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Direct determination of tagitinin C in *Tithonia diversifolia* leaves by on-line coupling of supercritical carbon dioxide extraction to FT-IR spectroscopy by means of optical fibres

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Abstract

Supercritical fluid extraction (SFE) with carbon dioxide as extraction medium was on-line coupled to a FT-IR spectrometer equipped with a Mercury Cadmium Telluride (MCT) detector using a tailor-made high-pressure fibre optic flow cell. This method was optimised and developed for the monitoring in real time and the quantification of dynamic extractions of tagitinin C from *Tithonia diversifolia* leaves.

In order to demonstrate the method ability to allow the direct quantification of tagitinin C in the extract medium the standard addition method was used. The area integration of curves obtained by plotting the absorbance of the highly specific C=O stretching vibration at 1668 cm^{-1} versus time (i.e. extractograms) was used as instrumental response.

The SFE/FT-IR process was successfully validated using the accuracy profile as decision tool. On this basis, a linear regression model was chosen for the calibration curve. The relative standard deviation for repeatability and intermediate precision were between 0.8 and 3.1 %, respectively. Moreover, the method was found to be accurate as the two-sided 95% beta-expectation tolerance interval did not exceed the acceptance limits of 85 and 115% on the analytical range investigated (500–2500 μg of added amount of tagitinin C).

The proposed method allowed the non-destructive extraction of tagitinin C and its on-line quantitative determination in less than 25 min thus facilitating the subsequent experiments or the pharmacological studies performed on this compound.

Keywords: On-line detection; Supercritical fluid extraction; FT-IR spectroscopy; Tagitinin C; *Tithonia diversifolia*; Accuracy profile

1. Introduction

Tithonia diversifolia (Hemsley) A. Gray (Asteraceae) is a shrub which is native to Mexico and also grows in parts of Africa and Asia. Extracts of this plant have been used traditionally for the treatment of diarrhea, fever and malaria. Recently, the antimalarial properties of *T. diversifolia* against *Plasmodium falciparum* were investigated in vitro by Goffin et al. [1]. Tagitinin C (Fig. 1) was identified as an active component against *Plasmodium*. An additional work of Gu et al. [2] showed significant antiproliferative activity of tagitinin C.

In the last decade, supercritical fluid extraction (SFE) processing with carbon dioxide has emerged as the alternative to the conventional solvent extraction (e.g. maceration, percolation, Soxhlet, microwave extraction and accelerated solvent extraction) of natural products for foods and medicines. Indeed, carbon dioxide is an inert, inexpensive, easily available, odourless, tasteless and environment-friendly solvent avoiding any solvent residue in the extract because of its gas state under the ambient conditions. Moreover, the near-ambient critical temperature of carbon dioxide (31.1 °C) makes it ideally suitable for the extraction of thermolabile natural products by reducing the risks of degradation or volatilisation of the compounds under investigation.

In addition, as on-line technique is less tedious and time-consuming than off-line analysis, SFE has been coupled to a

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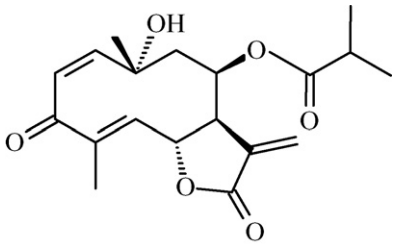

Fig. 1. Structure of tagitinin C.

wide range of detection systems. In cases of SFE-GC [3] and SFE/HPLC [4–8], trapping onto a solid phase adsorbent is the most common approach. The dynamic character of SFE also enables its direct coupling to continuous on-line systems as spectrophotometry [9], spectrofluorimetry [10], atomic absorption spectroscopy [11], proton nuclear magnetic resonance [12,13], mass spectrometry [14,15] and infrared spectroscopy [16–19].

The direct coupling of SFE/IR using the optical fibres was pioneered by Heglung et al. [16] in 1994 and applied to the extraction of caffeine from coffee and the extraction of total petroleum hydrocarbons (TPHs) in soil in a static extraction mode. In this latter application, only the quantitative determination of TPHs was carried out. A few years later, Current and Tilotta [18] realised the first quantitative determination of TPHs in a static extraction mode also using a simple IR filter spectrometer as detection system. In 2003, TPHs in soil were analysed by Liang and Tilotta [19] in a dynamic extraction mode by means of infrared filter photometry detection.

