Standardization of Sufoof-e-mohazzil: An Anti-Obese Unani Formulation

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Abstract

The use of traditional medicines has been very popular in most of the countries of Asia, Africa, Latin America and some other parts of the world from the time immemorial. But for the last few years the developed nations too have been tremendously growing interest in the use of traditional medicines. In India, traditional systems of medicine especially Unani System of Medicine has been practiced to great advantage in the treatment of several diseases for centuries. Today, everyone wants a safe and effective treatment of various ailments including Diabetes, Jaundice, Arthritis, Malaria, Filaria and Obesity etc. These expectations of the people lead to concern over the quality of these medicines. Thus, the quality standardization of the traditional medicines is evidently essential in the present scenario. Therefore, Sufoof-e-Mohazzil, a safe and effective anti-obese compound Unani formulation, was taken up for the standardization. To ascertain the quality of this Unani formulation, Physico-chemical parameters, Thin Layer Chromatography, HPTLC, UV Spectroscopy studies were carried out.

Key Words: Standardization, TLC, HPTLC, UV Spectroscopy

Introduction

Today while a large population of the world is suffering from obesity or the diseases occurred due to obesity, the role of anti-obese medicines has become very important in reducing one's weight safely. Sufoof-e-Mohazzil is one of the safe and effective drugs in the treatment of Saman-e-Mufrit (Obesity) (Kabiruddin, 1967). It is a powder drug listed under the sufoof category in National Formulary of Unani Medicines (part- I) (Anonymous, 2006). The present study was carried out on the drug prepared at DSRI, Ghaziabad. According to the formula of the drug (Table-1), Sufoof-e-Mohazzil is made up of six ingredients out of which five ingredients are from plant origin while one ingredient is from animal origin (Anonymous, 2007). A review of the literature indicated that no work was available on identity and Pharmacopoeial Standards of this compound formulation. In order to develop SOPs and Pharmacopoieal Standards, this drug was prepared at laboratory scale to analyze different parameters (Anonymous, 1966). The present paper describes the salient features of microscopical characteristic, physicochemical data, TLC, HPTLC studies, and UV Spectroscopic studies of Sufoof-e- Mohazzil.

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Materials and Methods

The ingredients Nankhwah (seed), Tukhm-e-Karafs (seed), Sumbul-ut-Teeb (rhizome), Gul-e-Surkh (flower), Marzanjosh (vegetative parts) and Luk Maghsool (resin) (Fig. – 1) were procured from local raw drug dealer and were identified botanically (Wallis, 1967; Trease & Evans, 1972) using pharmacognostical methods. The Sufoof-e-Mohazzil was prepared at DSRI, Ghaziabad as per the formulation given in National Formulary of Unani Medicine, Part-1 (Anonymous, 2006).

All the ingredients of pharmacopeial quality were taken and made free from all physical impurities and dried under the shade to remove moisture if any.

All the ingredients were crushed separately in an iron mortar to obtain coarse powder. The coarse powder was processed further in a grinder to get its fine form. The ingredients were then thoroughly mixed and sieved through mesh size 60. The powder was stored in a tightly closed plastic container free from moisture and kept in a cool, dry place.

Microscopy

5g of powder was taken and stirred thoroughly in ethanol for some time to remove Luk Maghsool. The supernatant was discarded and the residue was washed with distilled water. A little residue was stained with iodine solution and mounted in 50% glycerin to examine starch grains. Some of the residue was cleared by heating in chloral hydrate solution which was washed with distilled water, stained with safranine and then mounted in 50% glycerin. A little residue was boiled in 2% potassium hydroxide solution, washed with distilled water and mounted in 50% glycerin (Johnson, 1940; Wallis, 1967).

Chemical Analysis

The physico-chemical parameters of Sufoof-e-Mohazzil were analyzed by standard methods as per the WHO guideline (Anonymous, 1998) like removal of foreign matters, water, alcohol and petroleum ether (60-80^{o}) solubilities, total ash, acid insoluble ash and water soluble ash, loss on drying at 105^{o}c, pH values for 1% and 10% aqueous solutions (Anonymous, 1987) and volatile oil estimation (Anonymous, 2000).

