Research Article

# Quantitative Analysis of Iridin in the Different Species of Iris Plant by Reverse Phase High Pressure Liquid Chromatography

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**Abstract**

HPLC-UV-DAD method was developed for rapid identification and quantitation of Iridin in different species of *Iris* plant species growing wild in Kashmir valley. The analysis was performed by using Chromolith RP-18e analytical column at isocratic elution of methanol and water (30:70, v/v) with diode array detection at 265nm.The optimized method was successfully applied for the analysis of Iridin in 12 samples of five species of Iris collected from different eco-geographical zones. The identification of the investigated compounds was carried out by comparison of retention times and UV spectra with those obtained by injecting standards in the same conditions. The results showed that the content of *Iridin* greatly varied in five different *Iris* species. Furthermore, the content of *Iridin* varied within species growing at different eco-geographical zones. The concentration of *Iridin* ranged from 1.11% dry weight in IC-2 sample to 8.23% dry weight in IG-2 sample. The method established in this paper is simple and reliable and could easily be used for the qualitative analysis of Iridin in Iris species.

**Keywords:** Iris plant, chromatography, Iridin, Chromolith

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## Introduction

*Iris* is the largest and most complicated genus of family Iridacea [1]. The genus comprising of about 300 species is originated in Japan and in the Mediterranean [2], however the species of this plant are more concentrated in the south of equator and very widely distributed throughout the North Temperate Zone [3]. About twelve species of genus *Iris* are found all over India [4]. Their habitats are considerably varied ranging from cold regions into the grassy slopes, meadowlands, the Middle East and northern Africa, Asia and across North America. Intensive phytochemical investigations of various Iris species have resulted in the isolation of a variety of compounds including quinones, triterpenoids, flavonoids, isoflavonoids and stilbene glycosides [5]. Phenols, Flavonoids, isoflavonoids and their derivatues are important plant secondary metabolites [6-10] with structural diversity and are consumed by human as dietary constituents that have been found to have immense medicinal value [11]. During the last decade (1999-2008) over 90 flavonoid/ isoflavonoid constituents have been discovered and characterized, including 38 new compounds, from 15 species of Iris. The isoflavone rich dietary consumption is reported to reduce risk of cancer particularly breast and prostate cancer [12, 13]. The role of isoflavones in cancer [12] osteoporosis, cardiovascular diseases and menopausal symptoms in addition to their antioxidan(8). Antimicrobial [14] anti-inflammatory and estrogenic activities [12, 15] are well documented. It is anticipated that plants can act as potential source of bioactive compounds that can be used in near future for the development of new 'leads' to combat against dread full diseases such as cancer. The genus Iris is reported to contain volatile oils that can be used for medicinal purposes [16]. A potential antitumour agent benzoquinone, Irisoquinone having 3 - (10 - heptadecenyl) - 5-methoxy – 1 – 4 - benzoquinone structure has been isolated from the seed oil of Iris pallasi [17]. Apart from above a second antitumour benzoquinone, Irisoquin, 3 has been isolated from the related Iris species [18] and has various effective anticancer properties [19]. The seed oil of Iris Pseudocorous, Iris Sibrica, and Iris missouriansis has been found to contain appreciable amounts of phenols, quinines and resorcinols, substituted with homologous alkyl or alkenyl side chains [20]. Triterpenoids with an unusual spiro - bicyclic structure [21] and irisolidone, irigenin and iridia [22] have been isolated and characterized from rhizomes of Iris germanica.

The present work describes the qualitative as well as quantitative analysis of Iridin in some selected species of genus Iris growing wild in Kashmir valley. The analysis was performed using HPLC coupled with diode array detection. The present work is the first report of its kind in which Iridin was quantified in five species of Iris growing in Kashmir.

## Experimental Results

### *Chemicals and reagents*

Methanol and water were of HPLC grade and were purchased from Merck (Mumbai, India). Iridin purchased from Sigma Aldrich, India was used as an external standard for HPLC in the current study.

### *Plant material*

The plant material of *Iris* plant species were collected from different ecogeographical regions of Kashmir valley covering high altitude regions like Gulmarg, sonamarg, naranag etc and collected throughout the year (**Table 1**). The collected plant material was properly identified and the specimen vouchers were deposited in the Indian Institute of Integrative Medicine, Sanat Nagar, Srinagar.

