

# Standardization of Unani Ointments : 'Marham Quba'

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## Abstract

In India the Greco-Arab medicine that is derived from Greece about 2000 year ago is known as Unani medicine; and a number of herbal drugs (Unani drugs) and formulations are in place by various herbal pharmaceutical industries. During the past decade there is tremendous demand, especially from developed countries, for drugs of herbal origin and this revival of interest is mainly due to the current widespread belief that Traditional Herbal Medicines are safe and more dependable than synthetic drugs. The SOPs (Standard Operational Procedure) and standardization, which are essential for efficacy of every batch of a drug, have not yet been thoroughly investigated and Ointments that are one of the important groups have not been screened; therefore, the work on different ointments was started and in the present paper the standardization and quality assurance of 'Marham Quba' is reported. The present formulation is an important ointment of Unani System of Treatment, used as topical applicant for cuts, pains and abrasions etc. The parameters that are selected are those that are recommended by National Unani Pharmacopoeia Committee. 'Marham Quba' is a red, semisolid compound with strong smell of mustered oil. Its action is mentioned as 'Mubarrid' and 'Daf-e-Taffun', in Unani literature and the mode of administration is topical (Anonymous, 1971; Anonymous, 2008). The parameters that are studied are Total ash (32.77%), Acid insoluble ash (2.17%), Water soluble ash (0.9%), Alcohol soluble matter (13.73%), Water soluble matter (14.17%), Pet. ether soluble matter (36.34 %), Water content (7.64%), Loss on drying (10.56%), pH of 1% & 10% solution (9.84 & 10.15 respectively) and Congealing point (64-700C). Thin Layer Chromatography (TLC) profile are also used for finalizing the marker compounds. The heavy metals, Aflotoxins and Pesticidal residue are not detected. No microbes noted in the final product. In addition HPLC profile of 'Marham Quba' are also recorded for future reference.

**Keywords:** Marham Quba, Standardization, Quality control, Ointment

## Introduction

The use of herbs and their formulations to treat diseases has stood the test of time. The chemical constituents present in them are playing vital role in the physiological functions of living flora and hence they are believed to have better compatibility with the human body (Kamboj, 2000). That is why herbs and their products is now the centre of gravity for researches and application

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for treatment of various ailments. It is a point to note that the plants and their products are composed of many constituents and are therefore, capable of variation. The variability of the plant material is due to different conditions of growth, harvesting, drying, and storage. Regarding extracts we can say that the polarity of the solvent, the mode of extraction, and the instability of constituents may also influence the composition hence affecting the quality. In olden times, Hakims used to treat patients on individual basis and prepare drug according to the requirements of the consumer. Today herbal medicines however, are manufactured on a large scale in mechanical units, where manufacturers come across many problems such as non availability of good quality raw materials in bulk, and proper methodology for standardization, etc., (Harish Padh, 2001). The availability of SOP (Standard Operational Procedure) for manufacturing units on large scale production is also lacking. Marahim (Ointments) are the important preparation of Unani Medicine, used as topical applicant for cuts, pains and abrasions etc. Most of the ointments contain mineral and/or plant products that vary from formulation to formulation. No work on SOP and standardization of such type of drugs has been done till date, therefore, a series of work has been initiated to standardize the ointments for maintaining the quality and efficacy. For the present study 'Marham Quba' is selected and standardization is made. The part I of this series contains the SOP and standardization of 'Marham Kafoor' and communicated for the publication (Afaq *et al.* Hippocratic Journal of Unani Medicine). The work on others ointments will be reported else where. The parameters that are selected are those which are recommended by National Unani Pharmacopoea Committee.

## Materials and Methods

### *Raw Materails*

The formulation contains the ingredients (Table 1) that are mentioned in part V<sup>th</sup> of National Formulary of Unani Medicine (Anonymous, 2008). The raw materials were purchased from the market and their identity, purity and strength were checked as per reference (Anonymous, 1978 (a)), given in table 1. The commercial sample of Sendur and Seemab banafsi was standardized and their standards are quoted here. **Sendur ( $Pb_3O_4$ )**: Decomposition point 500°C, insoluble in alcohol and water, soluble in strong acid, Total Ash 99.0%. Water soluble and acid insolubles are negligible. Seemab banafsi,  $HgI_2O_6$ , colour change to yellow at 130°C, melting point 258°C, solubility in water 0.006 g/100 ml, solubility in alcohol 1 g/115 ml.