Preparation of extract for TLC & HPTLC

5 g of the drug sample was dissolved in 50 ml of pet. ether (60-80^{o}) and refluxed for 30 minutes on a water bath and filtered. The filtrate was

concentrated on water bath and reduced to 5 ml in a standard flask. This extract was used for Thin Layer Chromatography (Wagner, *et al*.,1984; Stahl, 1996).

2 g of the drug was dissolved in 50 ml of pet. ether (60-80^{o}) and refluxed for 15 minutes on water bath and filtered and used as such for HPTLC profile.

Camag HPTLC system was used for the purpose and the photographs were taken by ink jet printer.

Preparation of extract for U.V. Spectroscopic studies

1 g of the drug was dissolved in 100 ml of pet. ether and refluxed for 15 minutes on water bath and filtered. The solution was made up to 100ml in a volumetric flask. This solution was used for U. V. spectroscopic analysis and pure pet. ether was used as a blank solution (Willard *et al.*, 1965).

Observations

The Sufoof-e-Mohazzil is a yellowish brown powder. It has unpleasant smell and slightly bitter 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:

1. Papillose epidermal cells in surface view with striated cuticle and short glandular outgrowths; endosperm parenchyma tissue filled with oil droplets and aleurone grains (Nankhwah).
2. Epicarp tissue having stomata, striated papillose outgrowths, vittae, sclereids, endosperm parenchyma with aleurone grains and spheroidal crystals of calcium oxalate (Tukhm-e-Karafs).
3. Cork cells with parenchyma rich in starch and oil globules; fragments showing fibre, sclereids; vessels with scalariform thickenings (Sumbul-ut-teeb).
4. Parenchymatuos fragments of petals with vascular strands; the surface showing rectangular to radially elongated cells and stomata; simple trichomes and oval pollen grains (Gul-e-Surkh).
5. Stem fragments with quadrangular outline; tracheary strands showing spiral thickenings; leaf fragments with stoamata and glandular hairs (Marzanjosh).

The results observed for the physico-chemical data, heavy metals, aflatoxin level, pesticide residue and microbial load have been shown in table 2, 3, 4, 5 & 6 respectively.

Table 1: Formulation Composition

S. No.	Unani Name	Botanical/English Name	Part Used	Quantity
1.	Nankhwah	Trachyspermum ammi (L) Spr.	Seed	10 g
2.	Tukhm-e-Karafs	Apium graveolens L.	Seed	10 g
3.	Sumbul-ut-teeb	Valeriana jatamansi DC.	Rhizome	10 g
4.	Gul-e-Surkh	Rosa damascena Mill.	Flower	25 g
5.	Marzanjosh	Origanum vulgare L.	Vegetative parts	25 g
6.	Luk Maghsool	Cocus lacca	Resin	10 g

Table 2: Physico-Chemical Parameters

S. No.	Parameters	Values
1.	Alcohol soluble matter (%)	20.12 – 21.45
2.	Water soluble matter (%)	14.94 – 16.20
3.	Pet. ether (60-80°) soluble matter (%)	6.22 – 6.78
4.	pH of 1% aqueous solution	5.70 – 5.80
5.	pH of 10% aqueous solution	5.35 – 5.40
6.	Loss of weight on drying at 105° C (%)	8.58 – 9.10
7.	Total Ash (%)	8.10 – 9.32
8.	Water soluble ash (%)	1.50 – 1.92
9.	Acid insoluble ash (%)	2.45 – 2.82
10.	Volatile Oil (%)	0.62 – 0.74

