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Journal of Chromatography A, 1093 (2005) 1–10
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Single-drop liquid-phase microextraction for the determination of hypericin, pseudohypericin and hyperforin in biological fluids by high performance liquid chromatography

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Received 16 May 2005; received in revised form 6 July 2005; accepted 11 July 2005
Available online 16 August 2005

Abstract

The analysis of hypericin, pseudohypericin (collectively called in this study hypericins) and hyperforin in biological fluids is reported using single-drop liquid-phase microextraction in conjunction with HPLC-UV-fluorescence detection. A new option for analysis of the active principle constituents in biological samples is proposed, reducing the steps required prior to analysis. There are several parameters which determine the mass transfer such as the extraction solvent, drop and sample volumes, extraction time and temperature, pH and ionic strength, stirring rate and depth of needle tip in the bulk solution. These parameters were chosen to optimize the performance in the current study. The method was validated with respect to precision, accuracy and specificity. The intra-day precision values were below 2.3% for the high concentration level of control samples and 6.2% for the low level. The respective inter-day precision values were calculated to be below 4.4 and 7.1%, respectively, for the two concentration levels. Accuracy of the method, calculated as relative error, ranged from −2.6 to 7.0%. It was demonstrated that as long as the extraction procedure is consistently applied, quantitative analysis is performed accurately and reproducibly in human urine and plasma samples. Limits of quantitation (LOQs) in urine were calculated to be 3, 6 and 12 ng/ml for pseudohypericin, hypericin and hyperforin, respectively. Slightly higher limits were measured in plasma, i.e. 5, 12 and 20 ng/ml, for the respective analytes.

Keywords: Pseudohypericin; Hypericin; Hyperforin; Analysis; Biological fluids; Single-drop liquid-phase microextraction

1. Introduction

St John's wort (*Hypericum perforatum* L.) has been known since antiquity for many medicinal properties such as hepatic disorders and gastric ulcers. In the last two decades, anti-inflammatory [1], anti-microbial [2], anti-viral [3], anti-depressant [4] and cytotoxic [5] activities have also been attributed to the total extract or individual components.

In recent years, increased interest in hypericin, one of the major components of the plant, as a potential photosensitizing anticancer agent has arisen. Several studies established the powerful in vivo and in vitro antineoplastic activity of hypericin in the absence of or upon irradiation [6,7]. Associated experimental results suggest that hypericin has considerable potential for use as a sensitizer in the photodynamic therapy of cancer [8,9]. Recently, also, the possibility of using hypericin as a diagnostic tool for the fluorescence detection of flat neoplastic lesions in urine bladders has been investigated [10].

Anti-depressant applications of St John's wort medicinal products (e.g. Psychotonin®, Neuroplant®, Hyperforat®) have become increasingly popular in Europe, particularly in Germany, where physicians routinely prescribe herbal medicines. The anti-depressant activity was first attributed to hypericin, its derivatives and polyphenols flavonols [11,12], but recent pharmacological and clinical results focus on hyperforins, as the main active ingredients of the extract

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doi:10.1016/j.chroma.2005.07.038

