Pasteurization as a tool to control the bio-burden in solid herbal dosage forms: A pilot study of formulating Ashoka tablets with an industrial perspective

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Abstract

Irradiation and use of preservatives are routine procedures to control bio-burden in solid herbal dosage forms. Use of steam or pasteurization is even though reported in the literature, not many studies are available with respect to its application in reducing the bio-burden in herbal drug formulations. Hence, we undertook a series of studies to explore the suitability of pasteurization as a method to reduce bio-burden during formulation and development of herbal dosage forms, which will pave the way for preparing preservative-free formulations. Optimized Ashoka (Saraca indica) tablets were formulated and developed. The optimized formula was then subjected to pasteurization during formulation, with an aim to keep the microbial count well within the limits of pharmacopoeial standards. Then, three variants of the optimized Ashoka formulation - with preservative, without preservative and formulation without preservative and subjected to pasteurization, were compared by routine in-process parameters and stability studies. The results obtained indicate that Ashoka tablets manufactured by inclusion of the pasteurization technique not only showed the bio-burden to be within the limits of pharmacopoeial standards, but also exhibited the compliance with other parameters, such as stability and quality. The outcome of this pilot study shows that pasteurization can be employed as a distinctive method for reducing bio-burden during the formulation and development of herbal dosage forms, such as tablets.

Key words: Decontamination of herbs, microbial quality control, pasteurization, preservative-free, Saraca indica, stability studies

INTRODUCTION

Microbial contaminations have been inadvertently associated with herbal formulations. Microorganisms may originate from herbal raw materials, which are untreated and invariably get transferred to the final product depending on their nutritive properties and moisture contents. Herbal extracts can be contaminated with microorganisms that survive the primary processing and/or drying process; thus, microbial bio-burden is always a risk.[1] Many pharmacopoeias (European Pharmacopoeia, Indian Pharmacopoeia, United States Pharmacopeia) and regulatory agencies have included the limits for bio-burden, which are not uniform across the globe; however, they are unequivocal in setting a limit for bio-burden.

Inclusion of preservatives in several types of drug formulations has been of considerable value for many years. However, preservatives are often connected to the leading causes of adverse reactions. For example, parabens interact with mitochondrial cells and induce male infertility; sodium benzoate causes hyperactivity in...
children; potassium sorbates cause mutagenic effects in lymphocytes; butylated hydroxy anisole induces squamous cell papilloma of the nonglandular squamous portion of the stomach.[2–5] An alternative to the use of preservatives is the irradiation technique, which is widely employed to produce shelf-stable products in food industries.[6] Irradiation destroys and disrupts several micronutrients and macronutrients in the food in addition to its reported ill-health issues.[7–9] Besides these, the other technique widely described in the literature for reducing bio-burden in spices and other products is pasteurization, which employs moist heat or super-heated steam under pressure to destroy microbes. Moist heat kills microorganisms by denaturing cellular proteins.[10] This article describes the formulation and development of optimized Ashoka tablets using pasteurization to keep the microbial count well within limits.

**MATERIALS AND METHODS**

**Standardized aqueous extract of Ashoka bark**
The extract was supplied by the Phytochemistry Division, Research and Development Center, The Himalaya Drug Company (Makali, Bangalore, India). The extract was soft and found to contain 32-35% w/w of total solid content without any preservatives. The extract was subjected to detailed analytical studies and standardized with respect to markers such as, polyphenols and catechins.

**Chemicals**
Microcrystalline cellulose (MCC), maize starch, colloidal silicon dioxide, talc, magnesium stearate, and all other chemicals were of European Pharmacopoeia Grade.

**Pasteurizer**
A specially designed pasteurizer called Auraclave (M/s Machin Fabrik, Mumbai, India) was used for pasteurization. This technique, henceforth described here as auraclaving, works on the principle similar to that of autoclaving—employs moist heat in the form of steam under specific process conditions of temperature and pressure to reduce the microbial load.