Table 3: TLC Results

S. No.	Name of Drug	Extract	Solvent System	Spraying reagent	No. of Spots	Rf Values with colour
1.	Sufoof-e-Mohazzil	Pet. Ether (60-80o)	Toluene: Ethyl acetate (9:1)	2% Ethanolic Sulphuric acid	05	0.15 (pink) 0.29 (pinkish purple) 0.39 (yellow) 0.62 (pink) 0.74 (pink)
2.	Sumbul-ut-Teeb	-do-	-do-	-do-	08	0.07 (pinkish purple) 0.14 (blue) 0.17 (pink) 0.30 (pink) 0.34 (yellow) 0.39 (florescent green) 0.47 (pinkish purple) 0.50 (blue)
3.	Nankhwah	-do-	-do-	-do-	06	0.22 (brown) 0.30 (brown) 0.34 (yellow) 0.40 (yellow) 0.47 (pink) 0.75 (pink)
4.	Tukhm-e-Karafs	-do-	-do-	-do-	02	0.30 (brown) 0.37 (yellow)
5.	Gul-e-Surkh	-do-	-do-	-do-	07	0.15 (pink) 0.23 (brown) 0.29 (brown) 0.39 (pink) 0.41 (blue) 0.46 (pink) 0.69 (pinkish brown)
6.	Marzanjosh	-do-	-do-	-do-	07	0.16 (pinkish purple) 0.30 (brown) 0.35 (green) 0.41 (blue) 0.47 (pink) 0.53 (green) 0.74 (pink)
7.	Luk Maghsool	Acetone	-do-	-do-	02	0.15 (yellow) 0.67 (light pink)

Table 4: HPTLC Results

S. No.	Name of Drug	Extract	Solvent System	Developing reagent	Rf Values (main) with colour		
					UV254nm	UV 366 nm	After derivatisation
1.	Sufoof-e-Mohazzil	Pet Ether (60-80°)	Toluene: Ethyl acetate (9:1)	2% Ethanolic Sulphuric acid	0.66 Black	0.31 F. Red 0.37 F. Red 0.43 F. Blue 0.51 F. Red 0.72 F. Blue	0.16 Pink 0.31 Brown 0.60 Pink 0.72 Pink
2.	Sumbul-ut-Teeb	-do-	-do-	-do-	0.06 Black 0.31 Black 0.43 Black 0.69 Black	0.31 F. Red 0.35 F. Red 0.43 F. Blue 0.45 F. Blue 0.49 F. Red 0.72 F. Blue 0.77 F. Blue	0.15 Pink 0.31 Brown 0.49 Pink 0.70 Pink
3.	Nankhwah	-do-	-do-	-do-	0.08 Black 0.41 Black 0.46 Black 0.70 Black	0.38 F. Red 0.43 F. Blue 0.46 F. Blue 0.51 F. Red 0.72 F. Blue 0.78 F. Blue	0.15 Pink 0.34 Brown 0.45 Brown 0.49 Pink 0.67 Purple 0.73 Pink
4.	Tukhm-e-Karafs	-do-	-do-	-do-	0.06 Black 0.45 Black 0.51 Black 0.55 Black 0.68 Black	0.37 F. Red 0.41 F. Blue 0.49 F. Red 0.72 F. Blue 0.78 F. Blue	0.15 Pink 0.34 Brown 0.49 Pink 0.70 Pink
5.	Gul-e-Surkh	-do-	-do-	-do-	0.06 Black 0.46 Black 0.68 Black	0.37 F. Red 0.43 F. Blue 0.49 F. Red 0.72 F. Blue 0.78 F. Blue	0.15 Purple 0.34 Brown 0.45 Purple 0.49 Pink 0.70 Pink
6.	Marzanjosh	-do-	-do-	-do-	0.68 Black	0.31 F. Red 0.35 F. Red 0.41 F. Blue 0.45 F. Red 0.48 F. Red	0.15 Pink 0.34 Brown 0.49 Pink 0.70 Pink
7.	Luk Maghsool	Acetone	-do-	-do-	0.08 Black 0.46 Black 0.70 Black	0.11 Yellow 0.35 F. Red 0.37 F. Red 0.45 F. Blue 0.51 F. Red 0.74 F. Blue 0.80 F. Blue	0.16 Pink 0.34 Brown 0.49 Pink 0.70 Pink

Results and Discussion

Chemical Analysis

The physico-chemical data of the drug are shown in Table 2 .The water soluble extractives (14.94-16.20%) indicates the absence of any inorganic constituent. An 8.5-9% loss in weight on drying at 105º C shows that the moisture contents are very low in the drug. The low value of acid insoluble ash of the drug indicates that the drug is free from siliceous matter. The volatile oil is present due to the presence of Nankhwah and Tukhm-e-Karafs in the Sufoof.

