Standardization of Habb-e-Ustukhuddus: A Classical Unani Formulation

***Asma Sattar Khan,**
R.P. Meena,
Shoeb A. Ansari,
Mustehasan,
Mokhtar Alam,
Arshad Hashmi,
[1]Shams ul Arfin
and
[1]Aminuddin

Drug Standardization Research Institute,
PLIM Campus, Kamla Nehru Nagar,
Ghaziabad-201002

[1]Central Council for Research in Unani Medicine,
61-65 Institutional Area,
Janakpuri, New Delhi-110058

Abstract

Habb-e-Ustukhuddus is a safe and effective compound Unani formulation with high therapeutic value in hemiplegia (Falij), facial paralysis (Laqwa) and paralysis (Istirkha). SOP of the drug has been developed by preparing the drug at laboratory scale with the standard ingredients and by following the prescribed procedure. The formulation was studied on the basis of pharmacopoeial parameters such as organoleptic, microscopic, physico-chemical analysis, TLC/HPTLC, aflatoxin, heavy metals and level of microbial and pesticide contamination to prove its quality, safety and efficacy.

Keywords: SOP, TLC, HPTLC, UV Spectroscopy, Heavy metal

Introduction

The World Health Organization has reported that more than half of the population in developing countries rely on traditional medicines for their primary health care needs. For last few years developed countries have also been showing interest in traditional herbal medicines. This tremendous growth of herbal medicine consumption leads to concern over its safety issues. There is an urgent need to ensure the quality of these medicines to expand its acceptability worldwide. The Unani system of medicine is very popular traditional system and has wider reach among people. In order to enhance its reliability, several Unani Formulations for numerous diseases have been standardized so far and the exercise is constantly continued. Present work is based on this rationale.

Habb-e-Ustukhuddus is a Unani poly-herbal formulation, categorized as Habb (Anonymous, 2006). The drug is reputed for its demulcent action and is used in ailments of hemiplegia, facial paralysis, tremor, paralysis neurasthenia and epilepsy. It is tonic to the body as well as visceral organs, reduces flabbiness of the muscles and Munaqqi-e-Dimagh (clears toxic humours from brain) (Anonymous, 2003; Khan, 1933; Kabiruddin, 1929).

The drug was prepared at laboratory-scale at D.S.R.I., Ghaziabad. According to the formulation composition of the drug, Habb-e-Ustukhuddus is comprised of 12 ingredients of plant origin (Table I) as described in NFUM Part-IV.

In order to develop SOP and pharmacopoeial standards, the drug was subjected to microscopical and physico-chemical analysis. The present study deals with the preparation, microscopical characters, physico-chemical parameters, TLC & HPTLC profile, U.V. spectroscopic study and heavy metal estimations.

*Author for correspondence

Table I: Formulation Composition

S.No.	Ingredients	Botanical/English Name	Part used
1.	Turbud	*Operculina turpethum* (L.) S. Manso	Root
2.	Sibr	*Aloe vera* (L.) Burm.f.	Leaf extract
3.	Post-e-Halela Zard	*Terminalia chebula* (Gaertu) Retz.	Fruit Pericarp
4.	Post-e-Halela Kabuli	*Terminalia chebula* (Gaertu) Retz.	Fruit Pericarp
5.	Ustukhuddus	*Lavendula stoechas* L.	Flower
6.	Ghariqoon Safaid	*Agaricus alba* L.	Fruit body
7.	Bisfayez	*Polypodium vulgare* L.	Rhizome
8.	Aftimoon	*Cuscuta reflexa* Roxb.	Whole Plant
9.	Shahm-e-Hanzal	*Citrullus colocynthis* Sehrad	Fruit Pulp
10.	Qaranful	*Syzygium aromaticum* (L.) Merr & Perry	Floral bud
11.	Nana	*Mentha viridis* L.	Aerial Part
12.	Roughan-e-Badam	*Prunus amygdalus* Batsch.	Oil

Material and Methods

All the ingredients were procured from local raw drug dealer and identified botanically (Wallis 1967; Trease & Evans 1972) through pharmacognostical methods. Three batches of Habb-e-Ustukhuddus were prepared at DSRI, Ghaziabad as per the formulation composition given in NFUM, Part IV (Anonymous 2006). All the ingredients were of pharmacopoeial quality and free from physical impurities and dried under the shade to remove moisture, if any.

