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# Standardization and Assessment of Preformulation Parameters of Rasayana Tablet

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**ABSTRACT**
Rasayana tablet is a polyherbal preparation recommended as a tonic. It contains *Amla, Gokhru and Galo* in equal proportion. This preparation is generally marketed in the form of churna and tablet with several problems in weight hardness, disintegration time and friability. The present work is based on the Standardization of individual ingredients and formulation of Rasayana tablets with improved formulation parameters in order to compare it with the marketed formulation. Pharmacognostical parameters were also checked for individual crude drugs and marketed tablets like ash value, extractive value, loss on drying and powder microscopy. The preformulation parameters like bulk density, tap density, Carr's index, Hausner's ratio and angle of repose were also checked for laboratory granules. The tablets were prepared by wet granulation technique using sucrose (55 % solution) and starch (20 % solution) as binder. The designed formulations were evaluated for thickness, diameter, hardness, friability and disintegration time. The designed formulation was in conformity to the properties evaluated for the tablets and is discussed in detail.

**Keywords**: Rasayana tablet, wet granulation, density, Carr's index.

## INTRODUCTION

Ayurveda, literally the "science of life and longevity" in ancient Sanskrit, is the one of the oldest healing system, based on lifestyle, diet and herbs. Ayurvedic texts contained complete sections on anti-ageing. Ayurveda contains several such plants have been dealt with ageing. [1] Sushruta, ancient Indian physician defies Rasayana as a measure, which prolongs and provides positive health, improves mental faculties and provides resistance and immunity against diseases. Charaka Samhita, a great ancient classic treatise of Ayurveda states that the means of obtaining optimum nourishment to the dhatus are called Rasayanas. [2] Rasayana plants are said to possess properties such as antiageing, re-establish youth, strengthen life, brain power and prevent diseases all of which imply that they increase the resistance of the body against any onslaught. In many cases these natural products are used as an alternative to the conventional chemotherapy against a variety of diseases. It is well known that immune system plays an important role in biological adaptation contributing to maintenance of homeostasis and establishment of body's' integrity. Traditionally, 'Rasayana' drugs are used against a plethora of seemingly diverse disorders with no pathophysiological connections according to modern medicine. It has been reported that the 'Rasayanas' are rejuvenators, nutritional supplements and possess strong antioxidant activity. They also have antagonistic actions on the oxidative stressors, which give rise to the formation of different free radicals. They are used mainly in ageing, atherosclerosis, cancer, diabetes, rheumatoid arthritis, autoimmune and Parkinson's disease. Their antistress/adaptogenic actions have made them therapeutically more important. [3-5]

Certain of these plants are believed to promote positive health and maintain organic resistance against infection by re-establishing body equilibrium and conditioning the body tissues. [6] Modulation of immune responses to alleviate disease has been of interest for many years and the concept of rasayana in Ayurveda is based on related principles. [7] Indian medicinal plants are rich source of substances that are claimed to induce para-immunity; the non specific immunomodulation. [8]

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Ayurveda not only incorporates detoxification, antioxidant, *Vajikarana* (virilification) therapies, immunomodulation, Rasayana and adaptogens as well, but focuses on development of a specific lifestyle plan including individual dietary and exercise guidelines as well. Many of the traditional drugs are still proving useful beyond doubt and in future also, traditional knowledge will contribute pivotal role in ageing research. Plants with antioxidant activity are in high demand because of their anti-ageing effects. [9] The use of herbal tonics as a part of a daily health regimen is found everywhere in traditional medicine. [10] The Ayurvedic system contains several powerful antioxidant herbs as well as Rasayana drugs available to prevent degenerative diseases.

For example, Amla (*Emblica officinalis*), or Indian Gooseberry is prized for its concentrations of naturally occurring vitamin C. This herb has been taken for centuries as chief ingredient in Chyavanprash, fortified with other balancing herbs as an antioxidant to prevent the occurrence of free radical chain reactions. In the Ayurvedic textbooks, rejuvenation has been termed as Rasayana. According to the definition of Ayurveda, Rasayana is one, which destroys the old age and disease. Rejuvenating agents are taken so that you become healthy and give your services for others. It is taken so that you can explore the spiritual aspect of life and help the society, the sick, the hungry, the poor and the disabled. The Rasayana or rejuvenation therapy aims at keeping the enzymes in the tissue cells in their normal functioning condition. This prevents the process of ageing and makes the individual free from any disease even during an advanced age.