Thin Layer Chromatography Analysis

TLC was carried out on pet. ether (60-80º) extracts of the sufoof and its ingredients except Luk Maghsool which was extracted by acetone. TLC was performed on pre-coated plate of Silica Gel 60 F_{254} (E. Merck) using the solvent system of Toluene - Ethyl acetate (9:1). The TLC of Sufoof-e-Mohazzil shows five spots at R_f 0.15(pink), 0.29(pinkish purple), 0.39(yellow), 0.62(pink) and 0.74(pink) on spraying with 2% ethanolic sulphuric acid and heating the plate for about 10 minutes at 105º C in an oven (Table-7, Fig.- 2).

HPTLC profile

HPTLC of pet. ether (60 º -80º) extract of the drug was performed using TLC plate pre-coated with Silica Gel 60 F_{254} (E. Merck). CAMAG Linomat IV automatic sample spotter was used as an applicator. After developing the plate in the solvent system of Toluene - Ethyl Acetate (9:1), it was scanned through CAMAG TLC scanner. The plate was dipped in 2% elcoholic sulphuric acid and heated at 105º C on CAMAG TLC plate heater till the coloured spots appeared (Table-8; Fig.-3 (a,b,c).

HPTLC finger-printing of Sufoof-e-Mohazzil with its all six ingredients under U.V. 254 nm, 366 nm and after derivatization shows similar pattern as all major bands shown in the chromatogram of the ingredients are also present in the chromatogram of the sufoof (Fig. – 3 (a,b,c). This comparative HPTLC spectra of sufoof with its ingredients confirm the absence of any adulterants and also confirm that the sufoof is derived from a defined botanical species and all constituents are clearly characterized (Sethi, 1996).

U.V. Spectroscopic Studies

The study of the U V spectra of the drug Sufoof-e-Mohazzil and its ingredients shows that a single broad region (218.00 nm) appears in the spectrum of

sufoof is the characteristic one and shows the merger of characteristic peaks of its ingredients like peak no. 2 at 209 nm in Nankhwah, peak no.1 at 220 nm in Sumbul-ut-teeb, peak no. 2 at 209 nm in Marzanjosh, peak no.1 at 209 nm in Gul-e-Surkh, peak no. 1 at 210 nm in Tukhm-e-Karafs and peak no. 1 at 209 nm in Luk Maghsool (Fig. 4, 5, 6, 7, 8, 9, 10).

Fig. 1: Ingredients of Sufoof-e-Mohazzil (Nankhwah; Tukhm-e-Karafs; Sumbul-ut-Teeb; Gul-e-Surkh; Marzanjosh; Luk Maghsool)

