Estimation and quantitation of β-asarone from *Acorus calamus* rhizome and its formulations using validated RP-HPLC method

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### ABSTRACT

**Introduction:** *Acorus calamus* Linn. (*A. calamus*) has been found use in medicines to cure fevers, asthma, bronchitis and as an all-round sedative. β-asarone is an important phytochemical compound present richly in the rhizomes of *Acorus calamus* that imparts several therapeutic properties to the plant by the virtue of which the plant has occupied a significant therapeutic acclaim in ancient Ayurvedic text and is employed as one of the key ingredients in several traditional and herbal formulations. Thus, the study aims to develop and validate an efficient Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method for quantification of β-asarone from rhizomes of *A. calamus* of the wild and marketed variety and also intends to apply the validated method for the estimation of the biomarker from different formulations containing the rhizome as one of its ingredients. **Methods:** Separation was carried out on Cosmosil C₁₈ column eluted with mobile phase of methanol: distilled water (50:50, v/v) at flow rate of 1 mL/min. Detection was carried out at 304 nm using a photodiode array detector (PDA) and the method was validated as per International Conference on Harmonization (ICH) guidelines. Rhizome was collected from Kerala and also procured from the market. Commercial traditional and herbal formulations like Sarasvata Churna, Maanasmithra Vatakam, Khadiradi Gutika, Chandraprabha Bati, Sanjeevani Vati, Mahashankh Bati, Smritisagar Ras, Abana, Vacadi Taila, and Ashwagandharishtha were further subjected to RP-HPLC for separation and estimation of β-asarone. **Results:** The limit of detection (LOD) and limit of quantitation (LOQ) levels were found to be 0.025 µg/mL and 0.1 µg/mL, respectively. The content of β-asarone was found to be maximum in the sample collected from Kerala which was 0.2946±0.0152 mg/g. **Conclusion:** The developed method can be recommended for marker-based standardization and quality assurance of *A. calamus* and its formulations.

**Key words:** *Acorus calamus*, Formulations, Rhizome, RP-HPLC, Validation.

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### INTRODUCTION

*Acorus calamus* (*A. calamus*) Linn. (Araceae), commonly known as sweet flag or Bach is a widespread, semi-aquatic, aromatic plant of temperate to sub-temperate regions of Asia, North America and Europe growing along swampy and marshy areas.¹ ³ ⁴ *A. calamus* species exists in diploid (2n = 24), triploid (3n = 36), tetraploid (4n = 48) and hexaploid (6n = 72) varieties,⁴ out of which, the tetraploid variety is found throughout India. It has a lengthy branched underground rhizome from which long, erect, narrow aromatic leaves ascend.¹ ³ Rhizome of *A. calamus* is highly valued medicinal part and is traditionally used as a sedative, behavior modifying, agent anticonvulsant, anti-rheumatic, anti-spasmodic, antibiotic and as a memory booster and is reported to possess acetyl cholinesterase inhibitory, anti-diabetic, cytoprotective, hypolipidemic, bronchodilatory, anti-inflammatory, anti-diarrheal, anti-ulcer and analgesic properties.⁵ ⁴ ¹³ ¹⁴ The high therapeutic relevance, of the plant is principally attributed to the presence of the biomarkers α-asarone and β-asarone.¹³ β-asarone (cis-2,4,5-trimethoxy-1-propenylbenzene) (Figure 1), a sesquiterpenoid, is a major active principle found in oil of the rhizomes, along with a few fatty acids, terpenoids and flavonoids.¹⁵ Many scientists have reported various spectroscopic and chromatographic methods like NMR, TLC, HPTLC, HPLC, GC-MS, LC-MS, etc. for the identification, separation and quantitation of this biomarker from the rhizomes of the plant and other matrices.¹⁶ ⁻¹⁸ However, some of the methods are less sensitive and require the use of derivatizing agent, while other systems though are highly sensitive, they are relatively costly and increasingly complicated while some have long run time. Thus, the present study aims in development of a reliable, cost effective, rapid and validated analytical method for separation and quantification of β-asarone from rhizomes of *A. calamus* on a reversed-phase Cosmosil CN-MS (150×4.6 mm) column with photodiode array (PDA) using the guidelines by ICH. The validated method has also been extended as a quality control tool for various commercial Ayurvedic and herbal formulations of different matrices like Sarasvata Churna, Maanasmithra Vatakam, Khadiradi Gutika, Chandraprabha Bati, Sanjeevani Vati, Mahashankh Bati, Smritisagar Ras, Abana, Vacadi Taila, and Ashwagandharishtha containing the rhizomes of *A. calamus*.

