QUANTITATIVE DETERMINATION OF THREE CONSTITUENTS OF RASAYANA CHURNA (A CLASSICAL AYURVEDIC FORMULATION) BY A REVERSED PHASE HPLC

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ABSTRACT
India has long tradition of using Ayurvedic medicines as therapeutic agent. In comparison to rapid growth of herbal market, export rate of traditional Indian medicine is quite insignificant. It might be due to lack of proper quality control measures of raw materials and formulation. So, it is essential to ensure quality, safety and efficacy of raw materials and formulation. Rasayana Churna is a classical Ayurvedic formulation, which comprises dried powders of three well known rejuvenating drugs viz. dried stem of Guduchi (Tinospora cordifolia Miers.), dried fruit of Gokshur (Tribulus terrestris Linn.) and dried pericarp of Amalaki (Emblica officinalis Gaertn.) in equal proportion. It is used in Ayurveda as Rasayana to enhance general body resistance, promote longevity, as anti-stress and adaptogen. Therapeutic activity of Rasayana Churna may be attributed to cordifolioside A, diosgenin and gallic acid. Cordifolioside A is an active constituent of Guduchi (Tinospora cordifolia Miers.). Diosgenin is steroidal saponin present in Gokshur (Tribulus terrestris Linn.) and gallic acid is a phenolic compound present in Amalaki (Emblica officinalis Gaertn.). Several research work have reported the quantitative estimation of active constituent in individual drug but no established method was found for quantitative analysis of Rasayana Churna. This is the first attempt ever regarding quantitative estimation of Rasayana Churna by RP-HPLC. Various validated methods were used for estimation of cordifolioside A, diosgenin and gallic acid. These RP-HPLC methods can be used for routine quality control of raw materials and Rasayana Churna.

Keywords: Rasayana Churna, Reversed-phase HPLC, Cordifolioside A, Diosgenin, Gallic acid

INTRODUCTION
India has long tradition of using Ayurvedic medicines as therapeutic agent. In recent years, a renewed interest has been shown by many people throughout the world to know more about the contributions that have been made or that can be made in future by the Ayurvedic Medicine (Indian System of Medicine). The World Health Organization (WHO) estimated that 80 % of the population of developing countries relies on traditional medicines, mostly plant drugs, for their primary health care needs1,2. In comparison to rapid growth of herbal market, export rate of traditional Indian medicine is quite insignificant. It might be due to lack of proper quality control measures of raw materials and formulation. So, it is essential to ensure quality, safety and efficacy of raw materials and formulation3. Instrumental analytical methods play a significant role in assessing the authenticity and quality of herbal drugs hence these methods are widely used for quality control. The persistent and excessive stress of modern life and the explosion of conventional therapeutic drugs (chemical stressor) have made us recognize with new class of diseases, which we call the stress-related diseases4. In Ayurveda stress related diseases and their treatment with vitalizes and rejuvenators is well documented under the heading of Rasayana by charak5. The main purpose of Rasayana therapy is to prevent the process of ageing, minimize problems of elderly and enhance the immunity of the body. Rasayana Churna is a classical Ayurvedic formulation, which comprises dried powders of three well known rejuvenating drugs viz. dried stem of Guduchi (Tinospora cordifolia Miers.), dried fruit of Gokshur (Tribulus terrestris Linn.) and dried pericarp of Amalaki (Emblica officinalis Gaertn.) in equal proportion6,7. As name suggested, it is used in Ayurveda as Rasayana to enhance general body resistance, promote longevity, as anti-stress and adaptogen8,9. The therapeutic efficacy of Rasayana Churna might be due to presence of secondary metabolites like alkaloids, glycosides, steroids, flavonoids, tannins, terpenoids and phenolic compounds. As its evident that quantification of active constituents can play important role to ensure quality of raw materials and formulation. In the present study three active constituent’s viz. cordifolioside A, diosgenin and gallic acid were taken to establish quality of Rasayana Churna. Cordifolioside A is an active constituent of Guduchi (Tinospora cordifolia Miers.)10. Diosgenin is steroidal saponin present in Gokshur (Tribulus terrestris Linn.)11 and gallic acid is a phenolic compound present in Amalaki (Emblica officinalis Gaertn.).12 Several research work have reported the quantitative estimation of active constituent in individual drug but no established method was found for quantitative analysis of Rasayana Churna. This is the first attempt ever regarding quantitative estimation of Rasayana Churna by RP-HPLC (Reversed Phase – High Performance Liquid Chromatography). We have used validated RP-HPLC methods for the estimation of above mentioned active components respectively13,14. In previous research publication, Quality of raw materials and Rasayana Churna was already ensured by different physico-chemical parameters, heavy metal analysis and microbial load15.
**MATERIALS AND METHODS**

