HPLC determination of wedelolactone in a market herbal extract sample and its validation

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Abstract

Wedelolactone has a wide range of biological activities and used for the treatment various ailments. In the present study an attempt has been developed and validation for the quantification of wedelolactone. Analysis of wedelolactone was performed by HPLC using linear gradient and applied to the herbal extract samples from the local market. The linear regression data analysis for the calibration plots showed good linear relationship with $r = 0.9961$ in the concentration range $11.0 – 90.9 \, \text{ug.mL}^{-1}$. The present method was validated for precision, recovery, limit of detection and limit of quantitation. Statistical analysis of the data showed that the method is reproducible and selective for the estimation of wedelolactone.

Keywords: Wedelolactone, HPLC, method validation, herbal extract, limit of confidence

Introduction

Wedelolactone is coumestan derivative organic compounds contained in Eclipta alba L. Hask plant (figure 1). Traditionally, Eclipta alba L. Hask leaf widely used to treat shortness of breath, headache, toothache, bronchitis, menstrual disorders, and as a hair fertilizer (Unnikrishnan et al., 2007).

As a bioactive marker of Eclipta alba L. Hask plant, wedelolactone (figure 2) is the most sought after by industry and herbal medicines because it has various functions. From the results of pharmacological screening of plants or synthesis results have revealed that wedelolactone activity as antihapatotoxic (Franca, et al., 1995) immunomodulators (Jayathirtha & Mishra, 2004), antioxidants (Majumdar, 2008), anti-inflammation (inflammatory) (Arunachalam et al., 2009), antidote to snake venom toxins (Melo, et.al., 2010), Pithayanukul, et al., (2004), Diogo., et al. (2009)), an inhibitor of hepatitis C virus (Basu, 2008), analgesic (Sawant, et al., 2004) antosteoporetic (Annie, et al. 2006), suppress androgen activity and growth in prostate cancer cells synergistically (Lin, 2007), neuropharmacological (Prakash, 2008), anti-HIV (Tewtrakul, et al., 2007).

Several methods have been developed for wedelolactone determination. Patel & Mishra (2006) use a preparative thin layer chromatography technique. A mixture of toluene: acetone: formic acid (11: 6: 1) use as mobile phase and silica gel G as stationary phase. Measurements performed with spectrofluorometer instrument at an excitation wavelength of 384 nm and an emission wavelength of 458 nm. Spectrophotometric method for wedelolactone determination simultaneous with asiaticoside in polyherbal formulation developed by Pallavi Rai & Mishra (2007). Unnikrishnan et al. (2007) using high performance thin layer chromatography (HPTLC) technique to determine wedelolactone content using mobile phase (toluene: acetone: formic acid (11: 6: 1 v/v) same as Patel & Mishra (2006). The spots (RF 0.39) of the chromatogram were visualized in UV-254 and UV-