapeutic agents. Today estimate that about 80% of people in developing countries still relays on traditional medicine based largely on species of plants and animals for their primary health care. Herbal drugs referred as plants materials or herbals, involves the use of whole plants or parts of plants, to treat injuries or illnesses^[1]. These are generally regarded as safe based on their long-standing use in various cultures. However, there are case reports of serious adverse events after administration of herbal products. In a lot of cases, the toxicity has been traced to contaminants and adulteration. These drugs have been used more and more by the people without prescription for the past decades^[2]. In rural areas, there are additional cultural factors which encourage the use of botanicals, such as the environment and culture that is in other words, a 'man earth relationship.' It is a common belief that where an area gives rise to a particular disease, it will also support plants that can be used to cure it^[3]. Correct identification of the desired

plant, purification of the intermediates and the final products upto its expected standard of purity and uncompromising consistency of the product quality should be the basis of the production of herbal remedies^[4]. As the formulations are adulterated with steroids an attempt has made to know the presence of steroids in the formulations that are available in the local market. These steroids are consisting of significant side effects apart from reducing inflammation^[5]. There are few methods reported for determination of corticosteroids using several methods^[6-26]. The proposed method was based on the oxidation of corticosteroids with iron(III) in acidic medium and subsequent complexation of iron(II) with potassium hexacyanoferrate (III) which is simple, selective and precise. Usage of cheap and non-explosive chemicals make the method more advantageous. The method includes identification and determination of amount of corticosteroids in Ayurvedic formulations.



Scheme-1 Reaction sequence for the formation of products.

MATERIAL AND METHODS

Chemical Reagents:

All the chemicals used were of the analytical grade. The Ayurvedic formulations were purchased from local market regions of Kadapa and the pharmaceutical formulations Omnaacortil-10 containing Prednisolone 10 mg and Deflagard containing 6 mg Deflazacort from pharmacy. Chemical reagents Ferric chloride (96.00% purity), Potassium hexacyanoferrate (98% Purity), Sulphuric acid (98% purity) were purchased from Finar Chemicals Limited (Ahmedabad, Gujarat, IND) and Distilled water from distillation unit from our laboratory.

Apparatus: UV-Visible double beam spectrophotometer Systronics -2203 with 1 cm stoppered quartz cells was used for the absorbance measurements.

Reagents: Standard solutions of corticosteroids, 0.5% (w/v) solutions of iron(III) chloride and potassium hexacyanoferrate (III) were prepared in distilled water.

Methods for Identification of steroids:

The materials used for the present study was purchased from local market of Kadapa. About 11 Ayurvedic formulations of Anti - Rheumatoid category were purchased and were represented as AF1, AF2, AF3,...,AF11 respectively.

Salkowski test: Treat the extract with few drops of conc. sulphuric acid, red colour at lower layer indicates presence of steroids and formation of greenish yellow coloured lower layer indicates presence of triterpenoids.^[27]

Liebermann-Burchard test: Dissolve the extract in chloroform, and treat with few drops of acetic anhydride, boil and cool. Then add Conc. sulphuric acid from the sides of the test tube, brown ring is formed at the junction of two layers and upper layer turns green which shows presence of

steroids and formation of deep red colour indicates presence of triterpenoids.

Liebermann test: Mix 3 ml extract with 3 ml of acetic anhydride. Heat & cool. Add few drops of conc. sulphuric acid, blue colour appears indicates presence of steroids.^[28]

General Procedure: Appropriate volumes of working solutions of corticosteroids were transferred into a series of 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron(III) chloride (0.5% w/v, 2 ml), were added to each followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70±2°C for 30 minutes with occasional shaking and diluted to the mark of distilled water. The absorbance was measured at 780 nm against the reagent blank.

Analysis of tablets:

An accurately weighed amount of powdered tablets equivalent to pure form of corticosteroids as dissolved in distilled water (10 ml). The solution was filtered through a whatmann filter paper (no.41). Weight equivalent to 50 mg of Prednisolone and Deflazacort were dissolved in 50 ml of distilled water in 50 ml of volumetric flask which gives 1000 µg/ml. [stock solution -A]. 10 ml of above solution was taken and made up to 100 ml with distilled water in 100 ml standard flask which gives 100 µg/ml. [stock solution -B]. To get the resulting solution, 25 ml of stock -B solution was taken and made up to 100 ml with distilled water in 100 ml standard flask which gives 25 µg/ml concentration. 25 µg/ml concentration of drug was prepared and absorbance was measured at 780 nm, for selected Ayurvedic formulations. From obtained data, amount of corticosteroids in Ayurvedic formulation was determined using following formula

$$\frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times \text{weight of standard}$$

RESULTS AND DISCUSSION

The method was successfully employed for the determination of corticosteroids in pharmaceutical & Ayurvedic formulations. The method offers the advantages of simplicity, sensitivity and rapidity without the need of extraction. The results of Ayurvedic formulations were reported for steroids are reflecting the accuracy and precision.

