



Research Article

Estimation of Naturally Produced Water-Soluble Vitamins in Different Asavas and Arishtas Using Liquid Chromatography

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ABSTRACT

Self fermented polyherbal Ayurvedic preparations are called Asavas and Arishtas and are efficacious classical preparations. Insignificant efforts have been made to delve deep into scientific rationale of this ancient dosage form of Ayurveda. An investigative analysis using reversed phase chromatography was conducted to evaluate and estimate the presence of water-soluble vitamins in seven Asavas and Arishtas namely Drakshasava, Ashokarishta, Dashmulrishta, Khadirarishta, Lauhasava, Arjunarishta and Ashwagandharishta. The study revealed that these preparations contained water soluble vitamins B₁, B₂, B₃ & B₆ in significant concentrations. Reversed phase high performance liquid chromatography was carried out on an Agilent 1200 DAD system. Separation was carried out on an Inertsil ODS 3 V column (250 mm x 4.6 mm, 5 µm) at wavelength of 265 nm.

Keywords: Water- soluble vitamins, HPLC, Reversed phase chromatography, Asavas and Arishtas

1. Introduction

Although the past decade has seen a paradigm shift in consumer's inclination from western medicines to herbal medicines, yet there is a segment which is skeptical about using classical ayurvedic preparations due to lack of enough documentation to establish their safety and efficacy (Patwardhan et al., 2006).

Asava and *Arishtas* are unique dosage form contributed by Ayurveda and are supposed to have indefinite shelf life. In terms of current understanding, this phrase assumes more importance because this dosage form has an inherent attribute of continuous hydro-alcoholic extraction and probably formation of natural analogues of the chemical compounds present in the medicinal plants (Sekar and Mariappan, 2008). Flowers of *Woodfordia fruticosa* are used for fermentation to produce self generated alcohol in *Asavas* and *Arishtas* (Krores et al., 1993). These flowers contain yeast. Since water-soluble vitamins are also produced from yeast, it was hypothesized that *Asavas* and *Arishtas* may also contain vitamins. *Asava* and

Arishtas are used as natural supplements / tonic for boosting general health through ayurveda. The present study was undertaken in this background (API, 2003). Since the process of fermentation also results in generation of vitamins, the same principle was correlated with their efficacy by comparing them with the recommended dose of vitamins for a healthy living.

Considering HPLC as a powerful tool in the analysis of complex matrix, the vitamin analysis was carried out simultaneously using a simple and rapid HPLC separation method (Sharma et al., 2011).

2. Materials and methods

Vitamins B₁, B₂, B₃, B₅ & B₆ were obtained from Sigma-Aldrich. HPLC grade Methanol was obtained from Merck (Darmstadt, Germany). AR (Analytical Reagent) grade triethylamine, pentane sulphonic acid sodium salt & acetic acid were obtained from Qualigens (Mumbai, India). Seven samples of Dabur *Asava* and *Arishtas* were procured from local market.

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2.1. Mobile Phase

Water, Methanol & Acetic acid in the ratio of 73:26.5:0.5, with 1.92g Pentane sulphonic acid & 0.9 ml triethylamine were used in the study. Mobile phase solution were filtered with a 0.45 μ m membrane filter paper utilising a millipore filtration set and subsequently degassed for 15 minutes with an ultrasonic bath. The column was washed with at least 100 to 150 ml of mobile phase and then equilibrated at a flow rate of 1 ml per minute before injecting the standard mixture.

2.2. Standard preparation

All the stock solutions as well as dilutions were prepared in amber colored bottles. Stock solutions for vitamins B₁, B₂, B₃ & B₆ were prepared by dissolving reference/working standard in 2% acetic acid & 25% methanol. Stock solution for B₅ was prepared by dissolving reference material into mobile phase. A mixture of all the standards was prepared by taking a suitable aliquot to give a final concentration of: Vitamin B₁ (5 ppm), Vitamin B₂ (1 ppm), Vitamin B₃ (5 ppm) Vitamin B₅ (20 ppm) and Vitamin B₆ (2 ppm), against which the vitamins were quantified in the sample.

