



## ASSESSMENT OF QUALITY CONTROL PARAMETERS AND STANDARDIZATION OF PEPGARD SYRUP: A POLYHERBAL FORMULATION

Amit Patel<sup>1</sup>, Komal Hirani<sup>2</sup>, Vishva Bhuva<sup>3</sup>, Payal Panchal<sup>4</sup>

<sup>1,2,3,4</sup>R&D Department, Vital Care Pvt. Ltd, 361-362, Por-GIDC, Ramangamdi, Vadodara - 391243, Gujarat, India

Email: [rd@vitalcare.co.in](mailto:rd@vitalcare.co.in)

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## ABSTRACT

World Health Organization highlighting the necessity of quality and safety of herbal formulations also proposes guidelines for its standardization. Standardization assures the identity, determination of quality, purity of herbal formulation through its active or marker compounds. Peggard syrup is a proprietary Ayurvedic poly-herbal formulation widely used in clinical practice as antacid for treating Heartburn, Non-ulcer dyspepsia, Gastroesophageal reflux (GERD) and Drug-induced gastritis. Scientific standardization of Peggard Syrup is not yet studied. In the present study, we have standardized the Peggard Syrup using standard physicochemical protocols such as pH, Specific gravity, viscosity, Total sugar and Total sugar content. In addition, residue analyses such as heavy metal content, pesticide analysis, microbial load analysis and Accelerated stability study were also examined to strength the standardization process. Microbial load, heavy metals and Pesticide residues were found to be within the AYUSH permissible limits. Our results give an idea about the beneficial effect of Peggard Syrup.

**Keywords:** Standardization, Peggard Syrup, Accelerated stability studies, Physicochemical parameters.

## INTRODUCTION

The use of herbal medicines in human health care has developed substantially in both developed and devel-

oping countries because of its lesser side effect as compare to synthetic drug. Natural based customary

cures are energetically prescribed by World Health Organization (WHO) in light of their security, simple accessibility and minimal effort in the treatment of different sicknesses. In conventional framework, these drugs have a most extravagant bio-asset, for example, phenols, miniaturized scale and macronutrients and so on. They can go about as a nutraceutical, nourishment supplements and pharmaceutical intermediates and so on. [1] An herbal based formulation improves the quality of human life through its potent natural antioxidants effect [2] and by its potential bioactive compounds. [3] They provide remedy for various chronic diseases and metabolic disorders which are multifactorial and therapeutic intervention. [4]

Quality Evaluation of polyherbal formulations is of paramount importance in order to validate their suitability in modern system of medicine. [5] But we don't have a rigid quality control profiles for standardization of herbs and their formulations. It is mainly due to the lack of inadequate regulatory standards and implementation protocols. [6] Development of standards for plant-based drugs being a challenging task, it needs innovative and creative approaches. [7] At each and every step of standardization viz; identification, organoleptic, pharmacognostic, physicochemical, phytochemical, presence of xenobiotics, microbial load and toxicity needs special attention because of complex nature of plant-based medicines and the inherent variability of their constituents [4] of these, the phytochemical profile is of special significance since it has a direct bearing on the activity of the herbal drugs.

This is the first report on the standardization of Peggard syrup; a polyherbal formulation comprises variant proportion of herbs such as aqueous extract of *Glycerrhiza glabra* Linn., *Asparagus racemosus* Willd., *Centella asiatica* Linn., *Embellica officinale* Gaertn., *Ipomoea turpethum* Linn., *Syzygium aromaticum* (L.), *Fumaria officinalis* Linn., cold macerated extract of fresh herb *Coriandrum sativum* Linn. And powder of *Astoneman indicum*. Peggard syrup is a proprietary Ayurvedic poly-herbal formulation widely used in clinical practice as Antacid for treating Heartburn, Non-ulcer dyspepsia, Gastroesophageal reflux (GERD) and Drug-induced gastritis. In the present

study, we have elucidated the Determination of pH, Measurement of Specific Gravity, Measurement of Surface tension, Measurement of Viscosity, Total Sugar content, Total Solid content, Heavy metals analysis, Microbial analysis of Peggard syrup by using standard and modern techniques.