### *Preparation of Ointment*

Flaks of Soap were put in a pan and kept on burner till melts. In the content wax and mustered oil were added. When all the ingredients mixed properly then the pan was removed from the burner. Further "Sendur" and "Safeda kashgari" were mixed and stirred till it become semi solid. Lastly "Semab-e-banfshi" was added and mixed to make a homogenous mixture.

### *Physicochemical Parameters*

Physicochemical studies like total ash; acid insoluble ash; water soluble ash; alcohol, petrolium Ether and water soluble matter; water content; loss on drying and congealing point were determined quantitatively according to methods recorded in Indian Pharmacopoeia (Anonymous, 1978(a)), WHO guidelines (Anonymous, 1978(b), 2005) and methods mentioned by Afaq *et al* (1994). Thin Layer Chromatography was conducted taking the help of method mentioned by Harborne (1973). The HPLC methods determination of pesticidal residue and Atomic absorption method for Heavy metals determination was used. The presence of Aflotoxins and Microbial load were studied as per revised recommendation of WHO mentioned in its bulletin (Anonymous, 2005).

### *HPLC analysis*

Common pesticide (Chloropyriphos, DDT, Parathion, Malathion and Endosulphan) were obtained from Sigma-Aldrich and dissolved in acetonitrile (HPLC Grade). These standards were injected in the C18 column (30 cm) fitted in the HPLC instrument (Cyber lab, USA) and software driven peaks were obtained. The pressure was 6.5 Pa and temperature was 25°C. The Flow rate was 1.0 ml/min. The detector was UV and the wavelength was 254 nm. The mobile phased was acetonitrile: water (75:25). The drug dissolved in acetonitrile were also injected and the peaks appears were compared with the peaks of pesticides (Fig. 1; Table 5), considering the retention time in the same conditions. The general HPLC profile of drug were also recorded and given in figure (Fig.2), and the details of the 28 peaks are given in table 6.

## **Results and Discussion**

The present study is an attempt to ascertain the pharmacopoeial standards for the standardization of 'Marham Quba'. Total ash (32.77%), Acid insoluble ash (2.17%), Water soluble ash (0.9%), Alcohol soluble matter (13.73%), Water soluble matter (14.17%), Pet. ether soluble matter (36.34 %), Water content (7.64%), Loss on drying (10.56%), pH of 1% & 10% (9.84 & 10.15 respectively)

and Congealing point (64-70°C) are depicted in table 2. These parameters are considered as tools of checking the quality, identity, purity and strength of the ointment. The HPLC profile of the drug was recorded as the obtained graph can be compared with the batches in future. The HPLC pattern shows 28 peaks and peak number 11 is the major peak. The concentration of that compound is 86.801%. This peak is followed by peak number 6 (6.217%), peak number 15 (1.411%) and peak No. 12 (1.236%). The total concentration of two compounds (Peak No. 11 and 6) is 93.011%. The total concentration of compounds depicted in peak number 15 and 12 (2.647%). Other peaks show non significant concentration, so for checking the quality one should check peak number 11 and 6. The change in the profile of any batch will be a check point for low quality or adulteration. Thin Layer Chromatography (TLC) profile (Table 4) and Rf value obtained alongwith photographs of the TLC plate (Fig. 3) was also recorded for future refernce. The heavy metals, aflotoxins, pesticidal residue and microbial load were also studied and reported (Table 3a, 3b, 3c, 3d). No growth of any Fingi or Bacteria were observed in the cultural media and no aflotoxines (B1,B2,G1,G2) were detected. The limit of heavy metals were not considered in this formulation as lead and mercury are ingredients; whereas As and Cd may be impurities but in the limit. The HPLC analysis show no any common pesticide as in HPLC profile of drug non of the peak correspond to peak number 2,4,5,7, and 8 of soft ware driven HPLC graph of the mixture of different pesticides on the same instrument and same conditions (Fig.1; Table 5 ). The ointment is for topical use and contains heavy metals therefore used cautionary and kept out of reach of childrens to avoid any accident.