Except Sibr (*Aloe vera*) and Roughan-e-Badam, all the ingredients were crushed separately in an iron mortar to obtain coarse powders which were further processed in a grinder to obtain the fine forms. Sibr (*Aloe-vera*) was soaked in water for about 24 hours to make Rabeta (Adhesive). The powdered ingredients were added to Rabeta and mixed thoroughly to make lubdi mass. Roughan-e-Badam was then added to lubdi mass by continuous mixing. This lubdi mass was used to prepare Huboob by mechanical process. The huboob was dried under shade and stored in a tightly closed glass container free from moisture.

Microscopy

5 g of the powdered drug was taken and stirred gently with hot water in a beaker. The supernatant was discarded and the residue was washed with the distilled water. A little residue was stained with Iodine solution and mounted in 50% glycerine. Some of the residue was heated in chloral hydrate solution and mounted in 50% glycerine and a little residue was boiled in 2% potassium hydroxide solution, washed with distilled water and mounted in 50% glycerine (Johansen, 1940; Wallis, 1967).

Chemical Analysis

Physico-chemical parameters of Habb-e-Ustukhuddus was analysed by standard methods as per the WHO guidelines (Anonymous, 1998) such as removal of foreign matters, solubility in water, alcohol and petroleum ether (60-80^{o}), total ash, acid insoluble ash and water soluble ash, loss on drying at 105^{o}C, pH values of 1% and 10% aqueous solution (Anonymous, 1987), volatile oil estimation, microbial load, aflatoxins, pesticide residue (Anonymous, 2000) and heavy metal estimation (Sahito *et al.,* 2001).

Preparation of extract for TLC/HPTLC

Samples of all the three batches of the formulation were extracted with chloroform and alcohol. The extracts were concentrated and made up to 10ml in a volumetric flask separately. These solutions were used for the TLC/HPTLC finger print analysis by employing CAMAG Linomat IV sample applicator on aluminium TLC plate pre-coated with silica gel 60 F_{254} (E. Merck). The chromatograms were developed using the solvent system toluene: ethyl acetate in the ratio 8:2 & 1:1 respectively for chloroform and alcohol extracts. The plates were dried at room temperature and observed the spots at UV-254 and UV-366. Further the plates were dipped in 1% vanillin-sulphuric acid reagent and heated at 105^{0} C till coloured spots appeared. (Wagner *et al.*, 1984; Sethi, 1996; Stahl, 1996)

Preparation of extract for U.V. Spectroscopic studies

1g of the drug was extracted with 100 ml. Petroleum ether (60-80^{o}) by refluxing for 15 minutes on water bath and filtered. The solution was made up to 100 ml. in volumetric flask. This solution was used for U.V. analysis and pure petroleum ether (60-80^{o}) was used as blank solution. (Willard *et al.,* 1965)

Observations

Habb-e-Ustukhuddus is a brown colour pill, hard in texture with spicy smell and bitter in taste. The drug did not show any filth, fungus or objectionable matter while the sample was spread in a petridish.

Microscopy shows presence of following plant tissues

Cork cells are rectangular radially flattened, thin walled parenchymatous cells, vessels and fibres (Sham-e-Hanzal); brown polygonal epidermal cells, starchy parenchymatous cells and very large resin cells. Various rosettes like raphids (Turbud); barrel shaped cells their inner tangential and radial wall very thick, mesophyll tissue shrunken collapsed (Aftimoon); epidermal cells in surface view with uniformly thick walled cells, several of them divided by a thin septa and fragments of cris-cross fibre (Post-e-Halela Zard); spherical smooth pollen grains fragments of calyx tube with prominent nerves (Ustukhuddus); sclereids of various sizes, collenchyma and raphides (Post-e-Halela Kabuli); non septet fungal hyphae (Ghariqoon); isodiamatric shaped cells long trachieds with scalariform thickening, pigmented parenchymatous cells (Bisfayej); pollen grains and scaleranchymatous pericycle (Qaranfal); nonglandular trichomes diacystic stomata on fragments of leaf (Nana).