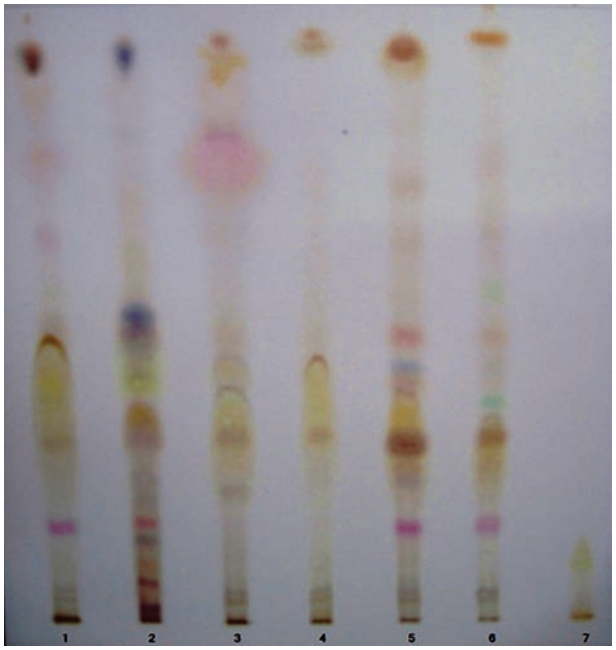


Fig. 2 : TLC of Sufoof-e-Mohazzil and its ingredients

1. Sufoof-e-Mohazzil; 2. Sumbul-ut-Teeb; 3.Nankhwah; 4.Tukhm-e-Karafs; 5. Gul-e-Surkh; 6.Marzanjosh; 7.Luk Maghsool

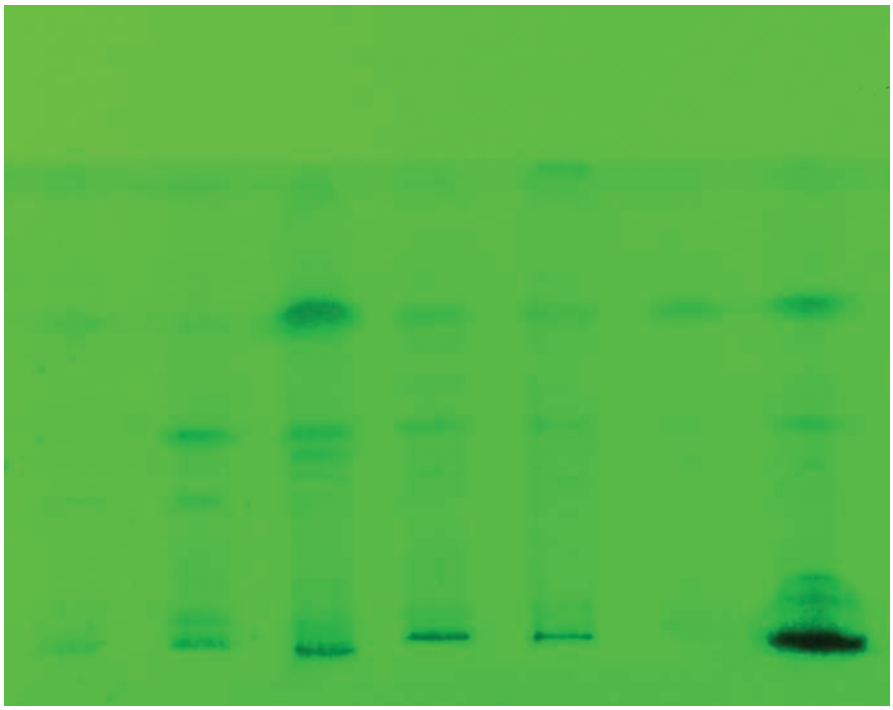