## Material and Methods

**Drugs and Chemicals:** Peggard syrup is a proprietary Ayurvedic poly-herbal formulation of Vital Care Pvt. Ltd., Vadodara. All other chemicals and solvents were of analytical grade obtained from Merck, SISCO and SD-fine chemicals, India.

### 1]. Physicochemical Parameters: [8, 9, 10, 11, 12]

**Description :** The general appearance, its visual identity is essential for consumer acceptance for control of lot-to-lot uniformity and for monitoring trouble free manufacturing. The control of the general appearance of syrup involves the measurement of colour, odour, taste etc.

**Determination of pH:** The pH value of a solution was determined potentiometrically by means of a glass electrode, a reference electrode and a digital pH meter. The pH meter was operated according to the manufacturer's instructions. First the apparatus was calibrated using buffer of 4, 9 and 7 pH. 1 ml syrup was taken in 100 ml demineralised water. The electrodes were immersed in the solution and measured the pH.

**Measurement of Specific Gravity:** To find the density of any substance, knowledge of volume and weight of that substance are known. Divided the weight of substance by its volume, density is obtained in unit gm/ml.

**Measurement of Viscosity:** Viscosity of any liquid is measured by comparing it with the viscosity of water. It is measured by Oswald viscometer. At first the viscosity of water is measured. Take water in Oswald viscometer fill up the water and shuck it up to mark A then note the time for water run up to mark A to B. Then do the same for any liquid. Viscosity of water is taken as standard. Viscosity of any liquid is measured by following formula

$$\frac{\eta_1}{\eta_2} = \frac{t_1}{t_2}$$

Where,  $\eta_1$  and  $\eta_2$  are the viscosity of water and liquid respectively while  $t_1$  and  $t_2$  are time taken for reaching water or liquid from mark A to mark B in second. Unit is given in terms of centipoises.

**Determination of total sugar and invert sugar:** Reducing and non-reducing sugar was estimated by titrimetric method using Benedict's quantitative reagent. The reagent was standardized using glucose stock solution (2mg/ml). Sample stock solution were prepared by weighing 5gm powder and macerated for 24h with 100ml distilled water for reducing sugar while for non-reducing sugar samples were boiled for 30 min with 5ml of 0.1N HCl on water bath with 25ml distilled water. After filtration the volume was adjusted to 100ml with distilled water. 5ml of each sample solution was then treated with fresh Benedict's reagent drop wise from burette in warm condition until blue colour changes to whitish. From burette volume amount of sugar was calculated using formula 5ml burette reading= 10mg sugar by calculation using dilution factor.

**Total Solid content:** 10ml of the samples were taken in tarred dish and evaporated at low temperature until the liquid was removed and then heated until the residue was apparently dried. Thereafter, it was transferred to an oven and dried to constant weight at 105°C.

## 2]. Heavy Metal analysis <sup>[13]</sup>

**Preparation of samples by acid digestion method:** Accurately weighed 2g of each sample of Peggard Syrup was taken in kjeldahl flask. Acid mixture of HNO<sub>3</sub>:HClO<sub>4</sub> (4:1) was added in the flask and heated continuously till the solution is colourless. The sample was then transferred in a 25ml volumetric flask and the volume was made-up with distilled water. Reagent blank was synchronously prepared according to the above procedure. The standards of Lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg) were prepared as per the protocol in the manual and the calibration curve was developed for each of them.

**Detection:** Then samples were analyzed for the presence of Pb, Cd, As and Hg using Atomic absorbance

spectrophotometer (AAS) 6300 (by SHIMADZU).