### MATERIALS AND METHODS

**Materials**

*Plant material*

Rhizomes of *A. calamus* were collected from Kerala and authenticated by Tropical Botanic Garden and Research Institute, Kerala (Authentication No. TGBRI/PS/669/2010) and the voucher specimens were also deposited for future reference. The collected material was cleaned; shade dried for ten days and kept in the oven at 45°C for 7 days. Dried rhizomes were powdered in a mixer grinder, sieved through 85 mesh (BSS) sieve and preserved in an air tight container at room temperature. Powder of dried rhizomes was also procured from local market.
was carried out at 304 nm at RT (22 °C). Detection phase Cosmosil C18 (Merck Specialities Private Limited (Mumbai, India). HPLC grade methanol and distilled water was procured from HPLC grade methanol and distilled water was procured from Natural Remedies Pvt. Ltd. Commercial formulations like Sarasvata Churna (Lion, Batch No. 161), Maanasmitra Vatakam (Nagarjunaa, Batch No. OSAV), Khadiradi Vati (Zandu, Batch No. CB007), Chandraprabha Bati (Zandu, Batch No. EH0002), Sanjeevani Vati (Baidyanath, Batch No. 130211), Mahashankh Bati (Baidyanath, Batch No. 130209), Smitisagar Ras (Baidyanath, Batch No. 130182), Abana (Himalaya Herbal Health Care, Batch No. 37400906B), Vacadi Taila (Ayurveda Pratishthan, Batch No. 0-29) and Ashwagandharishtha (Sandu, Batch No. 68) were purchased from local markets. HPLC grade methanol and distilled water was procured from Merck Specialities Private Limited (Mumbai, India).

Sample preparation
Different extraction techniques were employed for extraction of β-asarone depending on the matrix of the sample. Dried rhizome powder of A. calamus was extracted in methanol in the ratio 1:10 (w/v). It was vortexed for 60 s and kept standing for 12 hr. Resultant mixture was filtered through Whatman filter paper no.1 followed by filtration through millipore filter (0.45 µm). Similar procedure was applied for Sarasvata Churna. Tablets of Maanasmitra Vatakam, Khadiradi Guti Ka, Chandraprabha Bati, Sanjeevani Vati, Mahashankh Bati and Abana were crushed using mortar and pestle after removing the outer coat. Powder of the tablets was extracted in methanol in the ratio 0.5: 10 (w/v) for Sanjeevani Vati and 1: 10 (w/v) for other tablets. The further extraction procedure was as mentioned above. In case of oil, Vacadi taila (2.0 mL) was fractionated in methanol (5.0 mL) (v/v) into stoppered conical flask. The mixture was vortexed for 1 min and kept on the shaker for 6 hr at 65 rpm followed by overnight refrigeration at 4°C. Next day, the immiscible organic layer was separated, filtered through Whatman filter paper no.1 followed by filtration through millipore filter (0.45 µm). Water based formulation i.e. Ashwagandharishtha (2.0 mL) was extracted in petroleum ether (5.0 mL). The mixture was vortexed for 1 min and refluxed for 6 hr at 30% heat. The immiscible organic layer was separated and evaporated to dryness in water bath (80°C) and reconstituted with 500.0 µL of methanol. These samples were filtered through millipore filter (0.45 µm) and used for HPLC analysis.