**Table 1** Collection of samples of *Iris* plant species

| S. No. | Code | Samples | Sources | Collection date |
|---|---|---|---|---|
| 1 | IC-1 | *Iris* croceae | Sonamarg, | May, 2016 |
| 2 | IC-2 | *Iris* croceae | IIIM field, Srinagar, | April, 2016 |
| 3 | IC-3 | *Iris* croceae | Gulmarg, Kashmir, | June, 2016 |
| 4 | IE-1 | *Iris* ensata | Naranag, Kashmir, | May, 2016 |
| 5 | IE-2 | *Iris* ensata | IIIM field, Srinagar, | April, 2016 |
| 6 | IG-1 | *Iris* germanica | IIIM field, Srinagar, | April, 2016 |
| 7 | IG-2 | *Iris* germanica | Gulmarg, | June, 2016 |
| 8 | IG-3 | *Iris* germanica | Sonamarg, | May, 2016 |
| 9 | IK-1 | *Iris* kashmiriana | Srinagar | April, 2016 |
| 10 | IK-2 | *Iris* kashmiriana | Naranag, | May, 2016 |
| 11 | IS-1 | *Iris* spuria | Gulmarg, | June, 2016 |
| 12 | IS-2 | *Iris* spuria | Sonamarg, | May, 2016 |

### *Extract preparations from plant rhizome*

The shade dried underground part (rhizome) of different *Iris* plant species viz, *Iris kashmiriana, Iris croceae, Iris spuria, Iris ensata, Iris germanica* (2 Kg) was finely ground and soaked in hexane (5L x 2) at room temperature for 30 hrs. The defatted plant material was extracted with methanol (7.5L x 3) for 40 hrs at room temperature and the resulting extract was concentrated to a gum. The remaining plant material was extracted with methanol (10L x 4) at room temperature for 40 hrs, the resulting extract was reduced to a manageable residue to get gummy material. The final plant material was washed with water to prepare the aqueous extracts. The extract percentage hexane, methanol and water extract were calculated and recorded. The extracts were stored at 4deg until use.

### *Sample preparation for HPLC*

Stock solutions of extracts and the standard were obtained by dissolving 5 mg of each of the extract in 1.0 ml MeOH and 1.0 mg of the standard in 1.0 ml MeOH respectively. The resulting solutions were filtered through 0.45µm filter membrane. Working solutions of appropriate concentrations were prepared by diluting stock solutions. The stability of stock as well as working solutions was monitored and no change in concentration was observed.

### *HPLC conditions for quantitative analysis*

Quantitative HPLC analysis was performed on a Schimadzu Class VP HPLC system equipped with a binary pump (LC-10AT), an autosampler (SIL-10AD), a column oven (CTO-10AS), a Diode array detector (SPD-M10A), vacuum membrane degasser (DGU-14A) and a system integrator (SCL-10A) controlled by a Class VP software which was

used for data analysis and processing. Separation was carried out on a Chromolith RP-18e column (5 µm; 4.6 mm × 100 mm) with column oven temperature of 30°C using an isocratic solvent system consisting of methanol and water (30:70; v/v). Elution was performed at a flow rate of 0.6 ml/min and the injection volume was 5.0 µl. The analytes were monitored at 265 nm.

## Results and discussion

### *Optimization of HPLC conditions*

The optimization of HPLC conditions was performed using the standard solution of Iridin first and then solution of IC-3 sample. Peak resolutions were tested and compared using different solvent systems (acetonitrile-methanol, acetonitrile-water, and methanol-water) in varying proportions. The separation of Iridin was achieved on a RP-18e column using methanol-water (30:70; v/v) at a flow rate of 0.6 ml/min with a runtime of 55 minutes. Photodiode array detector (DAD) was used in HPLC analysis and the optimum monitor wavelength at 265nm was selected from the full range spectra.