## 3]. Pesticide residues

To determine the pesticide residues, 2g of each sample was extracted in Soxhlet apparatus with 150ml hexane. Traces of water and oil were removed from hexane extract. After oil removal, this extract was concentrated on rotary evaporator under reduced pressure and this concentrated extract was transferred to clean-up column. The elute was collected carefully and made up to 5ml with hexane. Aliquots of above concentrate were injected into pre-calibrated GC machine equipped with 63Ni electron capture detector. Operation temperature was programmed at 195°C, 200°C, 220°C for column, injector, and detector, respectively. Purified nitrogen gas was used as carrier gas at flow rate of 60 ml/min. Limit of detection was 0.1 to 0.5 ppb for organochlorine pesticides analyzed. Periodically procedural blanks were used to check cross contamination. Recovery studies with purified samples indicated that overall recovery value exceeded 80%. Identification and quantification were accomplished using known amount of external standard procured from Sigma-Aldrich. <sup>[14]</sup>

## 4]. Microbial Analysis <sup>[15, 16]</sup>

### a) Total Microbial count

**Preparation of sample:** Dissolve 10mg of the preparation being examined in buffered sodium chloride peptone solution pH 7 and adjust the volume 100ml with same medium.

**Examination of sample:** Total viable aerobic count in the sample was examined by using the plate count method by Digital colony counter.

**For bacteria:** Petri dishes of 10cm diameter were used, mixture of 1ml of the pre-treated preparation and 15ml of liquefied casein soyabean digest agar was added in each of Petri dishes at not more than 45°C. If necessary, dilute the pre-treated preparation so that a colony count of not more than 300 may be expected. Two Petri dishes for each sample were prepared using the same dilution and incubated at 30-40°C for 5 days. The number of colonies form was calculated using the digital colony counter. Results were calculated using plate with the greatest no. of colonies but taking 300 colonies per plate as the maximum consistent with

good evaluation.

**For fungi:** Proceed as described in the test for bacteria but sabouraud dextrose agar with antibiotic was used in place of soyabean digest agar and incubate the plates at 20-25°C for 5 days unless a more reliable count was obtained in shorter time. Results were calculated using the plates with not more than 100 colonies by using colony counter.

**b) Test for *Escherichia coli***

Take 10gm of powdered material and the volume made up to 100ml with lactose broth. This mixture was incubated at 35-37°C for 4 hrs. 1ml sample from this to 100ml MacConkey broth and incubated at 43-47°C for 24 hrs. Subculture was prepared and inoculated on MacConkey agar media, and incubated at 43-47°C for 24 hrs. Growth of red, generally non-mucoid colonies of Gram negative rods indicated the possible presence of *E. coli*.

**c) Test for *Salmonella typhimurium***

Take 10gm of powdered material and the volume made up to 100ml with lactose broth. This mixture was incubated at 35-37°C for 4 hrs. A further 10ml of this sample was taken in 100ml of tetrathionate bile brilliant green broth and incubated at 42-43°C for 18-24 hrs. 1ml of sample was taken from it and plated on a xylose lysine deoxycholate agar media and incubated at 35-37°C for 24 hrs. Well developed, red with or

without black centre colonies indicated the presence of *S.thyphi*.

**d) Test for *Pseudomonas aeruginosa***

0.1ml of the solution was pipetted out from SCDB and streaked onto Cetrimide agar plates to check for the presence of *Pseudomonas aeruginosa*, The plates were then inverted and incubated at 37°C for 18-24 hours and were then observed for fluorescence colonies under UV.

**e) Test for *Staphylococcus aureus***

0.1ml of the solution was pipetted out from SCDB and streaked onto Vogel-Johnson Agar Medium to check out the presence of *Staphylococcus aureus*. The plates were then inverted and incubated at 37°C for 18-24 hours and were then observed for typical black colonies surrounded by yellow zones if any.