Results and Discussion

Chemical Analysis

The physico-chemical data of the drug are shown in Table II. The water soluble extractives (23.93-24.25%) show the absence of any inorganic constituents. The moisture content in the drug is very low as the loss in weight on drying at 105^0 C occurs (5.02-5.38%). The low value of acid insoluble ash indicates that the drug is free from siliceous matter. The results of microbial studies are within the permissible limits while total fungal count is nil (Table-III). The results of aflatoxin

Table II: Physico-Chemical Paramaters

S.No.	Parameters	Results
1.	Alcohol Soluble matter %	10.92 - 11.58
2.	Water Soluble matter %	23.93 - 24.25
3.	Pet ether Soluble matter %	7.66 – 7.78
4.	Loss in weight on drying at 105ºC	5.02 - 5.38
5.	Total Ash %	7.38 – 7.88
6.	Water Soluble Ash %	4.11 – 4.29
7.	Acid Insoluble Ash %	1.42 - 1.80
8.	pH of 1% aqueous solution	4.08 - 4.24
9.	pH of 10% aqueous solution	4.20 - 4.40
10.	Volatile oil %	*Traces*

(Table-IV) and pesticide residue (Table-V) studies show that the drug is free from aflatoxin as well as pesticide residue. The content of heavy metal is below detectable limits (Table-VI).

Table III: Microbial Load

S.No.	Parameter Analyzed	Results	Permissible limit as per WHO
1.	Total Bacterial load	$3x10^3$ cfn/g	10^5 CFU/gm
2.	Total fungal count	< 10 cfu/gm	10^3 CFU/gm
3.	Enter obacteriaceae	Absent	Nil
4.	Escherichia coli	Absent	Nil
5.	Salmonella cpp.	Absent	Nil
6.	Staphoilococcus aureus	Absent	Nil

Table IV: Aflatoxin level

S.No.	Parameter Analyzed	Results	Detection limits
1.	B-1	Not detected	0.50 ppm
2.	B-2	Not detected	0.10 ppm
3.	G-1	Not detected	0.50 ppm
4.	G-2	Not detected	0.10 ppm

Table V: Pesticide Residue

S.No.	Parameter Analyzed	Results	Limit
1.	Chlorpyriphos	Not detected	0.20 mg/Kg
2.	DDT	Not detected	1.00 mg/Kg
3.	Endosulfan	Not detected	3.00 mg/Kg
4.	Malathon	Not detected	1.00 mg/Kg
5.	Parathion	Not detected	0.50 mg/Kg

Table VI: Heavy Metals

S.No.	Heavy Metal Analyzed	Results	Permissible limit as per WHO
1.	Arsenic	Not detected	3.00 ppm
2.	Cadmium	Not detected	0.30 ppm
3.	Mercury	0.0079	01.00 ppm
4.	Lead	Not detected	10.00 ppm

Table VII: TLC Results

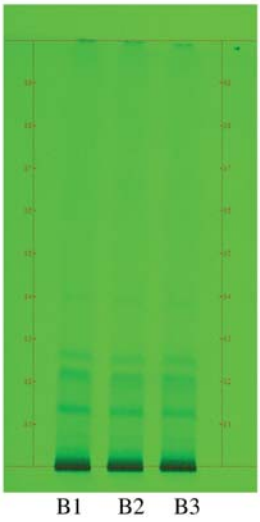
S.No.	Extract	Solvent System	Developing reagent	Rf Values with colour		
				UV254nm	UV366nm	After derivatisation
1.	Chloroform	Toluene: Ethyl acetate (8:2)	Vanillin – Sulphuric acid	0.40 Black	0.77 Red	0.91 Grey
				0.26 Black	0.70 Red	0.72 Grey
				0.22 Black	0.65 Red	0.48 Grey
				0.14 Black	0.62 Blue	0.37 Violet
					0.59 Red	0.26 Green
					0.54 Violet	0.22 Violet
					0.44 Violet	0.14 Violet
					0.40 Brown	
					0.32 Brown	
					0.24 Blue	
					0.17 Violet	
2.	Alcohol	Toluene: Ethyl acetate (1:1)		0.76 Black	0.95 Red	0.92 Grey
				0.66 Black	0.87 Fluorescent blue	0.85 Grey
				0.58 Black	0.76 Violet	0.74 Violet
				0.39 Black	0.69 Blue	0.66 Violet
				0.34 Black	0.63 Blue	0.58 Violet
				0.13 Black	0.56 Green	0.52 Grey
					0.46 Violet	0.48 Violet
					0.31 Green	0.28 Violet
					0.22 Brown	0.16 Green
					0.13 Red	0.13 Violet