2.3. Sample Preparation

About 5.0 ml of sample was taken into 25 ml amber colored volumetric flask. The sample was mixed well in the diluent by leaving the flask on a wrist-shaker for about 10 minutes under dark conditions. The solution thus obtained was filtered using 45 μ filter paper for injecting into the chromatograph.

2.4. Validation of method

The HPLC method was validated by defining the precision, linearity and recovery. Six determinations of the same solution were performed under same conditions to evaluate the method precision. Linearity of the method was performed by analyzing a standard solution by the proposed method in the concentration range. The accuracy of the proposed method was determined by a recovery study, while was carried out by adding standard solution in the *Asavas & Arishtas*.

3. Results & Discussion

Six determinations of the same solution were performed under same conditions to evaluate the method precision. The precision of the method for determination of vitamins in *Asavas & Arishtha's* is shown in Table 1. The linearity relationship between the concentration of the vitamins and the respective peak area was plotted at different concentrations (Figure 1). As seen from Table 2, the correlation co-efficient for all the vitamins is more than 0.998. To ensure the accuracy of the data obtained recovery of the vitamins in the same matrix were performed. The values have been tabulated in Table 3.

Table 1. Precision of the method for determination of vitamins

Sample No.	Vitamins	Mean area	%RSD
1	B ₁	112.83	2.75
2	B ₂	78.26	1.37
3	B ₃	51.75	1.35
4	B ₆	71.45	1.92

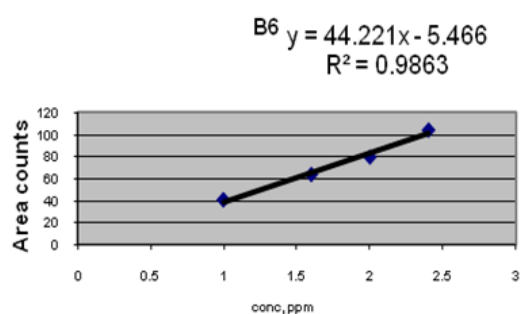
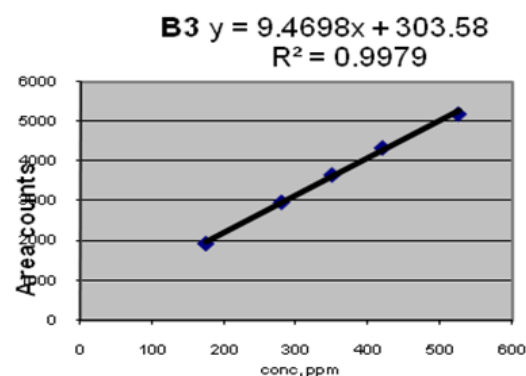
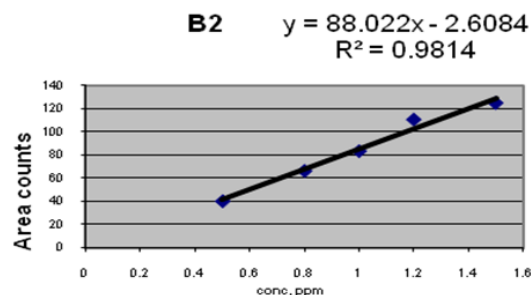
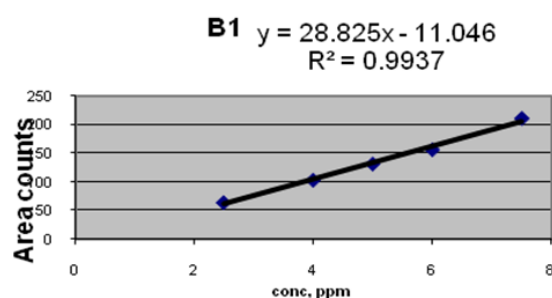


