# A Developed and Validated High-Performance Thin-Layer Chromatographic Method for the Quantitative Determination of Quercetin in *Satyrium nepalense* Tubers

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**Key Words**

**Bioflavonoid**
**Orchid**
**Biomarker**
**Herbal drugs**
**Method validation**

## Summary

***Satyrium nepalense* D. Don (Orchidaceae) is a reservoir of numerous chemical compounds such as flavonoids, glycosides, and unsaturated sterols/triterpenes. Thin-layer chromatography (TLC) and qualitative tests confirm the presence of quercetin in *S. nepalense* tubers – this is why we must pay attention to its chemistry. A simple, sensitive, and accurate method developed with a normal-phase high-performance thin-layer chromatography (HPTLC) has been optimised for the separation, identification, and quantitative estimation of quercetin in the methanol extract of *S. nepalense* tubers. Chromatography was performed by using aluminum TLC plates precoated with silica gel 60 $F_{254}$ using toluene–ethyl acetate–formic acid (7:5:1, *v*/*v*) as the solvent system at 25 ± 2°C. Linear ascending development was carried out in a saturated twin-trough glass chamber. Densitometric determinations were performed in reflectance–absorbance mode by using a deuterium lamp (D2) for excitation at 366 nm and a K400 filter in order to measure the emitted light by using a CAMAG TLC Scanner 3. *S. nepalense* (tuber) contained 40.28 mg/100 g extract DW of quercetin. The detector response was linear for concentrations with a range of 2–12 µg band$^{-1}$ through a correlation coefficient (*r*) of 0.97874 with respect to peak area. The method has a significant precision (0.71%) and repeatability (0.74%), as far as the percentage value; the standard deviation was found to be less than 2. The accuracy of this method was checked by doing some recovery experiment at three different levels, by using the standard addition method; in fact, values were discovered to be 99.50–100.24%. There are no reports concerning the quantitative assessment of quercetin in the *S. nepalense*. Hence, an efficient HPTLC method has been developed and validated. It is simple, fast, reliable, specific, precise, and useful for routine assays of *S. nepalense* extracts containing quercetin.**

## 1 Introduction

The global herbal drugs and nutraceutical market is growing and so is the incidence of adulteration. It is essential to detect the counterfeits and ensure quality, safety, and efficacy of herbal materials. Quercetin is a bioflavonoid which is commonly found in many herbal medicinal plants such as fruits and vegetables, not only in its free form but also as glycosides [1, 2]. It has anti-inflammatory, antioxidant, antihistamine, anti-edematous, anticancer, and direct radical scavenging effects; it stabilizes cell membranes; it inhibits the aging process of skin, cornea, and myocardium and positively affects the function of the cardiovascular system. Along with protein, fats, carbohydrates, vitamins, and trace elements, quercetin is recognized as an integral part of a healthy diet. Its daily dose ranges from 4 to 68 mg [3–5]. It takes part in the composition of many dietary supplements and specific medications. Quercetin is often used as a reference substance in order to determine the antioxidant activity of various objects [6, 7]. In an organism, quercetin could be conjugated with a sugar group, and for this reason, the absorption process could be enhanced. Its two forms, aglycone and glucosidal, are better absorbed than nonglucosic forms. In plants, quercetin is almost found exclusively bound to one or more sugar molecules, so-called quercetin-β-glycosides [8].

*Satyrium nepalense* D. Don (Orchidaceae) is a medicinal herb which contains quercetin in its tuberous part. The plant is found at the altitude of 2400–5000 m. The plant is used by local inhabitants of Uttarakhand (India) as traditional medicine against different types of minor illnesses. Decoction of tubers, roots, and stems of the plant has been used in various infectious diseases and also as a nutritional supplement since ancient times. It is also consumed as a food, tonic, in diarrhea, malaria, and dysentery. The methanol extract from the plant's part shows the number of secondary metabolites such as alkaloids, carbohydrates/glycosides, flavonoids, and unsaturated sterols/triterpenes previously studied [9]. Here, an attempt has been made to develop a high-performance thin-layer chromatographic (HPTLC) method for the identification and quantification of quercetin in the methanol extract of tubers of *S. nepalense*. Therefore, we describe in this paper a simple, sensitive, and accurate HPTLC method for the determination of quercetin.

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