**Formulation and optimization of Ashoka tablets**
An accurately weighed quantity of 40# sifted MCC (M/s Sigant Chemical Corporation Pvt. Ltd., Mumbai, India), talc (M/s Signet Chemical Corporation Pvt. Ltd., Mumbai, India), maize starch (M/s Signet Chemical Corporation Pvt. Ltd., Mumbai, India) and colloidal silicon dioxide (M/s Evonik Industries, Essen, Germany) were loaded into a rapid mixing granulator, and premixed for 10 min at slow impeller speed to ensure uniform mixing. To this premix, a weighed quantity of the Ashoka soft extract was added under continuous shear. Purified water was then added as a granulating fluid to this mixture at a slow rate with slow impeller speed to form desired wet mass. The wet mass was dried in fluid bed drier at 60°C until optimal moisture content was achieved. Dried granules were then passed through a 16# sieve to obtain uniform-sized granules. A homogenous lubricated blend was obtained by mixing 60# sifted magnesium stearate (M/s Signet Chemical Corporation Pvt. Ltd., Mumbai, India). This lubricated blend was then compressed with 13 mm biconvex punch with a target tablet weight of 500 mg with an active content of 225 mg. The batch size of each variant was adjusted to 8 kg.

**Following scheme of trial formulations were prepared**
- Ashoka soft extract without preservatives (C1)
- Ashoka soft extract containing preservatives (combination of methyl and propyl parabens at 0.25% and 0.025% concentration, respectively) (C2)
- Ashoka soft extract without preservatives and employing pasteurization (C3).

The granulation and tableting were done as per the procedure described previously.

**Pasteurization**
Pasteurization of the C3 granules was performed by auraclaving. The granules were loaded on to the Auraclave trays, and the process was initiated by introducing steam into the jacket, briefly. First, vacuum was created inside the chamber with the help of water ring type vacuum pump and then steam was introduced in the chamber up to the set value 95% of air removal from the chamber was ensured. After the completion of pasteurization process (121°C, 1.2 kg/cm² or 15 psi for 20 min), vacuum was created in the chamber up to a predetermined level, which ensured drying of the load on the tray. After the completion of vacuum drying time, the negative pressure in the chamber was brought to atmospheric pressure by injecting sterile air through the air filter. The sterilized load was then removed from the chamber and used for compression into tablets.

**Comparative evaluation of C1, C2, and C3**
The lubricated blends were evaluated for their well-established indicators of flow properties, such as, compressibility index and Hausner ratio. The compressed tablets were evaluated for physicochemical parameters, such as, hardness, weight variation, disintegration time, and friability.

**Stability studies**
The stability studies of the tablet formulations C1, C2, and C3 were performed as per International Conference on Harmonization (ICH) guidelines Q1A (R2).[10] Accelerated and real time stability studies were performed and the exact conditions and the results were tabulated. The samples were withdrawn at predetermined time intervals and evaluated.
for physical and chemical parameters and active marker content quantification apart from microbial analysis for enumeration.

**Statistical analysis**
The results were expressed as mean ± standard deviation and analyzed by one-way ANOVA with Tukey’s multiple comparison test using GraphPad Prism version 4.03 for Windows (San Diego, California, USA).

**RESULTS**

**Preformulation studies**
Ashoka soft extract was found to be compatible with the excipients used as per the drug-excipient compatibility study.

**Formulation and optimization of Ashoka tablets**
Formulations with various concentrations of excipients were prepared. An optimized formula was then chosen to evaluate the effectiveness of auraclaving in reducing the bio-burden.

**Comparative evaluation of C1, C2, and C3**
The detailed formulation composition of Ashoka tablets are given in Table 1. The lubricated blends were evaluated for their flow properties and physical properties of tablets, and the results are presented in Tables 2 and 3. The mean values of the evaluated parameters were almost similar in C1, C2, and C3. Analysis of tablets of C1, C2, and C3 for markers such as polyphenols and catechins showed their levels to be same in all [Table 4]. The results of microbial analysis are indicated in Table 5. The C1 tablets showed the highest microbial count, both in terms of total aerobic microbial count (TAMC) and total yeast and mold count (TYMC). In the case of C1 and C2, the bio-burden was significantly reduced with reference to both TAMC and TYMC. Notably, C3 showed a microbial limit < 10 cfu/g and was one of the best among all the tested optimized formulations [Table 5].

**Stability studies**
Inclusion of preservatives and pasteurization in formulations C2 and C3 yielded almost similar beneficial effects on the final product with respect to bio-burden. As expected, the formulation C1 failed to conform to microbial limits. However, analytical marker levels in C1, C2, and C3 were similar indicating that the pasteurization has no effect on the quality of the herbal material [Tables 6 and 7].