**5]. Accelerated Stability testing of Peggard syrup**

The Accelerated Stability study of prepared syrup was carried out for 3 months. The syrup was kept at 40°C  $\pm$ 2°C/75% RH  $\pm$  5% and syrup was stored in amber coloured bottle. The parameters evaluated every month were pH, Specific gravity, Viscosity, Total sugar, Total solids. The quantitative estimation of phytoconstituents, and microbial load was done at the beginning and at end of the 3 months period.<sup>[17]</sup>

## RESULT AND OBSERVATION

**Table 1:** Physicochemical analysis ‘Peggard Syrup’

Parameter	Peggard Syrup
<b>Description</b>	
Colour	Red
Taste	Characteristic
Odour	Characteristic
<b>Physicochemical parameters</b>	
pH	4.75 $\pm$ 0.020
Specific gravity	1.18 $\pm$ 0.005
Viscosity (poise)	181.09 $\pm$ 0.305
Total sugar (mg/5g/100ml)	36
Total solid content	37.02%

Peggard syrup has red colour and characteristic odour and taste. The pH of Peggard syrup was slightly acidic. Specific gravity, viscosity, total sugar and total solid of Peggard syrup were found in uniform.

**Table 2:** Heavy metal analysis Peggard Syrup

Sr. No.	Heavymetal	Limit	Peggard Syrup
1	Lead	10ppm	Nil
2	Cadmium	0.3ppm	Nil
3	Arsenic	3ppm	Less than 1.0 ppm
4	Mercury	1ppm	Less than 1.0 ppm

Results indicated that concentration of lead and cadmium in Peggard Syrup was not in detectable amount. Arsenic and mercury were below limit of detection in

the formulation. Result indicates that the heavy metal content in Peggard syrup was less than the prescribed limit.

**Table 3:** Pesticide analysis of Peggard Syrup

Name of Test	Method of Test	Limit	Test result
Aldrin	AOAC 2007.01/QuEChERS Method GC/LC-MS/MS	0.05 (max)	BLQ (LOQ 0.01)
Dieldrin	As Mentioned Above	0.05 (max)	BLQ (LOQ 0.01)
Lindane	As Mentioned Above	0.06 (max)	BLQ (LOQ 0.01)
Bromopropylate	As Mentioned Above	3.0 (max)	BLQ (LOQ 0.01)
Deltamethrin	As Mentioned Above	0.5 (max)	BLQ (LOQ 0.01)
2,4-DDT	As Mentioned Above	1.0 (max)	BLQ (LOQ 0.01)
4,4-DDT	As Mentioned Above	1.0 (max)	BLQ (LOQ 0.01)
2,4-DDE	As Mentioned Above	1.0 (max)	BLQ (LOQ 0.01)
4,4-DDE	As Mentioned Above	1.0 (max)	BLQ (LOQ 0.01)
2,4-DDD	As Mentioned Above	1.0 (max)	BLQ (LOQ 0.01)
4,4-DDD	As Mentioned Above	1.0 (max)	BLQ (LOQ 0.01)
Endrin	As Mentioned Above	0.05 (max)	BLQ (LOQ 0.01)
Hexachlorobenzene	As Mentioned Above	0.1 (max)	BLQ (LOQ 0.01)
Hexachlorobenzene isomer	As Mentioned Above	0.3(max)	BLQ (LOQ 0.01)
Alachlor	As Mentioned Above	0.02 (max)	BLQ (LOQ 0.01)
Cis-Chlordane	As Mentioned Above	0.05 (max)	BLQ (LOQ 0.01)
Trans-Chlordane	As Mentioned Above	0.05 (max)	BLQ (LOQ 0.01)
Alpha Endosulfan	As Mentioned Above	3.0 (max)	BLQ (LOQ 0.01)
Beta Endosulfan	As Mentioned Above	3.0 (max)	BLQ (LOQ 0.01)
Endosulfansulfate	As Mentioned Above	3.0 (max)	BLQ (LOQ 0.01)
Heptachlor	As Mentioned Above	0.05 (max)	BLQ (LOQ 0.01)
Cypermethrin	As Mentioned Above	1.0 (max)	BLQ (LOQ 0.01)
Ethion	As Mentioned Above	2.0 (max)	BLQ (LOQ 0.01)
Dichlorvos	As Mentioned Above	1.0 (max)	BLQ (LOQ 0.01)
Malathion	As Mentioned Above	1.0 (max)	BLQ (LOQ 0.01)
Methidathion	As Mentioned Above	0.2 (max)	BLQ (LOQ 0.01)
Fenitrothion	As Mentioned Above	0.5 (max)	BLQ (LOQ 0.01)
Parathion	As Mentioned Above	0.5 (max)	BLQ (LOQ 0.01)
Parathion Methyl	As Mentioned Above	0.2 (max)	BLQ (LOQ 0.01)
Phosalone	As Mentioned Above	0.1 (max)	BLQ (LOQ 0.01)
Piperonyl butoxide	As Mentioned Above	3.0 (max)	BLQ (LOQ 0.01)
Pirimiphos methyl	As Mentioned Above	4.0 (max)	BLQ (LOQ 0.01)
Chlorpyrifos	As Mentioned Above	0.2 (max)	BLQ (LOQ 0.01)