Fig. 3: (a) HPTLC of Sufoof-e-Mohazzil and its ingredients under UV 254 nm

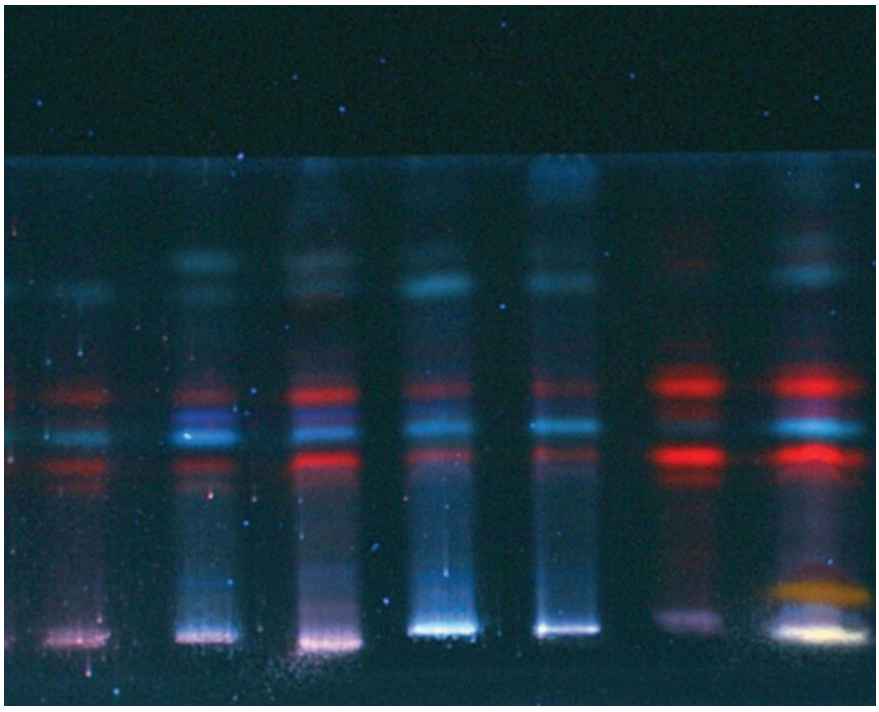


Fig. 3 : (b) HPTLC of Sufoof-e-Mohazzil and its ingredients under UV 366 nm

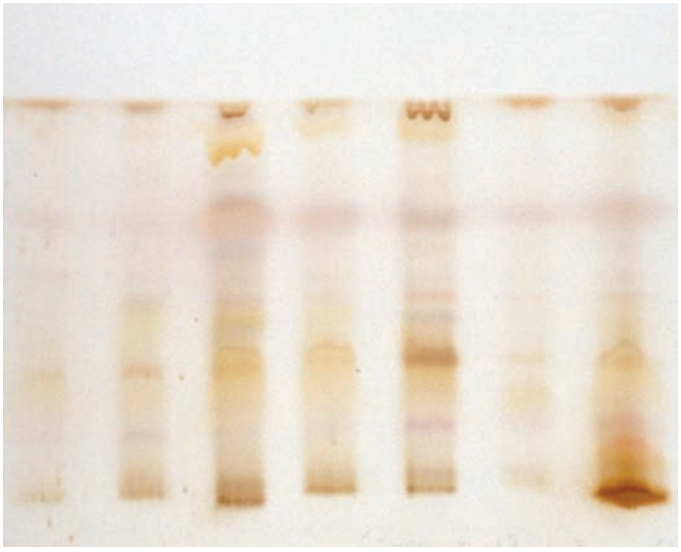
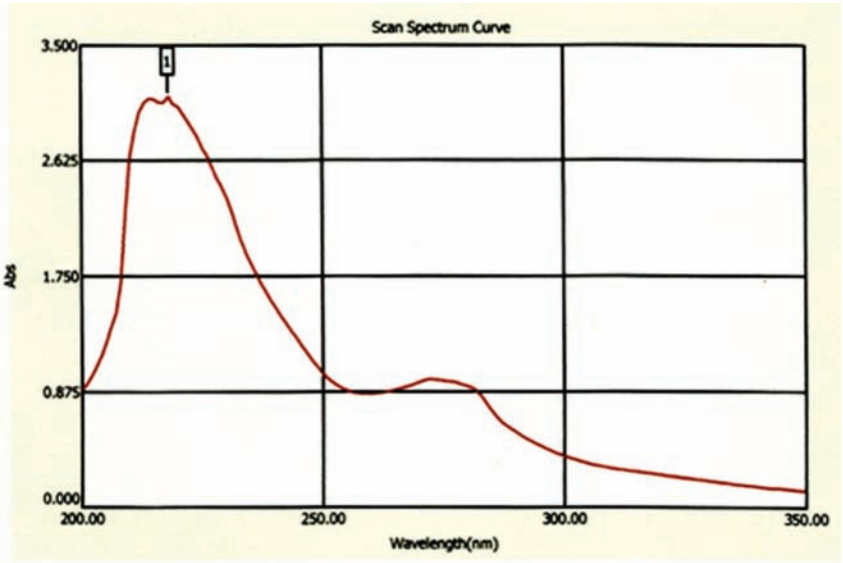


