

Abstract

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Title of the diploma thesis: Development of UHPLC method for the determination of withanolides in dietary supplements with Ashwagandha extract

In this diploma thesis, a new ultra high-performance liquid chromatography (UHPLC) method was developed and validated for the separation and determination of withaferin A, withanolide A, withanolide B, withanoside IV, withanoside V and withanone, which are contained in the extracts of *Withania somnifera*, commonly known as Ashwagandha. The developed method was subsequently used to determine the withanolides in ten dietary supplements: Ashwagandha BIO Organic (Blendea), Ashwagandha (ActiveLab), Ashwagandha (Kräuterhaus Sanct Bernahrd), Ashwagandha Extract Organic BIO (Viridian), Ashwagandha (Green Idea), Ashwagandha (ADVANCE nutraceuticals), Ashwagandha (Gym Beam), Ashwagandha (Webber Naturals), Ashwagandha (Vilgain) a Ashwagandha (Jíme zdravě).

A Kinetex F5 100 Å 150 x 4.6 mm analytical column with 2.6 µm particles was used for the analysis. The analysis was carried out by gradient elution with mobile phase A: acetonitrile, mobile phase B: ultrapure water. The analysis was carried out at a temperature of 30 °C and the flow rate of the mobile phase was 1.0 ml/min. Detection was performed with a diode array detector at two wavelengths of 215 nm (withaferin A, withanoside IV) and 228 nm (withanolide A, withanolide B, withanoside V, withanone).

Key words: UHPLC, withaferin A, withanolide A, withanolide B, withanoside IV, withanoside V, withanone, *Withania somnifera*, Ashwagandha, dietary supplements