HPTLC profile

TLC of all the three batches of Habb-e-Ustukhuddus were observed under UV 254nm, UV 366nm and after derivatization (Table-VII). Chromatogram of chloroform extract shows 04 spots under UV 254nm (Fig. 1), 11 spots under UV 366nm (Fig. 2) and 07 spots after derivatization (Fig. 3). The finger print of chloroform extract shows 10 peaks out of which peaks at R_f 0.15, 0.22, 0.25, 0.29 were major peaks whereas peaks at R_f 0.01, 0.04, 0.07, 0.12, 0.45 and 0.69 are relatively smaller peaks (Fig. 4). HPTLC Chromatogram of chloroform extract is shown in Fig. 5.

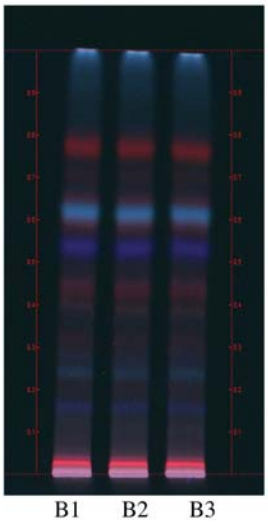
TLC of Chloroform extract of Habb-e-Ustukhuddus

Fig. 1



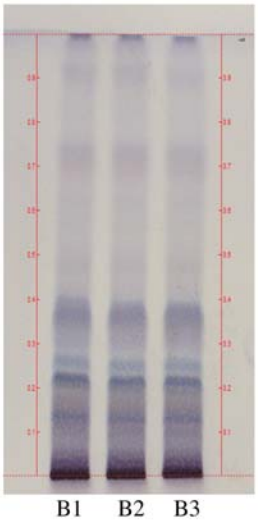
UV254nm

Fig. 2



UV366nm

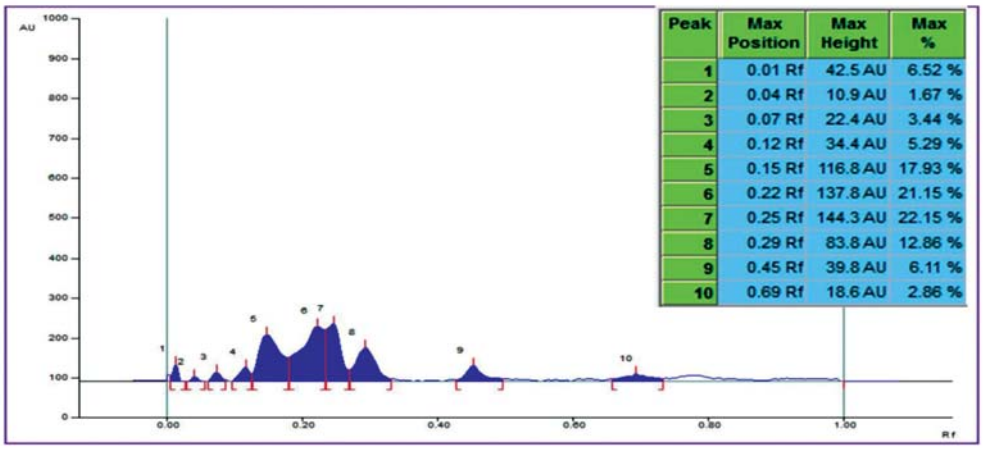
Fig. 3



After derivatization

*B1 – Batch1, B2 – Batch2, B3 – Batch3

Fig. 4

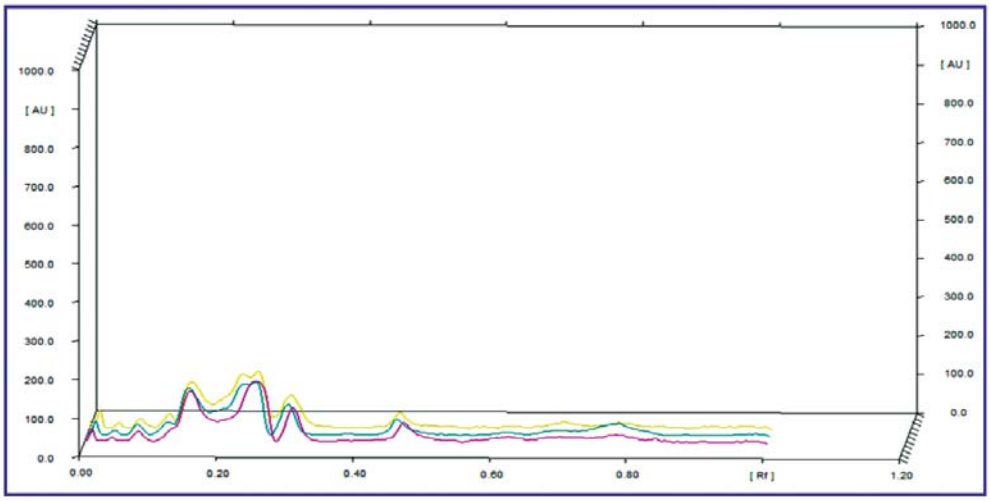


Peak	Max Position	Max Height	Max %