### *Calibration curves and recovery*

Methanol stock solution of the standard reference compound Iridin was prepared and diluted to appropriate concentrations for the construction of calibration curve. At least four concentrations of Iridin were injected in the concentration range of 0.08µg/ml to 0.96µg/µl. The calibration curve was constructed by plotting the peak area versus the concentration of each analyte with detector wavelength set at 265 nm. Excellent calibration curve was obtained for the standard within the concentration range of 0.08 µg to 0.96 µg/µl (r2 = curve coefficients > 0.998). The recovery study was within the concentration range of the calibration curve. The recovery of the method was estimated by spiking IK-1 sample with 2.0 µg/3.8 mg of the standard Iridin. The data from the recovery studies revealed that the recovery of the method was in the range of 98.2 to 101.2% evaluating the accuracy of the method.

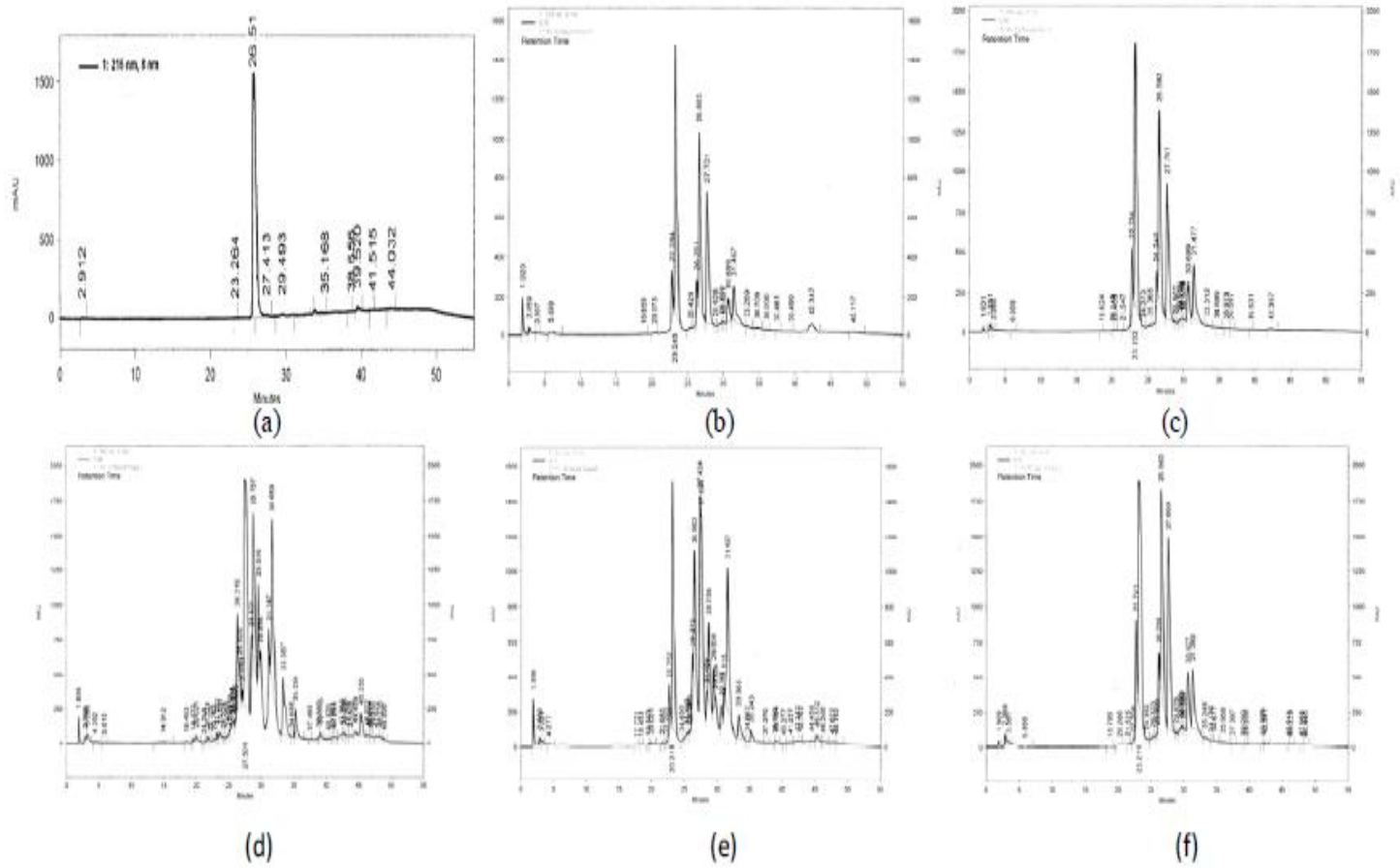
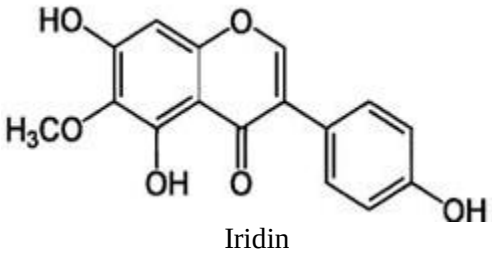