The preliminary tests are performed for the identification of steroids in collected Ayurvedic formulation and the results are shown in fig.1 & Table 1.

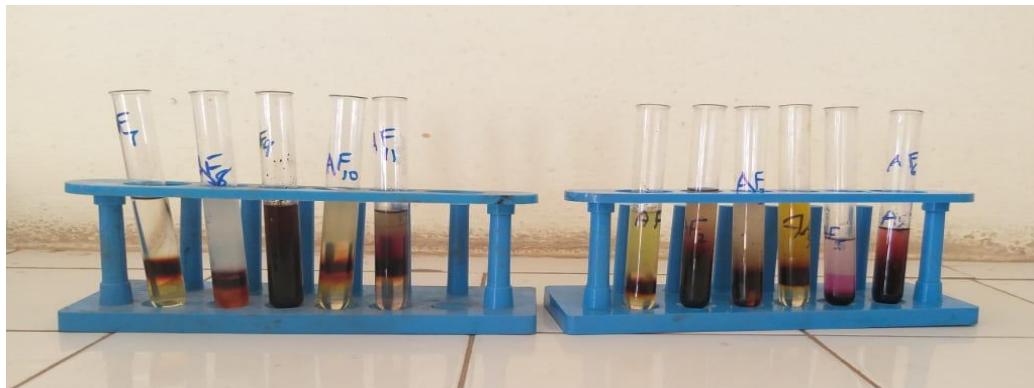


Figure 1: Results of tests for steroids in Ayurvedic formulations AF1 to AF11

Table 1: Results of presence of steroids in Ayurvedic formulations

S. No	Code	Category	Results		
			Salkowski test	Liebermann-Burchard test	Liebermann test
1)	AF1	Anti rheumatoid	+ve	+ve	+ve
2)	AF2	Anti rheumatoid	-ve	-ve	-ve
3)	AF3	Anti rheumatoid	-ve	-ve	-ve
4)	AF4	Anti rheumatoid	+ve	+ve	+ve
5)	AF5	Anti rheumatoid	-ve	-ve	-ve
6)	AF6	Anti rheumatoid	+ve	+ve	+ve
7)	AF7	Anti rheumatoid	-ve	-ve	-ve
8)	AF8	Anti rheumatoid	-ve	-ve	-ve
9)	AF9	Anti rheumatoid	-ve	-ve	-ve
10)	AF10	Anti rheumatoid	-ve	-ve	-ve
11)	AF11	Anti rheumatoid	+ve	+ve	+ve

In the proposed method, investigated corticosteroids are oxidized by iron(III) chloride in the acidic medium and produce iron(II). The iron (II) ion reacts with potassium hexacyanoferrate (III) and produces bluish green iron (II) ferricyanide complex with an absorbance maximum at 780 nm. The Absorption Spectra of selected Ayurvedic formulations are shown in Fig.2.