Gokhru (*Tribulus terrestris*), Guduchi (*Tinospora cordifolia*) are another Rasayana drugs. The Rasayana are the rejuvenative tonics that keep cells young and helped reverse age related damage. The objective is that all functions of the body are kept toned and working at optimum effectiveness. Ancient Ayurvedic physicians had developed certain dietary and therapeutic measures to arrest/delay ageing and rejuvenating whole functional dynamics of the body system. This revitalization and rejuvenation is known as the 'Rasayan chikitsa' (rejuvenation therapy). Adaptogenic herbs have the ability to work as a biological response modifier to environmental and psychological stress.

Rasayana tablet is one of the polyherbal formulation marketed by different companies as a tonic. It enhances the immune strength of the body. Certain class of drug which having a very potent rejuvenating effect, the powders or tablets of powders of such herbs are used as Rasayana drugs in Ayurveda. In the present study we selected Rasayana tablet which contains mainly dried powders of *Embelica Officinalis* (34 %), *Tribulus Terrestris* (33 %) *and Tinospora Cordifolia* (33 %).

*Embelica officinalis* is also known as *Phyllanthus embelica* belonging to family Euphorbiaceae, commonly known as Amla. It is widely used as a fruit and found in both wild and cultivated states. [11-13] It is effective in the treatment of amlapitta (peptic ulcer) [14] and in dyspepsia. [15] The fruit exhibit hypolipidaemic and antiatherosclerotic effects. [16-18] The fruit extract has antimutagenic activity on certain directly acting mutagens in some strains of *Salmonella Typhimurium*. [19] It also possesses so many activities like hepatoprotective, antioxidants, [20-21] cytoprotective [22] and anti-tumour activity. [23]

*Tribulus terrestris* commonly known as Gokhru belonging to family *Zygophyllaceae*, widely distributed throughout India. Roots and fruits useful in rheumatism, piles, renal and vesical calculi, menorrhagia, impotency, premature ejaculation, general weakness etc. It is a very good diuretic and tonic drug. [24] *Tribulus terrestris* L. (*Zygophyllaceae*) is an annual plant native of mediterranean region. In India, it is called Gokhru, an important herb commonly used in the folk medicine of many countries for different purposes. The fruits of the plant *Tribulus terrestris* has been shown to exhibit diuretic, [25] anti-urolithiatic, [26] CNS stimulant, [27] antimicrobial, [28] antifungal activities in rats, [29] antioxidant and antihypertensive activity in rat heart. [30-31]

*Tinospora cordifolia* commonly known as Galo belonging to family *Menispermaceae*, found throughout the tropical regions of the India. This multipurpose herb is useful in many illnesses. It improves body's defense mechanisms. It repairs the damaged cells. It gives new life to dying brain cells and rejuvenates the whole body. It is best anti-cancer, immunomodulator and memory enhancing herb. [32-34] Aqueous extract of the stem showed anti-inflammatory, analgesic and anti pyretic activity. [35] It also showed anti-oxidant activity and amelioration of cyclophasphamide induced toxicity. [36] The stem part is mainly used for immunomodulatory activity. [37]

The Rasayana tablet obtained from the market possess some problems of hardness, disintegration time and friability. So the present work was done to standardize and to improve the preformulation parameters of Rasayana tablet.

## MATERIALS AND METHODS

### Plant materials

Fresh fruits of *Embelica officinalis* (Amla) and *Tribulus terrestris* (Gokhru) were collected from local market of Rajkot. The stem of *Tinospora cordifolia* (Galo) was obtained from the medicinal garden of Saurashtra University, Rajkot, Gujarat, India. The authenticity of the freshly collected plant was confirmed by comparing their morphological characters with the description mentioned in the different standard texts and floras. Besides these, the identity of the plant was also confirmed from Botany Department, Saurashtra Univeristy, Rajkot. The required samples were thoroughly washed with running water to remove the adherent impurities and dried.

### Chemicals and reagents

Methanol, Silica gel-G, toluene, formic acid were obtained from E. Merck India Ltd. Sucrose and starch were obtained from Sisco Research Lab. Pvt. Ltd., Mumbai, India. All other chemicals and reagents were of analytical grade.