**Table 1:** Ingredients of ‘Marham Quba’

S. No	Unani Name	Botanical/ English Name	Part Used	Reference	Quantity
1	Safeda Kashgari	Zinc Oxide	Salt	IP; 1978, pp 550*	50 g
2	Sendur	Lead oxide Red (Pb3O4)	Lead oxide Red (Mineral)	-	50 g
3	Sabun	Soap	Soap	IP 1978, p.447*	200 ml
4	Roghane Sarsoon	Brassica juncea Hook f.	Mustard Seed oil	IP 1978, pp.320-321*	1.2 lit
5	Mom Asli (Pure Wax)	Beeswax	Wax from honey comb	IP, 1978, p. 62*	350 g
6	Seemab Banafshi	Mercuric Iodide (HgI2O6)	Mercuric Iodide (Mineral)		20 g

\*IP=Indian Pharmacopoeia

**Table 2:** Physicochemical Properties of Marham Quba

Parameter*	Marham Quba
Total ash	Not more than 32.77%
Acid insoluble ash	Not more than 2.17%
Water soluble ash	Not more than 0.9%
Alcohol soluble matter	Not less than 13.73%
Water soluble matter	Not less than 14.17%
Pet. ether soluble matter	Not less than 36.34 %
Water Content	Not more than 7.64%
Loss on dry	Not more than 10.56%
Congealing point	64 - 70 °C
pH 1%	9.84
pH 10%	10.15

\*Each parameter is mean of three experiments

**Table 3:** Heavy Metals (a), Microbial Load (b), Aflatoxin (c) and Pesticide residue (d) of 'Marham Quba'

## (a) Qualitative Analysis for Heavy Metals

S. No.	Test Parameters	Results*	Limits
1	Lead as Pb	172.093ppm	*Not more than 10 ppm
2	Mercury as Hg	51.221ppm	* Not more than 10 ppm
3	Arsenic as As	0.117ppm	Not more than 3.0 ppm
4	Cadmium as Cd	0.229ppm	Not more than 0.3 ppm

\*Note: Limit of Lead and Mercury is not applicable with this product as lead oxide Red and Mercuric Iodide is ingredient of the formulation; Arsenic and Cadmium are within limit.

## (b) Microbial Load (for three samples)

S. No.	Microbs	Result*	Limit
1	Total Bacterial Count	Nil	Not more than 105 /g
2	Total Fungal Count	Nil	Not more than 103/g
3	Enterobacteriaceae	Nil	Nil
4	Salmonella	Nil	Nil
5	Staphylococcus aureus	Nil	Nil

## (c) Aflatoxin (for three samples)

S. No.	Aflatoxin	Result*	Limit
1	B1	Not detected	Not more than 0.50 ppm
2	B2	Not Detected	Not more than 0.10 ppm
3	G1	Not Detected	Not more than 0.15 ppm
4	G2	Not Detected	Not more than 0.10 ppm

(d) Pesticide residue (for three samples)

S. No.	Pesticide	Result*	Limit
1	Chloropyrifos	Not detected	Not more than 0.2 mg/kg
2	DDT	Not detected	Not more than 1.0 mg/kg
3	Endosulphan	Not detected	Not more than 3.0 mg/kg
4	Malathion:	Not detected	Not more than 1.0 mg/kg
5	Parathion	Not detected	Not more than 0.5 mg/kg

Note. \*All result based on three experiments

**Table 4:** Thin Layer Chromatography Profile of Marham Quba

Drugs	Extract	Mobile Phase	Spraying Reagent	Observation
Marham Quba	Methanolic Extract	Toluene: Ethyl acetate: Diethylene (7:2:1)	Vanillin H <sub>2</sub> SO <sub>4</sub>	After spray of Vanalne Sulphuric acid two spots appears; Rf. 0.10, 0.90 (Bluish Gray)