Methods
HPLC analytical conditions
HPLC analysis was performed on JASCO’s HPLC system equipped with PU pumps (HG-1580-31) and a rhodyne injector, a reversed-phase Cosmosil C18 column (15x4.6 mm, 5.0 µm) and PDA detector (MD-1510). Samples were eluted using mobile phase of methanol: distilled water (50: 50, v/v), delivered at a flow rate of 1.0 mL/min. Detection was carried out at 304 nm at RT (22 ±1°C). The injection volume was 20 µL for all runs. Data acquisition and analysis were carried out using Jasco-Borwin Chromatography Software, version 1.5.

Preparation of standard solution
10.0 mg of β-asarone was accurately weighed and transferred to 10.0 mL standard volumetric flask. The content of the flask was initially dissolved in minimum quantity of methanol, followed by sonication and then diluted up to the mark with methanol. A stock solution of the standard with concentration of 1000.0 µg/mL was prepared. Working standard solution was prepared by serial dilution of the standard stock solution. Quality control samples were prepared at three concentrations of the linearity range.

Method Validation
The developed RP-HPLC method was validated as per ICH guidelines in terms of its sensitivity (LOD and LOQ), linearity, assay, accuracy, precision, stability and robustness.

Selectivity and specificity
In specificity study, an ultraviolet scan ranging from 200 to 400 nm in the time window of the analyte was performed using PDA detector with the aim of revealing eventual interfering compounds and evaluating the selectivity of the method. Specificity of the intended method was established by comparing the HPLC retention time and absorption spectra of target peak from the analysed samples with those of the reference compound.

System suitability
System suitability experiment was assessed by injecting five consecutive injections of β-asarone at concentration of 20.0 µg/mL. Values with relative standard deviation (RSD) of ≤ 2% for peak areas and retention time (Rt) were accepted.

Calibration curves
Linearity of β-asarone was determined at seven different equispaced concentrations in triplicate and plotted using linear regression of the mean peak area versus concentration. The linear regression equation was obtained using a least-square method and used to estimate the concentration of the reference compound in the analysed samples.

Sensitivity
LOD and LOQ were estimated by measuring the signal-to-noise ratio (S/N). Stock solution of the reference standard was serially diluted with methanol to prepare the series of samples with low concentrations and injected into the HPLC system. LOD and LOQ was considered at S/N of 3:1 and 10:1, respectively.

Precision
Intra-day precision was evaluated from replicates analysis (n=3) of the three quality control samples on the same day while inter-day precision was assessed by analyzing them on three consecutive days in triplicate (n=3). Accuracy values within the range of 85–115% and % RSD of ≤ 2 were considered as the acceptance criteria.

Stability
Long-term stock solution stability of β-asarone was tested at 4°C and samples were analyzed in triplicate after 15 days. Same samples in triplicate were also subjected to bench top and short-term stability testing at 0.0 h and 2.0 h at RT, and 0.0 h and 6.0 h at 4°C respectively. Values within a difference range of ±5% were accepted.

Accuracy
The accuracy of the method was evaluated by measuring the recovery of β-asarone using the standard additions method. Quality control samples...
at three concentrations were added to the known amount of *A. calamus* rhizome, extracted and analyzed. Each set of additions was repeated 3 times at each level. The results were calculated using the formula: Recovery (%) = [(amount found−original amount)/amount added] × 100 and expressed as the percentage of analyte recovered. Values within the range of 95-110% were accepted.

**Robustness**

Robustness of the method was assessed by deliberately modifying the experimental conditions in terms of four factors such as analyst (analyst 1 and 2), batch of column (columns of different lot/batch from same manufacturer (Cosmosil, K64005 and K65113)), mobile phase composition (methanol (50.0 ± 1); distilled water (50.0 ± 1), v/v) and flow rate (1.0 ± 0.1 mL/min). The chromatographic variations were evaluated by analyzing the effect on peak areas and R of the three QC samples of β-asarone in triplicate. The results were expressed in terms of % mean difference. Values within a difference range of ±5% were accepted.

**Estimation of β-asarone from the samples and formulations of *A. calamus***

Relative retention time and relative peak area of each characteristic peak from the samples of *A. calamus* and its formulations related to the peak from β-asarone were calculated for quantitative expression of the chemical properties in the chromatographic pattern of *A. calamus* using regression equation.