Fig 1. Linearity Graphs for B1, B2, B3 and B6

Table 2. Linearity relationship between the concentrations of vitamins

Sample No.	Vitamins	r ²	Concentration Range (ppm)
1	B ₁	0.994	2.5 – 7.5
2	B ₂	0.981	0.5 – 1.5
3	B ₃	0.998	175 - 525
4	B ₆	0.998	1.0 – 2.4

Table 3. Recovery of vitamins

Sample No.	Vitamins	Initial (ppm)	Fortification (ppm)	% Recovery
1	B ₁	61	85	72
2	B ₂	19	21	91
3	B ₃	1020	1009	101
4	B ₆	20	30	67

Vitamin estimation was carried out in *Drakshasava*, *Ashokarishta*, *Dashmulrishta*, *Khadirarishta*, *Lauhasava*, *Arjunarishta* and *Ashwagandharishta* from Dabur India Limited (Kalaiselvan et al., 2010). The values for all the vitamins in different *Asavas* & *Arishthas* as an average of 5 batches have been summarized in Table 4. Figure 2 showed the chromatogram indicating the separation of vitamins at 280, 234 & 261 nm. The findings show that Vitamin B₅ could not be detected in any of the samples. Interestingly, as given in Table 4, in most of the cases, the concentration of Vitamin was more than the recommended daily intake. All the *asavas-arishthas* were found to be rich source of Vitamin B₆ with estimated values much higher than the minimum recommended values (Khor and Siong, 1996). Although the study indicated presence of Vitamin B₉ (Folic acid) in many of the samples, but since the quantitation was beyond the scope of the study, it has not been reported.

Table 4. Vitamin estimation with respect to daily intake in *Asavas* and *Arishtas*

	Product	Vitamin B ₁ (mg)	Vitamin B ₂ (mg)	Vitamin B ₃ (mg)	Vitamin B ₆ (mg)
RDI*	-	1.5	1.7	2	2
Vitamin intake in single dose of 60 mL	<i>Drakshasava</i>	0.65	11.4	4.9	159
	<i>Ashokarishta</i>	2.2	10.9	24.3	5.5
	<i>Dashmulrishta</i>	1.6	10	52.4	58.2
	<i>Khadirarishta</i>	1.9	7.7	63	29
	<i>Lauhasava</i>	2.3	8.5	26.1	90.2
	<i>Arjunarishta</i>	12	1.8	11	18
	<i>Ashwagandharishta</i>	1	77	23.6	31.4

