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# Determination of Ethanol Content in Few Asava and Arista Dosage Forms by UV-Visible Spectroscopy

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**Abstract:** Ayurveda has a long and strong heritage use of herbal drugs and formulations to treat various diseases. It comprises various types of herbal medicines including the fermented forms namely asavas (fermented infusions) and aristas (fermented decoctions). These are considered as valuable therapeutics, thanks to their efficacy and desirable features. The present work describes, a novel UV-Visible spectrophometric method and validated for the determination of the ethanol content in few herbal dosage forms asavas and arishtas. The assay method used was the standard calibration curve method. The method consists of a color reaction of ethanol with potassium dichromate. The colorimetric quantification was based on the formation of chromate ions resulting from the oxidation of ethanol and potassium dichromate. The absorbance maxima of the chromate ions were found to be 578 nm.

**Keywords:** Asava; Arista; Ethanol; UV-Visible Spectroscopy

## 1. Introduction

In modern days, though the world is witnessing science taking quick strides towards modern medicine, the majority of the global population are rediscovering the traditional medicine systems to avoid side effects yielded with modern medicine. Ayurveda is one among those systems of medicines with several thousands of years of history developed through daily life experiences with mutual relationship between mankind and nature showing good efficacy and much safety than modern medicine, this leads to a resurgence of the ayurvedic system to limelight in the medical world. Finished herbal products are presented in various dosage forms like decoctions, herbal powders, alcoholic beverages, capsules, tablets, ointments, and creams.[1]

Asava (fermented infusion) and arista (fermented decoction) amid various ayurvedic dosage forms have a high acceptance rate due to their easy palatability, accelerated therapeutic action, and enhanced drug concentration.[2] Formulation of these dosage forms includes usage of jaggery or sugar solution for solubility of drug either in powder form or in form of decoction for a specified period during which it undergoes a process of fermentation generating alcohol. Thus, facilitating the extraction of active principles contained in the drugs. The alcohol generated also serves as a preservative.[3] Patient compliance of asavas and aristas made them desirable for prolonged consumption. Though self-generated alcohol employs long shelf life, it is limited to 5-12% to avoid the risk of misuse, overdose, addiction, and toxicity.[4] Therefore the present work was executed to check the volume of self-generated ethanol in selected asavas and aristas. Commercially available asavas and aristas like Ashokarista, Balarista, Chandanasava, Kumariasava, and Pippalayasava were selected and analyzed for self-generated alcohol content. Literature survey revealed that all the five asavas and aristas used in this work were not estimated in a common method instead they have been estimated in individual methods.[5-18] They are brownish liquids due to the presence of complex phytoconstituents such as tannins. Separation and assessment of alcohol content from the complex ayurvedic dosage forms are achieved with the combination of two simple methods: distillation and UV-Visible spectroscopy.[19] Solvent extraction method was employed to isolate alcohol from asavas and aristas. The extracted alcohol is oxidized with potassium dichromate and the resulting chromate ions were determined through the measurement of absorbance at 578 nm.[20]

## 2. Material and Methods

Traditionally prepared Ashokarista, Balarista, Chandanasava, Kumariasava, Pippalayasava were procured as free samples from Sri Venkateshwara Ayurvedic Hospital, Tirupati, AP, India. Other reagents were purchased from commercial supplier's potassium dichromate (Himedia laboratories), sulphuric acid and absolute ethanol (Merck chemicals). Distilled water was an in-house production at Sri Padmavathi School of Pharmacy.