**Statistical evaluation**

Microsoft Excel 2007 was used to determine mean, standard deviation, relative standard deviation and mean difference during the analysis.

**RESULTS AND DISCUSSION**

Plant constituents vary considerably depending on several factors like temperature, light, drying, packing, storage etc. which may impair not only the quality of phytotherapeutic agents but also their therapeutic value. Thus, standardization of raw material and the herbal preparations needs to be permanently carried in terms of quality specification, stability profiles and chemical analysis of analyte of interest using sensitive validated analytical methods.

HPLC is a unique, versatile, universal and well recognized tool for qualitative and quantitative evaluation of herbal products against their respective bioactive molecules in terms of quality and batch-to-batch reproducibility. Thus, in this contribution we have developed a simple, cheap and rapid chromatographic method using RP-HPLC for the estimation of β-asarone from the rhizome of *A. calamus* and various traditional and herbal formulations of different matrices.

**Method validation**

β-asarone was detected and quantified by RP–HPLC, using methanol and distilled water (50:50, v/v) as the mobile phase. β-asarone got separated showing a sharp peak at a retention time of 5.0 ± 0.05 min under optimized chromatographic conditions at wavelength maxima of 304 nm. The representative chromatogram and spectra is depicted in Figure 3. The separation of the marker compound in short time reduced the run length which enabled the rapid analysis of the samples. Thus, the method can be adapted by various industries for analysis of multiple samples in less time. The calibration curve of standard β-asarone showed good linearity relationship in the specified concentration range (0.25-200 µg/mL) with a correlation coefficient (r²) greater than 0.99 (Figure 2). The LOD and LOQ were found to be 0.025 µg/mL and 0.1 µg/mL, respectively, thus suggesting a high sensitivity of the method which can be successfully exploited for quantifying even low sample concentrations of β-asarone. The RSD for system suitability in terms of R and area were found to be less than 2% indicating the suitability of the method. The % RSD of inter- and intra-day analysis of standard and extract were also found to be less than 2 with a high repeatability in the R and response. As there was no significant difference in the inter- and intra-day analysis it indicates that the proposed method is very suitable for the analysis. Mean recovery for the quality control samples of β-asarone was found to be within the acceptance limit of >95% (97.4-101.23%) (Table 2). Stability of standard solution were checked for bench top (2 hr), short term (6 hr) as well as for long term (15 days) and the standard was found to be stable at these conditions. The results of evaluated parameters are given in Table 1. The method was also found to be robust for various parameters like change in analyst, batch of column, flow rate and mobile phase composi-

![Figure 2: Linearity plot for β-asarone](image-url)

**Figure 2: Linearity plot for β-asarone**

**Table 1: Results of validation of β-asarone using RP-HPLC technique in terms of linearity, sensitivity, system suitability, precision**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Linearity (n=3)</th>
<th>Sensitivity</th>
<th>System suitability (% RSD, n=5)</th>
<th>Precision (% RSD, n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration range (µg/mL)</td>
<td>0.25-200.0</td>
<td>LOD (µg/mL)</td>
<td>LOQ (µg/mL)</td>
<td>Area</td>
</tr>
<tr>
<td>Correlation coefficient (r²)</td>
<td>0.999</td>
<td>0.025</td>
<td>0.1</td>
<td>5.03</td>
</tr>
<tr>
<td>Regression equation</td>
<td>y=10338x +116886</td>
<td></td>
<td></td>
<td>2189837.62</td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>6278.71</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R (min)</td>
<td>1.14-1.73</td>
<td>Intra-day</td>
<td>0.78-1.98</td>
<td></td>
</tr>
<tr>
<td>Area</td>
<td>1.69-1.96</td>
<td>Inter-day</td>
<td>1.06-1.99</td>
<td></td>
</tr>
<tr>
<td>R</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