Sample Info : Vit-B complex

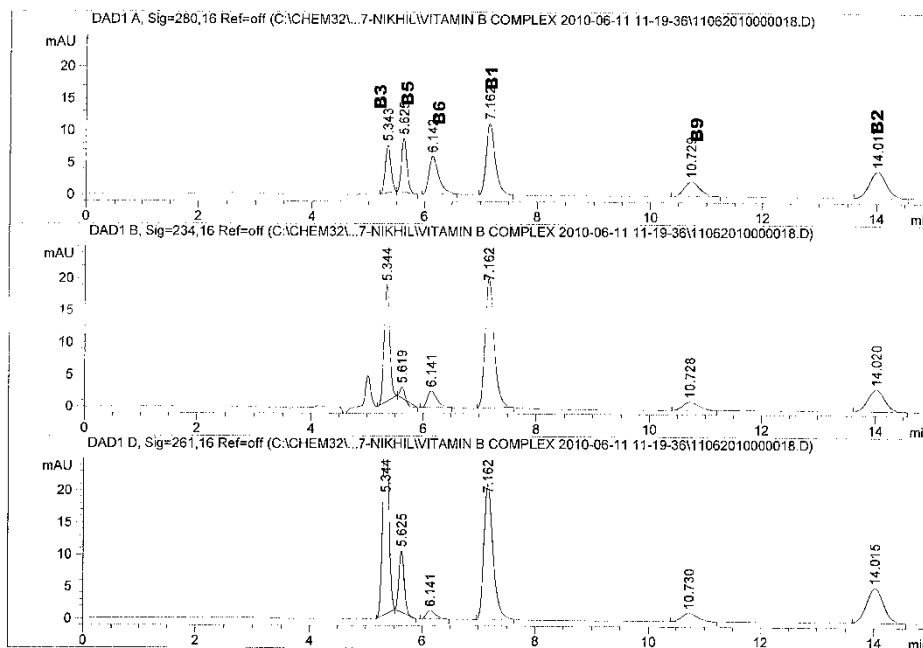


Fig 2. Chromatogram indicating the separation of vitamins at 280, 234 and 261 nm

The presence of water soluble vitamins in various concentrations justifies the claimed benefits of these Ayurvedic preparations (Asavas & Arishtha) as general tonics. The study provides a scientific evidence for the efficacy of these products for such usage. The methodology presented has an advantage in terms of ease of use & time of analysis over the other widely used methods which involve extraction, chemical reactions followed by fluorimetry for B1 & B2, spectrophotometry (for niacin & folic acid) & titrations for ascorbic acid & pyridoxine (Deutsch, 1984). Need for the use of hazardous chemicals such as cyanogen bromide for the estimation of niacin poses additional difficulties. Of late, it has been difficult to obtain this toxic and carcinogenic chemical. We hope that this work will act as a stimulant for other scientific studies on this unique form of Ayurvedic preparations.

4. Conclusion

The present study establishes the presence of good amount of vitamins from B complex range in the *Drakshasava*, *Ashokarishta*, *Dashmulrishta*, *Khadirarishta*, *Lauhasava*, *Arjunarishta* and *Ashwagandharishta* thereby supporting the usage of these *Asavas & Arishtas* in the form of general tonics suitable to be taken in routine life without any side effects due to its herbal base.

References

- Ayurvedic Formulary of India, Central Council for Research for Ayurveda and Siddha, 2nd Edn., Part 1, Ministry of Health and family Welfare, Government of India, **2003**, 113.
- Deutsch MJ. Vitamins and other nutrients. In: *Official Methods of Analysis of the AOAC*, Williams S ed, 14th edn, Association of Official Analytical Chemists, Washington, D.C. **1984**, 836-847.
- Kalaiselvan V.; Kumar K.; Shah AK.; Patel FB.; Shah CN.; Kalaivani M.; Rajasekaranm A, Quality assessment of different marketed brands of *Dasmoolaristam*, an Ayurvedic formulation. *Int. J. Ayur. Res.* **2010**, 1(1), 10-13.
- Khor SC.; Siong TE.; Development of a HPLC method for the simultaneous determination of several B-Vitamins and ascorbic acid, *Malaysian Journal of Nutrition*, **1996**, 2, 49-65.
- Krores BH.; Ajj VB.; Abeysekera AM.; Desilva KTD.; Labadie RP, Fermentation in traditional medicine: the impact of *Woodfordia fruticosa* flowers on the immunomodulatory activity and the alcohol and sugar content of *Nimbu arista*. *J. of Ethnopharmacol.* **1993**, 40, 117-125
- Patwardhan B.; Vaidya ADB.; Chorghade M, Ayurvedic and Natural Product drug discovery. *Curr. Sci.* **2006**, 86, 789-799.
- Sekar S.; Mariappan S, Traditionally fermented biomedicines, arishtas and asavas from Ayurveda. *Ind. J. of Trad. Know.* **2008**, 7(4):548-556.
- Sharma A.; Singh N.; Sharma M.; Singh R.; Kant KC, Comparative Study of Determination of Reducing Sugar by High-Performance Liquid Chromatographic and Titration Method in 'Chyawanprash: A Traditional Polyherbal Formulation. *Int. J. of Drug Discov. and Herbal Res.* **2011**, 1(4)106-111.