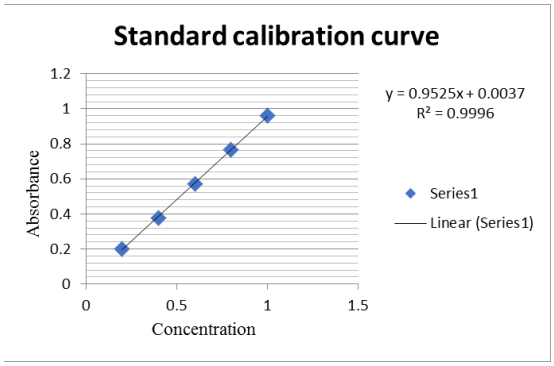



**Fig. 1.** Standard Calibration Curve of Absolute Ethanol

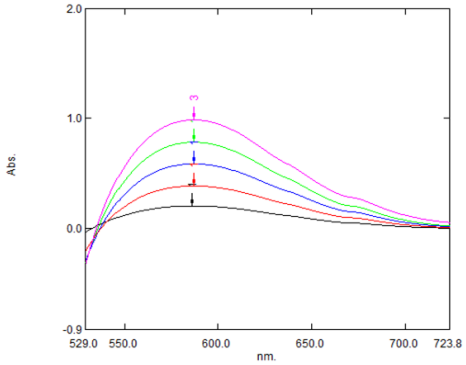



**Fig. 2.** Spectra of Calibration Curve

*2.1. Solvent extraction by distillation*[21]

Distillation apparatus was set up and 50 ml of the herbal dosage form was transferred accurately into the round bottom flask and heated at 600°C for 30 min. Then the product obtained was measured and stored in an amber colored bottle.

*2.2. Reagent preparation*[22]

Potassium dichromate solution was prepared by the adding 3.4 g of potassium dichromate with 32.5 ml of sulphuric acid and finally made up to 100 ml with distilled water.

*2.3. Stock solution and aliquots*

The stock solution concentration was maintained at 20 mg/ml. The aliquots were taken from the range of 0.2 to 1.0 mg/ml.

*2.4. Dichromate oxidation*

To prepare an aliquot of 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml, 0.1, 0.2, 0.3, 0.4 and 0.5 ml of stock solution and 5 ml of potassium dichromate solution were added to a 10 ml volumetric flask and the final volume was made up with distilled water and the same goes for the herbal dosage forms by taking 0.1 ml of the respective distilled product. The prepared aliquots and samples were shaken and kept for incubation (heating for 30 min at 60°C). After the incubation period, absorbance was checked at 578nm using Shimadzu UV-1800 UV Spectrophotometer. The absorbances of the aliquots were inserted in the standard calibration curve and by using the standard calibration curve the concentration of alcohol in the distilled products was calculated.

**Table 1.** Absorbances of Absolute Ethanol

| Concentration | Absorbance |
|---|---|
| 0.2 mg/ml | 0.202 |
| 0.4 mg/ml | 0.377 |
| 0.6 mg/ml | 0.572 |
| 0.8 mg/ml | 0.764 |
| 1 mg/ml | 0.961 |

**Table 2. Alcohol content of Herbal Samples**

| Samples | Absorbance | Ethanol Content (mg/ml) |
|---|---|---|
| Ashokarista | 0.850 | 0.9304 |
| Balarista | 0.665 | 0.6942 |
| Chandanasava | 0.466 | 0.4853 |
| Kumariasava | 0.542 | 0.5651 |
| Pippalayasava | 0.568 | 0.5924 |

*2.5. Method validation*[23-24] *(ICH Q2R1)*

*2.5.1. Linearity*

The linearity of the analytical procedure is its ability (within the given range) to obtain test results which are directly proportional to the concentration (amount) of an analyte in the sample.

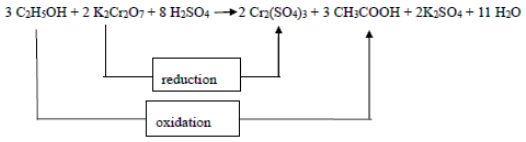
*2.5.2. Accuracy*

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value as an accepted reference value and value found.

*2.5.3. Precision*

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision maybe considered at three levels: repeatability, intermediate precision and reproducibility. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variations of a series of measurements.