Chlorpyrifos methyl	As Mentioned Above	0.1 (max)	BLQ (LOQ 0.01)
Chlorfenvinphos	As Mentioned Above	0.5 (max)	BLQ (LOQ 0.01)
Azinphos methyl	As Mentioned Above	1.0 (max)	BLQ (LOQ 0.01)
Fonofos	As Mentioned Above	0.05 (max)	BLQ (LOQ 0.01)
Diazinon	As Mentioned Above	0.5 (max)	BLQ (LOQ 0.01)
Dithiocarbamates	As Mentioned Above	2.0 (max)	BLQ (LOQ 0.01)
Fenvalerate	As Mentioned Above	1.5 (max)	BLQ (LOQ 0.01)
Permethrin	As Mentioned Above	1.0 (max)	BLQ (LOQ 0.01)
Pyrethrins	As Mentioned Above	3.0 (max)	BLQ (LOQ 0.01)
Quintozene	As Mentioned Above	1.0 (max)	BLQ (LOQ 0.01)

BLQ= Below Limit of Quantification LOQ= Limit of Quantification

Results indicated that concentration of Pesticide in Peggard Syrup was not in detectable amount. All the

pesticides are below limit of detection in the formulation. Result indicates that the pesticide content in Peggard syrup was less than the prescribed limit.

**Table 4:** Microbial analysis Peggard Syrup

Sr.No.	Microbial analysis	Limit	Peggard syrup
1.	Total Microbial count	1000 CFU/ml	4 CFU/ml
2.	Total Yeast & Mould count	100 CFU/ml	<10 CFU/ml
3.	Presence of <i>Staphylococcus aureus</i>	Absent/ml	Absent
4.	Presence of <i>Escherichia coli</i>	Absent/ml	Absent
5.	Presence of <i>Salmonella Spp.</i>	Absent/ml	Absent
6.	Presence of <i>Pseudomonas aeruginosa</i>	Absent/ml	Absent
7.	Presence of <i>Staphylococcus aureus</i>	Absent/ml	Absent

Results showed that total Microbial count was 4CFU/ml in Peggard syrup. It was less than prescribed limit. *E.coli*, *Salmonella spp.*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* were not found in detectable amount in the formulation.

### Accelerated stability study

#### 1. Evaluation of Physical parameters of Peggard Syrup

The results of the physical parameter evaluation are given in Table 5. There is very little alteration in the parameters even at the end of the 3months period.

**Table 5:** Physicochemical analysis in Accelerated stability study of Peggard Syrup

Parameters	Initial	First Month	Third Month	Sixth Month
pH	4.75±0.020	4.74±0.04	4.71±0.02	4.75±0.05
Specific gravity	1.18±0.005	1.18±0.06	1.17±0.03	1.18±0.01
Viscosity (poise)	181.09±0.305	181.08±0.305	181.08±0.305	181.09±0.305
Total sugar (g/ml at 20 <sup>o</sup> C)	36	36	36	36
Total solid content	37.02%	37.01%	37.03%	37.01%

#### 2. Microbial Load Analysis

The microbial load limit was also unaltered at the end of three months (Table 6).