1	0.01 Rf	42.5 AU	6.52 %
2	0.04 Rf	10.9 AU	1.67 %
3	0.07 Rf	22.4 AU	3.44 %
4	0.12 Rf	34.4 AU	5.29 %
5	0.15 Rf	116.8 AU	17.93 %
6	0.22 Rf	137.8 AU	21.15 %
7	0.25 Rf	144.3 AU	22.15 %
8	0.29 Rf	83.8 AU	12.86 %
9	0.45 Rf	39.8 AU	6.11 %
10	0.69 Rf	18.6 AU	2.86 %

HPTLC Finger printing of Chloroform extract

Similarly TLC of alcohol extract shows 06 spots under UV 254nm (Fig. 6), 10 spots under UV 366nm (Fig. 7) and 10 spots after derivatization (Fig. 8)). The finger print of Alcohol extract shows 11 peaks out of which peaks at R_f 0.01, 0.13, 0.69, 0.90 were major peaks whereas peaks at R_f 0.17, 0.34, 0.41, 0.49, 0.59, 0.63 and 0.78 are relatively smaller peaks (Fig. 9). HPTLC Chromatogram of Alcohol extract is shown in Fig. 10.

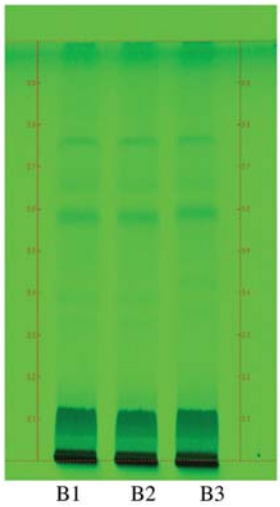
Fig. 5



HPTLC Chromatogram of Chloroform extract

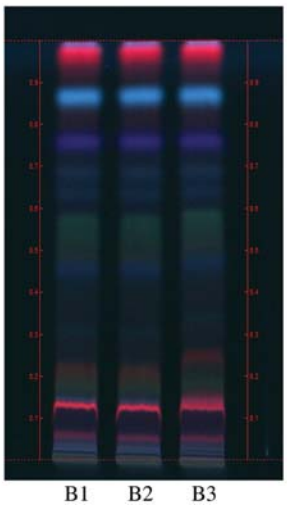
TLC of Alcohol extract of Habb-e-Ustukhuddus