### Powder microscopy

For the microscopic examination, individual crude drugs were shade dried, finely powdered. Then all the powders are separately treated with chloral hydrate. [38] For powdered microscopic study marketed Rasayana tablets were powdered in glass mortar and pestle. Preliminary analysis of the powder of *Embelica officinalis* fruit [11, 39], *Tribulus terrestris* fruit, [40] *Tinospora cordifolia* stem [41] and marketed Rasayana tablet powder were stained with concentrated hydrochloric acid and phloroglucinol, mounted in glycerine were observed under microscope. All the powder shows the character specified in the Ayurveda Pharmacopoeia. [42]

### Physico-chemical study

Physico-chemical parameter were determined as per Ayurvedic Pharmacopoeia guidelines and reported as total ash, water soluble ash, acid insoluble ash, water soluble extractive, methanol soluble extractive and loss on drying. [43] All the values comply with the standard value given in the standard book.

**Preparation of plant extract**

*Embelica officinalis* fruit, *Tribulus terrestris* fruit and *Tinospora cordifolia* stem were finely powdered (# 40). 400 g of each powder were separately shaken with 1500 ml methanol for 6 h and then kept it for 24 h. The methanolic extracts thus obtained were filtered and distilled on water bath. The last trace of solvent was evaporated under reduced pressure using rotatory evaporator. [44-45]

**Preparation of formulation**

100 mg of methanolic extract of each of *Embelica officinalis* fruit, *Tribulus terrestris* fruit, *Tinospora cordifolia* stem were taken for preparation of tablets by wet granulation technique for Batch RT1 using sucrose (55 %) solution as a binder and for Batch RT2 using starch (20 %) solution as binder. The wet mass was passed through # 30 to obtain granules. The granules were dried at 45$^{o}$ C in tray dryer. The granules of # 30/60 size were lubricated with 1 % magnesium stearate. The tablets with 10 kp crushing strength were prepared using single station tablet press (Cadmach Machines Ltd., India). Batch RT1 and Batch RT2 tablets were compared with the marketed tablet for hardness, disintegration time and friability. [47] Average weight (20 tablets) of RT1 and RT2 was 350 mg. Rasayana tablets were purchased from market manufactured by Ritesh Pharmaceuticals Ltd., Vadodara, Gujarat, India. The percentage of Amla, Gokhru and Guduchi powder were 34 %, 33 % and 33 % respectively. Average weight of the marketed Rasayana (20 tablets) was around 400 mg.

**Preformulation and Formulation Study**

RT1 and RT2 granules were analysed for preformulation parameter like bulk density, tap density, Carr's index, Hausner's ratio and angle of repose. [48] Also thickness, diameter, hardness, % friability and disintegration time of Rasayana tablets, RT1 tablets and RT2 tablets were performed.

**TLC Profile**

Rasayana tablet was powdered and treated with methanol to obtain methanolic extract of it; the same procedure was followed for obtaining methanolic extract of RT1 and RT2 methanolic extract for performing TLC. The TLC plate was prepared with silica gel-G, it was air dried and then kept it in the oven for 15 min. at 105°C. TLC plate was spotted with methanolic extract of *Embelica officinalis* fruit, methanolic extract of *Tribulus terrestris* fruit, methanolic extract of *Tinospora cordifolia* stem, methanolic extract of marketed Rasayana tablet, methanolic extract of RT1 and methanolic extract of RT2 respectively. Solvent system was toluene: ethyl acetate: formic acid (70:23:7) and it was run upto 80 % of the plate. Now TLC plate was air-dried, then kept in iodine chamber and observed visually. Different bands were observed for methanolic extract of *Embelica officinalis* fruit, *Tribulus terrestris* fruit, *Tinospora cordifolia* stem, marketed Rasayana tablet, RT1 tablet and RT2 tablet. [46]

## RESULT AND DISCUSSION

*Embelica officinalis* fruit, *Tribulus terrestris* fruit, *Tinospora cordifolia* stem powder were studied individually for its characteristics. Marketed Rasayana tablet powder was studied for comparision of individual powder characteristics as shown in Table 1. As per Indian Herbal Pharmacopoeia and Ayurvedic Pharmacopoeia of India of individual herb, various powder characteristics like epicarp with anamocytic stomata, tracheids, calcium oxalate crystals and prism shaped calcium oxalate, lignified fibers, sclereids, cork cells, non glandular trichomes, starch grains, pericyclic fibers, pitted and bordered pitted xylem vessel were also observed in the powder of Rasayana tablets. So we can say that the tablet obtained from the market contains all the three ingredients in the pure form which proves the qualitative standardization of the marketed Rasayana tablets of Ritesh Pharmaceuticals Ltd., Vadodara, India.