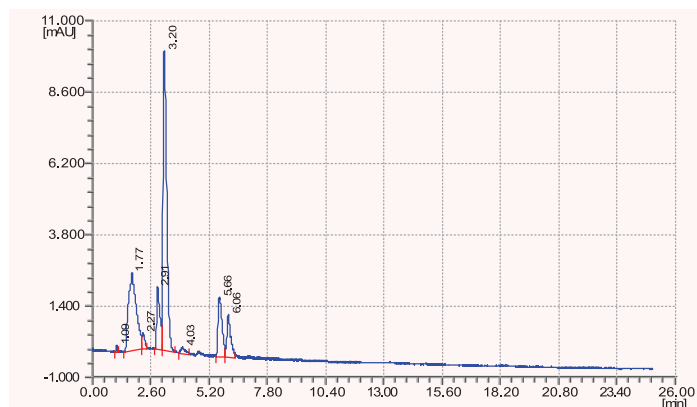


Fig. 1. HPLC of the Mixture of different pesticides

**Table 5:** HPLC Obtained Peaks of Pesticides

Peak	Retain. Time	Height	Area	Concentration
1	1.092	23	82.9	1.1967
2	1.768	261	6405.7	13.5796
3	2.268	54	378.2	2.8096
4	2.912	210	2042.2	10.9261
5	3.203	1009	11936.6	52.4974
6	4.030	21	294.6	1.0926
7	5.665	199	2523.1	10.3538
8	6.058	145	1701.5	7.5442

Note: Peak 2, 4, 5, 7 and 8 are the major pesticides

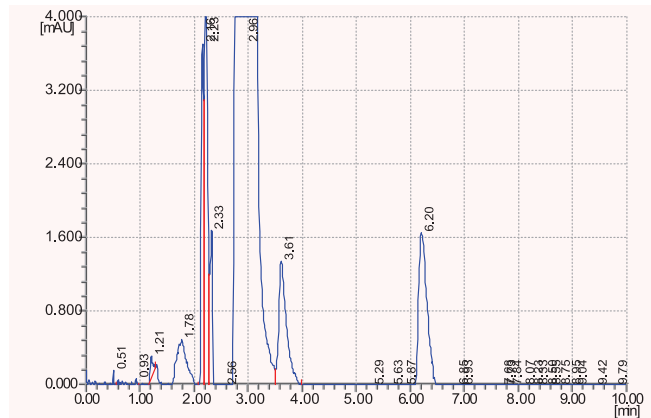
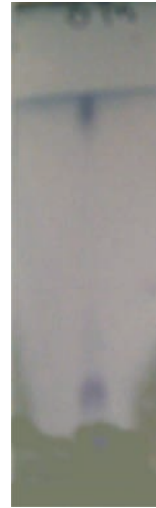


Fig. 2. HPLC profile of Marham Quba

Table 6: HPLC Obtained Peaks of Marham Quba

Peak	Retain. Time	Height	Area	Concentration
1	0.717	41	65.4	0.247
2	0.859	13	64.2	0.078
3	1.119	12	12.6	0.072
4	1.469	10	39.1	0.060
5	1.519	13	18.6	0.078
6	1.794	1031	12484.1	6.217
7	2.154	59	168.1	0.356
8	2.295	73	532.6	0.440
9	2.470	60	419.5	0.362
10	2.562	59	401.3	0.356
11	2.854	14395	133343.0	86.801
12	3.530	205	2732.6	1.236
13	4.005	136	1695.5	0.820
14	4.649	15	44.7	0.090
15	5.057	234	4720.1	1.411
16	5.800	18	60.2	0.109
17	6.527	15	41.6	0.090
18	6.627	20	43.0	0.121
19	6.877	15	100.9	0.090
20	7.177	30	394.7	0.181
21	8.267	11	97.2	0.066
22	8.400	16	56.2	0.096
23	8.442	16	36.5	0.096
24	8.525	23	34.6	0.139
25	8.667	20	42.6	0.121
26	9.142	23	43.6	0.139
27	9.312	10	22.2	0.060
28	9.445	11	42.1	0.066



Vanillin sulphuric acid

Fig. 3. Thin Layer Chromatography of 'Marham Quba'

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