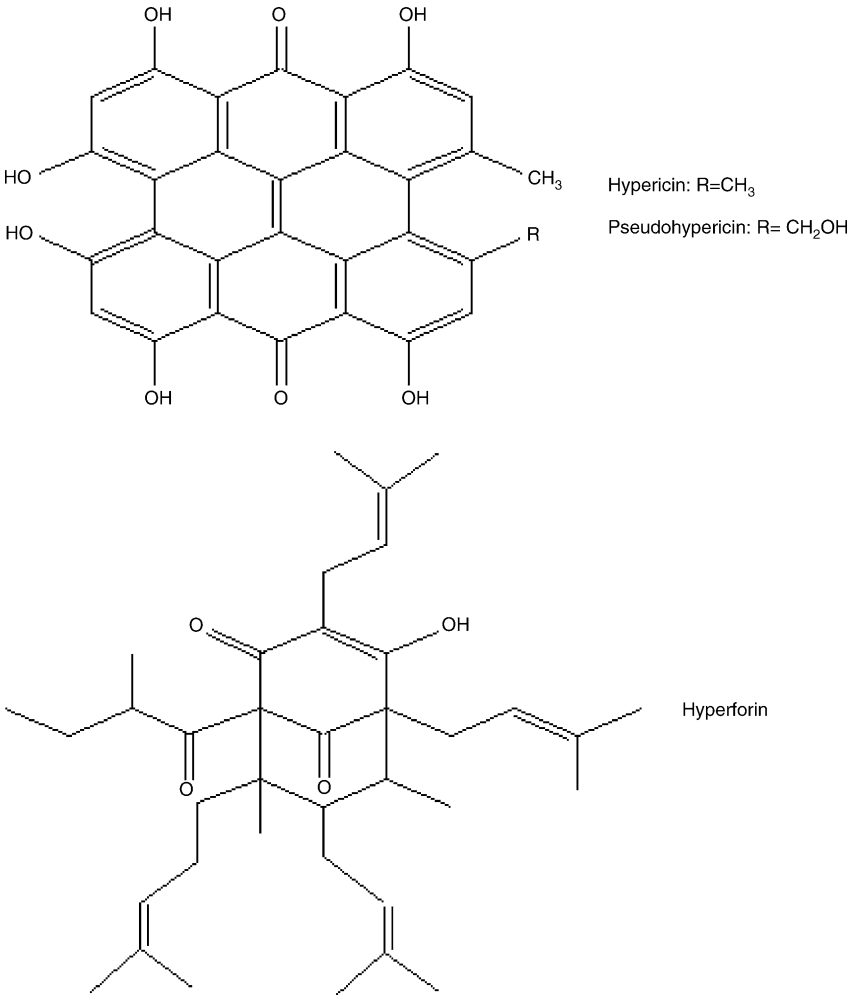

Fig. 1. Chemical structures of hypericin, pseudohypericin and hyperforin.

[13,14]. Thus, the standardisation of the extracts based on hypericin can no longer be proposed as a tool to evaluate potential benefits or risks of St. John's wort preparations. Jones et al. found that during a routine drug history, one in seven patients did not disclose that they were taking herbal medicines [15]. In another study, half of the outpatients reported that their doctor or pharmacist was unaware that they were taking St John's wort [16]. However, detailed information about the concurrent drug use is important because exposure to unknown drugs may hamper individualization of therapy and drug safety [17].

St. John's wort extracts are prescribed not only as herbal medicinal products but also as a top-selling botanical dietary supplement both standardised using the naphthodianthrones of the hypericin group, calculated as 0.2–1 mg hypericin daily dose. Finally, St. John's wort preparations have recently been used as an ingredient in some food products sold as functional foods [18].

A multitude of methods have been developed for the measurement of hypericin, pseudohypericin and hyperforin (Fig. 1). Some of them have been reported in the use, in a variety of biological media [19–27]. The methods employed hitherto in such matrixes require apolar organic solvents where hyperforin is unstable. A pretreatment step, most frequently solid-phase extraction, for clean up and preconcentration is necessary in order to detect low concentration levels.

During the last 10 years, with the upsurge of miniaturization in analytical chemistry several liquid–liquid extraction alternatives drew the attention of researchers. The major incentive behind this has been to speed up extractions, reduce the consumption of organic solvents and to facilitate towards automation. Liquid-phase microextraction, performed by using either a single drop of solvent [28–32] or a small length of porous hollow fiber-protected solvent [33], has shown to be an attractive alternative for sample preparation. In one of the single-drop modes, the so-called single-drop liquid-phase microextraction (SD-LPME), the organic micro droplet is placed into the aqueous sample and the analytes are extracted into the organic droplet (microextract) based on passive diffusion. It was reported that SD-LPME has comparable extraction efficiency and reproducibility with the widely used solid-phase microextraction.