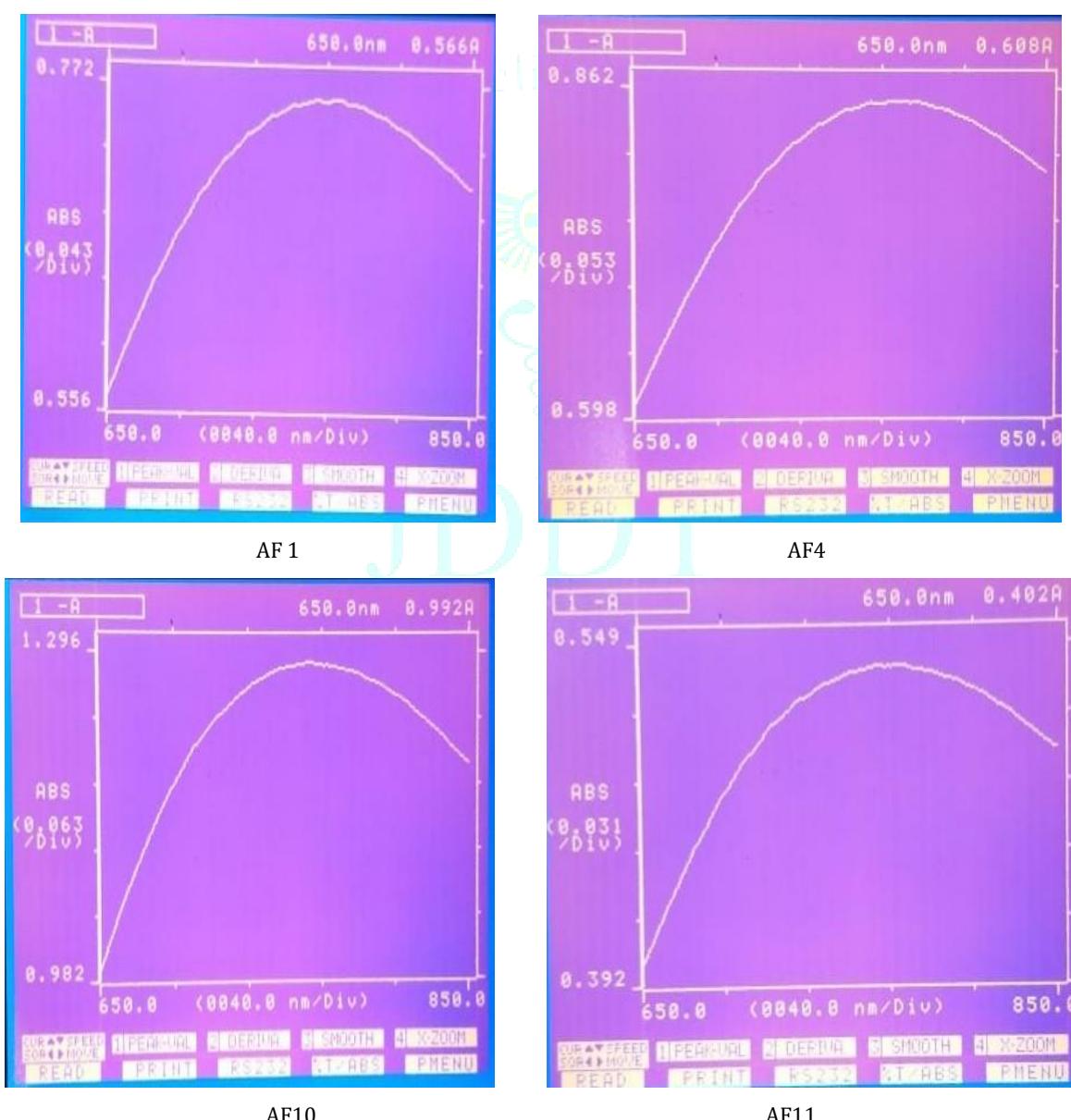


Figure 2: UV- Spectrums of selected Ayurvedic Formulations

A aliquots of each steroids (25 μ g/10 ml) were analysed by the proposed method, and the absorbance were determined. The standard deviation varied from 0.0008 to 0.0135 relative

standard deviation varied from 0.08 to 1.35% and relative error varied from 0.0018 to 0.0060 are shown in table 2.

Table 2. Absorbance replication of proposed procedure

Run	Prednisolone	Deflazacort	AF1	AF4	AF10	AF11
1	0.652	0.453	0.857	0.834	1.300	0.527
2	0.650	0.453	0.856	0.830	1.293	0.527
3	0.649	0.453	0.854	0.832	1.287	0.526
4	0.649	0.453	0.853	0.829	1.282	0.525
5	0.648	0.453	0.850	0.828	1.280	0.525
Average	0.649	0.453	0.853	0.831	1.288	0.526
S.D	0.0014	0	0.002	0.0135	0.0099	0.00089
R.S.D(%)	0.148	0	0.264	1.352	0.994	0.0089
R.E	0.0006	0	0.001	0.006	0.0042	0.0089

Concentration: 25 μ g/ml

The optical characteristics are given in table 3. The stability of the products was more than 2 hr. The amount of steroids present in selected Ayurvedic samples were determined and the results are shown in table 4.

Table 3. Results of Optical characteristics

Characteristics	Prednisolone	Deflazacort	AF1	AF4	AF10	AF11
Color	Bluish green					
Lambda _{max}	780	780	780	780	780	780
Stability	2	2	2	2	2	2

Table 4- Results of amount of steroids found in Ayurvedic formulations

S/No	Formulations	Mean absorbance	Amount of drug	
			Found using PF1	Found using PF2
1	AF1	0.854	13.1 mg	11.3 mg
2	AF2	0.830	13.1mg	10.9 mg
3	AF3	1.288	18.9 mg	17.0 mg
4	AF4	0.526	8.1 mg	6.9 mg
5	PF1	0.649	Label claim -10 mg	
6	PF2	0.453	Label claim - 6 mg	

PF1-Pharmaceutical Formulation 1 PF2- Pharmaceutical Formulation 2

CONCLUSION

Different Ayurvedic formulations were selected and evaluated for the presence of steroids using chemical tests and were analyzed by u. v. spectroscopy for determination of corticosteroids at 780nm. Among 11, we found 4 formulations containing steroids. Eventhough we don't know

the particular steroid by which the formulation was adulterated, but it is dangerous for humans as the steroids are harmful. The Advantage of the reported method for the detection of the steroids is low costing and rapid assay without sacrificing the sensitivity and accuracy. The consumption of very common chemicals like ferric chloride and Conc. Sulphuric acid which you can find even most

laboratories make this method very handy cost wise. The usage of the such cheaper chemical will definitely reflect on the cost of the method. As far as the time consumption is concerned the total time to carry out the complete experiment right from the beginning is less than single hour and it will add up in the values of the reported method. The cost and time is the most serious concern where we have focused while developing method without losing the basics interest like sensitivity and accuracy.

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Conflicts of Interest:

No conflicts of interest

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