**Reaction:**




$C_2H_5OH$ –Ethanol, $K_2Cr_2O_7$ –Potassium dichromate
$H_2SO_4$ –Sulphuric acid, $Cr_2(SO_4)_3$ –Chromium (III) sulphate

## 3. Results and Discussions

Instigating our study with the apprehension of the color reaction of ethanol with the potassium dichromate in the presence of sulphuric acid, we have developed a method, with which the ethanol content in asava and arista herbal dosage forms can be estimated. The results recorded in Table 1 are the absorbances of standard alcohol, which is commercially available absolute ethanol. The standard calibration curve plotted by the absorbances of the aliquots after oxidation has a

**Table 3.** Accuracy

| S.No | Sample | Level | Standard added (mg/ml) | Test added (mg/ml) | Amount Found | % Recovery |
|---|---|---|---|---|---|---|
| 1 | Ashokarishta | 50% | 0.2 | 0.5 | 0.441 | 88.25% |
| | | 100% | 0.4 | 0.5 | 0.447 | 89.4% |
| | | 150% | 0.6 | 0.5 | 0.478 | 95.60% |
| 2 | Balarishta | 50% | 0.2 | 0.5 | 0.504 | 100.8% |
| | | 100% | 0.4 | 0.5 | 0.523 | 104.6% |
| | | 150% | 0.6 | 0.5 | 0.528 | 105.6% |
| 3 | Chandanasava | 50% | 0.2 | 0.333 | 0.356 | 106.9% |
| | | 100% | 0.4 | 0.333 | 0.383 | 115% |
| | | 150% | 0.6 | 0.333 | 0.394 | 118.3% |
| 4 | Kumariasava | 50% | 0.2 | 0.26 | 0.261 | 100.38% |
| | | 100% | 0.4 | 0.26 | 0.274 | 105.3% |
| | | 150% | 0.6 | 0.26 | 0.284 | 109.2% |
| 5 | Pippalayasava | 50% | 0.2 | 0.41 | 0.397 | 96.8% |
| | | 100% | 0.4 | 0.41 | 0.418 | 101.9% |
| | | 150% | 0.6 | 0.41 | 0.426 | 103.9% |