Fig. 6



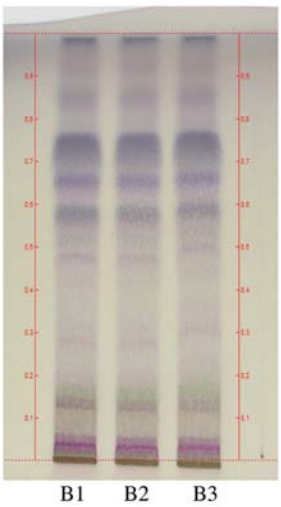
UV254nm

Fig. 7



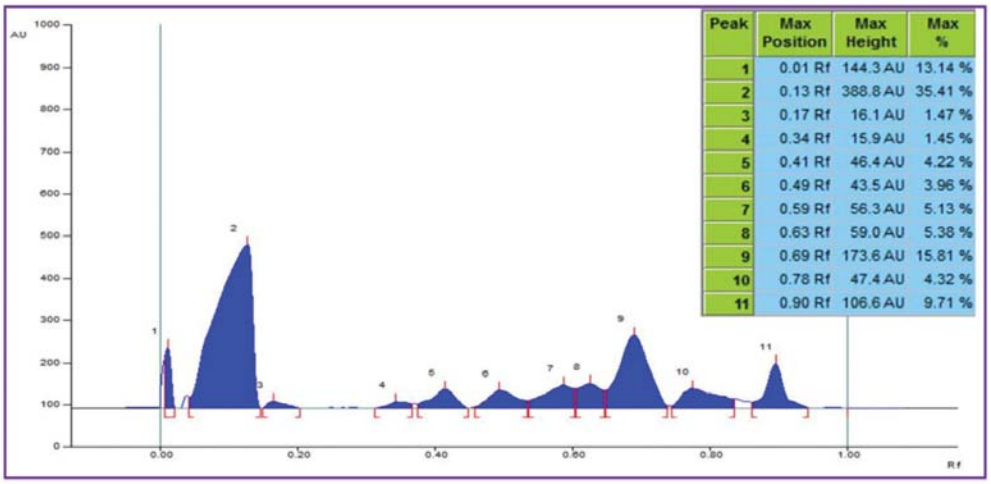
UV366nm

Fig. 8



After derivatization

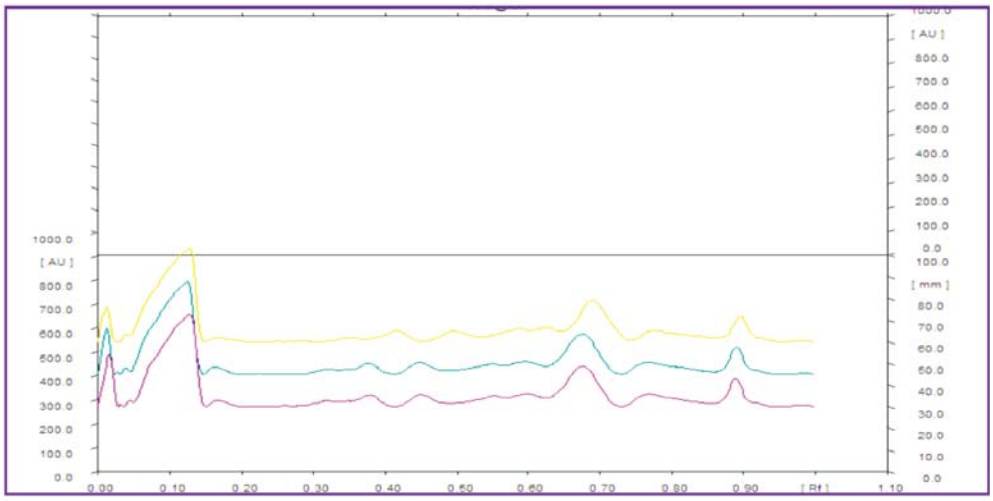
Fig. 9



Peak	Max Position	Max Height	Max %
1	0.01 Rf	144.3 AU	13.14 %
2	0.13 Rf	388.8 AU	35.41 %
3	0.17 Rf	16.1 AU	1.47 %
4	0.34 Rf	15.9 AU	1.45 %
5	0.41 Rf	46.4 AU	4.22 %
6	0.49 Rf	43.5 AU	3.96 %
7	0.59 Rf	56.3 AU	5.13 %
8	0.63 Rf	59.0 AU	5.38 %
9	0.69 Rf	173.6 AU	15.81 %
10	0.78 Rf	47.4 AU	4.32 %
11	0.90 Rf	106.6 AU	9.71 %