**Table 2: Recovery of β-asarone from the sample**

<table>
<thead>
<tr>
<th>Amount spiked (µg/mL)</th>
<th>Concentration after spiking (µg/mL)</th>
<th>Amount recovered (µg/mL)</th>
<th>% Recovery</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.35</td>
<td>30.35</td>
<td>29.56</td>
<td>97.4</td>
<td>1.11</td>
</tr>
<tr>
<td>7.5</td>
<td>37.5</td>
<td>37.14</td>
<td>99.04</td>
<td>1.54</td>
</tr>
<tr>
<td>160</td>
<td>190</td>
<td>183.407</td>
<td>96.53</td>
<td>0.87</td>
</tr>
</tbody>
</table>

RSD: Relative standard deviation
tion as the values obtained were within the acceptance limits (Table 3). Thus, the described HPLC method represents a reliable procedure for detection, separation and quantitation of β-asarone.

**Assay**

The validated method was further employed for separation and quantitation of β-asarone from the rhizome of *Acorus calamus* collected from Kerala and powder procured from the market to evaluate the variation in terms of the marker content. Chromatogram of the plant samples showed sharp peak for β-asarone at R$_t$ 5.02 and 5.04 min which was comparable with the standard β-asarone. Figure 3 demonstrates the clear separation of β-asarone. The content of β-asarone in the sample collected from Kerala was found to be higher than the marketed sample by 83.23%. The representative chromatograms and values are given in Figure 3 and Table 4 respectively.

**Method application**

With exponential increase and demand of Ayurvedic and herbal remedies for treatment of various ailments, validation is needed for identification, purity, stability data and scientific based evidence about efficacy of the formulations to produce results which are reliable, accurate and reproducible. The validated method was also extended to Ayurvedic, Siddha and herbal formulations of varied complex matrices like vati, churna, taila and arishtha which employ the rhizomes of *A. calamus* in either pure form, mixtures or water and alcoholic extracts. The observations revealed that the method successfully separated the biomarker from all the formulations suggesting the use of appropriate extraction technique and applicability of the method. The representative chromatograms for four formulations viz. Sarasvata Churna, Sanjeevani Vati, Ashwagandharishta and Vacadi Taila are depicted in Figure 3. Least content of β-asarone was found in Ashwagandharishta and maximum in Sanjeevani Vati, highlighting the evident matrix effect in water based formulation as compared to the solid ones. The content of β-asarone was found to be more in all the tested samples when compared with the expected label claim values. This may be owing to the presence of the marker in other plant ingredients in addition to *A. calamus* rhizome. Lesser content of β-asarone than the expected value was observed in Maanasmithra Vatakam which might be due to the reason of poor efficiency of extraction of the marker from the matrix (Table 4).

**Table 3: Results of robustness for β-asarone by variation in analyst, batch of column, flow rate and mobile phase composition**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Change</th>
<th>%RSD (n=3)</th>
<th>% Mean difference</th>
<th>%RSD (n=3)</th>
<th>% Mean difference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Analyst</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.11-1.85</td>
<td>0.93-1.72</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.18-1.79</td>
<td>1.02-1.29</td>
<td>-1.18-2.67</td>
<td>-0.03-(-4.72)</td>
<td></td>
</tr>
<tr>
<td><strong>Column</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K60005</td>
<td>0.69-1.67</td>
<td>1.38-1.74</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>K65113</td>
<td>0.46-1.67</td>
<td>0.99-1.52</td>
<td>0.40(-1.46)</td>
<td>-3.00-4.54</td>
<td></td>
</tr>
<tr>
<td>0.9 mL/min</td>
<td>1.00-1.99</td>
<td>0.75-1.40</td>
<td>-3.56-4.10</td>
<td>-1.84-2.67</td>
<td></td>
</tr>
<tr>
<td><strong>Flow rate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 mL/min</td>
<td>0.43-0.89</td>
<td>0.40-1.54</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>1.1 mL/min</td>
<td>0.93-1.63</td>
<td>0.97-1.85</td>
<td>-1.56-3.30</td>
<td>-2.75-1.35</td>
<td></td>
</tr>
<tr>
<td>49:51 (v/v)</td>
<td>1.01-1.50</td>
<td>0.60-1.23</td>
<td>-3.05-1.45</td>
<td>-1.67-2.72</td>
<td></td>
</tr>
<tr>
<td>51:49 (v/v)</td>
<td>1.36-1.88</td>
<td>0.93-1.67</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td><strong>Mobile phase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50:50 (v/v)</td>
<td>1.00-1.46</td>
<td>1.42-1.66</td>
<td>-46-3.91</td>
<td>-0.31-2.55</td>
<td></td>
</tr>
<tr>
<td>51:49 (v/v)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