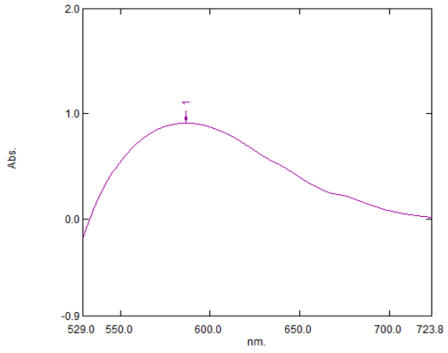



**Fig. 3.** Spectrum of Ashokarishta distilled product

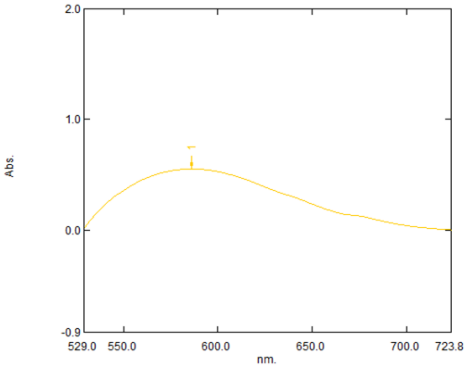



**Fig. 6.** Spectrum of Kumariasava distilled product

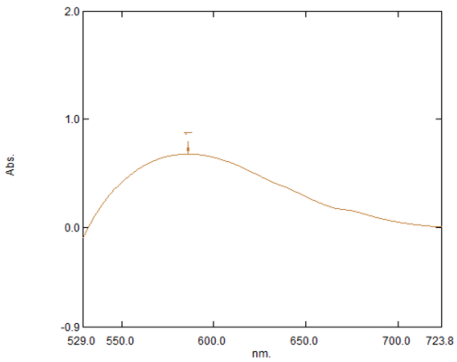



**Fig. 4.** Spectrum of Balarishta distilled product

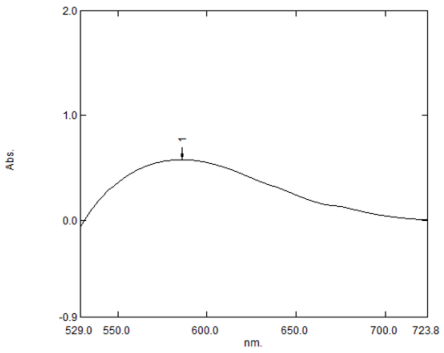



**Fig. 7.** Spectrum of Pippalayasava distilled product

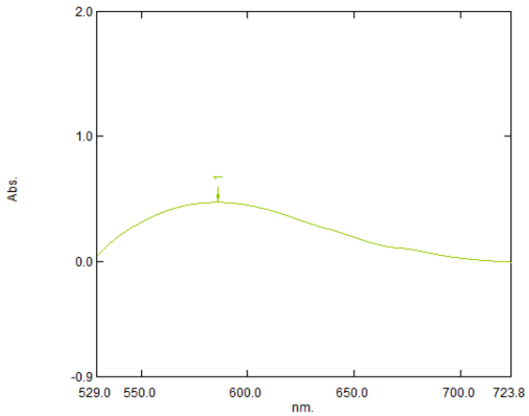



**Fig. 5.** Spectrum of Chandanasava distilled product

correlation constant (R2) of 0.9996 with the equation of line Y = 0.9525x + 0.0037 and was shown in Fig. 1. The spectra of calibration curve were given in Fig. 2. The absorbances and alcohol content of five herbal dosage forms used in this present study were reported in Table 2. The spectra of each dosage form were displayed in Figs. 3-7.

The alcohol content in Ashokarista was found to be relatively high and in Chandanasava it was relatively low. The concentration of ethanol found in all the five commercial herbal products lies within the limits as prescribed by AYUSH. Asava and arista dosage forms consists of sugars and nitrogen rich contents, which make them more prone to microbial growth but the ethanol produced by the self-fermentation helps to prevent microbial attack and improves the stability of these herbal formulations. Hence, presence of ethanol in these formulations increases their shelf life.[25]

$$Percent\ Recovery = \frac{Calculated\ Quantity\ of\ test\ samples}{Weight\ of\ test\ sample(\mu g/ml)} \times 100$$

**Table 4.** Intraday precision

| S.no | Samples | Absorbance at 578nm | | | Mean | % RSD |
|---|---|---|---|---|---|---|
| | | trial 1 | trial 2 | trial 3 | | |
| 1 | Ashokarishta | 0.096 | 0.097 | 0.097 | 0.096 | 0.597 |
| 2 | Balarishta | 0.616 | 0.618 | 0.618 | 0.617 | 0.187 |
| 3 | Chandanasava | 0.749 | 0.750 | 0.752 | 0.750 | 0.203 |
| 4 | Kumariasava | 0.403 | 0.404 | 0.404 | 0.403 | 0.143 |
| 5 | Pippalayasava | 0.579 | 0.579 | 0.579 | 0.579 | 0 |

**Table 5.** Inter day precision

| S.no | Samples | Absorbance at 578nm | | | Mean | % RSD |
|---|---|---|---|---|---|---|
| | | Day 1 | Day 2 | Day 3 | | |
| 1 | Ashokarishta | 0.901 | 0.901 | 0.901 | 0.901 | 0 |
| 2 | Balarishta | 0.803 | 0.804 | 0.804 | 0.803 | 0.071 |
| 3 | Chandanasava | 0.749 | 0.750 | 0.752 | 0.749 | 0.077 |
| 4 | Kumariasava | 0.431 | 0.432 | 0.432 | 0.431 | 0.133 |
| 5 | Pippalayasava | 0.639 | 0.640 | 0.640 | 0.639 | 0.090 |