In previous works, we have demonstrated the feasibility and usefulness of the FT-IR spectroscopy technique for the off-line analysis of tagitinin C in crude extracts obtained after conventional liquid solvent [20] or supercritical fluid extraction [21]. The main objective of the present work was to develop an on-line FT-IR detection coupled to dynamic supercritical fluid extraction for the direct determination of tagitinin C in the leaves of *T. diversifolia*. The validation of the proposed method was then performed using the concept of accuracy profile in order to demonstrate its ability to quantify [22]. This work is the first application of a validated on-line SFE/FT-IR method for the quantitative determination of a natural compound in plant materials in dynamic extraction mode.

2. Experimental

2.1. Chemicals and reagents

Methanol (p.a.), anhydrous Na_2SO_4 (p.a.), glass beads whose diameter were between 400 and 520 μm were obtained from Merck (Darmstadt, Germany).

The carbon dioxide, 99.98% (w/w), was purchased from Air liquide (Liège, Belgium).

Reference tagitinin C (97.2%) was extracted from the powder of *T. diversifolia* leaves (<63 μm) by SFE [21] and purified in our laboratory as described previously by Goffin et al. [1]. A stock mixture of tagitinin C, 0.5% (w/w), was prepared by thoroughly mixing it with anhydrous Na_2SO_4.

2.2. Plant material

The aerial parts of *T. diversifolia* (Asteraceae) were collected at the Democratic Republic of São Tomé e Principe in 1997. A voucher specimen (MM625) has been deposited at the Botanic Institute of the University of Coimbra. The leaves moisture content was 8.93 ± 0.24 wt.%.

Only the plant leaves were used in this work after being thoroughly grounded and sieved under 63 μm size.

2.3. High pressure fibre optic flow cell

The high-pressure fibre optic flow cell was a 0.125 in. stainless steel cross cell (Autoclave Engineers, Erie, PA, USA) with an optical path length of 1.5 mm.

Two 100 cm lengths of chalcogenide-glass (AsSeTe) infrared fibres (Amorphous materials, Garland, TX, USA) were used as input and ouput fibres in order to couple the IR cell located into the SFE system to the FT-IR spectrometer.

Plano/convex AMTIR (GeAsSe) lenses (amorphous materials) were used to focus the infrared beam into the fibre and the optical path of the instrument. The diameter and the focal length of the lenses were 2 and 1.5 in., respectively.

Optimisation of the IR beam transmission through optical fibres was performed by means of two self-centering lens mounts and two translating fibre holders mounted on a small optical rail (Thorlabs, Karlsfeld, Germany). This latter component of the system was fixed on a Bruker's QuickLock baseplate (Bruker, Ettlingen, Germany) located into the sample compartment of the FT-IR spectrometer.

2.4. Apparatus

An Ultra Centrifugal Mill ZM 100 (Retsch, Germany) was used to grind and sieve plant samples.

Supercritical fluid extractions were performed using a stainless steel vessel of 15 mL in a system designed by Separex (Champigneulles, France).

The quantification of tagitinin C was done using a Tensor 27 Fourier Transform Infrared spectrophotometer equipped with a liquid nitrogen-cooled Mercury Cadmium Telluride (MCT) detector (Bruker, Ettlingen, Germany).

The spectra acquisition and the data manipulation were carried out using OPUS, OPUS CHROM and OPUS 3D software from Bruker.

The validation was processed using the e·noval internet validation package (Version 1.1a; Arlenda, Liège, Belgium).

2.5. Experimental procedures

2.5.1. Quantification of tagitinin C by FT-IR

The background for all spectra, unless otherwise stated, was obtained through the atmospheric pressure. All spectra were the average of 16 co-added scans collected from 4000 to 900 cm^{-1} at 8 cm^{-1} resolution. An apodization function was used to bring the interferogram smoothly down to zero at the edges of the sample region. Unfortunately, the sidelobes suppression causes