Fig. 3 : (c) HPTLC of Sufoof-e-Mohazzil and its ingredients after derivatization

1. Sufoof-e-Mohazzil; 2. Sumbul-ut-Teeb; 3.Nankhwah; 4.Tukhm-e-Karafs; 5. Gul-e-Surkh; 6.Marzanjosh; 7.Luk Maghsool



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Scan Spectrum Performance
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Measure Mode : Abs
Interval : 1.00 nm
Speed : Medium
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Method File:

No.	P/V	Wavelength(nm)	Abs	Comment
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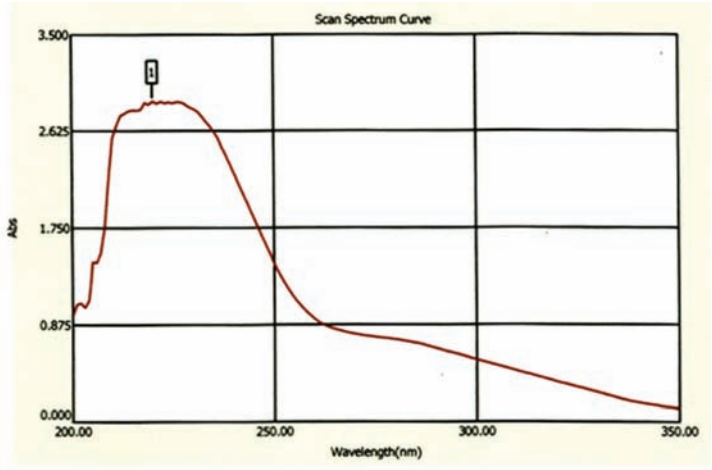
Fig. 4



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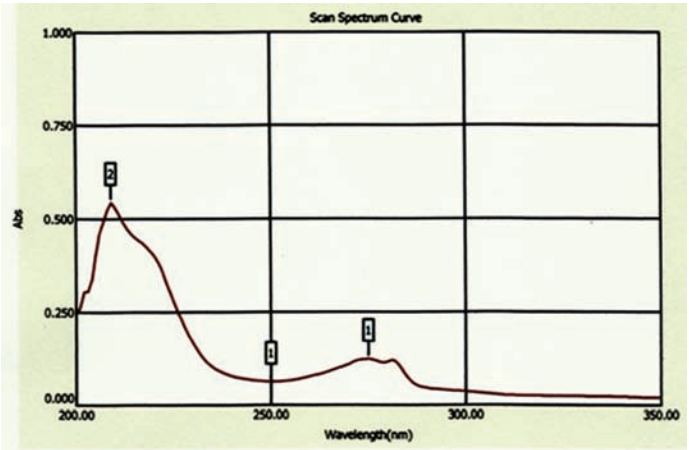
1	Peak	220.00	2.894	

Fig. 5



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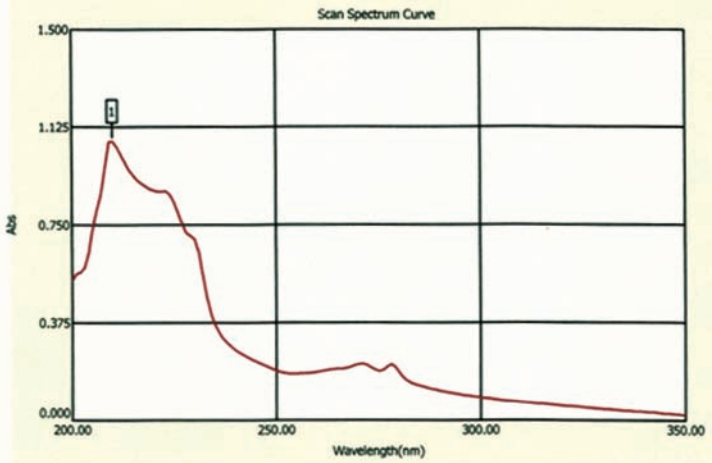
2	Peak	209.00	0.545	
1	Valley	250.00	0.063	