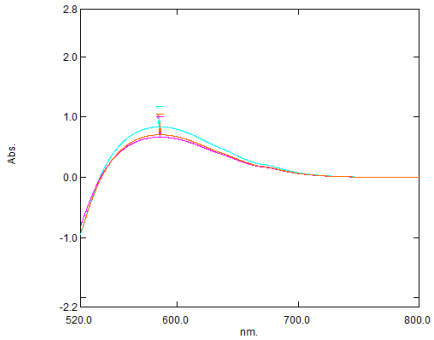



**Fig. 8.** Accuracy overlay spectra of Ashokarishta distilled product

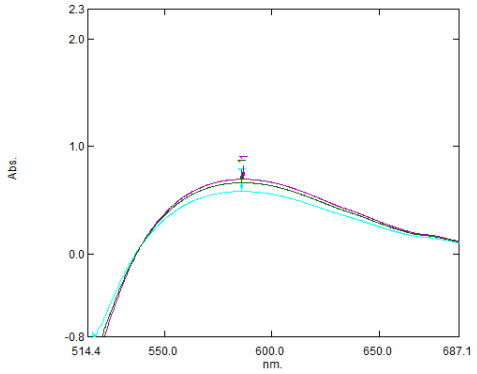



**Fig. 9.** Accuracy overlay spectra of Balarishta distilled product

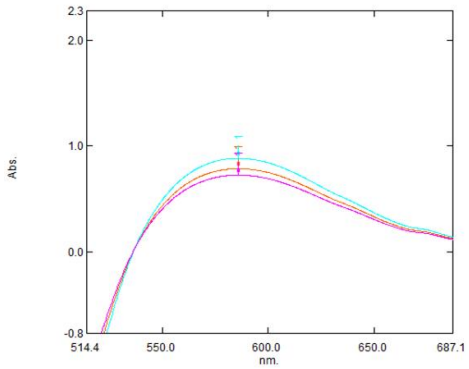



**Fig. 10.** Accuracy overlay spectra of Chandanasava distilled product

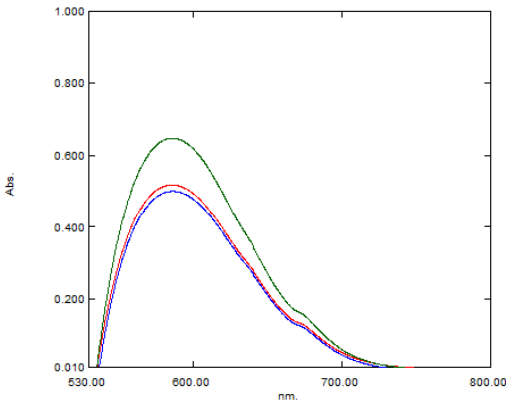



**Fig. 11.** Accuracy overlay spectra of Kumariasava distilled product

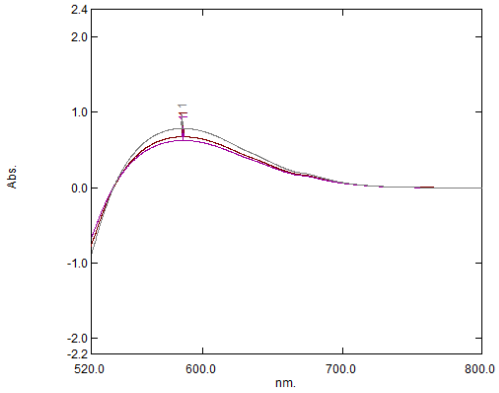



**Fig. 12.** Accuracy overlay spectra of Pippalayasava distilled product

### *3.1. Linearity*

In this experiment graph between concentration and absorbance of the respective asavas and aristas was plotted and a correlation coefficient (0.9996) was found.