**DISCUSSION**
Formation of Ashoka tablets as a prototype was designed with an aim to compare and evaluate the bio-burden

| Table 1: The detailed formulation composition of Ashoka tablets |
|---------------------------------------------------------------|
| **Ingredients** | **Quantity/tablet (mg)** |
| Active (herbal) | 225.00 |
| Microcrystalline cellulose | 225.00 |
| Maize starch | 030.00 |
| Colloidal silicon dioxide | 010.00 |
| Talc | 005.00 |
| Methyl paraben sodium | 001.25 |
| Propyl paraben sodium | 000.12 |
| Magnesium stearate | 005.00 |
| Total weight | 500.00 |

| Table 2: Precompression lubricated blend evaluation parameters (n=3) |
|------------------------------------------------------------------|
| **Formulation code** | **Compressibility index (%)±SD** | **Hausner ratio±SD** |
| C1 | 17.68±0.012 | 1.21±0.012 |
| C2 | 12.06±0.011 | 1.13±0.023 |
| C3 | 14.28±0.021 | 1.16±0.020 |

| SD: Standard deviation |

| Table 3: Physicochemical parameters of compressed tablets (n=3) |
|----------------------------------------------------------------|
| **Formulation code** | **Hardness (kg/cm²)±SD** | **Weight variation (mg)±SD** | **Disintegration time (min)** | **Friability (%) loss** |
| C1 | 09±0.02 | 501.50±2.5 | 12 | 0.03±0.01 |
| C2 | 07±0.02 | 501.37±2.9 | 14 | 0.12±0.01 |
| C3 | 09±0.12 | 500.12±3.0 | 18 | 0.05±0.02 |

| SD: Standard deviation |

| Table 4: Enumeration of analytical marker percentage assay (n=3) |
|----------------------------------------------------------------|
| **Formulation code** | **Ashoka tablets** | Polyphenols | Catechin |
| C1 | 11.70 | 0.33 |
| C2 | 11.91 | 0.33 |
| C3 | 11.74 | 0.34 |

| Table 5: Enumeration of total microbial load (n=3) |
|--------------------------------------------------|
| **Formulation code** | **TAMC (cfu/g)** | **TYMC (cfu/g)** |
| C1 | 35,000 | 1500M |
| C2 | 100 | <10 |
| C3 | <10 | <10 |

TAMC: Total aerobic microbial count, TYMC: Total yeast and mold count, cfu: Colony forming units
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Table 6: Summary of real-time stability studies of Ashoka tablets

| Parameters                  | Initial | C1 | C2 | C3 |
|-----------------------------|---------|----|----|----|
| Mean disintegration time (min) | 11.40±0.36 | 11.80±0.27 | 11.35±0.48 | 11.61±0.11 |
| NLT 6.75%                   | CP      | CP | CP | CP |
| Polyphenols (%)             | 0.33±0.02 | 0.33±0.02 | 0.33±0.02 | 0.33±0.02 |
| TLC fingerprint             | PR      | PR | PR | PR |
| Assay for total polyphenols (%) | 0.35±0.02 | 0.33±0.01 | 0.33±0.01 | 0.34±0.01 |

Accelerated and real time stability studies performed for 6 months as per ICH guidelines[11] revealed that the tablets of all three variants did not show any physical change during the study period and the active content was found to be more than 95% at the end of 6 months in accelerated conditions. C1 demonstrated the presence of maximum microbial colonies indicating the vital necessity for microbial control. C3 showed a remarkable microbial control, comparable if not more efficient than its counterpart C2.

Pasturization not only reduced the bio-burden to acceptable limits of pharmacopoeial standards, but also exhibited the compliance with other parameters, such as stability and quality. Pasteurization proved to be effective and produced significantly reproducible physicochemical/pharmaceutical parameters, and Ashoka tablets were stable under both, real and accelerated stability conditions (6 months).