Physico-chemical parameters of powdered *Embelica officinalis* fruit, *Tribulus terrestris* fruit, *Tinospora cordifolia* stem like total ash, water soluble ash, acid insoluble ash, water soluble extractive, ethanol soluble extractive and moisture content are shown in Table 2.

Preformulation parameters like bulk density, tap density, Carr's index, Hausner's ratio and angle of repose were obtained for batch RT1 and RT2 granules. Batch RT1 granules showed comparatively better flow property with compare to Batch RT2 granules. Moreover, the angle of repose of batch RT2 (32.84) was poor with compare to batch RT1 (24.52). Granules of batch RT1 was having less hausner's ratio than batch RT2 granules. Though both the batches were having good flow, batch RT1 was much improved than batch RT2.

All other evaluation parameters like thickness, diameter, hardness, % friability and disintegration time of Rasayana tablets were compared with tablets prepared from batch RT1 and RT2 granules. The results are shown in Table 4. % Friability and hardness of Batch RT1 were good as compared with marketed Rasayana tablet and Batch RT2. Also comparatively disintegration time for Batch RT1 tablets was just 2.5 ± 0.19 minutes compared to marketed Rasayana tablet (18 ± 0.47 min.) and Batch RT2 (4.43 ± 0.03 min.). It proved the better characteristics of tablet from batch RT1 than the other two tablets.

In TLC study, better separations of individual methanolic extracts were observed. In Table 5; Rf values of methanolic extract of *Embelica officinalis* fruit, methanolic extract of *Tribulus terrestris* fruit, methanolic extract of *Tinospora cordifolia* stem, methanolic extract of marketed Rasayana tablet, methanolic extract of RT1 and methanolic extract of RT2 are shown. Bands with similar Rf values were observed in marketed Rasayana tablet methanolic extract, RT1 methanolic extract and RT2 methanolic extract; when compared with methanolic extracts of *Embelica officinalis* fruit, *Tribulus terrestris* fruit and *Tinospora cordifolia* stem. So by this way we can also say that marketed Rasayana tablet and laboratory formulated (Batch RT1 and Batch RT2) tablets were qualitatively standardized.

*Embelica officinalis* fruit, *Tribulus terrestris* fruit and *Tinospora cordifolia* stem powders complies with guidelines of Indian Herbal Pharmacopoeia and Ayurvedic Pharmacopoeia of India for their qualitative standard parameters like ash value, extractive value and loss on drying. Powder characteristics of individual powder of herbs part were also seen in Rasayana tablet. A comparative TLC study of individual extract of herbs part, Rasayana tablet, Batch RT1 and Batch RT2 showed many same Rf values

**Table 1: Powder microscopy of *Embelica officinalis* fruit, *Tribulus terrestris* fruit, *Tinospora cordifolia* stem and marketed Rasayana tablet**

| Powder / Powder Characteristics | *Embelica officinalis* | *Tribulus terrestris* | *Tinospora cordifolia* | Marketed Rasayana Tablet |
|---|---|---|---|---|
| Epicarp with Anamocytic stomata | Present | --- | --- | Present |
| Tracheids | Present | --- | Present | Present |
| Calcium oxalate crystals | Present | | --- | Present |
| Lignified Fibers | Present | Present | --- | Present |
| Sclereids | Present | --- | --- | Present |
| Prism calcium oxalate | --- | Present | --- | Present |
| Cork cells | --- | --- | Present | Present |
| Non glandular trichomes | --- | Present | --- | Present |
| Pitted xylem vessel | --- | Present | --- | Present |
| Starch grains | --- | --- | Present | Present |
| Pericyclic fibres | --- | --- | Present | Present |
| Bordered pitted xylem vessel | --- | --- | Present | Present |