RSD: Relative standard deviation.

**Table 4: Results of assay and method application for β-asarone**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Content of β-asarone (mg/g or mg/mL)</th>
<th>Mean ± SD, n=3</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acorus calamus</em> rhizome (Kerala)</td>
<td>0.2946 ± 0.0152</td>
<td></td>
</tr>
<tr>
<td><em>Acorus calamus</em> rhizome (Marketed)</td>
<td>0.0494 ± 0.0133</td>
<td></td>
</tr>
<tr>
<td>Sarasvata Churna</td>
<td>0.1802 ± 0.0325</td>
<td></td>
</tr>
<tr>
<td>Mahashankh Bati</td>
<td>0.0173 ± 0.0034</td>
<td></td>
</tr>
<tr>
<td>Khadiradi Gutika</td>
<td>0.0022 ± 0.0001</td>
<td></td>
</tr>
<tr>
<td>Chandraprabha Bati</td>
<td>0.0194 ± 0.0014</td>
<td></td>
</tr>
<tr>
<td>Smritisagar Ras</td>
<td>0.0015 ± 0.0003</td>
<td></td>
</tr>
<tr>
<td>Maanasmithra Vatakam</td>
<td>0.0090 ± 0.0016</td>
<td></td>
</tr>
<tr>
<td>Abana</td>
<td>0.0203 ± 0.0094</td>
<td></td>
</tr>
<tr>
<td>Sanjeevani Vati</td>
<td>0.3902 ± 0.0265</td>
<td></td>
</tr>
<tr>
<td>Vacadi Taila</td>
<td>0.0327 ± 0.0121</td>
<td></td>
</tr>
<tr>
<td>Ashwagandharishta</td>
<td>0.0008 ± 0.00001</td>
<td></td>
</tr>
</tbody>
</table>

SD: Standard deviation.
Thus, the developed method has wide range of applications and can be easily applied for analysis of various matrices.

**CONCLUSION**

The described RP- HPLC method represents a reliable procedure for detection, separation and quantitation of β-asarone which has been fully validated as per ICH guidelines and offered adequate accuracy, sensitivity and stability. Thus, the developed method can be successfully applied to have a routine quality check of the plant extracts, phytopharmaceuticals or multiherb combinations containing A. calamus rhizome which will aid in their standardization. The rhizomes of A. calamus are widely used as an ingredient in various formulations and thus are in great demand. Due to this, there are increasing chances of some substitutes or adulterants being used in place of the plant. The developed method may aid in identifying the presence of adulterants and confirming the authenticity of the plant material used. The application of this method to other traditional and herbal formulations will possibly present abundant extended opportunities for checking their authenticity thus facilitating the escalation of their globalization and increased acceptance with patients and physicians.

**ABBREVIATION USED**


**REFERENCES**


**SUMMARY**

- RP-HPLC method was developed for estimation and quantitation of bioactive marker, β-asarone.
- The developed method was validated as per ICH guidelines in terms of sensitivity, accuracy, system suitability, stability, linearity, ruggedness and precision.
- The validated method was applied for the separation and quantitation of β-asarone from rhizome of Acorus calamus from various regions and from traditional and herbal formulations employing rhizome of Acorus calamus as one of its ingredients.
- Highest content of β-asarone was found in the rhizome collected from Kerala (0.2946 ± 0.0152 mg/g).
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PICTORIAL ABSTRACT

ABOUT AUTHOR

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