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## HPLC SEPARATION OF THE HYPERFORIN/ADHYPERFORIN MIXTURE ISOLATED FROM *HYPERICUM PERFORATUM L.*

The hyperforin/adhyperforin mixture isolated from *Hypericum perforatum L.* was separated by the HPLC method. Beside acetonitrile and water, the mobile phase used for the separation contained trifluoroacetic acid. Due to its volatility, trifluoroacetic acid is very convenient both for the analytical and preparative separation of hyperforin/adhyperforin.

Common St. John's wort, *Hypericum perforatum L.*, is one of the most often used medical plants in the phyto-therapy, its tradition as a medicinal plant stretching back to ancient times [1]. More recently, an increasing number of experimental and clinical studies performed with commercial preparations of extracts of *Hypericum perforatum L.* (common St. John's wort) have shown that these preparations are more effective than placebo for the treatment of mild to moderately severe depressive disorders [2, 3]. Hyperforin (C<sub>35</sub>H<sub>52</sub>O<sub>4</sub>) might make an important contribution to the antidepressant activity of hypericum extracts [4–8].

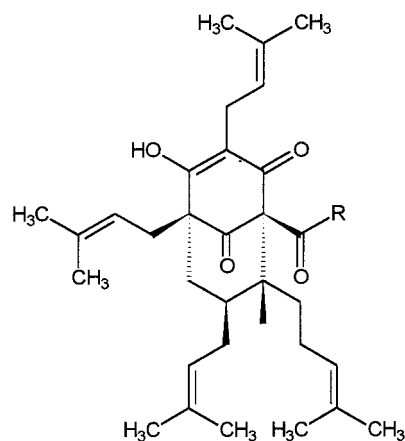
Gurevich was the first to describe the characteristics and isolation of hyperforin, and the structural determination was completed in 1983 [9–13]. According to Berghöfer [14], hyperforin is contained only in the buds, flowers and capsules in amounts of up to 2 to 4%. Ostrowski [15] proved the oral availability of hyperforin, while Maisenbacher and Kovar [16] described adhyperforin, a methyl analogue of hyperforin (Figure 1). Gorsubellin A, obtained from *Garcinia subelliptica*, has a structure very similar to that of hyperforin [17]. Attempts to synthesize hyperforin were described by Heidt [18].

The scope of this study was the separation of the hyperforin/adhyperforin mixture isolated from *Hypericum perforatum L.* by the HPLC method.

### EXPERIMENTAL CONDITIONS

The hyperforin/adhyperforin mixture isolated from St. John's wort, *Hypericum perforatum L.*, was used for the tests carried out in this study [19].

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R = CH(CH<sub>3</sub>)<sub>2</sub> = HYPERFORIN

R = CH(CH<sub>3</sub>)CH<sub>2</sub>CH<sub>3</sub> = ADHYPERFORIN

Figure 1. Hyperforin and adhyperforin structures

The HPLC separation of the hyperforin/adhyperforin mixture was obtained under the following conditions:

Apparatus: Knauer (two Knauer HPLC pumps 64; Shimadzu SPD-6A UV Spectrophotometric Detector; Knauer HPLC Software)

Column: Lichrosphere 60 RP-Select B (Merck 50829), 5 μm

Eluent: (A) Acetonitrile: water:trifluoroacetic acid = 89.9:10:0.1 (v/v)

(B) Acetonitrile:trifluoroacetic acid = 99.9:0.1 (v/v)

Gradient: 0 min 40:60 (A:B); 15–35 min 100:0

Flow Rate: 0.8 ml/min

Test samples: 20 μl

UV Detection: 272 nm

## RESULTS AND DISCUSSION

The HPLC chromatogram of the separation of the hyperforin/adhyperforin mixture is shown in Figure 2.

Beside acetonitrile and water, the mobile phase used for this separation contained trifluoroacetic acid. This is important for the preparative separation of the hyperforin/adhyperforin mixture because trifluoroacetic acid is very volatile and so can be quickly and easily evaporated from the separated product, which is not the case with many other acids. Although the separation of hyperforin/adhyperforin mixture was attempted using other mobile phases, complete separation was only realized under the above mentioned conditions. This work indicates the possibility of adding trifluoroacetic acid to the mobile phase for HPLC analysis of herbal extracts.

## ACKNOWLEDGMENT

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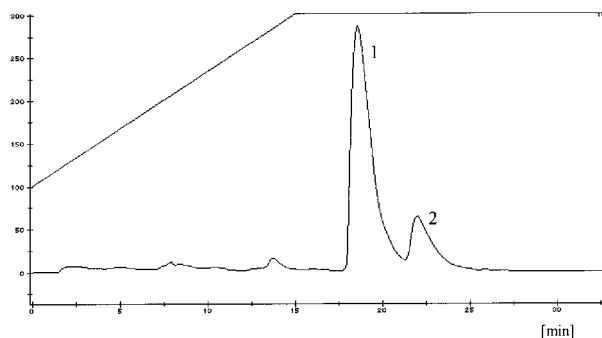


Figure 2. HPLC chromatogram of the separation of the hyperforin/adhyperforin mixture (1. hyperforin; 2. adhyperforin)

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## IZVOD

HPLC RAZDVAJANJE SMEŠE HIPERFORIN/ADHIPERFORIN, IZOLOVANE IZ *HYPERICUM PERFORATUM L.*

(Naučni rad)

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Smeša hiperforin/adhiperforin, izolovana iz *Hypericum perforatum L.*, razdvojena je metodom HPLC. Mobilna faza, koja je korišćena za ovo razdvajanje, pored acetonitrila i vode, sadrži trifluorsirćetnu kiselinu. Zbog svoje lake isparljivosti, trifluorsirćetna kiselina je pogodna kako za analitičko, tako i za preparativno hiperforin/adhiperforin razdvajanje.

Ključne reči: Hiperforin • Adhiperforin • HPLC • *Hypericum perforatum L.*

Key words: Hyperforin • Adhyperforin • HPLC • *Hypericum perforatum L.*