**Chemicals**

HPLC grade solvents and other analytical grade solvents were purchased from Merck Ltd., Mumbai, India. Cordifolioside A was purchased from Chromodex; India. Diosgenin was purchased from Sigma Aldrich; India. Gallic acid was purchased from Himedia Laboratories Pvt. Ltd.; India. Purity of Cordifolioside A was found to be > 60 %, diosgenin and gallic acid were found to be > 98 %.

**Test materials**

**Collection and authentication of raw materials**

Raw materials were collected from Atasurumba forest department, Vireshwar, Sabarkantha, Gujarat, India during the winter season. Authentication of raw materials was carried out at Department of Botany, The H.N.S.B. Ltd. Science College, Himmatnagar, Gujarat, India.

**Preparation of Rasayana Churna**

The Rasayana Churna was prepared as per the general method describe in Ayurvedic Formulary of India. All the ingredients were shade dried and powdered separately, passed through 80# sieve and then mixed together in equal proportions to get uniformly blended Churna. Rasayana Churna was finally passed through 80# sieve.

**Preparation of standard solution**

For Cordifolioside A: 10 mg Cordifolioside A was dissolved in 10 mL methanol.

For Diosgenin: 2.4 mg diosgenin was dissolved in 10 mL Acetonitrile: Methanol (8:2 v/v) solution.

For Gallic acid: 2.5 mg Gallic acid was dissolved in 25 mL Water: Acetonitrile: Glacial Acetic Acid (90: 8: 2 v/v/v) solution.

**Sample Preparation**

**For Gokshur powder and Rasayana Churna**

5 g of sample was refluxed with 50 mL of 10 % w/v sulphuric acid for 4 h. Content was transferred to separating funnel and extracted with 50 mL ethyl acetate (3 times). Collected ethyl acetate was passed through sodium sulphate bed. Dry residue was obtained after evaporation of ethyl acetate. 500 mg residue was dissolved in 50 mL of methanol. Solution was filtered using 0.22 mm filter paper and used as test solution.

**For Amalaki powder and Rasayana Churna**

100 mg sample was dissolved in 25 mL Water: Acetonitrile: Glacial Acetic Acid (90: 8: 2 v/v/v) solution. Solution was filtered using 0.22 mm filter paper and used as test solution.