CONCLUSION

The outcome of this pilot study shows that the pasteurization is an effective, reliable and validated approach for microbial reduction and can be employed as a distinctive method for reducing bio-burden in the formulation and development of herbal dosage forms, such as tablets. Given that the process of pasteurization does not involve the use of any chemicals, the approach becomes more relevant in the development of preservative-free formulations and can be employed without any regulatory concerns for product registration. However, it is important that the temperature and pressure be customized and validated for each individual product to maintain its integrity and efficacy.

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Table 7: Summary of accelerated stability studies of Ashoka tablets (40°C±2°C and 75%±5% RH)

| Parameters                      | Limits          | C1                | C2               | C3               | Initial                     | 1st month                     |
|---------------------------------|-----------------|-------------------|------------------|------------------|-----------------------------|-------------------------------|
| Description                     | Brown colored uncoated tablets | CP               | CP               | CP               | CP                          | CP                            |
| Mean disintegration time (min)  | NMT 30M         | 10.33±0.58        | 10.33±0.58       | 10.67±1.15       | 10.67±0.58                 | 10.33±0.58                    |
| Assay for total polyphenols (%) | NLT 6.75%       | 11.60±0.10        | 11.83±0.06       | 11.84±0.02       | 11.61±0.24                 | 11.80±0.17                    |
| Assay for catechin (%)          | NLT 0.135%      | 0.32±0.02         | 0.33±0.01        | 0.33±0.01        | 0.32±0.02                 | 0.31±0.02                     |
| TLC fingerprint                 | To match with a reference sample | PR               | PR               | PR               | PR                          | CP                            |
| Total viable aerobic count (cfu/g) | NMT 10^3 cfu/g | 34666.67±577.35 | 133.33±57.74    | 40.00±51.96      | 38333.33±577.35            | 10.00±0.00                    |
| Yeast and mold count (cfu/g)    | NLT 10^2 cfu/g  | 2166.67±1258.31  | 10.00±0.00       | 10.00±0.00       | 1333.33±577.35             | 10.00±0.00                    |
| 2nd month                       | CP              | CP                | CP               | CP               | CP                          | CP                            |
| C1                              | 13.67±0.58      | 13.67±0.58        | 14.67±1.53       | 12.33±0.58       | 12.67±1.15                 | 12.33±0.58                    |
| C2                              | 11.35±0.13      | 11.75±0.13        | 11.56±0.14       | 11.59±0.09       | 11.84±0.02                 | 11.65±0.02                    |
| C3                              | 0.32±0.01       | 0.32±0.02         | 0.32±0.02        | 0.33±0.01        | 0.32±0.02                  | 0.31±0.02                     |
| 3rd month                       | CP              | CP                | CP               | CP               | CP                          | CP                            |
| C1                              | 16.67±1.53      | 17.00±1.00        | 17.00±1.00       | 10.00±0.00       | 10.00±0.00                 | 10.00±0.00                    |
| C2                              | 11.60±0.02      | 11.63±0.05        | 11.63±0.05       | 10.00±0.00       | 10.00±0.00                 | 10.00±0.00                    |
| C3                              | 0.32±0.02       | 0.32±0.02         | 0.35±0.03        | 10.00±0.00       | 10.00±0.00                 | 10.00±0.00                    |
| 6th month                       | CP              | CP                | CP               | CP               | CP                          | CP                            |
| C1                              | 37500.00±100.00 | 10.00±0.00        | 10.00±0.00       | 32100.00±100.00  | 10.00±0.00                 | 40.00±51.96                   |
| C2                              | 1500.00±500.00  | 10.00±0.00        | 10.00±0.00       | 500.00±0.00      | 10.00±0.00                 | 500.00±0.00                   |
| C3                              | 1500.00±500.00  | 10.00±0.00        | 10.00±0.00       | 500.00±0.00      | 10.00±0.00                 | 500.00±0.00                   |

Real-time stability study: 25°C±2°C and 60%±5% RH

ICH: International Conference on Harmonization, SD: Standard deviation, CP: Complies, cfu: Colony forming units, LOD: Loss on drying, NMT: Not more than, NLT: Not less than, PR: Performed, RH: Relative humidity, TLC: Thin layer chromatography. Disintegration time, LOD estimation, total viable aerobic count (cfu/g) and yeast and mold count (cfu/g) were performed as per the pharmacopoeial criteria/methodology with respect to sample size, sampling and procedure. ICH guideline was followed with respect to stability studies. The values were expressed as mean±SD period.

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