Supercritical Fluid Extraction Directly Coupled with Reversed Phase Liquid Chromatography for Quantitative Analysis of Analytes in Complex Matrices

by

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(ABSTRACT)

The purpose of this research was to design a simple, novel interface for on-line coupling of Supercritical Fluid Extraction (SFE) with High Performance Reversed Phase Liquid Chromatography (HP-RPLC), and to explore its ability for quantitative analysis of analytes in different matrices. First, a simple interface was developed via a single one six-port injection valve to connect the SFE and LC systems. A water displacement method was utilized to eliminate decompressed CO_2 gas in the solid phase SFE trap and connection tubes. To evalute this novel hyphenated system, spiked polynuclear aromatic hydrocarbons (PAHs) in a sand matrix were used as target analytes with the achievement of quantitative results. Also PAHs in naturally contaminated soil were successfully extracted and quantitatively determined by this hyphenated system. Compared to the EPA method (Soxhlet extraction followed by GC-MS), on-line SFE-LC gave precise (4-10% RSD) and accurate results in a much shorter time.

Based on this hyphenated technique, a method for the extraction and analysis of hyperforin in St. John's Wort was developed under air/light free conditions. Hyperforin is a major active constituent in the antidepression herbal medicine — *Hypericum Perforatum* (St. John's Wort). Hyperforin is very sensitive to oxygen and light. There is no way to date to determine whether any degradation occurs during the sample-processing step in the analytical laboratory. On-line coupling of SFE-LC with UV absorbance/ electrospray ionization mass spectrometry (SFE-LC-UV/ESI-MS) provided

an air/light free extraction-separation-detection system, which addressed this issue. Mass spectral data on the extract confirmed the presence of the major degradation compounds of hyperforin (i.e. furohyperforin and two of its analogues). Thus, the degradation process must have occurred during plant drying or storage. The feasibility of quantitative extraction and analysis of hyperforin by on-line SFE-LC was made possible by optimizing the extraction pressure, temperature, and modifier content. High SFE recovery (~90%) relative to liquid-solid extraction was achieved under optimized conditions.

We then extended the interface's application to an aqueous sample by using a liquid-fluid extraction vessel. Quantitative extraction and transfer were achieved for the target analytes (progesterone, phenanthrene, and pyrene) spiked in water, as well as in real samples (urine and environmental water). During each extraction, no restrictor plugging was realized. Extraction temperature and pressure were optimized. Different amounts of salt were added to the aqueous matrix to enhance ionic strength and thus extraction efficiency. Methanol and 2-propanol were used as CO_2 modifiers. Two modifier modes were compared, e.g. dynamically mixing modifier with the CO_2 extraction fluid, and pre-spiking modifier in the extraction vessel. Surprisingly, we found pre-spiking the same amount of modifier in the vessel enhanced the recovery from ~70% to ~100% for progesterone, phenanthrene, and pyrene due to a "co-extraction effect".

The last phase of our work explored the disadvantages/limitations of this hyphenated technique through the analysis of more highly polar phenolic compounds in grape seeds. Five types of SFE trapping adsorbent materials were evaluated in an effort to enhance the collection efficiency for the polar components. Pure supercritical CO_2 was used first to remove the less polar oil in the seeds. Then methanol-modified CO_2 was

used to remove the polar components (e.g. phenolic compounds). Catechin and epicatechin (90%) were exhaustively extracted out of the de-oiled seed after 240 minutes with 40% methanol as modifier. Both singly linked (B-type) and doubly linked (A-type) procyanidins were identified by LC-ESI-MS, as well as their galloylated derivatives. Compared to the off-line SFE-LC approach, much less sample was required for extraction in the on-line method, since all the extracted components could be transferred to the LC column. Also, no extract processing/concentration step was needed in the on-line method. However, in the on-line mode, some polar compounds were lost (1) during the collection step (e.g. lower trapping efficiency on a single solid SFE trap when a high percentage modifier was used) and (2) during the water rinsing step (e.g. less retention of polar compounds on C18 trap). Therefore, this hyphenated technique is less desirable for the analysis of highly polar compounds.

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