Fig. 6



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1	Peak	210.00	1.071	

Fig. 7

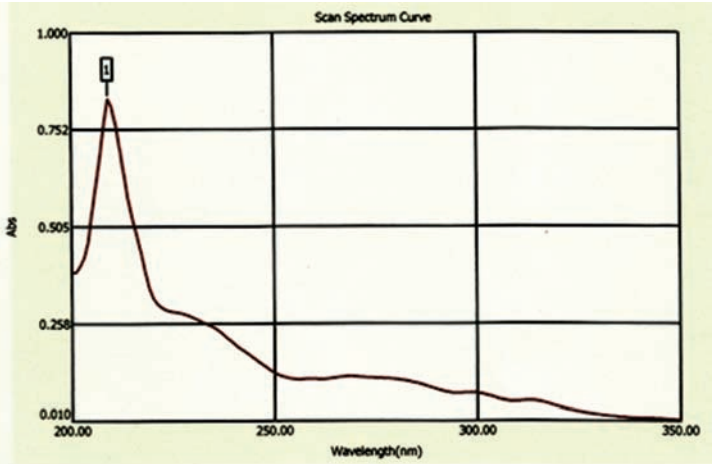

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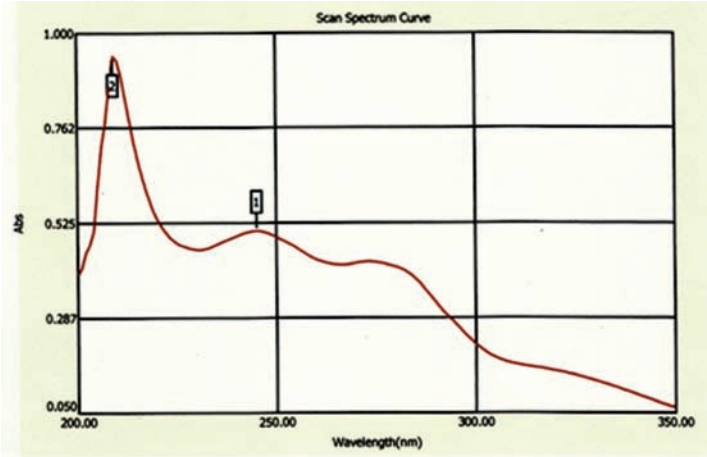
Fig. 8



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Scan Spectrum Performance
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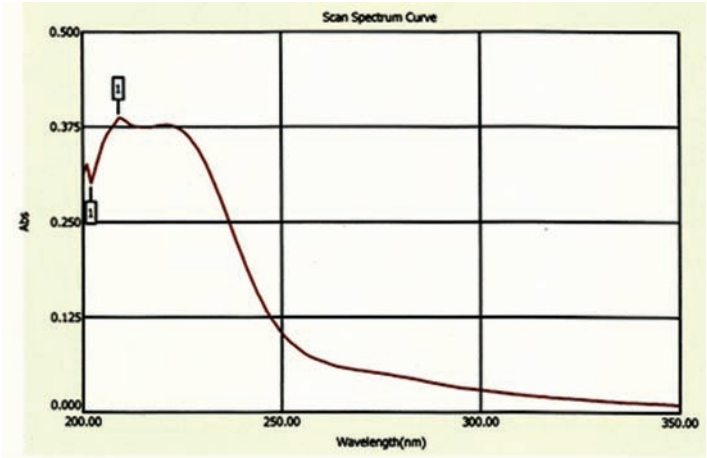
2	Peak	209.00	0.943	

Fig. 9



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1	Peak	209.00	0.388	
1	Valley	202.00	0.302	

Fig. 10

Conclusion

It is very difficult to identify the single drugs once they are powdered and mixed for preparing compound formulations. The present study, therefore, holds high significance as the microscopic features, various physico-chemical standards, the R_f values of TLC and HPTLC and U. V. Spectra provide criteria for easy identification of the drug Sufoof-e-Mohazzil and ensure the quality and efficacy of the medicine.

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