**Table 2: Physico-chemical parameters of powdered *Embelica officinalis* fruit, *Tribulus terrestris* fruit, *Tinospora cordifolia* stem**

| Physico-chemical parameters | *Embelica officinalis* (% w/w) | *Tribulus terrestris* (% w/w) | *Tinospora cordifolia* (% w/w) |
|---|---|---|---|
| Total Ash | 5 | 12 | 15 |
| Water soluble ash | 2 | 11.5 | 13 |
| Acid insoluble ash | 1.5 | 2.5 | 2 |
| Water soluble extractive | 56 | 24.8 | 12 |
| Ethanol soluble extractive | 43.2 | 14.4 | 18.4 |
| Loss on drying | 2 | 1.5 | 4 |

**Table 3: Preformulation parameters of Batch RT1 (Sucrose 55% solution as binder) and RT2 (starch 20 % as binder)**

| Types of Granules | Bulk density(g/ml) | Tapped density(g/ml) | Carr's index | Hausner's Ratio | Angle of repose |
|---|---|---|---|---|---|
| Laboratory Granules with sucrose (55%) (Batch: RT1) | 0.24 | 0.26 | 7.69 | 1.07 | 24.52 |
| Laboratory Granules with starch (20%) (Batch: RT2) | 0.34 | 0.38 | 10.53 | 1.12 | 32.84 |

**Table 4: Formulation Parameters of marketed Rasayana tablet, RT1 and RT2 tablets. (mean ± S. D.)**

| Parameters | Market Rasayana Tablet | Laboratory Tablets with sucrose (55 %) as binder (Batch: RT1) | Laboratory Tablets with starch (20 %) as binder (Batch: RT2) |
|---|---|---|---|
| Diameter (millimeters) (n = 20) | 10 ± 0.20 | 11 ± 0.68 | 11 ± 0.28 |
| Thickness (millimeters) (n = 20) | 4 ± 0.73 | 5 ± 0.24 | 4.5 ± 0.51 |
| Hardness (n = 20) | 3 ± 0.84 | 6 ± 0.07 | 4.5 ± 0.59 |
| % Friability | 0.81 % | 0. 63 % | 0. 72 % |
| Disintegration time (n = 6) | 18 ± 0.47 | 2.5 ± 0.19 | 4.43 ± 0.03 |

**Table 5: TLC of methanolic extract of *Embelica officinalis* fruit, *Tribulus terrestris* fruit, *Tinospora cordifolia* stem, marketed Rasayana tablet, RT1 tablet and RT2 tablet**

| Methanolic extract of / Rf Values of bands | *Embelica officinalis* fruit | *Tribulus terrestris* fruit | *Tinospora cordifolia* stem | Marketed Rasayana tablet | Laboratory Tablets with sucrose (55 %) (Batch: RT1) | Laboratory Tablets with starch (20 %) (Batch: RT2) |
|---|---|---|---|---|---|---|
| Band-1 | 0.032 | 0.329 | 0.202 | 0.107 | 0.211 | 0.213 |
| Band -2 | 0.106 | 0.585 | 0.372 | 0.214 | 0.586 | 0.583 |
| Band -3 | 0.213 | 0.787 | 0.457 | 0.585 | 0.455 | 0.455 |
| Band -3 | 0.319 | -- | 0.5 | 0.456 | 0.786 | 0.789 |
| Band -4 | -- | -- | -- | 0.786 | -- | -- |

indicating the presence of same phytoconstituents present in Rasayana tablets. Thus Rasayana tablet complies with the qualitative standard parameter in reference to laboratory scale formulation RT1 and RT2.

The tablets made with sucrose solution (55 %) as a binder (Batch RT1) have improved some parameters like % friability, disintegration time, weight variation as compare to marketed tablet. The tablets made with starch solution (20 %) as a binder (Batch RT2) have also improved same parameter as compared to marketed tablets but less than tablets made with sucrose solution (55 %) as a binder. Thus sucrose solution (55 %) as a binder (Batch RT1) was one of the best batches formulated as Rasayana tablet in this study. Sucrose solution (55 %) can be used as binder and further various preformulation and formulation parameters which are lacking in formulating traditional Ayurvedic drugs can be overcome by this type of comparative studies.

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