### *3.2. Accuracy*

Accuracy of this study was investigated by standard addition technique and the recovery range was found to be in accordance with ICH guidelines (Table 3). Recovery range of Ashokarista was less compared to other herbal products used in the work, due to the presence of high quantity of complex sugars (jaggery and dhataki). The accuracy overlay spectrums of herbals dosage forms are given from Figs. 8-12.

### *3.3. Precision*

Inter-day and intraday precision for Tri linear regression analysis method was calculated in terms of %RSD (Relative Standard Deviation). The experiment was repeated three times in a day for intraday and on three different days for inter-day, the values confirmed the precision of the method. Every trial had shown reproducible results. Table 4 shows intraday precision, the %RSD for the herbal dosages lies within the acceptance criteria. Whereas, Table 5 gives information about inter-day precision, and the %RSD was within the range prescribed.

The method validation provides the documentary evidence for the developed method.

## 4. Conclusions

Based on the literature works reviewed, a novel UV-Visible spectrophometric method was developed and validated for the determination of the ethanol content in few herbal dosage forms of asavas and aristas. The assay method used was the standard calibration curve method. The distilled products that are obtained after distillation of herbal dosage forms have shown similarities in the basic parameters (density, viscosity, surface tension, and pH) with that of the standard absolute ethanol (99.9%). The absorbances of the ethanol distillate from the herbal dosage forms were compared with that of standard calibration curve and the concentration of ethanol was calculated. As per AYUSH guidelines,[26] the self-generated ethanol content in asava and arista formulations should not exceed 12%v/v and none of the dosage forms had exceeded the limit. Hence, this study concludes that there will be no alterations in the pharmaceutical and therapeutic applications of these dosage forms as the ethanol was in its limits. The method used in this study is validated as per the ICH Guidelines (Q2R1) and can be used for regular analysis.

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## Conflicts of Interest

The authors declare no conflict of interest.

