

**DEVELOPMENT AND QUALITY ASSESSMENT OF POLYHERBAL ENTOBAN CAPSULES**Sadia Shakeel<sup>1</sup>, Zeeshan Ahmed Sheikh<sup>2</sup>, Somia Gul<sup>3</sup>, Khan Usmanghani<sup>2,3</sup><sup>1</sup> Dow College of Pharmacy, Dow University of Health Sciences, Karachi-Sind -75270 -Pakistan<sup>2</sup> Herbion Pakistan (Pvt.) Ltd., Karachi, Pakistan<sup>3</sup> Faculty of Pharmacy, Jinnah University for Women Karachi-Sind- 74600 - Pakistan**\*Corresponding author e-mail:** [sadia.shakeel@duhs.edu.pk](mailto:sadia.shakeel@duhs.edu.pk)**ABSTRACT**

The present study was conducted to develop and evaluate the quality control parameters of polyherbal Entoban capsules to reassure the safety and efficacy of developed dosage form. The flow property of powdered extract was evaluated by determining bulk density, tap density, Carr's index, Hausner's ratio and angle of repose. Various physicochemical parameters including weight variation and disintegration time were calculated. Alkaloids, tanning agents and microbial limit of the polyherbal formulation were also evaluated. The powder showed good flow property. Average weight of 20 capsules was between 450 mg and 550 mg (with a mean of 506 mg  $\pm$  10%). The maximum time for disintegration was 6 min. Both the alkaloids and tanning agents were within the specified limits. The developed formulation was in compliance of the permissible microbial limits. In the present study, the developed Entoban capsules were consistent with identity, quality, and purity specifications. So it can be concluded that developed formulation would provide an opportunity to validate its traditional claim regarding its therapeutic efficacy.

**Keywords:** Quality control; Entoban; polyherbal capsules; safety.**INTRODUCTION**

According to the World Health Organization (WHO), the utilization of plant based medicines has exceeded to that of conventional medicines all over the globe by two to three times. <sup>[1]</sup> Plant derived drug is still the mainstay of about 75 - 80% of the global populace for primary health care; owing to the general belief that herbal drugs are devoid of any side effects besides being economical and easily accessible. <sup>[1,2]</sup>

Traditional herbal products have a complex character and they are a heterogeneous combination of phytoconstituents. Due to this, they inflict integer challenges to meet the criteria of quality and safety. The majority of herbal products on the marketplace nowadays have not been subjected to drug regulatory process to reveal their safety and effectiveness. Some of them include mercury, lead, arsenic,

corticosteroids and poisonous organic substances in detrimental quantity. To develop community faith and to continue plant based product into mainstream of health care system, the manufacturers, the researchers and the regulatory agencies are obliged to apply meticulous scientific methodologies to make sure the quality and standardization of the traditional herbal products. In view of the fact that the distinctiveness of the finished products are not well defined and there are fundamentally no purification steps implicated in the productions of plant based drugs, the excellence and regularity of the products rely usually on the quality control of source materials. <sup>[3]</sup> Owing to poor quality control, it is not probable for most of the herbal drugs to establish their efficacy in clinical practice. Furthermore, the tribulations related with the preparation, storage, unpleasant taste and odor acts as an obstacle and interferes with the pharmacological activity of these

traditional dosage forms. Considering the clinical use of these drugs, focus has been shifted to the ease of medication rather than the traditional dosage form, which reduces the patient compliance. [4] With this background the present study was conducted to develop and evaluate the quality control parameters of polyherbal Entoban capsules in order to reassure the safety and efficacy of developed dosage form.

## MATERIALS AND METHODS

### 1. Selection and Evaluation of Herbs

The herbs used in capsules were tested for their prescribed parts, macro and microscopic descriptions. The herbs were dried and coarsely powdered in an electronic mixer, sieved through mesh no. 40 and stored in air tight, well closed container till further use

### 2. Preformulation parameters

#### **Bulk density and tap density and Carr's index** [5, 6]

A weighed quantity (15g) of powdered material was taken in a 50ml measuring cylinder. Initial volume ( $v_0$ ) was recorded. The contents were tapped and powdered volumes was recorded after 50 taps ( $v_{50}$ ).

Fluff density =  $w/v_0$  g/cc

Tapped density =  $w/v_{50}$  g/cc

Carr's index = Tapped density - Fluff density / Tapped density \* 100

Value for Carr's index below 15 indicate excellent flowing material and value over 20-30 suggested poor flowing material.

#### **Angle of repose** [6, 7]

A funnel was fixed at a particular height on a burette stand. A paper was placed under the funnel on the table. The powdered drug was passed gradually through the funnel until it forms a pile. The radius of the pile was noted down. Angle of repose of the powder material was calculated by using the formula:  $\tan\theta = h/r$   $\theta = \tan(h/r)$

where, h = height of the pile, r = radius.

Values for angle of repose  $< 30^\circ$  usually indicate a free flowing material and angle  $> 40^\circ$  suggest a poor flowing material.