**Table 6:** Microbial analysis in Accelerated stability study

Microbial analysis	Initial	Sixth Month	Limit
Total Microbial count	4 CFU/ml	10 CFU/ml	1000CFU/mlCFU/ml
Total Yeast & Mould count	<10 CFU/ml	<10 CFU/ml	100 CFU/ml
Presence of <i>Staphylococcus aureus</i>	Absent	Absent	Absent/ml
Presence of <i>Escherichia coli</i>	Absent	Absent	Absent/ml
Presence of <i>Salmonella Spp.</i>	Absent	Absent	Absent/ml
Presence of <i>Pseudomonas aeruginosa</i>	Absent	Absent	Absent
Presence of <i>Staphylococcus aureus</i>	Absent	Absent	Absent

## DISCUSSION

At the present time medicinal plants and herbal formulations are primarily used due to their less side effects and the presence of enormous level of active ingredients. But adulteration and misidentification of the herbs may lead to harmful effects in pharmaceutical industry which paves the way for the standardization of herbal drug. Standardization imparts information on chemical, biological, physicochemical profile and amount of heavy metals consistently. WHO and AYUSH insisted many guidelines to be followed for quality control for a better standardization of the drugs.<sup>[18]</sup> Ayurveda system of medicines comprises many numbers of safe and valuable herbal medicines have better therapeutic efficiency either at its raw state or processed form and are clinically used by the Ayurveda practitioners. One such formulation in clinical practice in modern era is Peggard syrup which comprises 9 herbs and has been used as Antacid for treating Heartburn, Non-ulcer dyspepsia, Gastroesophageal reflux (GERD) and Drug-induced gastritis. There were no scientific claims on the standardization of Peggard syrup. To fulfil this lacuna, in the present study, we have studied the organoleptic characters, physicochemical characters, Heavy metal analysis, Pesticide residue analysis and Accelerated stability study as per Ayurvedic pharmacopeia and compared with AYUSH standards.

Peggard syrup is red in colour, possess characteristic flavour and taste. The results of physiochemical parameters are presented in Table 1. The term total solid is applied to the residue obtained when the prescribed amount of the preparation is dried to constant weight under the specified condition. This parameter was important for the pharmacokinetic and pharmacodynam-

ics activity of drug because of the bioavailability condition. Result of total solid is found to be 37.02 %w/w which is quite normal. Viscosity is an important property of fluids which describes a liquids resistance to flow and is related to the internal friction within the fluid. viscosity and drug dissolution share an inverse relationship change in viscosity may affect the drug absorption by mechanisms like; Modification in gastric emptying rate, Modification in intestinal transit rate, Change in the rate of drug molecules from lumen to the absorbing membrane. The viscosity of Peggard syrup sample was 181.09poise. Which was quite high, but it is included in normal range. Here, the value of Specific gravity of Peggard syrup sample is found to be in normal range (1.18). The pH value indicates the relative concentration of hydrogen ion in the solution compared with that of standard solution that represents the relative acidity or alkalinity of solution. The pH of 10% solution of Peggard syrup was 4.75 which indicate that the Peggard syrup is quite acidic in nature.

Herbal materials are liable to contain heavy metals, microbial load and pesticide residues which accumulate from atmosphere, agricultural practices, such as spraying, treatment of soils during cultivation and administration of fumigants during storage, current practices of harvesting, handling and production which affects the health of the consumer. Especially, Heavy metals desire special attention because it will produce serious side-effects if consumed along with the crude drugs.<sup>[19]</sup> To overcome this deleterious effect, WHO and FAO recommended many regulations like analytical methodologies for the assessment of specific microbes. As per regulatory limits of heavy metals should be completely removed or should not be

present in herbal medicine by imposing regulatory limits. The determination of heavy metals will indicate the quality of production and harvesting practices of raw materials. The heavy metals such as Pb, Cd, Hg and As contents (Table 2), pesticide residue (Table 3) and total microbial count (Table 4) in Peggard syrup were found to be within the permissible limits as recommended by the AYUSH guidelines for herbal drugs.

## CONCLUSION

The present work was carried out for standardization of Peggard syrup. The prepared formulation was screened for various standardization parameters as per Ayurvedic pharmacopoeial standards. The research outcome of the standardization parameters can be used for evaluating the quality and purity of the formulations for the polyherbal formulation.

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