**Figure 1** Quantification of Iridin in various Iris plant species by RP-HPLC: Typical HPLC chromatograms for quantification of Iridin (a) Iridin marker (b) Iris Kashmiriana (c) Iris Ensata (d) Iris Spuria (e) Iris Germanica (f) Iris Crocea.

***Quantification of Iridin in five different species of Iris Plant***

Iridin was separated in all the samples using the developed HPLC method. Typical chromatograms of the different extracts and the mixed standard are shown in **Figure 1**. The identification of the investigated compounds was carried out by comparison of retention times and UV spectra with those obtained by injecting standards in the same conditions. The developed HPLC method was applied to analyze *Iridin* in twelve samples of five species of *Iris*.




Iridin

The data are summarized in **Table 2**. The results showed that the content of *Iridin* greatly varied in five different *Iris* species. Furthermore, the content of *Iridin* varied within species growing at different eco-geographical zones. The concentration of *Iridin* ranged from 1.11% dry weight in IC-2 sample to 8.23% dry weight in IG-2 sample **Figure 2**.

**Table 2** Content of *Iridin* in different tested samples of *Iris* on plant dry weight basis

| S. No. | CODE | % Content of *Iridin* |
|---|---|---|
| 1 | IC-1 | 1.113 |
| 2 | IC-2 | 2.289 |
| 3 | IC-3 | 3.948 |
| 5 | IE-1 | 2.751 |
| 5 | IE-2 | 1.984 |
| 6 | IG-1 | 6.552 |
| 7 | IG-2 | 8.232 |
| 8 | IG-3 | 6.216 |
| 9 | IK-1 | 6.436 |
| 10 | IK-2 | 5.040 |
| 11 | IS-1 | 1.795 |
| 12 | IS-2 | 1.648 |

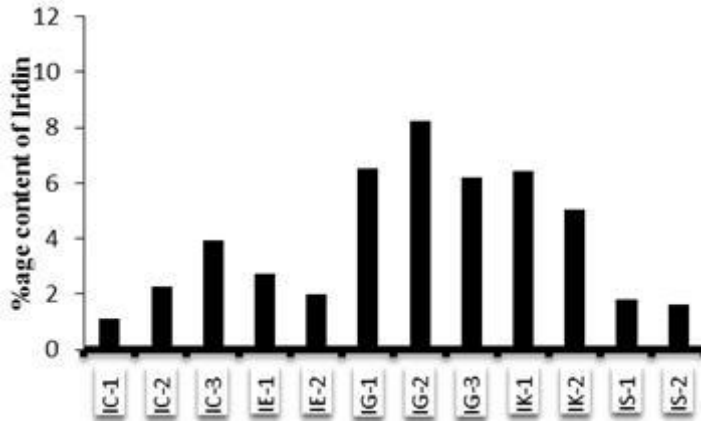



**Figure 2** Graph showing comparative Iridin content in different tested samples of Iris species collected from various geographical locations of Kashmir valley. Y=0.5123X-0.01, $R^2$=-0.337

## Summary and Conclusion

The present study demonstrates the quantification of Iridin in the different species of Iris plant growing under different eco-geographical conditions of Kashmir valley. The method is good, precise and accurate and can be used for routine quantification of Iridin in the different species of Iris plant.

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