Application of supercritical fluid extraction with modified CO₂ to biomarker analysis

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1. Introduction

Nowadays, the use of quinone profiles on environmental analysis is of interest to researchers. Usually in quinone analysis, organic solvent is used to extract quinone from environmental samples. However, this method has some disadvantages such as time consuming, the use of large quantities of various organic solvents and inaccuracy of sample preparation. These reasons motivated us to employ a new extraction method using supercritical carbon dioxide (ScCO₂). In this work, we investigated the feasibility of extracting quinones from activated sludge using ScCO₂, and then studied the effect of various parameters such as pressure, temperature and time on total quinone content.

2. Experimental Method

Approximately 0.1 g of dry weight activated sludge was placed into a 1 ml extraction vessel. This vessel was equipped with two high-pressure pumps, a back pressure regulator and an oven which controlled the temperature. All operations were performed in dynamic mode at the various desired conditions, where supercritical CO₂ and methanol as modifier were continuously mixed in line and passed over the sample inside the extraction vessel. Extracted quinones were trapped, collected and separated into ubiquinones (UQ) and menaquinones (MK) by using Sep-Pak® Plus Silica cartridges. After separation step, those species of UQ and MK were analyzed by HPLC.

3. Results and Discussions

Fig. 1. shows the effect of pressure on total quinone content. As the pressure was increased, the total quinone content also increased. Effect of pressure on extraction performance is well known by the fact that the pressure increases the density and subsequently increases the solvent power of the supercritical fluid.

Fig. 2. demonstrates that temperature played an important role in quinones extraction. Total quinone content increased with increasing temperature from 25 to 55°C and then decreased with further increase of temperature to 75°C.

Fig. 3. indicates that as the time was extended, the total quinone content also increased especially during 15 minutes extraction. There was no further improvement after 15 minutes extraction.

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