

Title Identification of steroid hormones in pomegranate (*Punica granatum*) using HPLC and GC–mass spectrometry

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### Abstract

Although it has been known that pomegranate (*Punica granatum* L.) contains several steroid hormones, concrete experimental proofs about that have not been published until now. In order to identify and evaluate the contents of steroid hormones including estrone in pomegranate, we analyzed pomegranate seed, fruit juice and commercial preparations. We developed a reproducible and sensitive method for separation and identification of steroid hormones in pomegranate samples using both high performance liquid chromatography (HPLC)–PDA and gas chromatography (GC)–MS. In case of HPLC, an isocratic elution method using 35% aqueous acetonitrile solution at 1.0 ml/min with photodiode-array (PDA) detection at 225 nm and 254 nm was found to optimally separate and identify the steroid hormones from the pomegranate samples with a run time of less than 30 min. The pomegranate samples were comparatively analyzed to the HPLC results by GC/FID or GC/MS detection on a HP-1 (30 m length, 0.32 mm I.D.) with helium as carrier gas under the oven temperature control as follows: start 220 °C for 5 min, raising 5 °C per min, final 280 °C for 10 min. The HPLC and GC methods were successfully applied to the identification of steroid hormones in pomegranate samples. Our results suggested that there were no steroid estrogens including estrone, estradiol and testosterone in pomegranate seed, fruit juice and preparations. Consequently, we assumed that the previously reported analysts of pomegranate were misunderstood their analytical results according to either the estrogen-like effects or similarity of peak retention time and R<sub>f</sub> values in experiments.