**RESULTS AND DISCUSSION**

Bauer and Titte16 and Springfield et al.17 reported that HPLC fingerprinting is the best way for chemical characterization. The present RP-HPLC methods were conducted to quantify three active constituents (viz. cordifolioside A, diosgenin and gallic acid) of Rasayana Churna which also play important role in therapeutic efficacy. Quantification of cordifolioside A was carried out in Guduchi powder and Rasayana Churna. Cordifolioside-A isolated from *Tinospora cordifolia* stems have been identified as active principles responsible for immune stimulant action18 and potential *in-vivo* radio-protective effect as well as *in-vitro* cytoprotective activity19. In study 0.57 % and 0.24 % cordifolioside A was found in Guduchi powder and Rasayana Churna respectively (Table 1). HPLC chromatograms of cordifolioside A standard, Guduchi powder and Rasayana Churna are shown in Figure 1, Figure 2 and Figure 3 respectively. Diosgenin is a naturally occurring steroidal saponin abundantly present in many medical plants and one of the active constituent of *Tribulus terrestris*. Diosgenin could stimulate lymphocyte transformation and enhance phagocytic capacity of macrophages *in-vitro*, and remarkably promoted the secretion of nitric oxide and TNF-α in macrophages. It could improve both specific and non-specific cellular immune responses20. Gokshur powder and Rasayana Churna was standardized by estimation of Diosgenin through HPLC. Diosgenin was found to be 1.69 % and 0.58 % in Gokshur powder and Rasayana Churna respectively (Table 2). HPLC chromatograms of gudicin standard, Gokshur powder and Rasayana Churna are shown in Figure 4, Figure 5 and Figure 6 respectively. The anti-oxidant and pro-oxidant mechanism of gallic acid is most likely due to the strong reducing power and weak metal chelating ability21. Amalaki powder and Rasayana Churna was standardized by estimation of gallic acid through HPLC. Gallic acid was found to be 11.02 % and 4.13 % in Amalaki powder and Rasayana Churna respectively (Table 3). HPLC chromatograms of gallic acid standard, Amalaki powder and Rasayana Churna are shown in Figure 7, Figure 8 and Figure 9 respectively.
Table 1: Percentage of cordifolioside A in Guduchi powder and Rasayana Churna

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the sample</th>
<th>Retention time (Min.)</th>
<th>Area (mV.s)</th>
<th>Percentage of cordifolioside A (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cordifolioside A (Standard)</td>
<td>9.724</td>
<td>4781</td>
<td>---</td>
</tr>
<tr>
<td>2</td>
<td>Guduchi powder</td>
<td>9.723</td>
<td>864</td>
<td>0.57 %</td>
</tr>
<tr>
<td>3</td>
<td>Rasayana Churna</td>
<td>9.713</td>
<td>375</td>
<td>0.24 %</td>
</tr>
</tbody>
</table>

Table 2: Percentage of diosgenin in Gokshur powder and Rasayana Churna

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the sample</th>
<th>Retention time (Min.)</th>
<th>Area (mV.s)</th>
<th>Percentage of diosgenin (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Diosgenin (Standard)</td>
<td>2.297</td>
<td>4924</td>
<td>---</td>
</tr>
<tr>
<td>2</td>
<td>Gokshur powder</td>
<td>2.327</td>
<td>3421</td>
<td>1.69 %</td>
</tr>
<tr>
<td>3</td>
<td>Rasayana Churna</td>
<td>2.314</td>
<td>1174</td>
<td>0.58 %</td>
</tr>
</tbody>
</table>

Table 3: Percentage of gallic acid in Amalaki powder and Rasayana Churna

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the sample</th>
<th>Retention time (Min.)</th>
<th>Area (mV.s)</th>
<th>Percentage of gallic acid (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gallic acid (Standard)</td>
<td>4.620</td>
<td>1081</td>
<td>---</td>
</tr>
<tr>
<td>2</td>
<td>Amalaki powder</td>
<td>4.690</td>
<td>4639</td>
<td>11.02%</td>
</tr>
<tr>
<td>3</td>
<td>Rasayana Churna</td>
<td>4.850</td>
<td>1759</td>
<td>4.13%</td>
</tr>
</tbody>
</table>

Figure 1: HPLC chromatogram of cordifolioside A (Standard)

Figure 2: HPLC chromatogram of Guduchi (Tinospora cordifolia Miers.) powder
Figure 3: HPLC chromatogram of Rasayana Churna for cordifolioside A

Figure 4: HPLC chromatogram of diosgenin (Standard)

Figure 5: HPLC chromatogram of Gokshur (Tribulus terrestris Linn.) powder
Figure 6: HPLC chromatogram of Rasayana Churna for Diosgenin

Figure 7: HPLC chromatogram of gallic acid (Standard)

Figure 8: HPLC chromatogram of Amalaki (Emblica officinalis Gaertn.) powder
CONCLUSION
Rasayana Churna was standardized by quantification of cordifolioside A, diosgenin and gallic acid. These RP-HPLC methods may be used for routine quality control of raw materials and Rasayana Churna.

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REFERENCES


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