## References

1 WHO Guidelines on Good Herbal Processing Practices for Herbal Medicines, Annex 1, 2018, 83. [Link]
2 Das C.; Ghosh G.; Das D. Ayurvedic Liquid Dosage Form Asava and Arista: An Overview. *Indian. J. Pharm. Edu. Res.*, 2017, **51**, 169-76. [Link]
3 Mendhe B.B.; Gupta P.; Mhaske S.; Shrivastava S. Evaluation of Ayurvedic Marketed Formulations Asava's and Arista's. *Res. J. Pharm.,* 2010, **3**, 915-916. [Link]
4 Selvan P.S.; Priya E.S. Determination of Ethanol Content in Ayurvedic Formulations Kumaryasava and Mustakarista by Gas Chromatography. *Indian J. Pharm. Sci.*, 2015, **77**, 120. [Link]
5 Ayurvedic Pharmacopoeia of India, Part I, Second Edition, 2008, p.34, 46, 56, 62, 75, 78.
6 Elamthuruthy A.T.; Shah C.R.; Khan T.A.; Tatke P.A.; Gabhe S.Y. Standardization of Marketed Kumariasava—An Ayurvedic Aloe Vera Product. *J. Pharm. Biomed. Anal.*, 2005, **37**, 937-941. [CrossRef]
7 Wadher S.J.; Puranik M.; Yeole P.G.; Lokh C.S. Determination of Ethanol in Abhayarishta by Gas Chromatography. *Indian J. Pharm. Sci.*, 2007, **69**, 152. [Link]
8 Parihar S.S.; Mishra S.K.; Himmat S.; Ambrish R. Standardization of Ashokarista Formulation by TLC Method. *Int. J. PharmTech Res.*, 2010, **2**, 1427-1430. [Link]
9 Shankar K.; Amritpal S. Standardization of Polyherbal Ayurvedic Formulation: Chandanasava. *Int. J. Res. Ayurveda Pharm*., 2011, **2**, 665-669. [Link]
10 Vora D.K.; Banerjee S.K.; Chhabra G.S. Method Development for Estimation of Alcohol in Ayurvedic Formulations using Gas Chromatography. *Bull. Pharm. Res.*, 2012, **2**, 34-37. [Link]
11 Kumar T.; Larokar Y.K.; Jain V. Standardization of Different Marketed Brands of Ashokarishta: An Ayurvedic Formulation. *J. Sci. Innov. Res.*, 2013, **2**, 993-998. [Link]
12 Chumbhale D.S.; Chaudhari S.R.; Upasani C.D. Standardization of Marketed Kumari Asava-A Polyherbal Ayurvedic Formulation. *Int. J. Pharma. Chem. Bio. Sci.*, 2014, **4**, 681-85. [Link]
13 Vinothkanna A.; Mariappan S.; Sekar S. Tracking the Organoleptic and Biochemical Changes in the Ayurvedic Polyherbal and Native Fermented Traditional Medicines: Balarishta and Chandanasava. *Int. J. Pharm. Pharm. Sci.*, 2014, **6**, 521-26. [Link]
14 Khan T.A.; Goheli A.K.; Mallya R.A.S.H.M.I. Standardisation and HPTLC Method Development of Marketed Ayurvedic Formulation—Balarishta. *Int. J. Pharm. Pharm. Sci.*, 2016, **8**, 201-209. [Link]
15 Mohini K.; Mohini U.; Ashwini C. Preliminary Studies of some Marketed Ayurvedic Preparations of Asavas and Arishtas. *Int. Res. J. Pharm*., 2017, **8**, 18-20. [Link]
16 Bahuguna Y.B.Y.; Kothiyal P.K.P.; Mehra S.M.S. Standardization and evaluation of marketed Kumari Asava of Baidyanath. *Int. J. Indig. Herbs Drugs*, 2017, 21-28. [Link]
17 Deepika S.; Rao S.; Valsan S.; Swati R. An Analytical Study on Pippalyasava. *J. Ayurveda Integr. Med. Sci.*, 2018, **3**, 57-62. [CrossRef]
18 Das C.; Ghosh G.; Bose A.; Das D. Analytical Methods for Standardization of Ayurvedic Asavas and Aristas: A Review. *Indian J. Pharm. Sci.*, 2019, **81**, 396-405. [Link]
19 Sayyad S.F.; Randive D.S.; Jagtap S.M.; Chaudhari S.R.; Panda B.P. Preparation and Evaluation of Fermented Ayurvedic Formulation: Arjunarishta. *J. Appl. Pharm. Sci.*, 2012, **2**, 22. [Link]
20 Sumbhate S.; Nayak S.; Goupale D.; Tiwari A.; Jadon R.S. Colorimetric Method for the Estimation of Ethanol in Alcoholic-Drinks. *J. Anal. Tech.*, 2012, **1**, 1-6. [Link]
21 The Ayurvedic Pharmacopoeia of India, Part-II, Volume-II, First Edition, 2008.
22 Sayyad S.A.F.; Chaudhari S.R.; Panda B.P. Quantitative Determination of Ethanol in Arishta by using UV-visible Spectrophotometer. *Pharm. Biol. Eval.*, 2015, **2**, 204-207. [Link]
23 Validation of Analytical Procedures: Methodology, ICH Harmonized Tripartite Guideline. 1995, EMEA.
24 Analytical Procedures and Method Validation for Drug and Biologics (Guidance for Industry), USFDA, 2015.
25 Gupta A.; Jaiswal M.; Pradeep Prajapati K. Shelf Life of Ayurvedic Dosage Forms-Traditional View, Current Status and Prospective Need. *Indian J. Traditi. Knowl*., 2011, **10**, 672-677. [Link]
26 Regulatory requirements for Ayurvedic, Siddha and Unani system of medicines.