HPTLC Finger Printing of Alcohol extract

Fig. 10



HPTLC Chromatogram of Alcohol extract

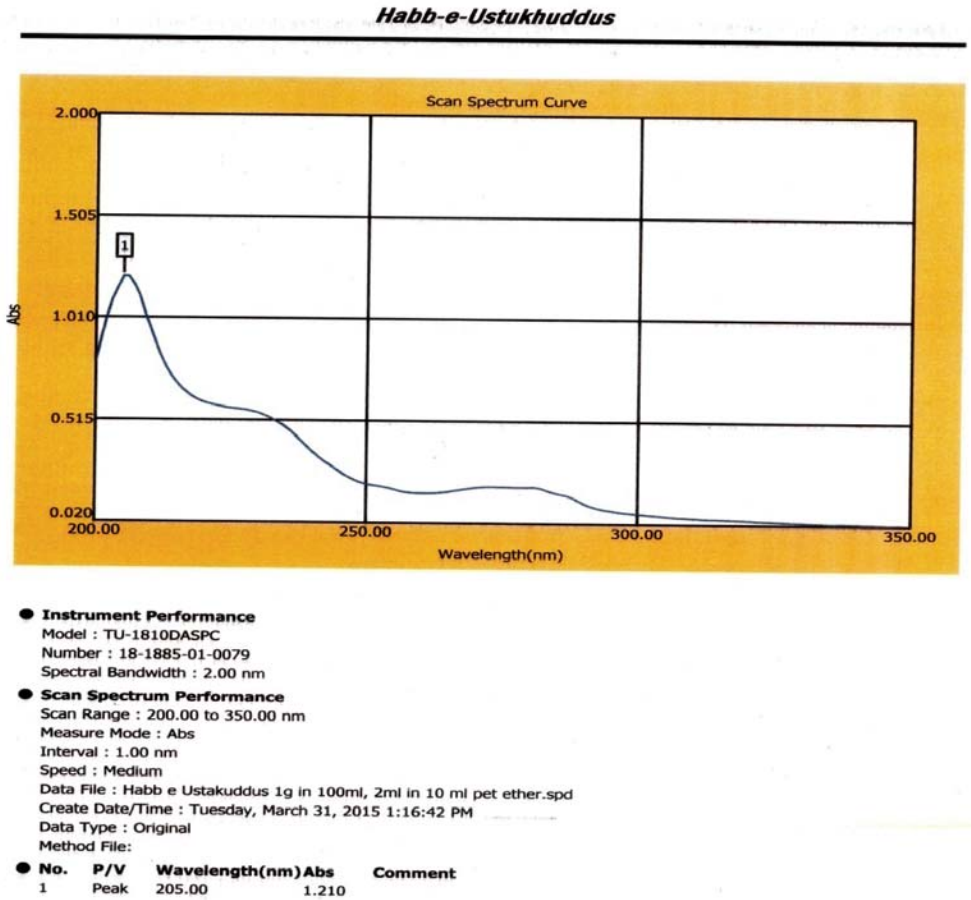
The HPTLC densitometry chromatograms of chloroform and alcohol extract of all the three batches were found to be similar when scanned at 254nm. It indicates batch to batch consistency of the compound formulation.

UV Spectoscopic Studies

The UV spectrum of Habb-e-Ustukhuddus has a single peak at 205nm with an absorbance of 1.210 %. Appearance of the single sharp peak without any noise fortifies the purity of the compound formulation (Fig. 11).

UV Spectrum of Habb-e-Ustukhuddus

Fig. 11



Conclusion

It is very difficult to identify the single drugs once they are powdered and mixed together for preparing compound formulation. The present study, therefore, holds high significance as the microscopic features, various Physico-chemical parameters, HPTLC profile, UV spectrum etc. provide criteria for easy identification of Habb-e-Ustukhuddus and ensure the quality and efficacy of the drug.

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