#### **Hausner's ratio** [6, 7]

The basic procedure was used to measure the unsettled apparent volume,  $V_0$  and the final tapped volume,  $V_f$ , of the powder. The Hausner's ratio was calculated as follows:

Hausner's ratio =  $V_0 / V_f$

Hausner's ratio between 1.00 and 1.11 shows excellent flow and value more than 1.60 shows very, very poor flow.

### 3. Composition of capsule

Each capsule contain:

|                            |       |
|----------------------------|-------|
| Holarrhena antidysenterica | 40 mg |
| Myrtus communis            | 40 mg |
| Symplocos racemosa         | 20 mg |
| Aluminum silicate          | 20 mg |
| Quercus infectoria         | 10 mg |
| Zingiber officinalis       | 10 mg |
| Helicteres isora           | 10 mg |
| Berberis aristata          | 10 mg |
| Butea frondosa             | 10 mg |
| Aegle marmelos             | 10 mg |
| Acacia Arabica             | 10 mg |
| Excipients                 | q.s.  |

#### **Preparation of formulation by Wet granulation method:**

All herbs were finely powdered (# 40), and taken for preparation of capsules by wet granulation technique using starch (20%) solution as binder. The wet mass was passed through # 30 to obtain granules. The granules were dried at  $45^\circ\text{C}$  in tray dryer. Diluents and preservatives were added and filled in capsules colored green-size '00' in capsule filling machine. The capsules were evaluated for different quality control parameters.

### 4. Quality control parameters

#### **Description**

Size, shape, colour were evaluated [3]

#### **Uniformity of weight**

Test for uniformity of weight was performed as per Indian pharmacopoeia (IP), 2007. [8] Randomly selected 20 capsules were weighed (individually and together) using electronic balance (Mettler Toledo B204-S, Switzerland).

#### **Determination of moisture content:**

The test was performed using Karl Fischer instrument. [9]

#### **Disintegration test**

Complete disintegration is defined as the state in which any residue of the unit, except fragments of the insoluble coating or capsule shell, remaining on the

screen of the test apparatus or adhering to the lower surface of the disc; if used is a soft mass having no palpably firm core. At least six capsules should be used to determine the disintegration time of capsules.<sup>[10]</sup> Disintegration test was performed using the digital microprocessor based disintegration test apparatus (Erweka ZT-2, Heuesnstanm, Germany). For the test, a 1000 ml beaker was filled with distilled water (approx. 900ml), equilibrated to  $37\pm 0.5^{\circ}\text{C}$ . Six capsules were subjected to the test. Time required for the last capsule to disintegrate was recorded.<sup>[10]</sup>

### 5. Qualitative Identification Reactions

#### Test for polysaccharides:

5 ml of the preparation was placed into a flat-bottomed 50 ml flask. 20 ml of 96% spirit was added and mixed; precipitated suspension was formed (polysaccharides).

#### Test for tanning agents:

The obtained solution was left for 1 hour for layer separation and filtered carefully through the folded paper filter (Solution A). 3 drops of Ferric chloride solution was added to 3 ml of the solution A. After shaking greenish-yellow color appeared (tanning agents).

### 6. Microbial Analysis<sup>[11, 12]</sup>

**Pre Treatment of the Sample:** 5 g of the sample was dissolved in 50 ml of buffered NaCl - peptone solution (pH-7.0) having no antimicrobial activity under the condition of test and in Lactose Broth having a pH of  $7.2 \pm 0.2$ . Then they were incubated at  $37^{\circ}\text{C}$  for 2 to 4 hours.

**Primary Treatment:** 0.1 of the sample was pipetted out from buffered NaCl - peptone solution and spreaded onto Soya bean Casein Digest Agar plates (SCDA) to determine the Total Bacterial Count and 0.1 ml onto Sabouraud's Dextrose Agar (SDA) having a pH range of  $5.6 \pm 0.2$  for Total Fungal Count. SCDA plates were then inverted and incubated at  $37^{\circ}\text{C}$  for 24 hours after which the number of colonies were counted and multiplied by the dilution factor and were represented in the form of CFU/g/ml. (Dilutions were performed when necessary) whereas the SDA plates were inverted and incubated at  $25^{\circ}\text{C}$  for 3 to 4 days after which the number of colonies were counted and multiplied by the dilution factor and were represented in the form of CFU/g/ml. (Dilutions were performed when necessary). 5 ml from Lactose Broth having a pH of  $7.2 \pm 0.2$  was pipetted out and transferred to both

Nutrient Broth (NB) and Soyabean Casein digest broth (SCDB). Both the flasks were then incubated at  $37^{\circ}\text{C}$  for 18 to 24 hours.

## RESULTS AND DISCUSSION

In the present study, standardized polyherbal mixture was formulated in hard gelatin capsule to replace the traditional liquid dosage form. Before converting the blended powder extract into dosage form it was passed through different procedures to estimate the flow ability of the powder extract that was necessary for getting pharmaceutically equivalent dosage. The flow property of powdered extract was evaluated by performing parameters like bulk density, tap density, Carr's index, Hausner's ratio and angle of repose. The powder showed good flow property. Table 1 depicts the report of various preformulation parameters.

To enhance the acceptability of the herbal medicine by consumers, many of the products have been formulated into conventional dosage forms such as tablets, capsules, suspensions, and powders. The present formulation is the combination of *Holarrhena antidysenterica* (Kura Chaal), *Myrtus communis* (Hab-ul-aas), *Symplocos racemosa* (Lodh Pathani), *Aluminum silicate* (Gil - e - Armani), *Quercus infectoria* (Mazu), *Zingiber officinalis* (Soanth), *Helicteres isora* (Maroor Phali), *Berberis aristata* (Zarishk), *Butea frondosa* (Kamarkas), *Aegle marmelos* (Belgiri) and *Acacia arabica* (Acacia). Proper and complete identification is one of the most important parameter incase of herbal medicine because the formulation cannot produce desired effects, if the herbs are not properly identified. The herbs used in capsules were properly identified. Various physicochemical parameters including physical appearance, weight variation and disintegration time were calculated for the polyherbal formulation. (Table 2) Weight variation is a direct indicator of the dosage form uniformity. Therefore, weights of the capsules have to be routinely measured to ensure the proper amount of the drug in each capsule.<sup>[13]</sup> Average weight of 20 capsules was between 450 mg and 550 mg with a mean of 506 mg  $\pm 10\%$ . The rate of absorption and bioavailability are dependent upon how fast the drug dissolves in GI fluid. This means that drugs administered orally in solid dosage forms (tablets, capsules, etc) than dissolve in the GI fluid before absorption.<sup>[14]</sup> Hence the rate of absorption and availability may be improved by improving the disintegration and the rate of dissolution of drug. In present study, six capsules were taken to determine the rate at which the active drug substance dissolved in the fluid of gastrointestinal tract. The maximum time for

disintegration was 6 min. Table 3 illustrates the determination of different components in capsules. Total alkaloids as berberine hydrochloride should not be less than 0.100 %. Total tanning agent as gallic acid should not be less than 1.5 %. Both the alkaloids and tanning agents were within the specified limits.

Herbal materials usually contain an integer quantity of bacteria and moulds, frequently instigating in soil or derivative of fertilizers. Bacteria and fungi form the naturally occurring microflora of curative plants, aerobic spore - forming bacteria often prevail. The presence of *Escherichia coli*, *Salmonella* spp. and moulds may be a sign of deprived quality of manufacturing and harvesting practices. *Salmonella* and *Shigella* species must not be present in herbal medicines proposed for internal use, at any stage. Other microorganisms should also be evaluated comply with limits set out in international pharmacopoeias. <sup>[15]</sup> Bioburden Analysis included total viable aerobic count, *E.coli*, *Salmonella*, *S.aureus* and fungi are depicted in Table 4. WHO has specified the limits for the presence of contaminants

like four pathogenic micro-organisms viz *E. coli*, *Staphylococcus aureus*, *P. aeruginosa* & *Salmonella enteric* along with yeast-molds. <sup>[16]</sup> The developed formulation was found in full compliance of the permissible microbial limits.

### CONCLUSIONS

In the present study, the developed polyherbal Entoban capsules were consistent with identity, quality, and purity specifications. So it can be concluded that developed formulation would provide an opportunity to validate its traditional claim regarding its therapeutic efficacy.

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None

### AUTHORS' STATEMENT

The authors declare that there is no conflict of interests.

**Table 1: Preformulation studies**

| Characterization Parameters | Data obtained |
|-----------------------------|---------------|
| Bulk density                | 0.65 ± 0.01   |
| Tap density                 | 0.78 ± 0.02   |
| Compressibility index       | 19.31 ± 2.63  |
| Hausner's ratio             | 1.24 ± 0.08   |
| Angle of repose             | 29.82 ± 0.75  |

**Table 2: Physicochemical parameters**

| Characterization Parameters<br>(n=20) | Data obtained                                 |
|---------------------------------------|-----------------------------------------------|
| Physical appearance                   | Green capsules filled with brown color powder |
| Average weight                        | 506mg ± 10%                                   |
| Moisture contents                     | 2.05% ± 0.5                                   |
| Disintegration Time                   | 06 minutes                                    |

**Table 3: Determination of different components**

| Quantitative determination                 | Specified limit                                                             | Quantity present |
|--------------------------------------------|-----------------------------------------------------------------------------|------------------|
| Total alkaloids as Berberine Hydrochloride | Total alkaloids as Berberine hydrochloride should not be less than 0.100 %. | 0.311 %          |
| Total tanning agent as gallic Acid         | Total tanning agent as gallic acid should be not be less than 1.5 %         | 2.780 %          |

**Table 4: Admissible contents for 1g of preparation**

| Microbial Analysis         | Limit CFU/g                | Observation |
|----------------------------|----------------------------|-------------|
| Total aerobic viable count | not more than $10^4$ CFU/g | Comply      |
| Salmonella                 | Absent                     | Absent      |
| Escherichia coli           | Absent                     | Absent      |
| Staphylococcus aureus      | Absent                     | Absent      |
| P. aeruginosa              | Absent                     | Absent      |
| Total fungal count         | not more than $10^2$ CFU/g | Comply      |

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